European Commission

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

METCONAZOLE

Volume 3 – B.9 (PPP) – BAS 555 01 F

Rapporter Member State : Belgium
Co-Rapporter Member State : United Kingdom

Version History

When	What
January 2004	Draft Assessment Report (DAR) – prepared by RMS BE in the context of the inclusion of the a.s. in Annex I to Council Directive 91/414/EEC. A revised version of the initial DAR was issued in December 2004. Addenda to the initial DAR were issued in August 2004, January 2005 and September 2005.
January 2018	Draft Renewal Assessment Report (DRAR) – prepared by RMS BE in the context of the application for renewal of approval of the a.s. according to Reg (EU) No 844/2012. *Note: The DRAR is a stand-alone document containing the evaluations already displayed in the original DAR dated January 2004, as well as the new assessments. The revision of the initial DAR has been done in accordance with SANCO/10180/2013 rev.1 (March 2013), with changes in the original text – resulting from assessment of new studies (or reconsideration of old studies or studies that were not yet previously peer-reviewed) – being highlighted by means of yellow shading.*

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

Introduction

Metconazole has previously been evaluated as a plant protection product and was included in the Annex I of the Council Directive of 15 July 1991 concerning placing of plant protection products on the market (91/414/EEC) in 2005. This active substance is an approved active substance under Regulation (EC) 1107/2009 (repealing Commission Directive 91/414/EEC) as specified in Commission Implementing Regulation (EU) No. 540/2011 of 25 May 2011. A draft assessment report (DAR) on metconazole (January 2004) is available.

In this report new data for the renewal of the approval of metconazole has been evaluated only. Studies and investigations already assessed within the EU DAR (2004) have been re-evaluated in this report. The conclusions have been updated to meet current scientific standards.

This document has been drafted for the application for renewal of the registration of metconazole under Commission Regulation (EU) 844/2012. This document reviews the ecotoxicological properties, including additional data and risk assessments, for metconazole. Changes as compared to the first version are highlighted by means of yellow shading, in order to facilitate the lecture and to draw the attention to parts which were re-assessed by the RMS.

The European Commission review report for metconazole (SANCO/10027/2006 final, dated 23 May 2006), and in particular background documents A, B and C to the review report are considered to provide the relevant review information.

Where appropriate this document refers to the Annex I Inclusion Directive for metconazole (Directive 2006/74/EC of 21 August 2006).

This document covers hazard and risk assessments which were not part of the original dossier and which are necessary to reflect changes

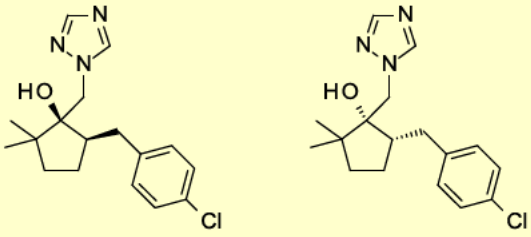
- In requirements under Commission Regulation (EU) No 283/2013, and the associated Annex, which repeals Commission Regulation (EU) No 544/2011 which, under Regulation (EC) 1107/2009, replaced the requirements of Annex II to Directive 91/414/EEC;
- in scientific and technical knowledge since the first inclusion;
- to representative uses.

The use pattern evaluated under the previous EU review of metconazole is included in Appendix 1 of the updating statement. The use pattern for evaluation for renewal of the registration is provided in Volume 1.

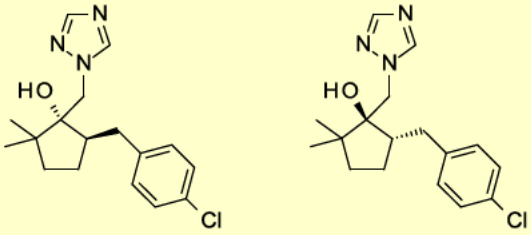
Metconazole is a triazole fungicide which is used in a broad range of crops for the control of a broad range of important pathogens. Metconazole is active against different fungal stages both on the plant surface and in the plant tissue. After application to the plant, the active ingredient is taken up via the leaf and then translocated via the transpiration flow. Due its mobility, it shows systemic and translaminar activity. By that, it can control fungal stages which have already become established in deeper tissue layers. Metconazole is thus suitable for preventative and curative treatments. Furthermore, metconazole is used a plant growth regulator in oilseed rape.

The primary mode of action of metconazole is the blocking of ergosterol biosynthesis through inhibition of cytochrome P450 sterol 14α-demethylase (CYP51). The depletion of ergosterol and accumulation of non-functional 14 α -methyl sterols results in inhibition of growth and cell membrane disruption. Because of the mode of action triazoles belong to the demethylation inhibitors (DMI). DMIs and morpholines together are named sterolbiosynthesis inhibitors (SBI).

The structural formula of the cis- and trans-isomers of metconazole is shown below:



cis-isomers
Reg.no. 4079468



trans-isomers
Reg.no. 4079654

This document reviews the ecotoxicology section of the **formulation BAS 555 01 F** (marketed under the trade name Caramba 90), an emulsifiable concentrate (EC) formulation containing 90 g/L of the active substance metconazole. The intended uses for the renewal approval are summarized in Table B.9-1 below.

Table B.9-1: Use pattern of BAS 555 01 F for use as foliar spray, considered in the risk assessment.

Crop	Application time (BBCH growth stage)	Max. number of applications	Interval between applications [d]	Application rate per treatment	
				Metconazole [kg a.s./ha]	BAS 555 01 F [L/ha]
Cereals (winter/spring)	30 - 69	1 - 2	21	0.090	1.0
Winter oilseed rape	13 – 20 (autumn) 21 – 71 (spring) [1)]	1 - 2 [2)]	min. 90 [2)]	0.072	0.8
	21 – 71 (spring)	1 - 2	14	0.072	0.8

[1)] *According to the GAP a first application in autumn (between BBCH 13 and BBCH 20) is followed by a second application in spring (between BBCH 21 and BBCH 71)*

[2)] *According to the GAP a minimum interval of 90 days should be considered. However, for calculation of the PEC$_{SW}$ values used throughout this volume, an interval of 150 days was used, which is assumed to account for the interval between the first application in autumn and the second application in spring.*

The ecotoxicological properties and risk assessment of the formulation BAS 555 01 F, the active ingredient metconazole and its metabolites, based on this use pattern, are evaluated in this document. For this purpose, ecotoxicological endpoints, generated from tests with the active substance and/or the formulated product, and metabolites, will be compared with predicted environmental concentrations estimated to arise following the use of BAS 555 01 F as a fungicide against a wide range of pathogens.

According to the specification of purity of the active substance metconazole (please refer to Volume 3, Section B.1 and Volume 4, Section C.1.2), the minimum content of metconazole is 940 g/kg (sum of *cis*- and *trans*-isomers) with a *cis*-metconazole level of not less than 800 g/kg. This is equivalent to an isomeric composition of 85:15 *cis:trans*. The majority of the studies with the active substance were performed with metconazole 85:15 *cis:trans*. However, in some studies with aquatic organisms, birds and other terrestrial vertebrates, test material with a different isomer ratio (95% *cis*) was used. As the toxicity results for the respective species were all in the same range, independent of the test material isomeric ratio, there are no indications that there are differences in toxicity between metconazole 85:15 *cis:trans* and 95% *cis*. Therefore, all available studies with metconazole are considered relevant and are taken into account in the risk assessment.

Several studies are available in which a potential shift in isomeric composition in soil, water and sediment were investigated (please refer to Volume 3 (CA), Section B.8 for details). In the aerobic soil metabolism study by Dalkmann P. and Kibat H. (2015; CA7.1.1.1/01) and the soil photolysis study by Knight L.(2015b; CA7.1.1.3/01), no shift in isomeric ratio *cis:trans* was observed during the incubation period. Similarly, in the water-sediment study by Knight L. (2015c; CA7.2.2.3/01), there was no change in enantiomer ratio of metconazole during the whole 99 day incubation period. Consequently, the available toxicity studies with the active substance metconazole are relevant for the respective compartments.

The studies to determine the ecotoxicological endpoints for the formulation BAS 555 01 F were performed with either BAS 555 01 F or BAS 555 00 F. Both formulations differ in active substance content (90 g a.s./L for BAS 555 01 F and 60 g a.s./L for BAS 555 00 F) and composition of the co-formulants. For details on the composition of the different formulations, reference is made to Volume 4, Section C.1.3.2. The studies performed with BAS 555 00 F were higher tier studies either with bees or earthworms. Based on a comparison of available laboratory toxicity data for BAS 555 01 F and BAS 555 00 F, studies with the latter formulation are therefore considered to be representative for formulation BAS 555 01 F. For details, reference is made to Section B.9.6.1.2 and B.9.8.1. Details on the specific batches and formulations used in the different studies are provided in Section 9.15. For details on the composition of the different formulations, reference is made to Volume 4, Section C.1.3.2.

The metabolites of metconazole which require ecotoxicological assessment are listed in Table B.9-2.

Table B.9-2: Metabolites of metconazole which require ecotoxicological assessment

Compartment	Metabolites
Soil	M555F020 (1,2,4-triazole)
Groundwater	M555F020 (1,2,4-triazole)
Surface water	M555F013 cis, M555F020 (1,2,4-triazole)
Sediment	M555F013 cis, M555F020 (1,2,4-triazole)

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

B.9.1.1. Effects on birds

B.9.1.1.1. Acute oral toxicity to birds

A standard guideline study with the representative formulation BAS 555 01 F is not available. However, BAS 555 01 F was tested within an investigative study that aimed to evaluate the toxicity of a tank mix of BAS 555 01 F and the formulation BAS 507 01 F, in direct comparison to the two underlying products. A summary of this study is included below.

Report:	**CP10.1.1.2/01. [redacted] (2008)** **Mixture of BAS 507 01 F and BAS 555 01 F - Acute toxicity in the bobwhite quail (*Colinus virginianus*) after single oral administration (LD_{50})**
Report No.:	2008/1032655
Guidelines:	EPA 71-1, EPA 850.2100, EPA 540/9-82-024, EPA 540/9-85-007, EPA 712-C-96-139
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and methods:	
Test substance:	- BAS 555 01 F (containing 84.6 g/L of the active substance BAS 555 F; Batch no.: 200002) - BAS 507 01 F (containing 49.0 g/L of the active substance BAS 480 F and 137.4 g/L of the active substance BAS 505 F; Batch no.: 350084) - Tank mix of BAS 507 01 F and BAS 555 01 F (mixture of 30 g BAS 507 01 F and 20 g BAS 55501 F)
Test species:	Bobwhite quail (*Colinus virginianus*)
Sex, weight, age:	5 male and 5 female birds per test group (40 birds in total); mean body weight per treatment between 182.68 and 189.02 g; before first egg-laying season, approximately 6 months old at dosing
Applied concentrations:	0 (control) and 2000 mg formulation/kg bw (nominal concentrations)
Preparation of the birds:	birds were acclimated for 21 days. The acclimation period was followed by an adaptation period to the test cages of 14 days, including a fasting period of about 27 to 29 h, prior to dosing.
Type of application:	the formulations were dosed directly without carrier. The control group were dosed with drinking water. The birds were administered the test substance once by gavage into the crop in a total amount of 200 mg of the respective formulation per kg body weight
Time of exposure:	Single application, monitoring during 14 days after dose administration.
Housing conditions:	stainless steel wire mesh cages (length 0.59 x width 0.45 x height 0.26 m) with wire mesh floors (mesh size 10 x 15 mm), floor area about 0.27 m^2 for up to 5 birds. Males and females were caged separately.
Test conditions:	Temperature: 21.2 ± 0.5 °C

	Relative humidity: 52 ± 5% Photoperiod: 8:16 hours light:dark
Light intensity:	4-9 lux, produced by warm-light fluorescent lamps (the light intensity was adjusted to these comparably low values to avoid aggressive behaviour of the birds)
Analytical method:	no analytical determinations of the test substance in the carrier were necessary since the test substance was applied without carrier

Test procedure:
After dosing, the birds were observed for regurgitation for at least 1 hour after dosing. No bird regurgitated parts of the test substance. An observation period of 14 days followed, during which the following observations were made: the birds were monitored 3 times on the day of dosing for mortality and clinical signs, and daily thereafter. The mean food consumption was calculated from the weekly food consumption per cage. The body weight was monitored on days 7 and 14 after dosing. Gross post-mortem examination was performed on birds that died during the study and all birds sacrificed at the end of the study.

Statistics: No statistical calculation of the LD_{50} was performed since only one dose was tested. Food consumption was not examined statistically, since the food consumption was evaluated only per cage not per animal. Body weights were examined using the Dunnett-Test. The statistical evaluation was performed, using the INSTEM-Toxicology-data system.

Findings:
In the test group 1 (2000 mg BAS 555 01 F/kg body weight) 2 males and 4 females died on day 1 and 2. Liquid stools were observed up to 5 days after dosing and furthermore apathy was seen in 3 males and 4 females in this test group. In the first week after dosing the food consumption in males and females was decreased by 57% and 54%, female food consumption recovered during the second week, so that there was no difference compared to the control. The mean food consumption of the males was decreased to 80% of the control over the whole 14 day period. Due to the low number of surviving animals the body weights of group 1 could not be evaluated statistically. The post mortem examination showed in 4 cases a liquid content of the gut, one of these animals showed also an abnormal coloration of the liver compared with an erosion of the glandular stomach.

In the test group 2 (2000 mg BAS 507 01 F/kg body weight) no specimen died. No substance related effects were observed in this test group regarding clinical signs. No effects were observed regarding food consumption, compared to the control during the whole test period. The body weight of this treatment group was in a normal range of the control group (181.5 g – 197.4 g). After the 14 days test period the post-mortem-examination showed no substance-related effects.

The test group 3, exposed to the tank-mix of 30 g BAS 507 01 F + 20 g BAS 555 01 F with a dose of 2000 mg tank mix/kg body weight, caused no mortality. Liquid stools were observed until day 3 after dosing. Food consumption was decreased to 60% and 77% of the control groups in the first week after dosing in males and females, respectively. A significant reduction was apparent for males only. During the second week the food uptake totally recovered, so that there was no observed difference to the control. After the 14 days test period the post-mortem-examination showed no substance-related effects.

Table B.9.1.1.1-1: Acute toxicity of the single formulations BAS 555 01 F, BAS 507 01 F and the tank mix BAS 555 01 F + BAS 507 01 F to bobwhite quail

Group No.	Dose (mg/kg b.w.)	Mortality (males)	Mortality (females)	Mortality (total)	LD_{50} [mg/kg b.w.]
0 (control)	0	0 / 5	0 /5	0 / 10	--
1 (BAS 555 01 F)	2000	2 / 5	4 / 5	6 / 10	< 2000
2 (BAS 507 01 F)	2000	0 / 5	0 / 5	0 / 10	> 2000
3 (Mixture of 30 g BAS 507 01 F + 20 g BAS 555 01 F)	2000	0 / 5	0 / 5	0 / 10	> 2000

b.w. = body weight

Table 9.1.1.1-2: Mean food consumption and mean body weight of Bobwhite quail (*Colinus virginianus*) for the different test items in the acute toxicity test with BAS 555 01 F, BAS 507 01 F and the tank mix BAS 555 01 F + BAS 507 01

Group No.	Dose (mg/kg b.w.)	Mean food consumption (g/bird/day)				Mean body weight (g)					
		Day 1 to 7		Day 8 to 14		Day 0		Day 7		Day 14	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
0 (control)	0	12.5	11.8	11.9	11.1	184.5	181.50	196.18	188.58	197.36	190.40
1 (BAS 555 01 F)	2000	5.32	5.4	14.1	15.8	189.02	181.18	179.20	182.20	188.0	189.90
2 (BAS 507 01 F)	2000	11.3	10.8	13.4	12.4	186.80	180.02	193.28	182.20	194.32	188.86
3 (Mixture of 30 g BAS 507 01 F + 20 g BAS 555 01 F)	2000	7.5	9.1	14.2	12.7	182.68	177.94	171.76	17.76	182.96	183.24

Conclusions:
In an acute toxicity test with bobwhite quail, the 14-day LD_{50} was > 2000 mg tank mix BAS 507 01 F + BAS 555 01 F /kg body weight, < 2000 mg formulation/kg body weight for BAS 555 01 F and > 2000mg formulation/kg body weight for BAS 507 01 F. The NOEL values were determined as < 2000 mg formulation/kg body weight for BAS 555 01 F, ≥ 2000mg formulation/kg body weight for BAS 507 01 F and < 2000 mg tank mix BAS 507 01 F + BAS 555 01 F /kg body weight.

RMS comments:
The study was carried out in agreement with the validity criteria of the US-EPA 71-1 test guideline, and is therefore considered acceptable for use in the risk assessment.

Due to the purpose of the study, no definite endpoint was derived for the BAS 555 01 F. However, considering that for this formulation at 2000 mg product/kg bw a mortality of 60% was observed, a LD50 < 2000 mg product/kg bw was determined. Taking into account that no mortalities occurred for the mixture of BAS 555 01 F and BAS 507 01 F, an LD_{50} for the mixture was set at > 2000 mg mixture/kg bw. As this mixture contained 40% BAS 555 01 F, it is reasonable to assume that the LD_{50} for BAS 555

01 F will be > 800 mg product/kg bw. Consequently, the following will be considered in the risk assessment:

800 mg formulation/kg bw (equivalent to 64.8 mg a.s./kg bw) < LD_{50} (*Colinus virginianus*) < 2000 mg formulation/kg bw (equivalent to 162 mg a.s./kg bw)

NOEL (*Colinus virginianus*) < 2000 mg formulation/kg bw (equivalent to < 162 mg a.s./kg bw)

B.9.1.1.2. Short-term dietary toxicity and reproductive toxicity to birds

No studies with the representative formulation are available. Reference is made to Volume 3 (AS), Section B.9.1.1 for the toxicity endpoints for the active substance metconazole.

B.9.1.2. Effects on terrestrial vertebrates other than birds

Mammalian acute oral, short-term dietary and long-term reproduction studies have been carried out with metconazole, and are summarized in Volume 3 (AS and PPP) Section B.6 on Mammalian toxicology. An overview of the studies that are relevant for the ecotoxicological risk assessment is given in Table B.9.2.2.1-1 and Table B.9.2.2.1-2 in Section B.9.2.2.1.

The applicant submitted a position paper in which the available chronic toxicity endpoints are discussed, and an endpoint for use in the chronic risk assessment for mammals is derived. This position paper is summarized below.

Report:	**CP10.1.2.2/01. Anonymous (2015)** **BAS 555 F - Ecologically relevant chronic toxicity endpoint for the wild mammalian reproductive risk assessment**

Report No.: 2015/1192591
Guidelines: None
GLP: No

Previous evaluation: None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application

Objective:
The current EU accepted endpoint for risk assessment for wild mammals is the NOAEL of 4 mg/kg bw/d. This endpoint is based on rabbit developmental toxicity data. The aim of this work is to propose a refined wild mammal reproductive risk endpoint for Metconazole according to EFSA Guidance on Risk Assessment for Birds and Mammals (EFSA 2009) of 8 mg/kg bw/d based on the overall lowest ecologically relevant NOAEL, seen in the rat reproduction study ([redacted] 1992a).

Executive summary:
In the current document, all critical reproduction and developmental toxicity studies are evaluated with the special focus on the ecological relevance of the findings.

In two rat 2-generation reproduction toxicity studies ([redacted] 1992a; [redacted], 2002), similar effects including prolonged gestation time, increased number of late embryonic deaths as well as body weight effects were observed at the higher doses. The overall lowest NOAEL of these studies is 8 mg/kg bw/d. The observed effects on reproduction may lead to population effects and are therewith of potential ecological relevance. This is in line with the evaluation stated in the DAR 2005. Noteworthy, such two-generation rat studies using dietary dosing regimen are considered to more closely represent exposure to wild animals in natural conditions than the gavage administration employed in the rat and rabbit developmental studies. In the wild, mammals are exposed to residues of plant protection products via their food. Hence, the twogeneration rat study allows evaluating potential trans-generational effects on population relevant parameters under a realistic dietary route of exposure, while gavage dosing is known to result in high systemic levels that can induce adverse effects not observed via dietary exposure (EFSA/2009/1438).

In three rat developmental toxicity studies ([redacted] 1991b; [redacted] 1992b; [redacted], 2002), similar effects including increased post-implantation loss, increased resorptions, skeletal anomalies and maternal toxicity showing body weight effects and clinical signs were observed at the higher doses. A

slight developmental effect, the increase of bilateral dilatation of the ureter observed in one study at 24 mg/kg bw/d, could not be confirmed during microscopical examination, by Wilsons serial sectioning, which is considered to be the superior method. In the DAR this observation was described as "barely perceptible". Hence, this effect is not considered to be of ecotoxicological relevance as this equivocal change is unlikely to have an effect on population development and reproduction. Therewith the overall lowest NOAEL of the rat developmental toxicity studies is 12 mg/kg bw/d.

In four rabbit developmental toxicity studies ([redacted] 1991a, [redacted] 1992a, [redacted] 1992b; [redacted] 1997, similar effects including maternal and foetal body weight effects, increased resorptions and late embryonic deaths were observed at the higher doses. Increased embryonic death and post-implantation loss, maternal body weight loss as well as an increased incidence of hydrocephaly at 10 mg/kg bw/d led to the EU accepted endpoint of 4 mg/kg bw/d. However, the observed maternal body weight loss was only transient and general less than 10% and the foetal changes were only slight and not statistically significant (increased late embryonic deaths: 1.0 compared to 0.5 in the control; increased post-implantation loss: 21.4% compared to 15.5% in the control). Hence, these findings at 10 mg/kg bw/d are not considered to be ecologically relevant as they are unlikely to have a notable effect on population development and reproduction. Furthermore, the increased incidence of hydrocephaly at 10 mg/kg bw is considered equivocal as it does not show a clear dose-response relationship (see and lies often within historical control ranges. An increased incidence of visceral/skeletal anomalies and hydrocephaly was observed at doses above 10 mg/kg bw/d. However, at 10 mg/kg bw/d only a low incidence of hydrocephaly was seen: 4 out of 714 foetuses from 3 out of 5 studies. When all available studies are considered, including preliminary studies and comparative studies, confirmed hydrocephaly was noted at 10 mg/kg bw in 4 out of 9 studies with 5 out of 1058 foetuses affected. During the EU review process the notifier proposed that the occurrence of spurious malformations in the older studies ([redacted] 1991a, 1992a and 1992b), even at low doses or in the controls, without similar findings at higher doses, resulted from a high spontaneous rate of malformation in the animals of this source at this time period. It was also suggested that these animals were of suboptimal health, as indicated by the high variability in pregnancy rate, maternal mortality, and abortion. It was further noted that the most recent study ([redacted] 1997) showed no developmental toxicity. Overall, it is highly unlikely that the low and equivocal incidence of hydrocephaly seen at 10 mg/kg bw/d would lead to population effects and therefore is not considered to be of ecological relevance. Hence, an overall lowest, ecologically relevant NOAEL of 10 mg/kg bw/d based on the rabbit developmental studies is considered justified.

Table 9.1.2-1: Rabbit developmental studies – incidence of hydrocephaly: number of litters (foetuses) affected

Report author and year BASF Report code	Code Isomer	Dose level (mg a.s./kg bw/day)										
		0	0.5	1	2	4	5	10	20	25	40	62.5
[redacted] 1997 MK-432-015	WL148271 *cis/trans*	0	-	-	-	-	0	1(1)	0	-	0	-
[redacted] 1991a MK-432-003 (main study)	WL148271 *cis/trans*	0	-	-	-	1(1)	-	0	-	4(4)	-	0
[redacted] 1991a MK-432-003 (additional study)	WL148271 *cis/trans*	0	-	-	0	0	-	2(2)	-	-	-	-
[redacted] 1992a MK-432-007	WL136184 *cis*	0	-	-	0	0	-	0	-	-	3(3)	-
[redacted] 1992b MK-432-010	WL136184 *cis*	1(1)	0	1(1)	0	-	-	1(1)	-	-	1(1)	-

- : test dose level not investigated; 0: zero incidence

Comparing the NOAEL of reproduction and developmental toxicity studies, the overall lowest ecologically relevant **NOAEL is 8 mg/kg bw/d** based on the rat reproduction study (Willoughby, 1992a) using the realistic dietary route of exposure. The endpoint of 8 mg/kg bw/d is considered justified to be used for the long-term risk assessment for wild mammals.

RMS comments:
The position paper provides a comprehensive overview of the main results of the available 2-generation reproduction studies with rats and the developmental toxicity studies with rats and rabbits.

It is noted that the rabbit developmental toxicity study by [redacted] (1990) was not included in the discussion. This was however a preliminary study, in which only doses of 10 mg a.s./kg bw/day and higher were tested. In the position paper, it is stated that 'Preliminary studies that do not provide additional information were not included'. Based on the doses tested and the results of the study by [redacted] (1990), RMS agrees that not including this study in the discussion does not influence the outcome of the assessment.

Whether the argumentation provided by the applicant to demonstrate that the endpoints are (not) ecologically relevant is considered acceptable is discussed in Section B.9.2.2.1.

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

B.9.2.1. Risk assessment for birds

The risk assessment for effects on birds has been updated according to the latest **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)**[1].

B.9.2.1.1. Toxicity

A study on the acute toxicity of the representative formulation BAS 555 01 F to birds is available and was summarized in Section B.9.1.1.1. For the toxicity endpoints for the active substance metconazole, reference is made to Volume 3 (AS), Section B.9.1.1. All available endpoints are summarized in Table B.9.2.1.1-1 below.

Toxicity of metconazole:

Avian acute oral, short-term dietary and reproduction studies have been carried out with metconazole. Short-term dietary toxicity data are not routinely used for an assessment according to the EFSA Guidance Document on birds and mammals (2009). However, according to the EFSA Guidance Document, when the mode of action and/or results from mammalian studies indicated a potential for the dietary LC_{50} measured by a short-term study to be lower than the LD_{50} based on an acute oral study, the dietary studies should be considered. If the dietary LC_{50} is lower than the acute gavage LD_{50}, then the dietary LC_{50} should be used in the acute risk assessment. For metconazole, there are no direct indications based on the mode of action or results from the mammalian studies that there is a potential for the dietary LC_{50} to be lower than the acute LD_{50}. Nevertheless, dietary toxicity studies are available for Bobwhite quail (*Colinus viginianus*) and Mallard duck (*Anas platyrhynchos*). As the available acute toxicity studies were all performed with Bobwhite quail, only for this species a comparison between the LD_{50} and LC_{50} can be made. The LC_{50} for Bobwhite quail is 167.85 mg a.s./kg bw/day, which is lower than the lowest available LD_{50} for this species of 787 mg a.s./kg bw. However, in the dietary toxicity study ([redacted] 1991a; CA8.1.1.2/01), the majority of the deaths occurred only after 3 to 5 days. This indicates that, when exposed to a dose equal to the LC_{50}, the tested birds would have to ingest 3 to 5 times the daily dose of 167.85 mg a.s./kg bw/day for mortality to occur, which corresponds to a total dose of 503.55 to 839.25 mg a.s./kg bw. This total dose is in the same range as the LD_{50} from the acute toxicity study. Therefore, it is considered not necessary to base the acute risk assessment for birds on the dietary LC_{50} instead of the acute LD_{50}. Only the acute and reproduction endpoints will thus be used in the present risk assessment.

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

Table B.9.2.1.1-1: Summary of endpoints for birds

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Bobwhite quail (*Colinus virginianus*)	BAS 555 F (metconazole) 83:17 *cis:trans*	Single dose (Acute oral toxicity)	LD_{50} NOEL	**787 mg a.s./kg bw** 423 mg a.s./kg bw	CA8.1.1.1/01 [redacted] 1992a
Bobwhite quail (*Colinus virginianus*)	BAS 555 F (metconazole) 85:15 *cis:trans*	Single dose (Acute oral toxicity)	LD_{50} NOEL	**798 mg a.s./kg bw** < 450 mg a.s./kg bw	CA8.1.1.1/02 [redacted] 1998
Bobwhite quail (*Colinus virginianus*)	BAS 555 F (metconazole) 95% *cis*	Single dose (Acute oral toxicity)	LD_{50} NOEL	**875 mg a.s./kg bw** < 450 mg a.s./kg bw	CA8.1.1.1/02 [redacted] 1998
Bobwhite quail (*Colinus virginianus*)	BAS 555 01 F	Single dose (Acute oral toxicity)	LD_{50} NOEL	**800 mg product/kg bw < LD_{50} < 2000 mg product/kg bw** **(64.8 mg a.s./kg bw < LD_{50} < 162 mg a.s./kg bw)** < 2000 mg a.s./kg bw (< 162 mg a.s./kg bw)	CP10.1.1.2/01 [redacted] 2008
Bobwhite quail (*Colinus virginianus*)	BAS 555 F (metconazole) 83:17 *cis:trans*	5 days (Short term dietary toxicity)	LC_{50} NOEL	1057 mg a.s./kg diet (167.85 mg a.s./kg bw/d) < 163 mg a.s./kg diet (<25.88 mg a.s./kg bw/d)	CA8.1.1.2/01 [redacted] 1991a
Mallard duck (*Anas platyrhynchos)*	BAS 555 F (metconazole) 83:17 *cis:trans*	5 days (Short term dietary toxicity)	LC_{50} NOEL	> 5200 mg a.s./kg diet (> 537.93 mg a.s./kg bw/d) 1300 mg a.s./kg diet (300.42 mg a.s./kg bw/d)	CA8.1.1.2/01 [redacted] 1991b
Mallard duck (*Anas platyrhynchos)*	BAS 555 F (metconazole) 95% *cis*	24 weeks (Sub chronic and reproduction)	NOEL EC_{10} EC_{20}	60 mg a.s./kg diet (= 9.33 mg a.s./kg bw/d) NE NE	CA8.1.1.3/02 [redacted] 1992c
Bobwhite quail (*Colinus virginianus*)	BAS 555 F (metconazole) 83:17 *cis:trans*	22 weeks (Sub chronic and reproduction)	NOEL EC_{10} EC_{20}	60 mg a.s./kg diet **(= 6.19 mg a.s./kg bw/d)** NE NE	CA8.1.1.3/03 [redacted] [redacted] 1999

Note: ND – could not be determined; NE – not estimated; ***bold*** *– endpoint used for the current risk assessment*

Two acute toxicity studies for birds are available ([redacted] 1992a, CA8.1.1.1/01; [redacted] 1998; CA8.1.1.1/02), in which the acute toxicity of metconazole to Bobwhite quail was tested for different isomeric compositions of the active substance. Based on the results from these studies, there was no difference in acute toxicity of metconazole 83:17 *cis:trans*, metconazole 85/15 *cis:trans* and metconazole 95% *cis*. According to the EFSA Guidance Document (2009) (Section 2.4), in cases where more than one acute study on the same species is available, it is permissible to use the geometric mean of all available endpoints for the acute assessment, except when the most sensitive endpoint is more than a factor of 10 below the geometric mean of all endpoints. As shown in Table B.9.2.1.1-2, an overall **geomean LD_{50} of 819 mg a.s./kg bw** can be calculated based on the available acute endpoints for Bobwhite quail. As the most sensitive endpoint (787 mg a.s./kg bw) is not more than a factor 10 below the geomean LD_{50}, the geomean endpoint is acceptable for use in the risk assessment.

Table B.9.2.1.1-2: Calculation of the relevant avian toxicity endpoint for the acute risk assessment of metconazole

Reference	**CA8.1.1.1/01** [redacted] **1992a**	**CA8.1.1.1/02** [redacted] **1998**	**CA8.1.1.1/02** [redacted] **1998**
Test species	*Colinus virginianus*	*Colinus virginianus*	*Colinus virginianus*
Test substance	metconazole 83:17 *cis:trans*	metconazole 85:15 *cis:trans*	metconazole 95% *cis*
Experimentally obtained LD_{50} [mg a.s./kg b.w.]	787	798	875
Birds tested/group [no.]	10	10	10
Mortality at highest dose level [%]	100	100	100
Overall geometric mean LD_{50} [mg a.s./kg b.w.]	819		

Two studies investigating the sub-chronic and reproductive toxicity of metconazole to birds are available. Both for Bobwhite quail and Mallard duck, a NOEC of 60 mg a.s./kg diet was derived. Based on data on the food consumption and the bodyweight of the birds, this NOEC was converted to a NOEL of 6.19 mg a.s./kg bw/day for Bobwhite quail and a NOEL of 9.33 mg a.s./kg bw/day for Mallard duck. In line with the EFSA Guidance Document (2009), the overall lowest **NOEL of 6.19 mg a.s./kg bw/day** will be used in the risk assessment.

Toxicity of the representative formulation BAS 555 01 F:

For birds, no definitive/standard guideline study with the formulated product BAS 555 01 F is available. However, the product was tested within an investigative study done for France ([redacted] 2008; CP10.1.1.2/01) that aimed to evaluate the toxicity of a tank mix of BAS 555 01 F and BAS 507 01 F, in direct comparison to the two underlying products. For this purpose, the mix as well as the two products were tested separately, but only a single dose was tested (limit dose of 2000 mg mix or product /kg bw). In case of the tank mix and BAS 507 01 F no mortalities were observed. In case of BAS 555 01 F six of ten animals died at limit dose. Consequently, a $LD_{50} < 2000$ mg product/kg bw was determined for BAS 555 01 F. Due to the different purpose of the study, no definite endpoint was derived. However, considering the findings for the product at 2000 mg product/kg bw, a mortality of 60% (6/10 animals died) was observed. It is therefore reasonable to assume that the LD_{50} is close to 2000 mg/kg bw. This assessment is supported by the results for the mixture. Considering an $LD_{50} > 2000$ mg mixture/kg bw for a mixture that contains 40% BAS 555 01 F, it is reasonable to assume the LD_{50} for BAS 555 01 F will be > 800 mg product/kg bw.

Based on the available data, the acute toxicity of BAS 555 01 F seems to be slightly higher compared to the toxicity of the active substance. Therefore, a risk assessment will be performed for BAS 555 01 F using the worst-case estimated **LD_{50}** from the study by [redacted] (2008) of **> 800 mg product/kg bw**.

Toxicity of metconazole metabolites:

In order to assess the possible relevance of metabolites in food items of wild birds and mammals, the occurrence of soil, water and plant metabolites was taken into consideration.

In the laboratory soil degradation studies summarized in Volume 3 (AS) Section B.8.1 (Fate and behaviour in soil), no metabolites exceeding 10% TAR were found. However, metabolite M555F020 (1,2,4-triazole) was appointed to an unidentified peak found in one of the available aerobic metabolism studies (Gedik and Fullard, 2002; see Volume 3 (AS) Section B.8.1.1.2.1.2). Based on this observation, **M555F020** was identified as a relevant **metabolite in soil**. In the studies summarized in Volume 3 (AS) Section B.8.2 (Fate and behaviour in water and sediment), no metabolites exceeding 10% TAR were found in water. Nevertheless, based on the available data, metabolites **M555F020 (1,2,4-triazole) and M555F013** were considered as relavant **metabolites in surface water**. Based on the above, M555F020 and M555F013 have to be considered for the environmental risk assessment. An assessment for secondary poisoning for earthworm-eating and fish-eating animals might be necessary for these metabolites.

The toxicity profile of metabolite M555F020 (1,2,4-triazole) is well investigated, and is currently under EU evaluation in the context of the request for confirmatory data for the "Triazole Derivative Metabolites". The available studies have been evaluated by RMS UK, and were summarized in a revised Addendum for the confirmatory data from May 2016 (see also EFSA, 2016[2]). Unfortunately, the assessment is not yet finalized (final conclusions are expected to be published mid 2018). However, the endpoints for 1,2,4-triazole listed in the revised Addendum indicate that this metabolites is slightly less toxic compared to the parent metconazole (e.g. endpoints for rats for 1,2,4-triazole: LD_{50} of 1648 mg/kg bw and $NOEL_{2\text{-generation}}$ = 15.4 mg/kg bw/day, compared to for mectonazole: LD_{50} > 500 mg/kg bw and $NOEL_{2\text{-generation}}$ = 8 mg/ kg bw/day). Further, this metabolite was identified in the rat metabolism studies with metconazole (summarized in Volume 3 (AS), Section B.6.1) and in the hen metabolism study (Jalal F., 2006a; see Volume 3 (AS) Section B.7.2.2 for a summary). It is therefore likely that this metabolite will have been formed in the mammalian and avian reproductive toxicity studies and hence the risk assessment for the active substance will also cover the risk from this metabolite. Finally, M555F020 is more hydrophilic than the parent metconazole, and would therefore be more rapidly excreted when ingested by animals. Although no study to identify the log P_{OW} for 1,2,4-triazole is available within the renewal dossier for metcoanzole, a value of -0.62 to -0.71 at a pH range of 5-9 is reported in the revised Addendum for the confirmatory data for the Triazole Derivative Metabolites (RMS UK, May 2016). This value still needs to be confirmed (see Volume 3, Section B.2). However, it seems that a secondary poisoning assessment is not necessary for M555F020, as according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), such an assessment is only required for substances with a log P_{OW} greater than 3.

[2] European Food Safety Authority (2016). Technical Report: Outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for triazole derivative metabolites in light of confirmatory data. EFSA Supporting publication 2016:EN-1080

For metabolite M555F013 (one of the carboxy-metabolites), no toxicity data is available. This metabolite was however identified in the rat metabolism studies with metconazole (summarized in Volume 3 (AS), Section B.6.1) and in the hen metabolism study (Jalal F., 2006a; see Volume 3 (AS) Section B.7.2.2 for a summary). It is therefore likely that this metabolite will have been formed in the mammalian and avian reproductive toxicity studies and hence the risk assessment for the active substance will also cover the risk from this metabolite. Further, the carboxy metabolite M555F013 is more hydrophilic than the parent compound metconazole, and would therefore be more rapidly excreted when ingested by animals. An experimental log P_{OW} value is not available. However, a QSAR calculation using ACD/Labs was submitted by the applicant to estimate the logD values (pH dependent logP values) for M555F013 (BASF DocID 2017/1199065; see Volume 3, Section B.2 for further details). The maximum estimated log P_{OW} was 2.31 at pH 4. Although an experimentally derived log P_{OW} is preferred over a QSAR calculation, this indicates that an assessment of the potential risk through secondary poisoning might not be required (calculated value is is below the trigger value of 3).

In the available **plant metabolism** studies performed in wheat, oilseed rape and peas, two metabolites, i.e. **M555F034** (triazolyl acetic acid) and **M555F035** (triazolyl alanine), were considered to be relevant (see Volume 3 (AS), Section B.7.2.1). These metabolites were mainly formed in seed, in quantities exceeding 10% of the TRR (M555F035) or close to 10% of the TRR (M555F034).

The toxicity profiles of M555F034 and M555F035 are well investigated. Studies on the acute and chronic toxicity of these metabolites to rats were previously evaluated on EU level, as reviewed in the DAR for the active substance epoxiconazole (Germany, March 2006) or the revised Addendum for the confirmatory data for the Triazole Derivative Metabolites (UK, May 2016). Reference is made to the these documents for the respective study summaries. In addition, toxicity studies with birds are available as well. These studies were also previously evaluated on EU level, as reviewed in the DAR of for epoxiconazole (Germany, March 2006). A summary of these studies was included in Vol. 3 (AS) Section B.9.1.1. The available metabolite toxicity endpoints for rats and birds are summarized and compared to the active substance toxicity in Table B.9.2.1.1-3. Based on these endpoints, the metabolites M555F034 and M555F035 show a significantly lower toxicity to rats and birds compared to the parent metconazole. Consequently, it can be concluded that for these two metabolites, the risk to birds is covered by the assessment for the parent metconazole.

Table B.9.2.1.1-3: Comparison of the acute and chronic toxicity endpoints for birds and mammals for metconazole and its metabolites triazolyl acetic acid and triazolyl alanine.

Substance	Test species	Endpoint [1]	Reference
Mammals - Acute oral toxicity			
Metconazole (BAS 555 F)	Rat	LD_{50} > 500 mg/kg	[redacted] 2005a
Triazolyl-acetic acid (M555F034)	Rat	LD_{50} > 5000 mg/kg	[redacted] 1984 [2] (BASF DocID 1984/100342)
Triazolyl alanine (M555F035)	Rat	LD_{50} > 2000 mg/kg	[redacted] G.R., 1980 [2] (BASF DocID 1980/1000167)
	Rat, Mouse	LD_{50} > 5000 mg/kg	[redacted] 1982 [2] (BASF DocID 1986/1000485)
Mammals – Reproductive toxicity			
Metconazole (BAS 555 F)	Rat	$NOAEL_{2\text{-}generetion}$ = 8 mg/kg bw/d	[redacted] 1992a (MKB-430-003)
Triazolyl-acetic acid (M555F034)		$NOAEL_{1\text{-}generetion}$ = 100 mg/kg bw/d	[redacted] (2010) [3] BASF DocID 2010/1225534
Triazolyl alanine (M555F035)	Rat	$NOAEL_{2\text{-}generetion}$ = 100 mg/kg bw/d	[redacted] (1986) [2] BASF DocID 1986/1000486
Birds – acute toxicity			
Metconazole (BAS 555 F)	Bobwhite quail	787 mg a.s./kg bw	CA8.1.1.1/01 [redacted] 1992a
Triazolyl-acetic acid (M555F034)	Bobwhite quail	LD_{50} > 2000 mg/kg bw	CA8.1.1.1/03 [redacted] 2003
Birds – short-term dietary toxicity			
Metconazole (BAS 555 F)	Bobwhite quail	LC_{50} > 167.84 mg/kg bw/d	CA8.1.1.2/01 [redacted] 1991a
	Mallard duck	LC_{50} > 537.93 mg/kg bw/d	CA8.1.1.2/02 [redacted] 1991b
Triazolyl alanine (M555F035)	Bobwhite quail	LC_{50} > 500 mg/kg bw/d	CA8.1.1.2/03 [redacted] [redacted] 1983
	Mallard duck	LC_{50} > 500 mg/kg bw/d	CA8.1.1.2/04 [redacted] 1983

[1]The lowest relevant endpoint from the available studies is reported; [2]Refer to the DAR of the active substance epoxiconazole (Germany, March 2006) for a study summary and evaluation; [3]Refer to the revised Addendum for the confirmatory data for the Triazole Derivative Metabolites (UK, Mat 2016) for a study summary and evaluation

B.9.2.1.2. Exposure

Exposure of birds will be predominantly dietary, through the consumption of residues on food items. Direct exposure of birds to BAS 555 01 F applications is considered unlikely, since at the time of application and for a short period thereafter, most birds will leave the immediate vicinity of spray operations in response to the human disturbance.

B.9.2.1.3. Risk assessment for metconazole

The endpoints for the active substance used in the risk assessment for birds are shown in Table B.9.2.1.3-1.

Table B.9.2.1.3-1: Summary of the endpoints used in the risk assessment for birds

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Bobwhite quail (*Colinus virginianus*)	BAS 555 F (metconazole)	Single dose (Acute oral toxicity)	LD_{50}	819 mg a.s./kg bw	Geomean endpoint calculated based on the endpoints from [redacted] (1992a) and [redacted] (1998)
Bobwhite quail (*Colinus virginianus*)	BAS 555 F (metconazole) 83:17 *cis:trans*	22 weeks (Sub chronic and reproduction)	NOEL	6.19 mg a.s./kg bw/day	CA8.1.1.3/01 Dighe R.P., 1994

The LD_{50}/10 is considered as an endpoint in the reproductive assessment to take account of the possibility of reproductive impairment due to sub-lethal effects on pair formation and breeding site selection, incubation, parental care of nestlings and survival of fledgling birds (see Appendix J – Phase-specific approach for reproductive risk assessment of the EFSA guidance document). For the screening assessment, the lowest of either the LD_{50}/10 or the NOEL from the avian reproduction studies should be considered as worst-case toxicity endpoint. For metconazole, the LD_{50}/10 (81.9 mg a.s./kg bw/day) exceeds the NOEL of 6.19 mg a.s./kg bw/day. Consequently, the NOEL is used as relevant worst-case endpoint in the risk assessment.

Acute risk assessment for birds – Screening step

The intended use patterns for the representative formulation BAS 555 01 F are shown in Table B.9-1. For the proposed use in oilseed rape, BAS 555 01 F can either be applied once in autumn (at BBCH 13-20) followed by a second application in spring (at BBCH 21-71), or can be applied two times in spring (at BBCH 21-71) with a 14-day interval. In the first case, the interval between the two applications is of such a length (min. 90 days) that both applications can be considered as independent in the context of the risk assessment for birds[3]. Taking this into account, the screening step crop grouping and critical use patterns relevant for the uses of BAS 555 01 F are given in Table B.9.2.1.3-2 . Note that for the proposed use in oilseed rape, only the worst case use rate of 2 x 0.072 kg a.s./ha is considered further in the risk assessment below. However, to cover also the single application in earlier growth stages, worst case scenarios for BBCH 13-20 are included in the calculations.

[3] The Multiple Application Factor (MAF) calculated using the equations given in Appendix H of the EFSA Guidance Document, based on a default DT_{50} of 10 days and an interval of 150 days is equal to 1.0.

Table B.9.2.1.3-2: Screening step crop groupings and critical use patterns considered in the risk assessment for the use of metconazole in BAS 555 01 F.

Crop group	GAP crop species	EU region	Application time (BBCH growth stage)	Indicator species	Critical use pattern		
					Rate (kg a.s./ha)	Max no. of applications	App. interval (days)
Cereals	Cereals	North, Central & South	30 - 69	Small omnivorous bird	0.090	2	21
Oilseed rape	Winter oilseed rape	North, Central & South	13 – 20 (autumn)	Small omnivorous bird	0.072	1	-
			21 – 71 (spring)		0.072	1	-
			21 – 71		0.072	2 [1)]	14 [1)]

[1)] *for oilseed rape, only the worst case use rate of 2x0.072 kg a.s./ha is used for the risk assessment; to cover also the single application in earlier growth stages, worst case scenarios for BBCH 13-20 were included in the calculations*

The acute 'Daily Dietary Dose' (DDD) is calculated by multiplying the shortcut value (SV) based on the 90th percentile residues by the application rate in kg a.s./ha. When more than one application is intended, this product is further multiplied with an appropriate multiple application factor for 90th percentile residue data (MAF_{90}).

$$DDD_{multiple\ applications} = application\ rate\ [kg\ a.s./ha]\ x\ SV\ x\ MAF_{90}$$

The acute risk to birds is assessed by calculation of an acute Toxicity:Exposure ratio (TER_A) according to the following equation:

$$TER_A = \frac{LD_{50}[mg/kg\ bw]}{DDD[mg/kg\ bw]}$$

The daily dietary dose (DDD) based on the above equation and TER values for the relevant indicator species for acute exposure to metconazole following the proposed uses of BAS 555 01 F are given in Table B.9.2.1.3-3 below. Shortcut values were derived from Table 6 of the EFSA Guidance Document. MAF_{90} values were calculated as described in Appendix H of the EFSA Guidance Document.

The TER_A values for all proposed uses exceed the Annex VI trigger of 10, indicating **an acceptable acute risk to birds** from exposure to metconazole following the proposed uses of BAS 555 01 F in cereals and oilseed rape.

It is noted that even when a more conservative approach is followed in the acute risk assessment for birds (i.e. the lowest available LD_{50} of 787 mg a.s./kg bw is used instead of the geomean LD_{50} of 819 mg a.s./kg bw), the calculated TER_A values at the screening step are between 50.1 and 57.4 and thus largely exceed the trigger of 10.

Table B.9.2.1.3-3: Screening step – estimates of acute exposure to metconazole and the risk to birds from such exposure following application of BAS 555 01 F in cereals and oilseed rape.

Crop group	Indicator species	Shortcut value (mg a.s./kg bw)	App. rate (kg a.s./ha)	MAF	DDD (mg a.s./kg bw)	LD_{50} (mg a.s./kg bw)	TER_A
Cereals	small omnivorous bird	158.8	0.090	1.1	15.72	819	52.1
Oilseed rape	small omnivorous bird	158.8	0.072	1.2	13.72	819	59.7

Note: TER shown in bold falls below the relevant trigger

Reproductive risk assessment for birds – screening step

The screening step crop groupings and critical use patterns relevant to the uses of BAS 555 01 F are given in Table B.9.2.1.3-2 above.

The long-term 'Daily Dietary Dose' (DDD) is calculated by multiplying the shortcut value (SV) based on the mean residues by the application rate in kg a.s./ha. When more than one application is intended, this product is further multiplied with an appropriate multiple application factor for mean residue data (MAF_m). The f_{twa} based upon a default DT_{50} of 10 days is 0.53, as given in the EFSA Guidance Document.

$$DDD_{\text{multiple applications}} = \textit{application rate [kg a.s./ha]} \times SV \times MAF_m \times f_{twa}$$

Long-term risk is assessed by comparing the long-term DDD with the worst case NOEL from the reproduction studies, expressed as daily dietary dose, to give a long-term Toxicity:Exposure Ratio (TER_{LT}):

$$TER_{LT} = \frac{NOEL[mg/kg\ bw/day]}{DDD[mg/kg\ bw/day]}$$

Daily dietary doses and TER values for long-term exposure to metconazole following use of BAS 555 01 F according to the proposed uses are given in Table B.9.2.1.3-4 below. Shortcut values were derived from Table 10 of the EFSA Guidance Document. MAF_m values were calculated as described in Appendix H of the EFSA Guidance Document.

The TER_{LT} values for all proposed uses are below the Annex VI trigger of 5, indicating **a potential reproductive risk to birds** from exposure to metconazole following the proposed uses of BAS 555 01 F in cereals and oilseed rape. Further consideration is thus necessary.

Table B.9.2.1.3-4: Screening step – estimates of long-term exposure to metconazole and the risk to birds from such exposure following application of BAS 555 01 F in cereals and oilseed rape.

Crop group	Indicator species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg a.s./ha)	MAF	f_{twa}	Long-term DDD (mg a.s./kg bw/day)	NOEL (mg a.s./kg bw/day)	TER_{LT}
Cereals	small omnivorous bird	64.8	0.090	1.2	0.53	3.71	6.19	**1.67**
Oilseed rape	small omnivorous bird	64.8	0.072	1.4	0.53	3.46	6.19	**1.79**

Note: TER shown in bold falls below the relevant trigger

Reproductive risk assessment for birds – Tier 1

For the reproductive risk assessment, the TER_{LT} values for metconazole for the use in cereals and oilseed rape at the screening step are less than the relevant trigger value. Consequently, Tier 1 assessments are required for these uses. The Tier 1 assessment initially requires identification of the appropriate crop groupings and generic focal bird species in Annex I of the EFSA Guidance Document. The Tier 1 crop groupings and critical use pattern relevant to the uses of BAS 555 01 F are given in Table B.9.2.1.3-5.

Table B.9.2.1.3-5: Tier 1 crop groupings and critical use patterns considered in the risk assessment for the use of metconazole in BAS 555 01 F.

Tier 1 crop group	GAP crop species	EU region	GAP growth stage window	Critical use pattern		
				Rate (kg a.s./ha)	Max no. of applications	App. interval (days)
Cereals	Cereals	North, Central & South	30 - 69	0.090	2	21
Oilseed rape	Winter oilseed rape	North, Central & South	13 – 20 (autumn)	0.072	1	-
			21 – 71 (spring)	0.072	1	-
			21 – 71	0.072	2 [1)]	14 [1)]

[1)] *for oilseed rape, only the worst case use rate of 2x0.072 kg a.s./ha is used for the risk assessment; to cover also the single application in earlier growth stages, worst case scenarios for BBCH 13-20 were included in the calculations*

The generic focal species relevant for the proposed uses in cereals and oilseed rape are considered with the worst-case application rate to calculate long-term DDD and TER values as shown in Table B.9.2.1.3-6 and Table B.9.2.1.3-7 for the proposed use in cereals and oilseed rape, respectively.

Table B.9.2.1.3-6: Tier 1 – estimates of long-term exposure to metconazole and the risk to birds from such exposure following application of BAS 555 01 F in cereals

Crop grouping/ growth stage	Generic focal species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg a.s./ha)	MAF	f_{TWA}	DDD (mg a.s./kg bw/ day)	NOEL (mg a.s./kg bw/day)	TER_{LT}
Cereals BBCH 30-39	Small omnivorous bird "lark"	5.4	0.090	1.2	0.53	0.309	6.19	20.03
Cereals BBCH ≥ 40	Small omnivorous bird "lark"	3.3				0.189		32.77

Note: TER shown in bold falls below the relevant trigger

Table B.9.2.1.3-7: Tier 1 – estimates of long-term exposure to metconazole and the risk to birds from such exposure following application of BAS 555 01 F in oilseed rape

Crop grouping/ growth stage	Generic focal species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg a.s./ha)	MAF	f_{TWA}	DDD (mg a.s./kg bw/ day)	NOEL (mg a.s./kg bw/day)	TER_{LT}
Oilseed rape Late – late (with seeds) (BBCH 30-90)	Small insectivorous bird "dunnock"	2.7	0.072	1.4	0.53	0.144	6.19	42.91
Oilseed rape Early shoots (BBCH 10-19)	Large herbivorous bird "goose"	15.9				0.849		7.29
Oilseed rape BBCH 10-29	Small omnivorous bird "lark"	10.9				0.582		10.63
Oilseed rape BBCH 30-39	Small omnivorous bird "lark"	3.3				0.176		35.11
Oilseed rape BBCH ≥ 40	Small omnivorous bird "lark"	2.7				0.144		42.91
Oilseed rape BBCH 10-19	Medium herbivorous / granivorous bird "pigeon"	22.7				1.213		5.10
Oilseed rape BBCH 20-29	Medium herbivorous / granivorous bird "pigeon"	3.5				0.187		33.10
Oilseed rape BBCH 30-39	Medium herbivorous / granivorous bird "pigeon"	1.1				0.059		105.33
Oilseed rape BBCH ≥ 40	Medium herbivorous / granivorous bird "pigeon"	0.9				0.048		128.74
Oilseed rape BBCH 10-19	Small insectivorous bird "wagtail"	5.9				0.315		19.64
Oilseed rape BBCH 20-29	Small insectivorous bird "wagtail"	2.8				0.150		41.38

Note: TER shown in bold falls below the relevant trigger

Based on the Tier 1 assessment, the long-term TER values of the different exposure scenarios for metconazole following the proposed uses of BAS 555 01 F exceed the Annex VI trigger value of 5, indicating **an acceptable reproductive risk to birds** from the proposed uses of BAS 555 01 F in cereals and oilseed rape.

Risk to birds through drinking water

According to the EFSA Guidance Document, two scenarios need to be considered for assessing the risk via the consumption of drinking water: the leaf scenario and the puddle scenario.

The ***leaf scenario*** is relevant for birds taking water that is collected in leaf whorls after application of a pesticide to a crop and subsequent rainfall or irrigation. This scenario applies to leafy vegetables forming heads or with a morphology that facilitates collection of rain/irrigation water sufficiently to attract birds. Since none of the proposed crop uses falls into these categories, the leaf scenario does not apply to the use of BAS 555 01 F.

The ***puddle scenario*** is relevant for birds taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all intended uses of BAS 555 01 F and should therefore be assessed.

The predicted environmental concentration in puddles is calculated as follows:

$$PEC_{puddle} = \frac{AR/10}{1000(w + K_{OC} \times s)}$$

Where:

AR	application rate (g/ha); divisor of 10 to achieve rate in mg/m²
w	0.02 (pore water term; volume)
s	0.0015 (soil term: volume, density, organic carbon content)

When multiple spray applications are considered, a MAF based on the DT_{50} in soil (single first order kinetics, geometric mean as used for PEC_{gw} and PEC_{sw}) may be applied to achieve the effective application rate AR_{eff}.

$$AR_{eff} = AR \times MAF_m = AR \times \frac{1 - e^{-nki}}{1 - e^{-ki}}$$

Where:

k	$\ln(2)/DT_{50}$ (rate constant)
n	number of applications
i	application interval (d)

According to the EFSA Guidance Document, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/day) does not exceed 50 in the case of less sorptive substances (K_{OC} < 500 L/kg) or 3000 in the case of more sorptive substances ($K_{OC} \geq$ 500 L/kg). For metconazole, the geometric mean K_{OC} is determined as 1071 L/kg (see Volume 3 (AS), Section B.8.1.2 on Fate and behaviour). The ratios of effective application rate to the relevant endpoint for the proposed uses of BAS 555 01 F are shown in Table B.9.2.1.3-8.

Table B.9.2.1.3-8: Ratios of effective application rate to endpoints for metconazole following the use of BAS 555 01 F in winter and spring cereals, and winter oilseed rape.

Intended use	App. rate (g a.s./ha)	MAF [1]	AR_{eff} (g a.s/ha)	LD_{50} (mg a.s./kg bw)	Ratio of AR_{eff} to LD_{50}	NOEL (mg a.s./kg bw/day)	Ratio of AR_{eff} to NOEL	Ratio trigger
Cereals	90	1.86	167.0	819	0.204	6.19	26.99	3000
Oilseed rape	72	1.90	136.9	819	0.167	6.19	22.12	3000

[1] *Calculated (for cereals and oilseed rape) based on the geometric mean normalized field DT_{50} = 93.6 days for metconazole in soil (see Volume 3 (AS) Section B.8.1)*

The resulting ratio is clearly below the trigger value of 3000 indicating that the **acute and long-term risk to birds via the consumption of drinking water (puddle scenario) can be considered acceptable** without further calculations.

Effects of secondary poisoning

According to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), substances with a log P_{OW} greater than 3 have potential for bioaccumulation. The log P_{OW} of metconazole was determined to be 3.85 (see Volume 3, Section B.2). Therefore, an assessment of the risk from bioaccumulation has to be performed.

Food chain from earthworm to earthworm-eating birds

For the effects of secondary poisoning on earthworm-eating birds, the dry soil approach was followed as recommended by the EFSA Guidance Document. According to this approach, the bioconcentration factor for the earthworm is calculated as follows:

$$BCF_{earthworm} = \frac{0.84 + 0.012\,P_{OW}}{f_{OC} \times K_{OC}}$$

With:

K_{OC} = organic carbon adsorption coefficient

f_{OC} = organic carbon content of soil (take 0.02 as a default value)

The estimated residues in earthworms are then calculated as:

$$PEC_{earthworm} = PEC_{soil} \times BCF_{earthworm}$$

The $PEC_{earthworm}$ is then converted to daily dose by multiplying with 1.05, and compared with the relevant long-term NOAEL. The multiplicator is based on a 100 g bird eating 104.6 g worms per day.

Table B.9.2.1.3-9 shows the parameters used to calculate the $BCF_{earthworm}$. The geometric mean K_{OC} for metconazole was derived from Volume 3 (AS), Section B.8.1.2 on Fate and behaviour. Table B.9.2.1.3-10 shows the TER values for the risk after exposure to metconazole for earthworm-eating birds for the different proposed uses of BAS 555 01 F. The PEC_{soil} value used is the maximum accumulated PEC_{soil} values as calculated in the fate and behaviour section (see Volume 3 (PPP), Section B.8.1). The calculated TER exceeds the trigger of 5, indicating an acceptable risk.

Table B.9.2.1.3-9: Parameters used in the calculation of the earthworm bioconcentration factor ($BCF_{earthworm}$).

Parameter	Value
Log P_{OW}	3.85
P_{OW}	7090
K_{OC} (geometric mean value) (L/kg)	1071
f_{OC}	0.02
$BCF_{earthworm}$	**4.01**

Table B.9.2.1.3-10: Estimates of exposure to metconazole through bioconcentration in earthworms, and the risk from such exposure following the application of BAS 555 01 F according to the proposed uses.

Substance	BCF_{worm}	PEC_{soil} [1] (mg/kg)	PEC_{worm}	DDD	NOEL (mg a.s./kg bw/day)	TER	Trigger
Metconazole	4.01	0.071	0.284	0.299	6.19	20.73	5

[1] *Worst-case accumulated PEC_{soil} values, calculated for twofold application of 72 g a.s./ha to oilseed rape (first application in autumn followed by a second application in spring).*

Food chain from fish to fish-eating birds

According to the EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (2013)[4], the assessment for effects of secondary poisoning for fish-eating birds should follow two steps. In a first step, the regulatory acceptable concentration for secondary poisoning (RAC_{SP}) for birds eating fish out of surface water contaminated with a plant protection product is calculated using the following formula:

$$RAC_{SP} = \frac{NOAEL_{bird}}{5 \times 0.159 \times BCF_{fish} \times BMF}$$

Where: RAC_{SP}: regulatory acceptable concentration in water for secondary poisoning (mg/L)
NOAEL: relevant long-term no-adverse-effect level for birds (mg/kg bw/day)
BCF_{fish}: whole body bioconcentration factor in fish (L/kg)
BMF: biomagnification factor (kg/kg)
5: assessment factor (AF) for the chronic risk assessment for terrestrial vertebrates
0.159: multiplication factor for conversion of residue in fish to daily dose for birds, based on a 1000-g bird eating 159 g of fish per day

For the active substance matconazole, two fish bioaccumulation studies were conducted with Bluegill Sunfish. From these studies, the highest steady state bioconcentration factor (BCF), corrected to 5% lipid content, was 105.1 (see Volume 3 (AS), Section B.9.2.2.3). The biomagnification factor (BMF) was derived from Table 25 of the EFSA Guidance Document on aquatic organisms. As the fish BCF is below 2000, a BMF of 1 is used in the risk assessment.

Table B.9.2.1.3-11 shows the calculated RAC_{SP} value for secondary poisoning for fish-eating birds for metconazole. As a second step in the assessment, this RAC_{SP} value is compared to the 21-day TWA PEC_{SW}. For the proposed uses of BAS 555 01 F, the RAC_{SP} exceeds the 21-day TWA PEC_{SW}, indicating that the risk through secondary poisoning for fish-eating birds is acceptable. No further assessment is thus required.

[4] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013; 11(7):3290. 268 pp.

Table B.9.2.1.3-11: Estimates of exposure and risk to metconazole through bioconcentration in fish following the application of BAS 555 01 F in cereals and oilseed rape.

BCF_{fish}	Multiplication factor	Assessment factor	BMF	NOEL (mg a.s./kg bw/day)	RAC_{SP} (mg/L)	21-day TWA PEC_{SW} [a] (mg/L)	Acceptable risk?
105.1	0.159	5	1	6.19	0.074	0.025	yes

[a] *the worst-case Step 1 21-day TWA PEC_{SW} for the application of 2 x 90 g a.s./ha in cereals. This value covers the Step 1 21-day TWA PEC_{SW} for the proposed uses in oilseed rape.*

Biomagnification in terrestrial food chains

As also discussed in Section B.9.3.3, metconazole shows very short clearance times in the available fish bioconcentration studies (CA8.2.2.3/01 [redacted] 1996; CA8.2.2.3/02 [redacted] M., 2002; see Volume 3 (AS) Section B.9.2.2.3 for a summary). Based on these two BCF studies, despite the relatively high lipophilicity of metconazole, it can be concluded that the potential for bioaccumulation is low, due to the low accumulation and rapid excretion of metconazole from fish. No further assessment on biomagnification is thus required.

Conclusion

The acute and long-term risk of metconazole to birds is acceptable following the intended uses of BAS 555 01 F in cereals and oilseed rape.

The risk to birds through drinking water, secondary poisoning and biomagnification in the food chain can also be considered acceptable.

B.9.2.1.4. Risk assessment for the representative formulation BAS 555 01 F

The endpoint for the formulation BAS 555 01 F used in the acute risk assessment for birds is shown in Table B.9.2.1.4-1.

Table B.9.2.1.4-1: Summary of the formulation endpoints used in the risk assessment for birds

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Bobwhite quail (*Colinus virginianus*)	BAS 555 01 F	Single dose (Acute oral toxicity)	LD_{50}	> 800 mg product/kg bw	CP10.1.1.2/01 [redacted] 2008

Acute risk assessment for birds – Screening step

The intended use patterns for the representative formulation BAS 555 01 F are shown in Table B.9-1. BAS 555 01 F is intended to be used at a maximum single application rate of 1.0 L/ha in cereals and 0.8 L/ha in oilseed rape. Taking into account the density of the formulation of 1.046 g/cm³, this is equivalent to an application rate of 1.046 kg formulation/ha and 0.8368 kg formulation/ha in cereals and oilseed rape, respectively.

For the proposed use in oilseed rape, BAS 555 01 F can either be applied once in autumn (at BBCH 13-20) followed by a second application in spring (at BBCH 21-71), or can be applied two times in spring (at BBCH 21-71) with a 14-day interval. In the first case, the interval between the two applications is of such a length (min. 90 days) that both applications can be considered as independent in the context of the risk assessment for birds[5]. Taking this into account, the screening step crop grouping and critical use patterns relevant for the uses of BAS 555 01 F are given in Table B.9.2.1.4-2. Note that for the proposed use in oilseed rape, only the worst case use rate of 2 x 0.8368 kg BAS 555 01 F/ha is considered further in the risk assessment below. However, to cover also the single application in earlier growth stages, worst case scenarios for BBCH 13-20 are included in the calculations.

Table B.9.2.1.4-2: Screening step crop groupings and critical use patterns considered in the risk assessment for the use of BAS 555 01 F.

Crop group	GAP crop species	EU region	Application time (BBCH growth stage)	Indicator species	Critical use pattern		
					Rate (kg form./ha)	Max no. of applications	App. interval (days)
Cereals	Cereals	North, Central & South	30 - 69	Small omnivorous bird	1.046	2	21
Oilseed rape	Winter oilseed rape	North, Central & South	13 – 20 (autumn)	Small omnivorous bird	0.8368	1	-
			21 – 71 (spring)		0.8368	1	-
			21 – 71		0.8368	2 [1)]	14 [1)]

[1)] for oilseed rape, only the worst case use rate of 2x0.8368 kg form./ha is used for the risk assessment; to cover also the single application in earlier growth stages, worst case scenarios for BBCH 13-20 were included in the calculations

[5] The Multiple Application Factor (MAF) calculated using the equations given in Appendix H of the EFSA Guidance Document, based on a default DT_{50} of 10 days and an interval of 150 days is equal to 1.0.

The acute 'Daily Dietary Dose' (DDD) is calculated by multiplying the shortcut value (SV) based on the 90th percentile residues by the application rate in kg a.s./ha. When more than one application is intended, this product is further multiplied with an appropriate multiple application factor for 90th percentile residue data (MAF_{90}).

$$DDD_{multiple\ applications} = application\ rate\ [kg\ a.s./ha]\ x\ SV\ x\ MAF_{90}$$

The acute risk to birds is assessed by calculation of an acute Toxicity:Exposure ratio (TER_A) according to the following equation:

$$TER_A = \frac{LD_{50}[mg/kg\ bw]}{DDD[mg/kg\ bw]}$$

The daily dietary dose (DDD) based on the above equation and TER values for the relevant indicator species for acute exposure to BAS 555 01 F following the proposed uses are given in Table B.9.2.1.4-3 below. Shortcut values were derived from Table 6 of the EFSA Guidance Document. MAF_{90} values were calculated as described in Appendix H of the EFSA Guidance Document.

The TER_A values for all proposed uses are below the Annex VI trigger of 10, indicating **a potential acute risk to birds** from exposure to BAS 555 01 F following the proposed uses in cereals and oilseed rape. Further consideration is thus necessary.

Table B.9.2.1.4-3: Screening step – estimates of acute exposure to BAS 555 01 F and the risk to birds from such exposure following application in cereals and oilseed rape.

Crop group	Indicator species	Shortcut value (mg/kg bw)	App. rate (kg form./ha)	MAF	DDD (mg form./kg bw)	LD_{50} (mg form./kg bw)	TER_A
Cereals	small omnivorous bird	158.8	1.046	1.1	182.7	> 800	**> 4.38**
Oilseed rape	small omnivorous bird	158.8	0.8368	1.2	159.5	> 800	**> 5.02**

Note: TER shown in bold falls below the relevant trigger

Acute risk assessment for birds – Tier 1

For the acute risk assessment, the TER_A values for BAS 555 01 F for the use in cereals and oilseed rape at the screening step are less than the relevant trigger value. Consequently, Tier 1 assessments are required for these uses. The Tier 1 assessment initially requires identification of the appropriate crop groupings and generic focal bird species in Annex I of the EFSA Guidance Document. The Tier 1 crop groupings and critical use pattern relevant to the uses of BAS 555 01 F are given in Table B.9.2.1.4-4.

Table B.9.2.1.4-4: Tier 1 crop groupings and critical use patterns considered in the risk assessment for the use of BAS 555 01 F.

Tier 1 crop group	GAP crop species	EU region	GAP growth stage window	Critical use pattern		
				Rate (kg form./ha)	Max no. of applications	App. interval (days)
Cereals	Cereals	North, Central & South	30 - 69	1.046	2	21
Oilseed rape	Winter oilseed rape	North, Central & South	13 – 20 (autumn)	0.8368	1	-
			21 – 71 (spring)	0.8368	1	-
			21 – 71	0.8368	2 [1)]	14 [1)]

[1)] *for oilseed rape, only the worst case use rate of 2x0.8368 kg form./ha is used for the risk assessment; to cover also the single application in earlier growth stages, worst case scenarios for BBCH 13-20 were included in the calculations*

The generic focal species relevant for the proposed uses in cereals and oilseed rape are considered with the worst-case application rate to calculate acute DDD and TER values as shown in Table B.9.2.1.4-5 and Table B.9.2.1.4-6 for the proposed use in cereals and oilseed rape, respectively.

Based on the Tier 1 assessment, the acute TER values of the different exposure scenarios for BAS 555 01 F following the proposed uses exceed the Annex VI trigger value of 10, indicating **an acceptable acute risk to birds** from the proposed uses of BAS 555 01 F in cereals and oilseed rape.

Table B.9.2.1.4-5: Tier 1 – estimates of acute exposure to BAS 555 01 F and the risk to birds from such exposure following application in cereals

Crop grouping/ growth stage	Generic focal species	Shortcut value (mg form./kg bw/day)	App. rate (kg form./ha)	MAF	DDD (mg form./kg bw/ day)	LD_{50} (mg form./kg bw)	TER_A
Cereals BBCH 30-39	Small omnivorous bird "lark"	12.0	1.046	1.1	13.81	> 800	> 57.94
Cereals BBCH ≥ 40	Small omnivorous bird "lark"	7.2			8.28		> 96.56

Note: TER shown in bold falls below the relevant trigger

Table B.9.2.1.4-6: Tier 1 – estimates of acute exposure to BAS 555 01 F and the risk to birds from such exposure following application in oilseed rape

Crop grouping/ growth stage	Generic focal species	Shortcut value (mg form./kg bw/day)	App. rate (kg form./ha)	MAF	DDD (mg form./kg bw/ day)	LD_{50} (mg form./kg bw)	TER_A
Oilseed rape Late – late (with seeds) (BBCH 30-90)	Small insectivorous bird "dunnock"	7.4	0.8368	1.2	7.43	> 800	107.66
Oilseed rape Early shoots (BBCH 10-19)	Large herbivorous bird "goose"	39.0			39.16		20.43
Oilseed rape BBCH 10-29	Small omnivorous bird "lark"	24.0			24.09		33.20
Oilseed rape BBCH 30-39	Small omnivorous bird "lark"	7.2			7.23		110.65
132.78Oilseed ra14.33pe BBCH ≥199.17 40331.95	Small omnivorous bird "lark"	6.0			6.02		132.78
Oilseed rap398.34e BBCH 10-19	Medium herbivorous / granivorous bird "pigeon"	55.6			55.83		14.33
Oilseed rape BBCH 20-29	Medium herbivorous / granivorous bird "pigeon"	4.0			4.01		199.17
Oilseed rape BBCH 30-39	Medium herbivorous / granivorous bird "pigeon"	2.4			2.41		331.95
Oilseed rape BBCH ≥ 40	Medium herbivorous / granivorous bird "pigeon"	2.0			2.01		398.34
Oilseed rape BBCH 10-19	Small insectivorous bird "wagtail"	10.9			10.95		73.09
Oilseed rape BBCH 20-29	Small insectivorous bird "wagtail"	7.7			7.73		103.47

Note: TER shown in bold falls below the relevant trigger

Conclusion

The acute risk of BAS 555 01 F to birds is acceptable following the intended uses in cereals and oilseed rape.

B.9.2.2. Risk assessment for terrestrial vertebrates other than birds

The risk assessment for effects on birds has been updated according to the latest **EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)**[6].

B.9.2.2.1. Toxicity

Mammalian acute oral, short-term dietary and long-term reproduction studies have been carried out with metconazole. From section B.6 on Mammalian toxicology, the acute and reprotoxicity endpoints listed in Table B.9.2.2.1-1 and Table B.9.2.2.1-2, respectively, were derived for the ecotoxicological risk assessment.

Table B.9.2.2.1-1: Summary of acute oral toxicity endpoints for mammals.

Organism	Test substance	Timescale (Test type)	Vehicle	Endpoint	Toxicity value	Reference
Rat	BAS 555 F (metconazole) 80:15 *cis:trans*	Single dose (Acute oral)	Solution in corn oil	♂ LD_{50} ♀ LD_{50}	**727 mg a.s./kg bw** **595 mg a.s./kg bw**	[redacted] 1990a
Rat	BAS 555 F (metconazole) 85:15 *cis:trans*	Single dose (Acute oral)	Solution in corn oil	LD_{50}	**> 500 mg a.s./kg bw** and < 2000 mg a.s./kg bw	[redacted] 2005a
Rat	BAS 555 F (metconazole) 95% *cis*	Single dose (Acute oral)	Solution in corn oil	♂ LD_{50} ♀ LD_{50}	**1627 mg a.s./kg bw** **1312 mg a.s./kg bw**	[redacted]., 1991
Mouse	BAS 555 F (metconazole) 80:15 *cis:trans*	Single dose (Acute oral)	Solution in corn oil	♂ LD_{50} ♀ LD_{50}	718 mg a.s./kg bw **410 mg a.s./kg bw**	[redacted] 1990a

Note: endpoints in bold are considered in the risk assessment.

Table B.9.2.2.1-2: Summary of short-term dietary and long-term reproduction endpoints for mammals.

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Rat	BAS 555 F (metconazole) 85:15 *cis:trans*	28-day dynamic exposure	NOAEL	9.1 mg a.s./kg bw/day	[redacted] 1990 (MK-420-002)
Rat	BAS 555 F (metconazole) 99% *cis*	28-day dynamic exposure	NOAEL	27.3 mg a.s./kg bw/day	[redacted] 1991a (MK-420-003)
Dog	BAS 555 F (metconazole) 85:15 *cis:trans*	28-day dynamic exposure	NOAEL	3.7 mg a.s./kg bw/day	[redacted] 1991a (MK-420-004)
Rat	BAS 555 F (metconazole) 80:20 *cis:trans*	90-day dynamic exposure	NOAEL	6.4 mg a.s./kg bw/day	[redacted] 1991b (MK-425-002)

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

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Rat	BAS 555 F (metconazole) 99% *cis*	90-day dynamic exposure	NOAEL	28.8 mg a.s./kg bw/day	[redacted] 1992 (MK-425-004)
Mouse	BAS 555 F (metconazole) 80:20 *cis:trans*	90-day dynamic exposure	NOAEL	4.6 mg a.s./kg bw/day	[redacted] (MK-425-003)
Dog	BAS 555 F (metconazole) 85:15 *cis:trans*	90-day dynamic exposure	NOAEL	2.6 mg a.s./kg bw/day	[redacted] 1991b (MK-425-001)
Dog	BAS 555 F (metconazole) 85:15 *cis:trans*	1-year dynamic exposure	NOAEL	10 mg a.s./kg bw/day	[redacted] 1992a (MK-427-002)
Rat	BAS 555 F (metconazole) 95% *cis*	2-generation reproduction study	NOAEL[1]	**8 mg a.s./kg bw/day**	[redacted] ., 1992a (MKB-430-003)
Rat	BAS 555 F (metconazole) 85:15 *cis:trans*	2-generation reproduction study	NOAEL[1]	10 mg a.s./kg bw/day	[redacted] 2002 [3]
Rat	BAS 555 F (metconazole) 80:15 *cis:trans*	Developmental toxicity study	NOAEL[2]	12 mg a.s./kg bw/day	[redacted] 1991b (MK-432-005)
Rat	BAS 555 F (metconazole) 95% *cis*	Developmental toxicity study	NOAEL[2]	6 mg a.s./kg bw/day	[redacted], 1992b,c,d [4]
Rat	BAS 555 F (metconazole) 85:15 *cis:trans*	Developmental toxicity study	NOAEL[2]	16 mg a.s./kg bw/day	[redacted] 2002 [5]
Rabbit	BAS 555 F (metconazole) 80:15 *cis:trans*	Developmental toxicity study	NOAEL[2]	10 mg a.s./kg bw/day	[redacted] ., 1997 (MK-432-015)
Rabbit	BAS 555 F (metconazole) *cis*	Developmental toxicity study	NOAEL[2]	10 mg a.s./kg bw/day	[redacted] 1990 (MK-432-002)
Rabbit	BAS 555 F (metconazole) 85:15 *cis:trans*	Developmental toxicity study	NOAEL[2]	4 mg a.s./kg bw/day	[redacted] 1991a (MK-432-003)
Rabbit	BAS 555 F (metconazole) 95% *cis*	Developmental toxicity study	NOAEL[2]	4 mg a.s./kg bw/day	[redacted] 1992a and 1992b [6]

Note: [1] endpoint for reproduction parameters only; [2] endpoint for developmental or fetal parameters; [3] Submitted as a summary: [redacted] 2015a (DocID 2015/1087913); [4] In Volume 3 (AS) Section B.6.6.1, the studies by [redacted] 1992b (MK-432-009), 1992c (MK-432-008) and 1992d (MK-432-006) are discussed together to derive an overall NOAEL from these studies; [5] Submitted as a summary: [redacted] 2015 (DocID 2015/1087909); [6] In Volume 3 (AS) Section B.6.6.2, the studies by [redacted] 1992a (MKB-432-007) and 1992b (MKB-432-010) are discussed together to derive an overall NOAEL from both studies; ***bold*** *– endpoint used for the current risk assessment*

Toxicity of metconazole:

For the active substance metconazole, three **acute toxicity** studies with rats and one study with mice are available (see Table B.9.2.2.1-1). In the original DAR and in the List of Endpoints from the previous EFSA Conclusion for metconazole (2006)[7], the overall lowest LD_{50} of 410 mg a.s./kg bw for mice was used in the risk assessment. However, according to the new EFSA Guidance Document for birds and mammals (2009)[8] (Section 2.4), it is permissible to use the geometric mean of the available endpoints for the acute assessment in case acute toxicity data is available for more than one species. Further, when more than one acute toxicity study on the same species is available, Section 2.4 of the EFSA Guidance Document (2009) states that the geometric mean of the endpoints for the same species can be calculated. This calculated endpoint is then used in the overall geometric mean calculation. When calculating a geometric mean endpoint, it is important that the studies are equivalent in terms of guideline and in particular the vehicle/solvent used.

In the available acute toxicity study with mice ([redacted] 1990a), females were more sensitive than males, with a difference in LD_{50} value of >25%. According to Section 2.1.1 of the EFSA Guidance Document (2009), in this case the lower LD_{50} value should be used for risk assessment purposes. Therefore, the LD_{50} value for female mice of 410 mg a.s./kg bw is used in the geometric mean calculation. In the acute toxicity studies with rats, the difference in LD_{50} for males and females was <25%. Therefore, in line with the recommendations of Section 2.1.1 of the EFSA Guidance Document (2009), the geometric mean of the LD_{50} values for both sexes can be used in the risk assessment.

Combining the relevant endpoints for both rats and mice, an overall geometric mean LD_{50} is calculated, as shown in Table B.9.2.2.1-3. The endpoint for the most sensitive species (LD_{50} = 410 mg a.s./kg bw) is less than a factor of 10 below the overall geomean LD_{50}. Therefore, according to the EFSA Guidance Document (2009), it is permissible to use this **geometric mean LD_{50} of 566.7 mg a.s./kg** bw in the risk assessment.

Table B.9.2.2.1-3: Calculation of the geometric mean as the relevant mammalian toxicity endpoint for the acute risk assessment of metconazole

Test species	**Rat**			**Mice**
Reference	[redacted] 1990a	[redacted] 2005a	[redacted] 1991	[redacted] 1990a
Metconazole *cis*:*trans* ratio	80:15	85:15	95:5	80:15
Experimental LD_{50} [mg a.s./kg bw]	♂: 727 ♀: 595	♂ & ♀: > 500 [1]	♂: 1627 ♀: 1312	♂: 718 ♀: 410
Geometric mean LD_{50} of both sexes [mg a.s./kg bw]	657.7	500	1461.0	410 [2]
Species-specific LD_{50} (geometric mean) [mg a.s./kg bw]	783.2			410
Overall geometric mean of LD_{50} [mg a.s./kg bw] to be used in risk assessment	**566.7**			

[1] The lower end of the range (500 < LD50 < 2000 mg a.s./kg bw) is used for the calculations

[4] According to the EFSA Guidance Document (2009), the most sensitive endpoint hase to be taken into account when the endpoints of the two sexes in an acute oral study differ more than 25%. If the difference is less than 25%, the endpoint of the two sexes combined can be used.

[7] EFSA Scientific Report (2006) 64, 1-71. Conclusion regarding the peer review of the pesticide risk assessment of the active substance metconazole.

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

Following the EFSA Guidance Document (2009), all available short-term dietary and long-term reproduction studies are considered to determine the ecotoxicologically relevant endpoint for **reproductive/developmental toxicity** to mammals. In Table B.9.2.2.1-5, the main findings for the endpoints that are considered relevant for reproductive performance are summarized for these studies.

The lowest endpoints (below 10 mg a.s./kg bw/day) from the short-term repeated-dose oral toxicity studies with rats, mice and dogs were the following: a NOAEL of 9.1 mg a.s./kg bw/day (1990; rats, 28-day), 3.7 mg a.s./kg bw/day (1991a; dogs, 28-day), 4.6 mg a.s./kg bw/day (1991; mice, 90-day), 6.4 mg a.s./kg bw/day (1991b; rats, 90-day) and 2.6 mg a.s./kg bw/day (1991b; dogs, 90-day). The endpoints from the studies by (1991a), (1991) and (1991b) were based on effects in haematology, clinical chemistry and/or organ weights. Such effects are however not considered relevant for reproductive performance according to the EFSA Guidance Document (2009). Effects on body weight, on the other hand, are considered by the EFSA Guidance Document (2009) as relevant for the reproductive performance. In these three studies, no effects on body weight were observed up to a concentration of 37.8 mg a.s./kg bw/day (1991a), 50.5 mg a.s./kg bw/day (1991) or 19.2 mg a.s./kg bw/day (1991b). In contrast, the NOAEL from the studies by (1990) and (1991b) were based on effects on body weight in the next higher dose.

In the study by (1990), the NOAEL was set at 9.1 mg a.s./kg bw/day, based on a statistically significant effect on body weight of 14% at the next higher treatment dose of 90.4 mg a.s./kg bw/day. In the study by (1991b), the NOAEL was set at 2.6 mg a.s./kg bw/day. At the next higher treatment dose of 24.3 mg a.s./kg bw/day (600 ppm), a small but statistically significant effect on body weight (8%) was observed. As this effect was only small (less than 10%), it could potentially be considered of low ecotoxicological relevance. However, a dose response was observed, with effects on body weight increasing to 21% at the highest tested dose (6000 ppm). The dose spacing in this study was however rather wide, with the effects on body weight observed only at a dose of 10 times the NOAEL. Taking into account the wide dose spacing and the only small effect on body weight seen at 24.3 mg a.s./kg bw/day, the NOAEL from the study by . (1990) of 9.1 mg a.s./kg bw/day is also considered sufficiently protective for the observed effects on body weight in the dog in (1991b). Based on the above, the lowest NOAEL from the available <u>short-term repeated-dose oral toxicity studies</u>, relevant for ecotoxicology, is an <u>NOAEL of 9.1 mg a.s./kg bw/day</u> from the 28-day study with rats 1990).

Two <u>2-generation studies with rats</u> are available. The study by (1992a) was already evaluated in the original DAR for metconazole, and resulted in a <u>NOAEL of 8 mg a.s./kg bw/day</u>. This endpoint was based on a decreased body weight gain of F1 weanlings, increased gestation length and recuded post-implantation survival in F2 at the higher test concetration of 32 mg a.s./kg bw/day. For active substance renewal, an extensive summary of a second study has been submitted (2002). This study is owned by Kureha, and consequently the full study report is not available to the applicant and the RMS. As discussed in Volume 3 (AS) Section B.6.6.1.2, this study is therefore considered as complementary information only. From this study, a NOAEL of 10 mg a.s./kg bw/day was derived, based on decreased viability index, reduced pup body weight, a prolonged duration of gestation and increased maternal mortality during delivery at the higher dose of 49 mg a.s./kg bw/d. The results from the study by (2002) confirm those from the study by (1992a).

For the rat, three developmental toxicity studies are available ([redacted] 1991b; [redacted] 1992b,c,d; [redacted] 2002). The lowest endpoint from these studies was a NOAEL of 6 mg a.s./kg bw/day, which was derived from the study by [redacted] (1992b,c,d). This endpoint was based on increased bilateral dilatation of the ureter compared to the control at the higher tested doses of 24 and 60 mg a.s./kg bw/day. In the position paper submitted by the applicant (Anonymous, 2015; KCP10.1.2.2/01 – See Section B.9.1.2 for a summary), it is argued that, the apparent increase of bilateral dilatation of the ureter at 24 mg a.s./kg bw at visceral inspection was barely perceptible when examined microscopically using Wilson's serial sectioning technique, and that the study author did not attribute this finding to the test material administration. In Volume 3 (AS) Section B.6, the RMS is however of the opinion that despite being only a small effect at 24 mg a.s./kg bw/day, the presence of similar findings in the other available rat developmental toxicity studies (e.g. [redacted] 1991b at the top-dose of 75 mg a.s./kg bw/day), and the obvious dose-dependent increase in the study by [redacted] (1992b,c,d) could not exclude a substance-related effect. Therefore, the NOAEL for developmental toxicity was set at 6 mg a.s./kg bw/day (see Volume 3 (AS) Section B.6.6.2.1 for further details). The applicant further argues in the position paper that the small incidence of bilateral ureter dilatation seen at 24 mg a.s./kg bw/day should not be considered to be ecotoxicologically relevant as this equivocal change is unlikely to have an effect on population development and reproduction. RMS agrees with this argument, and therefore proposes an ecotoxicologically relevant NOAEL for developmental toxicity of 24 mg a.s./kg bw/day from the study by [redacted] (1992b,c,d). Taking the above into account, the lowest relevant NOAEL from the rat developmental toxicity studies is 12 mg a.s./kg bw/day, from the study by [redacted] (1991b).

For the rabbit, 5 developmental toxicity studies are available ([redacted]., 1997; [redacted] 1990; [redacted] 1991a; [redacted] 1992a and b). The lowest endpoint from these studies was a NOAEL of 4 mg a.s./kg bw/day, which was derived from both the studies by [redacted] (1991a) and [redacted] (1992a and b). This endpoint was based on either an increased incidence of embryonal deaths and post-implantation losses ([redacted] 1991a) or an increased incidence of hydrocephaly ([redacted] 1991b; [redacted] 1992a and b) compared to the control at the higher tested doses of 10 and 40 mg a.s./kg bw/d.

The applicant submitted an argumentation to demonstrate that the datasets for the four developmental toxicity studies wit rabbits can be merged to derive an overall endpoint for rabbits. This argumentation is included below (*text in italic*):

There are four rabbit developmental studies available, which were conducted according to very similar protocols. According to Section 2.4.3 of the EFSA Guidance Document (2009), such studies can be merged and a combined NOAEL can be derived. Three studies ([redacted] 1991a, 1992a and 1992b) are in compliance with method B.31 of directive 87/302/EEC and one study ([redacted]., 1997) was done according to OECD TG 414. All four studies used nulliparous female rabbits (New Zealand White), administered the dose in similar vehicles (0.5% carboxymethylcellulose or 1% methylcellulose) by gavage and basically addressed the same endpoints. Notably, more animals (n=25) were treated over a longer gestation period (GD 6-28) in the study by [redacted]. (1997) compared to the other studies (n=16-20, GD 7-19). Overall, these slight differences are – if at all – only supposed to increase the susceptibility of the study, meaning, the study by [redacted]. (1997) may be more sensitive than the other studies. However, since the overall merged NOAEL is not above the NOAEL of this study, a merging is still considered justified.

Table B.9.2.2.1-4 lists the available rabbit developmental studies and gives information on dose levels used and the overall NOAELs in each study as stated in the DAR 2005. The EU agreed rabbit

developmental NOAEL was set at 4 mg/kg bw/d. In the study by [redacted] (1997) an intermediate dose level of 5 mg/kg bw/d was investigated. This dose level showed no maternal, foetal or developmental effects. Therefore, it is feasible to slightly change the rabbit NOAEL$_{development}$ to 5 mg/kg bw/d.

Table B.9.2.2.1-4: Dose level spacing for rabbit developmental studies with metconazole. The NOAEL of each study is printed bold and the proposed overall NOAEL based the complete dataset is highlighted with a grey background

Reference	Code Isomer	Dose level (mg/kg bw/d)									
[redacted]., 1997 MK-432-015	WL148271 *cis/trans*					5	**10**	20		40	
[redacted] 1991a MK-432-003 (main & additional test)	WL148271 *cis/trans*			2	**4**		10		25		62.5
[redacted] 1992a MK-432-007	WL136184 *cis*			2	**4**		10			40	
[redacted] 1992b MK-432-010	WIL136184 *cis*	0.5	1	2			**10**			40	

RMS notes that the study by [redacted] (1990) is not considered in the argumentation provided. However, this was preliminary study in which only doses of 10 mg a.s./kg bw/day and higher were tested. Based on the tested doses and the findings of the study by [redacted] (1990), including this study in Table B.9.2.2.1-4 would not alter the outcome of the assessment. Overall, RMS agrees with the argumentation as submitted by the applicant. The overall NOAEL from the rabbit developmental toxicity studies is therefore considered to be 5 mg a.s./kg bw/day.

Further, in the position paper submitted by the applicant (Anonymous, 2015; KCP10.1.2.2/01 – See Section B.9.1.2 for a summary), an argumentation is provided to demonstrate that the effects seen in the rabbit developmental toxicity studies at 10 mg a.s./kg bw/day should not be considered ecotoxicologically relevant. The position paper states the following (*text in italic*):

In four rabbit developmental toxicity studies ([redacted] 1991a, [redacted] 1992a, [redacted] 1992b; [redacted] 1997, similar effects including maternal and foetal body weight effects, increased resorptions and late embryonic deaths were observed at the higher doses. Increased embryonic death and post-implantation loss, maternal body weight loss as well as an increased incidence of hydrocephaly at 10 mg/kg bw/d led to the EU accepted endpoint of 4 mg/kg bw/d. However, the observed maternal body weight loss was only transient and general less than 10% and the foetal changes were only slight and not statistically significant (increased late embryonic deaths: 1.0 compared to 0.5 in the control; increased post-implantation loss: 21.4% compared to 15.5% in the control). Hence, these findings at 10 mg/kg bw/d are not considered to be ecologically relevant as they are unlikely to have a notable effect on population development and reproduction. Furthermore, the increased incidence of hydrocephaly at 10 mg/kg bw is considered equivocal as it does not show a clear dose-response relationship and lies often within historical control ranges. An increased incidence of visceral/skeletal anomalies and hydrocephaly was observed at doses above 10 mg/kg bw/d. However, at 10 mg/kg bw/d only a low incidence of hydrocephaly was seen: 4 out of 714 foetuses from 3 out of 5 studies. When all available studies are considered, including preliminary studies and comparative studies, confirmed hydrocephaly was noted at 10 mg/kg bw in 4 out of 9 studies with 5 out of 1058 foetuses affected. During the EU review process the notifier proposed that the occurrence of spurious malformations in the older studies ([redacted] 1991a, 1992a and 1992b), even at low doses or in the controls, without similar findings at higher doses, resulted from a high spontaneous rate of malformation in the animals of this source at

this time period. It was also suggested that these animals were of suboptimal health, as indicated by the high variability in pregnancy rate, maternal mortality, and abortion. It was further noted that the most recent study ([redacted] 1997) showed no developmental toxicity. Overall, it is highly unlikely that the low and equivocal incidence of hydrocephaly seen at 10 mg/kg bw/d would lead to population effects and therefore is not considered to be of ecological relevance. Hence, an overall lowest, ecologically relevant NOAEL of 10 mg/kg bw/d based on the rabbit developmental studies is considered justified.

RMS agrees with the argumentation provided in the position paper. The NOAEL for ecotoxicologically relevant effects from the rabbit developmental toxicity studies is considered to be 10 mg a.s./kg bw/day.

Overall, based on all of the above, the lowest ecotoxicologically relevant NOAEL from the short-term dietary and long-term reproduction studies is 8 mg a.s./kg bw/day (obtained from the 2-generation study with rats by [redacted] 1992a). This value will be used in the chronic risk assessment for mammals.

Toxicity of the representative formulation BAS 555 01 F:

For the formulation BAS 555 01 F, an acute oral toxicity study was performed with rats ([redacted] 1997). The LD_{50} derived from this study was 3536 mg product/kg bw for males and 2102 mg product/kg bw for females. Based on the active substance content of 90 g a.s./L and a density of 1.046 g/cm², this corresponds to an LD_{50} of 304 mg a.s./kg bw for males and 181 mg a.s./kg bw for females. The acute toxicity of BAS 555 01 F thus seems slightly higher compared to the toxicity of the active substance. Therefore, a risk assessment will be performed for BAS 555 01 F using the lowest **LD_{50}** value from the study by [redacted] (1997) of **2102 mg product/kg bw**.

Toxicity of metconazole metabolites:

The potential risk from metconazole metabolites to birds and mammals is discussed in detail in Section B.9.2.1.1. Based on the performed assessment, it was concluded that for the metabolites M555F020 (1,2,4-triazole) and M555F013, which were relevant in soil and/or water, an assessment of the risk through secondary poisoning was not necessary, since their log P_{OW} value was below the trigger of 3. It should be noted that for M555F013, an experimental log P_{OW} value was not available, but QSAR calculations indicated that this value is not expected to exceed 2.31. Further, for the metabolites M555F034 (triazolyl acetic acid) and M555F035 (triazolyl alanine), which are relevant in plants, toxicity data is available that indicates that their toxicity is significantly lower compared to the parent metconazole. Consequently, for these metabolites the risk to mammals can be considered covered by the risk assessment for the parent metconazole.

Table B.9.2.2.1-5: Summary of the main findings for the endpoints relevant for reproductive performance for the available short-term dietary and long-term reproduction studies with mammals.

Endpoint	Relevant studies	Findings
Body weight change[1], behavioural effects and systemic toxicity[2]	28-day oral toxicity in rats Metconazole 85:15 *cis:trans* [redacted] *1990*	No treatment-related effects at 30 and 100 ppm. NOEAL set at 100 ppm (9.1 mg a.s./kg bw/d) based on a reduced feed consumption at 1000 ppm (90.5 mg a.s./kg bw/d) (♂) and 3000 ppm (♂,♀), leading to reduced body weights and body weight gains from wk1-4 (3000 ppm ♂, ♀) and from week 2-4 (1000 ppm ♂). Effects were also seen in haematology, clinical chemistry and organ weight at 1000 and 3000 ppm.
	28-day oral toxicity in rats Metconazole 99% *cis* [redacted] *1991a*	No treatment-related effects at 30, 100 and 300 ppm. The NOAEL was set at 300 ppm (27.3 mg a.s./kg bw/d) based on decreased feed intake at 1000 ppm (89.3 mg a.s./kg bw/d) (♂) and 10000 ppm (♂,♀) at all sampling times. Further, body weight and body weight gain was impaired at 1000 ppm and above on wk1-4 in both ♂ and ♀. Effects in haematology and clinical chemistry were seen at the top dose of 10000 ppm.
	28-day oral toxicity in the dog Metconazole 85:15 *cis:trans* [redacted] *1991a*	No treatment related effects on body weight and feed consumption at 100 ppm (3.7 mg a.s./kg bw/d) and 1000 ppm (37.8 mg a.s./kg bw/day). in the top-dose (10000 ppm) ♂, body weight decreases of 15% (wk1), 20% (wk2), 23% (wk3) and 24% (wk4) were recorded. Body weight losses in the ♀ at this dose-level were hardly interpretable (variation too high at the start of the study), but when comparing to wk –1, bw losses were noted at the top-dose whereas animals gained weight in the other dose-groups. The study NOAEL was set at 100 ppm based on an increased alkaline phosphatase activity and increased thyroid weight in females at 1000 ppm.
	90-day oral toxicity in rats Metconazole 80:20 *cis:trans* [redacted] *1991b*	No treatment-related effects on body weight and feed consumption at 30 ppm, 100 ppm (6.4 mg a.s./kg bw/d) and 300 ppm (19.2 mg a.s./kg bw/d). Both body weights and feed consumption were decreased during the whole treatment period at 1000 ppm (♂) and 3000 ppm (♂,♀). The study NOAEL was set at 100 ppm based on an increased WBC and hepatocellular fatty vacuolation observed at 300 ppm.
	90-day oral toxicity in rats Metconazole 99% *cis* [redacted] *1992*	No treatment related effects at 50, 150 and 450 ppm. NOAEL set at 450 ppm (28.8 mg a.s./kg bw/day) based on effects on feed consumption and body weight at 1350 ppm (88.6 mg a.s./kg bw/d) and 4050 ppm. Feed consumption was altered in all top-dose (4050 ppm) animals during the whole treatment, and at several sampling times at 1350 ppm, the effect being slightly more pronounced in the ♂. Body weight decrease was observed throughout treatment duration in both sexes at 1350 ppm and above. In addition, there were effects in haematology, clinical chemistry and organ weight at 1350 ppm and above.
	90-day oral toxicity in mice Metconazole 80:20 *cis:trans* [redacted] *1991*	No treatment-related effects on body weight and feed consumption at 30 (4.6 mg a.s./kg bw/d) and 300 ppm (50.5 mg a.s./kg bw/day). Feed consumption was impaired in all animals treated at 2000 ppm. As a consequence, body weight was decreased at this dose-level during the whole treatment period, leading to a significant drop in bw gain.

Endpoint	Relevant studies	Findings
		The study NOAEL was set at 300 ppm, based on decreased cholesterol levels, increased AST/ALT and creatinine level, increased liver and spleen weights, liver hypertrophy/vacuolation and spleen lymphoid hyperplasia at 2000 ppm.
	90-day oral toxicity in the dog Metconazole 85:15 *cis:trans* [redacted] *1991b*	No treatment related effects at 60 ppm. NOAEL was set at 60 ppm (2.6 mg a.s./kg bw/d) based on a decreased feed consumption, calculated over the whole treatment period, at 600 ppm (24.3 mg a.s./kg bw/d) (♀) and 6000 ppm (♂,♀). Consequently, body weights of animals at 600 ppm and 6000 ppm were decreased by 8% and 21%, respectively. In addition, there were effects in haematology and clinical chemistry at 600 ppm and above.
	1-year oral toxicity in the dog Metconazole 85:15 *cis:trans* [redacted] *1992*	No treatment related effects at 30 and 300 ppm. Feed consumption, body weight and body weight gain were globally unaltered in either sex over the whole treatment period. However, the ♂ showed a dose-dependent bw gain reduction when calculated over the first 13 weeks at 1000 ppm and above. The NOAEL was set at 300 ppm (10 mg a.s./kg bw/d) based on an increased alkaline phosphatase activity at 1000 ppm (36.5 mg a.s./kg bw/d) and above.
	2-generation study in rats Metconazole 95% *cis* [redacted] *1992a*	No effects on bodyweight gain of the parent animals were observed for doses up to 32 mg a.s./kg bw/d. Bodyweight gain of the F_0 generation ♀ was lower in the of 48 mg a.s./kg bw/d treatment (top-dose) than in the controls during the maturation and gestation periods, but the deficit was recovered during the lactation period. Feed consumption and food conversion efficiency were unaffected by treatment. For the F1 generation, inter-group differences in body weight gain were insignificant in the ♂, but decreased body weight gain was observed in top-dose ♀ during maturation (especially during wk 5-14), but not during gestation nor lactation.
	2-generation study in rats Metconazole 85:15 *cis:trans* [redacted] *2002*	No effects on body weight gain were observed up to 10 mg a.s./kg bw/d. Mean body weight gains of the parent animals of the F0 generation at the top-dose (49 mg a.s./kg bw/d) were significantly decreased at wk 1 and 2: up to -16.7% for P♂ and -12.1% for P♀. P♀ body weight gains were also decreased at wk1-10 (-8.4%) and during the gestation (-13%). Mean body weight gains were also decreased at the top-dose in the F_1♂ (wk0-4) and F_1♀ wk0-10.
	Developmental toxicity in rats Metconazole 80:15 *cis:trans* [redacted] *1991a*	No effects were seen at 12 mg a.s./kg bw/d. At 30 and 75 mg a.s./kg bw/d, there was a dose-related decrease of mean bodyweight gain during the first 2 days of dosing. The differences were restored at termination at 30 mg a.s./kg bw/d but not at the top-dose of 75 mg a.s./kg bw/d. Feed consumption was slightly reduced on the first 2 days of treatment at the top-dose. At the top-dose, the fetal body weight was also decreased.
	Developmental toxicity in rats Metconazole 95% *cis* [redacted] *1992b,c,d*	No effects were seen at 6 and 24 mg a.s./kg bw/d. At the top-dose of 60 mg a.s./kg bw/d, bodyweight gain of ♀ was lower than that of controls, mainly during the second half of the treatment period. Bodyweights were only minimally (about 3%) lower during this period, while feed consumption was slightly reduced during the treatment period, but not thereafter.
	Developmental toxicity in rats Metconazole 85:15 *cis:trans* [redacted] *2002*	No effects were seen at 1, 4 and 16 mg a.s./kg bw/d. At the top-dose of 64 mg a.s./kg bw/d, the overall mean bodyweight was lower compared to the control.

Endpoint	Relevant studies	Findings
	Developmental toxicity in rabbits Metconazole 80:15 *cis:trans* [redacted] *1997*	No treatment related effects on body weight were seen at 5, 10 and 20 mg a.s./kg bw/d. Does in the 40 mg a.s./kg bw/d dosage group lost weight on gestation days 24-29. The NOAEL for maternal toxicity was set at 10 mg a.s./kg bw/d based on a significant increase in alkaline phosphatase activity at 20 mg a.s./kg bw/d.
	Developmental toxicity in rabbits Metconazole *cis* [redacted] *1990*	Mean food consumption and body weight (days 7-9) of the does were decreased in both the 28 and 80 mg a.s./kg bw/d. In addition, clinical signs of toxicity (e.g. cold ears, reduced or altered fecal output) were observed at 10, 28 and 80 mg a.s./kg bw/d. Therefore, the NOAEL for maternal toxicity was set at < 10 mg a.s./kg bw/d.
	Developmental toxicity in rabbits Metconazole 85:15 *cis:trans* [redacted] *1991c*	No treatment related effects on food consumption and body weight were found at 2, 4 and 10 mg a.s./kg bw/d. For dams with live young at 4, 10 and 25 mg a.s./kg bw/d, although there appeared to be a slight dose-related bodyweight loss during days 7 to 9, bodyweight gain thereafter was similar or superior to the control. At the highest dose of 62.5 mg a.s./kg bw/d, a significant decrease in mean bodyweight gain was observed on days 7-9, and again betweend days 24-29. At 25 and 62.5 mg a.s./kg bw/d, there was an initial slight decrease in food consumprion. Based on these observations, the parental NOAELwas set at 25 mg a.s./kg bw/d.
	Developmental toxicity in rabbits Metconazole 95% *cis* [redacted] *1992a and b*	No treatment-related effects on food consumption and body weight were found at 0.5, 1, 2 and 4 mg a.s./kg bw/d. The maternal NOAEL was set at 4 mg a.s./kg bw/d based on a significant reduction in food consumption and bodyweight gain at 10 and 40 mg a.s./kg bw/d.
Indices of gestation, litter size, pup and litter weight[3]	2-generation study in rats Metconazole 95% *cis* [redacted] *1992a*	Gestation length of was slightly, but statistically significantly, increased for F0 females receiving 48 mg a.s./kg bw/d and for F1 females receiving 32 or 48 mg a.s./kg bw/d. No effects were observed up to 8 mg a.s./kg bw/d. The gestation index was unaffected by treatment for both generations. Post-implantation survival was statistically significantly reduced compared to the control in the F1 generation in females receiving 48 mg/kg bw/d, and in the F2 generation in females receiving 32 and 48 mg a.s./kg bw/d, resulting in a slight reduction in litter size. No effects were observed up to 8 mg a.s./kg bw/d. Bodyweights of offspring at birth was unaffected by treatment.
	2-generation study in rats Metconazole 85:15 *cis:trans* [redacted] *2002*	No treatment-related effects were observed up to 10 mg a.s./kg bw/day. For the F0 generation, the gestation index in the top-dose group (49 mg a.s./kg bw/d) was significantly lower than that in the control group, and duration of gestation was significantly prolonged as compared to control. For the F1 generation, the gestation index at the top-dose was significantly lower than that in the control group, and duration of gestation showed a tendency to be prolonged as compared to control (however not statistically significant). In the F1 litters, the litter size on d0 was decreased by about 11% at 49 mg a.s./kg bw/d, though without attaining significance. At the same dose, mean body weights of both F_1 ♂ and ♀ pups were slightly but not significantly lower on LD14 and LD21 than those in the control group (up to –6.5%). In the F2 litters, the litter size was unaffected by treatment. The mean body weights of the F2 pups at 49 mg a.s./kg bw/d were however significantly lower on LD0, 14 and 21 compared to the control group.

Endpoint	Relevant studies	Findings
	Developmental toxicity in rats Metconazole 80:15 *cis:trans* [redacted] *1991a*	No treatment-related effects were observed at 12 and 30 mg a.s./kg bw/d. At the top-dose of 75 mg a.s./kg bw/d, the combination of a low fetal weight and low litter size resulted in a significantly reduced litter weight.
	Developmental toxicity in rats Metconazole 95% *cis* [redacted] *., 1992b,c,d*	No treatment-related effects were observed at 6 and 24 mg a.s./kg bw/d. The litter size and fetal weight were reduced at 60 mg a.s./kg bw/d.
	Developmental toxicity in rats Metconazole 85:15 *cis:trans* [redacted] *2002*	No treatment-related effects were observed at 1, 4 and 16 mg a.s./kg bw/d. At 64 mg a.s./kg bw/d, the litter size and mean fetal weight were reduced. Mean litter weight and gravid uterus weight in this group were also markedly lower than in the control.
	Developmental toxicity in rabbits Metconazole 80:15 *cis:trans* [redacted] *1997*	No treatment-related effects were observed at 5, 10 and 20 mg a.s./kg bw/d. Treatment-related reductions in live litter size, fetal body weight were found at 40 mg a.s./kg bw/d.
	Developmental toxicity in rabbits Metconazole *cis* [redacted] *1990*	No treatment-related effects were observed at 10 mg a.s./kg bw/d. The litter size and litter weight was significantly reduced at 28 and 80 mg a.s./kg bw/d. The fetal NOAEL was therefore set at 10 mg a.s./kg bw/d. Mean fetal weight was significantly lower than in the control in the 80 mg a.s./kg bw/d treatment group.
	Developmental toxicity in rabbits Metconazole 85:15 *cis:trans* [redacted] *1991c*	No treatment-related effects were observed at 2 and 4 mg a.s./kg bw/d. At 10 and 25 mg a.s./kg bw/d, the mean litter size was slightly lower compared to the control. At the highest dose of 62.5 mg a.s./kg bw, the mean litter size was markedly reduced. At 62.5 mg a.s./kg b.w./d, there was a marked (and highly significant) reduction in mean litter weight. This was not only due to the lower mean litter size but was also a reflexion of a concomitantly lower mean fetal weight. At the other doses, litter weights were slightly lower than controls, and the findings were mainly related to the decreased litter sizes, since fetal weights were unaffected.
	Developmental toxicity in rabbits Metconazole 95% *cis* [redacted] *1992a and b*	No treatment-related effects were observed at 0.5, 1, 2, 4 and 10 mg a.s./kg bw/d. At the top dose of 40 mg a.s./kg bw/d, an increased number of late embryonic deaths resulted in a markedly reduced mean litter size. In additions, the fetal weight and mean litter weight were notably lower in top-dose animals. The fetal NOAEL was therefore set at 10 mg a.s./kg bw/d.
Indices of viability, pre- and post-implantation loss	2-generation study in rats Metconazole 95% *cis* [redacted] *1992a*	Post-implantation survival was statistically significantly reduced compared to the control in the F1 generation in females receiving 48 mg/kg bw/d, and in the F2 generation in females receiving 32 and 48 mg a.s./kg bw/d. No treatment-related effects were observed up to 8 mg a.s./kg bw/d The live birth index was slightly reduced at 48 mg a.s./kg bw/d in both the F1 and F2 generation, but subsequent survival of offspring was unaffected by treatment.
	2-generation study in rats Metconazole 85:15 *cis:trans* [redacted] *2002*	The number of live litters born was reduced in both the F1 and F2 generation in the 49 mg a.s./kg bw/d group. Offspring survival (viability index) of the F2 litters in the 49 mg a.s./kg bw/d treatment group was significantly decreased on d0 compared to that in the control. For the F1 litters, offspring survival was unaffected by treatment.

Endpoint	Relevant studies	Findings
	Developmental toxicity in rats Metconazole 80:15 *cis:trans* [redacted] *1991a*	No treatment-related effects at 12 and 30 mg a.s./kg bw/d. At the top-dose of 75 mg a.s./kg bw/d, there was a significant increase in post-implantation loss (408% of controls), involving both early and late deaths, resulting in a reduced mean litter size at this dosage
	Developmental toxicity in rats Metconazole 95% *cis* [redacted] *1992b,c,d*	No treatment-related effects at 6 and 24 mg a.s./kg bw/d. At the top-dose of 60 mg a.s./kg bw/d, the number of resorptions was increased with a consequent increase in post-implantation loss and a decrease in viable litter size.
	Developmental toxicity in rats Metconazole 85:15 *cis:trans* [redacted] *2002*	No treatment-related effects at 1, 4 and 16 mg a.s./kg bw/d. At the top-dose of 64 mg a.s./kg bw/d, there was a clear increase in post-implantation loss, resulting in a significantly lower live litter size.
	Developmental toxicity in rabbits Metconazole 80:15 *cis:trans* [redacted] *1997*	No treatment related effects at 5 and 10 mg a.s./kg bw/d. The NOAEL was set at 10 mg a.s./kg bw/d, as the 20 and 40 mg a.s./kg bw/d dosage of the test substance were associated with an increase in post-implantation loss (as evidenced by increases in the number of does with with any resorptions and the percentage of dead or resorbed conceptuses per litter).
	Developmental toxicity in rabbits Metconazole *cis* [redacted] *1990*	No treatment related effects at 10 mg a.s./kg bw/d. The NOEAL for fetal toxicity was set at 10 mg a.s./kg bw/d based on an increase in the value for late embryonic deaths and post-implantation loss at 28 and 80 mg a.s./kg bw.
	Developmental toxicity in rabbits Metconazole 85:15 *cis:trans* [redacted] *1991c*	No treatment-related effects were observed at 2 and 4 mg a.s./kg bw/d. At 10 and 25 mg a.s./kg bw/d, values for late embryonic death were slightly higher than in the controls and this was reflected in the higher post-implantation loss. At the highest dose of 62.5 mg a.s./kg bw, there was a statistically significant increase in late embryonic deaths which resulted in a markedly increased post-implantation loss. Based on these findings, the fetal NOAEL was set at 4 mg a.s./kg bw/d.
	Developmental toxicity in rabbits Metconazole 95% *cis* [redacted] *1992a and b*	No treatment-related effects were observed at 0.5, 1, 2, 4 and 10 mg a.s./kg bw/d. At the top dose of 40 mg a.s./kg bw/d, an increased number of late embryonic deaths resulted in a markedly increased post-implantation loss. The fetal NOAEL was therefore set at 10 mg a.s./kg bw/d.
Embryo/foetal toxicity including teratological effects	Developmental toxicity in rats Metconazole 80:15 *cis:trans* [redacted] *1991a*	No effects were observed at 12 mg a.s./kg bw/d. At 30 and 75 mg a.s./kg bw/d, there was a dose-related increase in the incidence of fetuses with skeletal variations. This was mainly due to increased ossification in the axial skeleton. These changes particularly included lumbar ribs, one extra pre-sacral vertebra, cervical ribs and variant sternebrae. Also at 75 mg a.s./kg bw/d, less frequent observations noted included distorted (thoracic) ribs and caudal vertebra/disc irregularities.
	Developmental toxicity in rats Metconazole 95% *cis* [redacted] *., 1992b,c,d*	There were no treatment-related increases in the incidence of fetal external, visceral or skeletal malformations up to 60 mg a.s./kg bw/d. At 24 and 60 mg a.s./kg bw/d, at necropsy of the litters, bilateral dilatation of ureter occurred more frequently than in the controls. At the top-dose of 60 mg a.s./kg bw/d, a increased bilateral dilatation of the kidney was also observed. This effects resulted in a NOAEL for developmental toxicity of 6 mg a.s./kg bw/d.

Endpoint	Relevant studies	Findings
	Developmental toxicity in rats Metconazole 85:15 *cis:trans* [redacted] *2002*	At 64 mg/kg bw/d, a dosage where fetal weight was significantly low, there was a notable increase in the incidence of minor skeletal and visceral anomalies compared with the control.
	Developmental toxicity in rabbits Metconazole 80:15 *cis:trans* [redacted] *1997*	There were no treatment-related effects on fetal weight, gross external or soft tissue or skeletal malformations up to 40 mg a.s./kg bw/d, the highest dose tested. The development NOAEL was therefore set at 40 mg a.s./kg bw/d
	Developmental toxicity in rabbits Metconazole *cis* [redacted] *1990*	There were no clear treatment-related effects on the incidence of malformed fetuses up to 80 mg a.s./kg bw/d.
	Developmental toxicity in rabbits Metconazole 85:15 *cis:trans* [redacted] *1991c*	There were no treatment-related effects at 2 and 4 mg a.s./kg bw/d. At 10 and 25 mg a.s./kg bw/d, an increased incidence of hydrocephaly was detected. At 62.5 mg a.s./kg bw/d the percentage of skeletal anomalies was noticeably higher than in the control, which was principally due to the incidence of cranial anomalies and cervical ribs. Therefore, the developmental NOAEL was set at 4 mg a.s./kg bw/d.
	Developmental toxicity in rabbits Metconazole 95% *cis* [redacted] *1992a and b*	There were not treatment-related effects at 0.5, 1, 2 and 4 mg a.s./kg bw/d. There was an increased incidence of hydrocephaly/hydranencephaly at 10 and 40 mg a.s./kg bw/d. Further, there was also an increased proportion of fetuses with both visceral and skeletal anomalies at 40 mg a.s./kg bw/d. The developmental NOAEL was therefore set at 4 mg a.s./kg bw/d.
Number aborting and number delivering early	2-generation study in rats Metconazole 95% *cis* [redacted] *1992a*	Parturation was normal for all tested doses (up to 48 mg a.s./kg bw/d)
	2-generation study in rats Metconazole 85:15 *cis:trans* [redacted] *2002*	Parturation was normal for doses up to 10 mg a.s./kg bw/d. At the top-dose of 49 mg a.s./kg bw/d, there was an increased maternal death during delivery (5 females of the F0 generation and 4 females of the F1 generation).
	Developmental toxicity in rabbits Metconazole 80:15 *cis:trans* [redacted] *1997*	No effects on the number aborting and number delivering early were observed for any of the tested doses.
	Developmental toxicity in rabbits Metconazole *cis* [redacted] *1990*	No effects on the number aborting and number delivering early were observed for the 10 and 28 mg a.s./kg bw/d treatment groups. At the highest dose of 80 mg a.s./kg bw/d, 4/5 pregnant females aborted.
	Developmental toxicity in rabbits Metconazole 85:15 *cis:trans* [redacted] *1991c*	No treatment-related effects on the number aborting and number delivering early were observed at 2, 4, 10 and 25 mg a.s./kg bw/d. One abortion occurred at 10 mg a.s./kg bw/d (day 19/21), but it was considered unlikely to be related to treatment. At the highest test dose of 62.5 mg a.s./kg bw/d, there were 3 abortions.
	Developmental toxicity in rabbits Metconazole 95% *cis* [redacted] *1992a and b*	No effects on the number aborting and number delivering early were observed for any of the tested doses (up to 40 mg a.s./kg bw/d).

Endpoint	Relevant studies	Findings
Systemic toxicity and effects on adult body weight	2-generation study in rats Metconazole 95% *cis* [redacted]., *1992a*	No effects on bodyweight gain of the parent animals were observed for doses up to 32 mg a.s./kg bw/d. Bodyweight gain of the F_0 generation ♀ was lower in the of 48 mg a.s./kg bw/d treatment (top-dose) than in the controls during the maturation and gestation periods, but the deficit was recovered during the lactation period. Feed consumption and food conversion efficiency were unaffected by treatment. For the F1 generation, inter-group differences in body weight gain were insignificant in the ♂, but decreased body weight gain was observed in top-dose ♀ during maturation (especially during wk 5-14), but not during gestation nor lactation.
	2-generation study in rats Metconazole 85:15 *cis:trans* [redacted] *2002*	No effects on body weight gain were observed up to 10 mg a.s./kg bw/d. Mean body weight gains of the parent animals of the F0 generation at the top-dose (49 mg a.s./kg bw/d) were significantly decreased at wk 1 and 2: up to -16.7% for P♂ and -12.1% for P♀. P♀ body weight gains were also decreased at wk1-10 (-8.4%) and during the gestation (-13%). Mean body weight gains were also decreased at the top-dose in the F_1♂ (wk0-4) and F_1♀ wk0-10.
	Developmental toxicity in rats Metconazole 80:15 *cis:trans* [redacted] *1991a*	No effects were seen at 12 mg a.s./kg bw/d. At 30 and 75 mg a.s./kg bw/d, there was a dose-related decrease of mean bodyweight gain during the first 2 days of dosing. The differences were restored at termination at 30 mg a.s./kg bw/d but not at the top-dose of 75 mg a.s./kg bw/d.
	Developmental toxicity in rats Metconazole 95% *cis* [redacted]., *1992b,c,d*	At the top-dose of 60 mg a.s./kg bw/d, bodyweight gain of ♀ was lower than that of controls, mainly during the second half of the treatment period.
	Developmental toxicity in rats Metconazole 85:15 *cis:trans* [redacted] *2002*	No effects were seen at 1, 4 and 16 mg a.s./kg bw/d. At the top-dose of 64 mg a.s./kg bw/d, the overall mean bodyweight was lower compared to the control.
	Developmental toxicity in rabbits Metconazole 80:15 *cis:trans* [redacted] *1997*	No treatment related effects on parent body weight were seen at 5, 10 and 20 mg a.s./kg bw/d. Does in the 40 mg a.s./kg bw/d dosage group lost weight on gestation days 24-29. The NOAEL for maternal toxicity was set at 10 mg a.s./kg bw/d based on a significant increase in alkaline phosphatase activity at 20 mg a.s./kg bw/d.
	Developmental toxicity in rabbits Metconazole *cis* [redacted] *1990*	Mean body weight (days 7-9) of the does were decreased in both the 28 and 80 mg a.s./kg bw/d. In addition, clinical signs of toxicity (e.g. cold ears, reduced or altered fecal output) were observed at 10, 28 and 80 mg a.s./kg bw/d. Therefore, the NOAEL for maternal toxicity was set at < 10 mg a.s./kg bw/d.
	Developmental toxicity in rabbits Metconazole 85:15 *cis:trans* [redacted] *1991c*	No treatment related effects on body weight were found at 2, 4 and 10 mg a.s./kg bw/d. For dams with live young at 4, 10 and 25 mg a.s./kg bw/d, although there appeared to be a slight dose-related bodyweight loss during days 7 to 9, bodyweight gain thereafter was similar or superior to the control. At the highest dose of 62.5 mg a.s./kg bw/d, a significant decrease in mean bodyweight gain was observed on days 7-9, and again betweend days 24-29.
	Developmental toxicity in rabbits Metconazole 95% *cis* [redacted] *1992a and b*	No treatment-related effects on food consumption and body weight were found at 0.5, 1, 2 and 4 mg a.s./kg bw/d. The maternal NOAEL was set at 4 mg a.s./kg bw/d based on a significant reduction in food consumption and bodyweight gain at 10 and 40 mg a.s./kg bw/d.

Endpoint	Relevant studies	Findings
Indices of post-natal growth[4], indices of lactation and data on physical landmarks	2-generation study in rats Metconazole 95% *cis* [redacted] *1992a*	While bodyweights of offspring at birth were unaffected by treatment, subsequent bodyweight gain of pups of the F1 generation dosed at 8, 32 or 48 mg/kg bw/d were decreased when calculated until weaning. The reductions at 8 mg/kg bw/d and above were small (5-7%). Bodyweight gain of the pups of the F2 generation up to weaning at 48 mg a.s./kg bw/d was slightly lower than that of the controls. For both the F1 and F2 generation, the rate of physical development, as assessed by the timing of onset and completion of pinna unfolding, hair growth and tooth eruption was unaffected by treatment. The onset of eye opening in offspring receiving 32 or 48 mg/kg bw/d occurred slightly earlier than in the control group and just outside the lower limit of the background data
	2-generation study in rats Metconazole 85:15 *cis:trans* [redacted] *2002*	In the F1 litters, at 49 mg a.s./kg bw/d, mean body weights of both F_1 ♂ and ♀ pups were slightly but not significantly lower on LD14 and LD21 than those in the control group (up to –6.5%). In the F2 litters, the mean body weights of the F2 pups at 49 mg a.s./kg bw/d were significantly lower on LD0, 14 and 21 compared to the control group.
Survival and general toxicity up to sexual maturity	2-generation study in rats Metconazole 95% *cis* [redacted] *1992a*	The live birth index was slightly reduced at 48 mg a.s./kg bw/d in both the F1 and F2 generation, but subsequent survival of offspring was unaffected by treatment.
	2-generation study in rats Metconazole 85:15 *cis:trans* [redacted]	At the top-dose (49 mg a.s./kg bw/d), relative spleen weights of F_1 ♂ and ♀ pups were significantly increased and absolute (but not relative) brain weights were significantly decreased. For F_2 ♀ pups in the 49 mg a.s./kg bw/d group, the relative spleen weight was significantly higher than those in the control group. Absolute brain weights of F_2♂ and ♀ pups in the 45 mg a.s./kg bw group were significantly lower than those in the control group; however, no significant differences were noted in the relative weights of their brains. No treatment-related effects for macroscopic and microscopic findings were reported.

[1] This is included as an indicator of parental effects with potential to disrupt reproduction. It is considered in the reproductive assessment for mammals but not for birds, where $LD_{50}/10$ is used instead

[2] Effects derived from absorption of the substance that causes modification of an organ or an apparatus (biochemical, physiological and/or morphological). Examples include behavioural or physiological impairment (e.g. reduced locomotive activity, altered reflexes).

[3] Any effects in foetal body weight should be evaluated in the context of all pertinent data including other developmental effects as well as maternal toxicity.

[4] For example body weight gain, ear and eye opening, tooth eruption, hair growth and effects on sexual maturation such as age and body weight at vaginal opening or balano-preputial separation.

[5] The studies by [redacted] 1992a and 1992b are discussed together, as they were also considered together in Volume 3 (AS) Section B.6.6.2 to derive an overall NOAEL from both studies

B.9.2.2.2. Exposure

Exposure of mammals will be predominantly dietary, through the consumption of residues on food items. Direct exposure of birds to BAS 555 01 F applications is considered unlikely, since at the time of application and for a short period thereafter, most mammals will leave the immediate vicinity of spray operations in response to the human disturbance.

B.9.2.2.3. Risk assessment for metconazole

The endpoints for the active substance used in the risk assessment for mammals are shown in Table B.9.2.2.3-1.

Table B.9.2.2.3-1: Summary of the endpoints used in the risk assessment for mammals

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Rat / Mouse	BAS 555 F (metconazole)	Single dose (Acute oral)	LD_{50}	566.7 mg a.s./kg bw	Geomean endpoint calculated based on the studies referenced in Table B.9.2.2.1-3
Rat	BAS 555 F (metconazole)	2-genereation reproduction study	NOAEL	8 mg a.s./kg bw/day	[redacted] 1992a

Acute risk assessment for mammals – Screening step

The intended use patterns for the representative formulation BAS 555 01 F are shown in Table B.9-1. For the proposed use in oilseed rape, BAS 555 01 F can either be applied once in autumn (at BBCH 13-20) followed by a second application in spring (at BBCH 21-71), or can be applied two times in spring (at BBCH 21-71) with a 14-day interval. In the first case, the interval between the two applications is of such a length (min. 90 days) that both applications can be considered as independent in the context of the risk assessment for mammals[9]. Taking this into account, the screening step crop grouping and critical use patterns relevant for the uses of BAS 555 01 F are given in Table B.9.2.2.3-2. Note that for the proposed use in oilseed rape, only the worst case use rate of 2 x 0.072 kg a.s./ha is considered further in the risk assessment below. However, to cover also the single application in earlier growth stages, worst case scenarios for BBCH 13-20 are included in the calculations.

[9] The Multiple Application Factor (MAF) calculated using the equations given in Appendix H of the EFSA Guidance Document, based on a default DT_{50} of 10 days and an interval of 150 days is equal to 1.0.

Table B.9.2.2.3-2: Screening step crop groupings and critical use patterns considered in the risk assessment for the use of metconazole in BAS 555 01 F.

Crop group	GAP crop species	EU region	Application time (BBCH growth stage)	Indicator species	Critical use pattern		
					Rate (kg a.s./ha)	Max no. of applications	App. interval (days)
Cereals	Cereals	North, Central & South	30 - 69	Small herbivorous mammal	0.090	2	21
Oilseed rape	Winter oilseed rape	North, Central & South	13 – 20 (autumn)	Small herbivorous mammal	0.072	1	-
			21 – 71 (spring)		0.072	1	-
			21 – 71		0.072	2 [1)]	14 [1)]

[1)] *for oilseed rape, only the worst case use rate of 2x0.072 kg a.s./ha is used for the risk assessment; to cover also the single application in earlier growth stages, worst case scenarios for BBCH 13-20 were included in the calculations*

The acute 'Daily Dietary Dose' (DDD) is calculated by multiplying the shortcut value (SV) based on the 90th percentile residues by the application rate in kg a.s./ha. When more than one application is intended, this product is further multiplied with an appropriate multiple application factor for 90th percentile residue data (MAF_{90}).

$$DDD_{\text{multiple applications}} = \textit{application rate [kg as/ha]} \times SV \times MAF_{90}$$

The acute risk to mammals is assessed by calculation of an acute Toxicity:Exposure ratio (TER_A) according to the following equation:

$$TER_A = \frac{LD_{50}[mg/kg\ bw]}{DDD[mg/kg\ bw]}$$

The daily dietary dose (DDD) based on the above equation and TER values for the relevant indicator species for acute exposure to metconazole following the proposed uses of BAS 555 01 F are given in Table B.9.2.2.3-3 below. Shortcut values were derived from Table 6 of the EFSA Guidance Document. MAF_{90} values were calculated as described in Appendix H of the EFSA Guidance Document.

Table B.9.2.2.3-3: Screening step – estimates of acute exposure to metconazole and the risk to mammals from such exposure following application of BAS 555 01 F in cereals and oilseed rape.

Crop group	Indicator species	Shortcut value (mg a.s./kg bw)	App. rate (kg a.s./ha)	MAF	DDD (mg a.s./kg bw)	LD_{50} (mg a.s./kg bw)	TER_A
Cereals	Small herbivorous mammal	118.4	0.090	1.1	11.72	566.7	48.3
Oilseed rape	Small herbivorous mammal	118.4	0.072	1.2	10.23	566.7	55.4

Note: TER shown in bold falls below the relevant trigger

The TER_A values for all proposed uses exceed the Annex VI trigger of 10, indicating **an acceptable acute risk to mammals** from exposure to metconazole following the proposed uses of BAS 555 01 F in cereals and oilseed rape.

It is noted that even when a more conservative approach is followed in the acute risk assessment for birds (i.e. the lowest available LD_{50} of 410 mg a.s./kg bw is used instead of the geomean LD_{50} of 566.7 mg a.s./kg bw), the calculated TER_A values at the screening step are between 35.0 and 40.1 and thus also exceed the trigger of 10.

Reproductive risk assessment for mammals – screening step

The screening step crop grouping and critical use patterns relevant to the uses of BAS 555 01 F are given in Table B.9.2.2.3-2 above.

The long-term 'Daily Dietary Dose' (DDD) is calculated by multiplying the shortcut value (SV) based on the mean residues by the application rate in kg a.s./ha. When more than one application is intended, this product is further multiplied with an appropriate multiple application factor for mean residue data (MAF_m). The f_{twa} based upon a default DT_{50} of 10 days and a 21-day exposure is 0.53, if a long term toxicity endpoint is considered, as given in the EFSA Guidance Document.

$$DDD_{\text{multiple applications}} = \textit{application rate [kg a.s./ha]} \times SV \times MAF_m \times f_{twa}$$

Long-term risk is assessed by comparing the long-term DDD with the ecotoxicologically relevant worst case NOAEL from the reproduction studies, expressed as daily dietary dose, to give a long-term Toxicity:Exposure Ratio (TER_{LT}):

$$TER_{LT} = \frac{NOEL[mg/kg\ bw/day]}{DDD[mg/kg\ bw/day]}$$

The indicator species that is relevant for the proposed use is considered with the worst case application rate to calculate long-term DDD and TER values as shown in Table B.9.2.2.3-4 below. Shortcut values were derived from Table 12 of the EFSA Guidance Document. MAF_m values were calculated as described in Appendix H of the EFSA Guidance Document.

The TER_{LT} values for all proposed uses are below the Annex VI trigger of 5, indicating **a potential reproductive risk to mammals** from exposure to metconazole following the proposed uses of BAS 555 01 F in cereals and oilseed rape. Further consideration is thus necessary.

Table B.9.2.2.3-4: Screening step – estimates of long-term exposure to metconazole and the risk to mammals from such exposure following application of BAS 555 01 F in cereals and oilseed rape.

Crop group	Indicator species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg a.s./ha)	MAF	f_{twa}	Long-term DDD (mg a.s./kg bw/day)	NOEL (mg a.s./kg bw/day)	TER_{LT}
Cereals	Small herbivorous mammal	48.3	0.090	1.2	0.53	2.76	8	**2.89**
Oilseed rape	Small herbivorous mammal	48.3	0.072	1.4	0.53	2.58	8	**3.10**

Note: TER shown in bold falls below the relevant trigger

Reproductive risk assessment for mammals – Tier 1

For the reproductive risk assessment, the TER_{LT} values for metconazole for the use in cereals and oilseed rape at the screening step are less than the relevant trigger value. Consequently, Tier 1 assessments are required for these uses. The Tier 1 assessment initially requires identification of the appropriate crop groupings and generic focal mammal species in Annex I of the EFSA Guidance Document. The Tier 1 crop groupings and critical use pattern relevant to the uses of BAS 555 01 F are given in Table B.9.2.2.3-5.

Table B.9.2.2.3-5: Tier 1 crop groupings and critical use patterns considered in the risk assessment for the use of metconazole in BAS 555 01 F.

Tier 1 crop group	GAP crop species	EU region	GAP growth stage window	Critical use pattern		
				Rate (kg a.s./ha)	Max no. of applications	App. interval (days)
Cereals	Cereals	North, Central & South	30 - 69	0.090	2	21
Oilseed rape	Winter oilseed rape	North, Central & South	13 – 20 (autumn)	0.072	1	-
			21 – 71 (spring)	0.072	1	-
			21 – 71	0.072	2 [1)]	14 [1)]

[1)] *for oilseed rape, only the worst case use rate of 2x0.072 kg a.s./ha is used for the risk assessment; to cover also the single application in earlier growth stages, worst case scenarios for BBCH 13-20 were included in the calculations*

The generic focal species relevant for the proposed uses in cereals and oilseed rape are considered with the worst-case application rate to calculate long-term DDD and TER values as shown in Table B.9.2.2.3-6 and Table B.9.2.2.3-7 for the proposed use in cereals and oilseed rape, respectively.

Table B.9.2.2.3-6: Tier 1 – estimates of long-term exposure to metconazole and the risk to mammals from such exposure following application of BAS 555 01 F in cereals

Crop grouping/ growth stage	Generic focal species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg a.s./ha)	MAF	f_{TWA}	DDD (mg a.s./kg bw/ day)	NOEL (mg a.s./kg bw/day)	TER_{LT}
Cereals BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9	0.090	1.2	0.53	0.109	8	73.56
Cereals BBCH ≥ 40	Small herbivorous mammal "vole"	21.7				1.242		6.44
Cereals BBCH 30-39	Small omnivorous mammal "mouse"	3.9				0.223		35.84
Cereals BBCH ≥ 40	Small omnivorous mammal "mouse"	2.3				0.131		60.77

Note: TER shown in bold falls below the relevant trigger

Table B.9.2.2.3-7: Tier 1 – estimates of long-term exposure to metconazole and the risk to mammals from such exposure following application of BAS 555 01 F in oilseed rape

Crop grouping/ growth stage	Generic focal species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg a.s./ha)	MAF	f_{TWA}	DDD (mg a.s./kg bw/ day)	NOEL (mg a.s./kg bw/day)	TER_{LT}
Oilseed rape BBCH 10-19	Small insectivorous mammal "shrew"	4.2	0.072	1.4	0.53	0.224	8	35.65
Oilseed rape BBCH ≥ 20	Small insectivorous mammal "shrew"	1.9				0.102		78.81
Oilseed rape BBCH ≥ 40	Small herbivorous mammal "vole"	18.1				0.967		8.27
Oilseed rape All season	Large herbivorous mammal "lagomorph"	14.3				0.764		10.47
Oilseed rape BBCH 10-29	Small omnivorous mammal "mouse"	7.8				0.417		19.20
Oilseed rape BBCH 30-39	Small omnivorous mammal "mouse"	2.3				0.123		65.11
Oilseed rape BBCH ≥ 40	Small omnivorous mammal "mouse"	1.9				0.102		78.81

Note: TER shown in bold falls below the relevant trigger

Based on the Tier 1 assessment, the long-term TER values of the different exposure scenarios for metconazole following the proposed uses of BAS 555 01 F exceed the Annex VI trigger value of 5, indicating **an acceptable reproductive risk to mammals** from the proposed uses of BAS 555 01 F in cereals and oilseed rape.

Reproductive risk assessment for mammals – Higher Tier

For the reproductive risk assessment, the TER_{LT} values for metconazole for the use in cereals and oilseed rape exceed the relevant trigger at Tier 1, indicating an acceptable risk. However, in the dossier submitted by the applicant, the Tier 1 risk assessment was performed with a NOAEL of 5 mg a.s./kg bw/day instead of the NOAEL of 8 mg a.s./kg bw/day as used above. This NOAEL of 5 mg a.s./kg

bw/day resulted in an unacceptable risk for the "small herbivorous mammal (vole)" scenario in cereals, and triggered the submission of additional data that allows to further refine the risk assessment. The additional data provided consisted of a position paper to demonstrate that the NOAEL of 8 mg a.s./kg bw/day is the ecotoxicologically relevant NOAEL for the reproductive risk assessment for mammals (CP10.1.2.2/01 Anonymous, 2015; also discussed in Section B.9.2.2.1), and residue decline studies.

Two studies have been submitted, which investigated the residue behaviour of metconazole in either wheat or peas under field conditions in Germany, the Netherlands, Italy and Spain (CA8.1/01 Martin T., 2015; CA8.1/02 Moreno S. and Galvez O., 2015; please refer to Volume 3 (AS) Section B.9.1.4 for a summary). In total, 16 GLP field residue trials in early growth stages of plants were conducted to obtain foliar residue decline data. For each trial, metconazole was applied once at a nominal rate of 0.09 kg a.s./ha at growth stage BBCH 11-13. Based on the measured residue data, DT_{50} values were calculated for each trial site (CA8.1/03 Delgado Cartay M.D., 2015; see Volume 3 (AS) Section B.9.1.4 for a summary). The calculated DT_{50} values are shown in Table B.9.2.2.3-8. These DT_{50} values are considered applicable for all zones in the EU since there are neither clear differences between the DT_{50} values calculated for trials in the Northers vs. Southern zone nor between the DT_{50} values calculated for trials in wheat vs. peas.

Table B.9.2.2.3-8: Foliar residue decline trials with metconazole: DT_{50} in wheat and peas.

Plant	Trial	Location	Kinetic model	DT_{50} (days)[1)]
Wheat	L130737	Germany	SFO[2)]	5.4
	L130738	Germany	SFO[2)]	5.8
	L130739	Netherlands	SFO[2)]	4.1
	L130740	Netherlands	SFO[2)]	3.6
	L130741	Spain	SFO[2)]	3.7
	L130742	Spain	SFO[2)]	4.2
	L130743	Italy	SFO[2)]	8.8
	L130744	Italy	SFO[2)]	4.6
Pea	L130765	Germany	SFO[2]	3.8
	L130766	Germany	SFO[2]	3.9
	L130767	Netherlands	SFO[2]	4.1
	L130768	Netherlands	SFO[2]	3.8
	L130769	Spain	SFO[2)]	6.2
	L130770	Spain	SFO[2)]	2.4
	L130771	Italy	SFO[2)]	3.6
	L130772	Italy	SFO[2)]	4.0
			Number of suitable trials:	16
			Highest DT_{50} (days):	8.8
			Geometric mean DT_{50} (days):	**4.31**
			Median DT_{50} (days):	4.05

[1] For details on calculation of DT_{50} values for BAS 555 F (metconazole) on young plants (wheat & peas), please refer to the report by Delgado Cartay M.D. (2015; CA8.1/03, see Volume 3 (AS) Section B.9.1.4 for a summary);
[2] Single first-order kinetics

The field trials were specifically designed to obtain data suitable for use in higher tier risk assessments for birds and mammals. Herbivorous / omnivorous birds and mammals may feed on grass and weed leaves growing between crop plants, e.g. in oilseed rape fields, with a clear preference for early growth stages of grass and weeds up to BBCH 30 (see Appendix F of the EFSA Guidance Document for birds and mammals, 2009). In addition, it is assumed that mammals will not eat large leaves. Hence, the field trials on foliar residue decline of metconazole were specifically targeted to cover early growth stages. Wheat and pea were considered by the applicant to be suitable surrogate plants for monocotyledonous and dicotyledonous weeds at young stages.

Overall, RMS considers the available residue decline studies acceptable and suitable for use in a higher tier risk assessment. As a relatively high number of field trials is available (16), the geometric mean DT_{50} of 4.31 days could be used to replace the standard DT_{50} of 10 days for green plant material consumed by herbivorous mammals. Based on this metconazole-specific DT_{50} value, a refined MAF x twa factor could be calculated in accordance with the recommendations from Appendix H of the EFSA Guidance Document for birds and mammals (2009).

The applicant performed calculations to determine the MAF and twa factor for an exposure period of 21 days for metconazole in plant material food items, to refine the risk assessment for the "small herbivorous mammal (vole)" scenario for the proposed use in cereals. The information provided by the applicant is included below (*text in italic*), and is considered acceptable. As, based on the ecotoxicologically relevant NOAEL of 8 mg a.s./kg bw/day used in the risk assessment performed by the RMS, these calculation is not required to demonstrate an acceptable long-term risk to mammals, it is included for information only.

Calculation of MAF and twa for an exposure period of 21 days

The calculation of MAF and twa factor is conducted in accordance with the recommendations from Appendix H of the EFSA Guidance Document for birds and mammals (2009). In a refined exposure assessment, the (time weighted) average factor over a 21 d time interval (twa 21 d) is calculated to translate residue decline following peak exposure into a constant exposure concentration. The refined value is used in the higher tier reproductive risk assessments for the feed item plant material (grasses and non-grass herbs).

For the calculations, an EXCEL spreadsheet was developed that describes the actual concentration in feed item from the days after first treatment (DAFT) up to 200 DAFT. According to Appendix H of the EFSA Guidance Document (2009), dissipation between the application events according to single first order kinetics (SFO) was introduced in the EXCEL spreadsheet. The geometric mean DT_{50} value of 4.31 d for metconazole derived from field studies conducted in peas and wheat are considered for the calculation approach as well as the build-up of residues through multiple applications.

The calculations follow the basic formula assuming single first order dissipation kinetic:

$$C_{act}(t) = C_0 * e^{-k*t}$$

$C_{act\,(t)}$ *actual concentration at time t*
C_0 *initial concentration*
k *degradation rate constant (= ln(2) / DT_{50})*
t *time t*

Furthermore, the established spreadsheet calculates - one after the other in a resolution of 0.1 d time steps - the average concentration factors for a 21 d time period, starting from the time of the first treatment (0 DAFT) up to 200 DAFT and scans for the maximum of the resulting twa values (moving time-frame approach) (EFSA 2009/1438 (Appendix H)). The high resolution of 0.1 d time steps leads to precise results even under consideration of short DT_{50} values. The calculation of the twa, 21 d is described in the equation below.

Calculation of the twa over 21 d using a "moving time frame" approach:

$$twa, 21d = max\left[\frac{1}{21 * 10}\sum_{t=t_j, step\ 0.1}^{t_{j+20.9}} C_{act}(t)\right] for\ j = 0.05, \quad , (200 - 21 - 0.05)$$

twa, 21 d (21) *maximum average concentration in feed item for a 21 d interval*
$C_{act\,(t)}$ *actual concentration at time t*
t *time*
t_j *start time point for integration*
j *time step running variable*

The calculated maximum twa 21 d factor (including MAF) for metconazole in plant material food items for the cereal scenario (based on 2 applications, an application interval of 21 days and the a DT_{50} value of 4.31 days) is given below in Table B.9.2.2.3-9.

Table B.9.2.2.3-9: Maximum 21 d twa value for the food item grasses and non-grass herbs based on residue decline data for metconazole in wheat (monocot) and pea (dicot) plants

Crop	BBCH	Interval	No. of applications	Geometric mean DT_{50} [days]	Maximum 21 d twa factor including MAF
Cereals	≥ 40	21	2	4.31	**0.2957**

Based on the calculated maximum twa 21 d factor (including MAF), the risk assessment for the "small herbivorous mammal (vole)" scenario is refined. The calculated refined DDD and TER values are shown in Table B.9.2.2.3-10. The refined TER value exceeds the Annex VI trigger value of 5, indicating an acceptable reproductive risk to mammals for the "small herbivorous mammal (vole)" scenario for the proposed use of BAS 555 01 F in cereals.

Table B.9.2.2.3-10: Refined reproductive dietary risk assessment for the "small herbivorous mammal (vole)" scenario for the proposed use of BAS 555 01 F in cereals

Crop grouping/ growth stage	Generic focal species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg a.s./ha)	Max. 21d MAF x f_{TWA}	DDD (mg a.s./kg bw/ day)	NOEL (mg a.s./kg bw/day)	TER_{LT}
Cereals BBCH ≥ 40	Small herbivorous mammal "vole"	21.7	0.090	0.2975	0.581	8	13.77

Risk to mammals through drinking water

There are two scenarios provided in the EFSA Guidance Document for assessing the risk via the consumption of drinking water: the leaf scenario and the puddle scenario.

The ***leaf scenario*** is not deemed relevant for small mammals.

The ***puddle scenario*** is relevant for mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all intended uses of BAS 555 01 F and should therefore be assessed.

The predicted environmental concentration in puddles is calculated as follows:

$$PEC_{puddle} = \frac{AR/10}{1000(w + K_{OC} \times s)}$$

Where:
AR application rate (g/ha); divisor of 10 to achieve rate in mg/m²
w 0.02 (pore water term; volume)
s 0.0015 (soil term: volume, density, organic carbon content)

When multiple spray applications are considered, a MAF based on the DT_{50} in soil (single first order kinetics, geometric mean as used for PEC_{gw} and PEC_{sw}) may be applied to achieve the effective application rate AR_{eff}.

$$AR_{eff} = AR \times MAF_m = AR \times \frac{1 - e^{-nki}}{1 - e^{-ki}}$$

Where:
k $\ln(2)/DT_{50}$ (rate constant)
n number of applications
i application interval (d)

According to the EFSA Guidance Document, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/day) does not exceed 50 in the case of less sorptive substances (K_{OC} < 500 L/kg) or 3000 in the case of more sorptive substances (K_{OC} ≥ 500 L/kg). For metconazole, the mean K_{OC} is determined as 1071 L/kg (see Volume 3 (AS), Section B.8.1.2 on Fate and behaviour). The ratios of effective application rate to the relevant endpoint for the proposed uses of BAS 555 01 F are shown in Table B.9.2.2.3-11.

Table B.9.2.2.3-11: Ratios of effective application rate to endpoints for metconazole following the use of BAS 555 01 in winter and spring cereals, and winter oilseed rape.

Intended use	App. rate (g a.s./ha)	MAF [1]	AR_{eff} (g a.s/ha)	LD_{50} (mg a.s./kg bw)	Ratio of AR_{eff} to LD_{50}	NOEL (mg a.s./kg bw/day)	Ratio of AR_{eff} to NOEL	Ratio trigger
Cereals	90	1.86	167.0	566.7	0.295	8	20.88	3000
Oilseed rape	72	1.90	136.9	566.7	0.242	8	17.11	3000

[1] *Calculated (for cereals and oilseed rape) based on the geometric mean normalized field DT_{50} = 93.6 days for metconazole in soil (see Volume 3 (AS) Section B.8.1)*

The resulting ratio is clearly below the trigger value of 3000 indicating that the **acute and long-term risk to mammals via the consumption of drinking water can be considered acceptable** without further calculations.

Effects of secondary poisoning

According to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), substances with a log P_{OW} greater than 3 have potential for bioaccumulation. The log P_{OW} of metconazole was determined to be 3.85 (see Volume 3, Section B.2). Therefore, an assessment of the risk from bioaccumulation has to be performed.

Food chain from earthworm to earthworm-eating mammals

For the effects of secondary poisoning on earthworm-eating mammals, the dry soil approach was followed as recommended by the EFSA Guidance Document. According to this approach, the bioconcentration factor for the earthworm is calculated as follows:

$$BCF_{earthworm} = \frac{0.84 + 0.012\ P_{OW}}{f_{OC} \times K_{OC}}$$

With:

K_{OC} = organic carbon adsorption coefficient

f_{OC} = organic carbon content of soil (take 0.02 as a default value)

The estimated residues in earthworms are then calculated as:

$$PEC_{earthworm} = PEC_{soil} \times BCF_{earthworm}$$

The $PEC_{earthworm}$ is then converted to daily dose by multiplying with 1.28, and compared with the relevant long-term NOAEL. The multiplicator is based on a 10g mammal eating 12.8 g worms per day.

Table B.9.2.2.3-12 shows the parameters used to calculate the $BCF_{earthworm}$. The geometric mean K_{OC} for metconazole was derived from Volume 3 (AS), Section B.8.1.2 on Fate and behaviour. Table B.9.2.2.3-13 shows the TER values for the risk after exposure to metconazole for earthworm-eating mammals for the different proposed uses of BAS 555 01 F. The PEC_{soil} values used are the maximum accumulated PEC_{soil} values as calculated in the fate and behaviour section (see Volume 3 (PPP), Section B.8.1). The TERs exceed the trigger of 5, indicating an acceptable risk.

Table B.9.2.2.3-12: Parameters used in the calculation of the earthworm bioconcentration factor ($BCF_{earthworm}$).

Parameter	Value
Log P_{OW}	3.85
P_{OW}	7090
K_{OC} (geometric mean value) (L/kg)	1071
f_{OC}	0.02
$BCF_{earthworm}$	**4.01**

Table B.9.2.2.3-13: Estimates of exposure to metconazole through bioconcentration in earthworms, and the risk from such exposure following the application of BAS 555 01 F according to the proposed uses.

Substance	BCF_{worm}	PEC_{soil} [1] (mg/kg)	PEC_{worm}	DDD	NOEL (mg a.s./kg bw/day)	TER	Trigger
Metconazole	4.01	0.071	0.284	0.364	8	21.98	5

[1] *Worst-case accumulated PEC_{soil} values, calculated for twofold application of 72 g a.s./ha to oilseed rape (first application in autumn followed by a second application in spring).*

Food chain from fish to fish-eating mammals

According to the EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (2013)[10], the assessment for effects of secondary poisoning for fish-eating mammals should follow two steps. In a first step, the regulatory acceptable concentration for secondary poisoning (RAC_{SP}) for mammals eating fish out of surface water contaminated with a plant protection product is calculated using the following formula:

$$RAC_{SP} = \frac{NOAEL_{mammal}}{5 \times 0.138 \times BCF_{fish} \times BMF}$$

Where: RAC_{SP}: regulatory acceptable concentration in water for secondary poisoning (mg/L)
NOAEL: relevant long-term no-adverse-effect level for birds (mg/kg bw/day)
BCF_{fish}: whole body bioconcentration factor in fish (L/kg)
BMF: biomagnification factor (kg/kg)
5: assessment factor (AF) for the chronic risk assessment for terrestrial vertebrates
0.138: multiplication factor for conversion of residue in fish to daily dose for mammals (it is noted that in the EFSA Guidance Document on Risk Assessment for Birds and Mammals a value of 0.142 is reported)

For the active substance matconazole, two fish bioaccumulation studies were conducted with Bluegill Sunfish. From these studies, the highest steady state bioconcentration factor (BCF), corrected to 5% lipid content, was 105.1 (see Volume 3 (AS), Section B.9.2.2.3). The biomagnification factor (BMF) was derived from Table 25 of the EFSA Guidance Document on aquatic organisms. As the fish BCF is below 2000, a BMF of 1 is used in the risk assessment.

Table B.9.2.2.3-14 shows the calculated RAC_{SP} value for secondary poisoning for fish-eating mammals for metconazole. As a second step in the assessment, this RAC_{SP} value is compared to the 21-day TWA PEC_{SW}. For the proposed uses of BAS 555 01 F, the RAC_{SP} exceeds the 21-day TWA PEC_{SW}, indicating that the risk through secondary poisoning for fish-eating mammals is acceptable. No further assessment is thus required.

Table B.9.2.2.3-14: Estimates of exposure and risk to metconazole through bioconcentration in fish following the application of BAS 555 01 F in cereals and oilseed rape.

BCF_{fish}	Multiplication factor	Assessment factor	BMF	NOEL (mg a.s./kg bw/day)	RAC_{SP} (mg/L)	21-day TWA PEC_{SW} [b] (mg/L)	Acceptable risk?
105.1	0.138	5	1	8	0.110	0.025	yes

[a] *the worst-case Step 1 21-day TWA PEC_{SW} for the application of 2 x 90 g a.s./ha in cereals. This value covers the Step 1 21-day TWA PEC_{SW} for the proposed uses in oilseed rape).*

Biomagnification in terrestrial food chains

As also discussed in Section B.9.3.3, metconazole shows very short clearance times in the available fish bioconcentration studies (CA8.2.2.3/01 [redacted] 1996; CA8.2.2.3/02 [redacted] M., 2002; see Volume 3

[10] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013; 11(7):3290. 268 pp.

(AS) Section B.9.2.2.3 for a summary). Based on these two BCF studies, despite the relatively high lipophilicity of metconazole, it can be concluded that the potential for bioaccumulation is low, due to the low accumulation and rapid excretion of metconazole from fish. No further assessment on biomagnification is thus required.

Conclusion

The acute and long-term risk of metconazole to mammals is acceptable following the intended uses of BAS 555 01 F in cereals and oilseed rape.

The risk to mammals through drinking water, secondary poisoning and biomagnification in the food chain can also be considered acceptable.

B.9.2.2.4. Risk assessment for the representative formulation BAS 555 01 F

The endpoints for the active substance used in the risk assessment for birds are shown in Table B.9.2.2.4-1.

Table B.9.2.2.4-1: Summary of the formulation endpoints used in the risk assessment for mammals

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Rat	BAS 555 01 F	Single dose (Acute oral)	LD_{50}	2102 mg product/kg bw (= 181 mg a.s./kg bw)	Bradley D., 1997

Acute risk assessment for mammals – Screening step

The intended use patterns for the representative formulation BAS 555 01 F are shown in Table B.9-1. BAS 555 01 F is intended to be used at a maximum single application rate of 1.0 L/ha in cereals and 0.8 L/ha in oilseed rape. Taking into account the density of the formulation of 1.046 g/cm³, this is equivalent to an application rate of 1.046 kg formulation/ha and 0.8368 kg formulation/ha in cereals and oilseed rape, respectively.

For the proposed use in oilseed rape, BAS 555 01 F can either be applied once in autumn (at BBCH 13-20) followed by a second application in spring (at BBCH 21-71), or can be applied two times in spring (at BBCH 21-71) with a 14-day interval. In the first case, the interval between the two applications is of such a length (min. 90 days) that both applications can be considered as independent in the context of the risk assessment for mammals[11]. Taking this into account, the screening step crop grouping and critical use patterns relevant for the uses of BAS 555 01 F are given in Table B.9.2.2.4-2. Note that for the proposed use in oilseed rape, only the worst case use rate of 2 x 0.8368 kg BAS 555 01 F/ha is considered further in the risk assessment below. However, to cover also the single application in earlier growth stages, worst case scenarios for BBCH 13-20 are included in the calculations.

[11] The Multiple Application Factor (MAF) calculated using the equations given in Appendix H of the EFSA Guidance Document, based on a default DT_{50} of 10 days and an interval of 150 days is equal to 1.0.

Table B.9.2.2.4-2: Screening step crop groupings and critical use patterns considered in the risk assessment for the use of BAS 555 01 F.

Crop group	GAP crop species	EU region	Application time (BBCH growth stage)	Indicator species	Critical use pattern		
					Rate (kg form./ha)	Max no. of applications	App. interval (days)
Cereals	Cereals	North, Central & South	30 - 69	Small omnivorous bird	1.046	2	21
Oilseed rape	Winter oilseed rape	North, Central & South	13 – 20 (autumn)	Small omnivorous bird	0.8368	1	-
			21 – 71 (spring)		0.8368	1	-
			21 – 71		0.8368	2 [1)]	14 [1)]

[1)] *for oilseed rape, only the worst case use rate of 2x0.8368 kg form./ha is used for the risk assessment; to cover also the single application in earlier growth stages, worst case scenarios for BBCH 13-20 were included in the calculations*

The acute 'Daily Dietary Dose' (DDD) is calculated by multiplying the shortcut value (SV) based on the 90th percentile residues by the application rate in kg a.s./ha. When more than one application is intended, this product is further multiplied with an appropriate multiple application factor for 90th percentile residue data (MAF_{90}).

$$DDD_{\text{multiple applications}} = \textit{application rate [kg as/ha]} \times SV \times MAF_{90}$$

The acute risk to mammals is assessed by calculation of an acute Toxicity:Exposure ratio (TER_A) according to the following equation:

$$TER_A = \frac{LD_{50}[mg/kg\ bw]}{DDD[mg/kg\ bw]}$$

The daily dietary dose (DDD) based on the above equation and TER values for the relevant indicator species for acute exposure to BAS 555 01 F are given in Table B.9.2.2.4-3 below. Shortcut values were derived from Table 8 of the EFSA Guidance Document. MAF_{90} values were calculated as described in Appendix H of the EFSA Guidance Document.

Table B.9.2.2.4-3: Screening step – estimates of acute exposure to BAS 555 01 F and the risk to mammals from such exposure following application in cereals and oilseed rape.

Crop group	Indicator species	Shortcut value (mg/kg bw)	App. rate (kg form./ha)	MAF	DDD (mg form./kg bw)	LD_{50} (mg form./kg bw)	TER_A
Cereals	Small herbivorous mammal	118.4	1.046	1.1	136.2	2102	15.43
Oilseed rape	Small herbivorous mammal	118.4	0.8368	1.2	118.9	2102	17.67

Note: TER shown in bold falls below the relevant trigger

The TER_A values for all proposed uses exceed the Annex VI trigger of 10, indicating **an acceptable risk to mammals** from exposure to BAS 555 01 F following the proposed uses in cereals and oilseed rape.

Conclusion

The acute risk of BAS 555 01 F to mammals is acceptable following the intended uses in cereals and oilseed rape.

B.9.3. EFFECTS ON AQUATIC ORGANISMS

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

The representative formulation for Annex I renewal of metconazole (BAS 555 01 F) is different from the one used for the initial Annex I inclusion. Therefore, new acute toxicity studies with the formulation BAS 555 01 F on fish, aquatic invertebrates and algae have been submitted. A summary of the available studies is provided below.

Report:	**CP10.2.1/01. [redacted] (2003)** **BAS 555 01 F - Acute toxicity study on the rainbow trout (*Oncorhynchus mykiss*) in a static system over 96 hours**

Report No.:	2003/1014059
Guidelines:	OECD 203, EEC 92/69 A V C 1, EPA 72-1, EPA 540/9-82-024
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and methods:	
Test substance:	BAS 555 01 F (Batch No.: R2066-079, contains 89.2 g/L of the active ingredient metconazole)
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms, age, weight, length, loading:	10 fish per aquarium (50 L), 1 aquarium and per treatment and control. Fish approximately 3 months old; mean weight: 1.03 g (0.59-1.51 g); Mean length: 5.0 cm (4.4-5.7 cm); Loading of 0.2 g fish/L
Type of test:	96h static acute toxicity test
Applied and measured concentrations:	
Nominal test concentrations:	0 (control), 1, 2.2, 5, 10 and 22 mg formulation/L (corresponding to 0, 0.085, 0.19, 0.43, 0.85 and 1.88 mg a.s./L)
Mean measured concentrations:	0 (control), 0.84, 1.99, 4.63, 9.67 and 21.59 mg formulation/L (corresponding to 0, 0.072, 0.17, 0.40, 0.83, 1.84 mg a.s./L)
Test conditions:	50 L glass aquaria, non-chlorinated charcoal filtered tap water Temperature: 11-12°C pH: 8.2-8.5 Oxygen content: 8.3-11.2 mg/L dissolved oxygen Water hardness: approximately 250 mg/L $CaCO_3$ Photoperiod: 16:8 hours light:dark Light intensity: approximately 82 – 280 lux
Analytical methods:	HPLC with UV-detection
Statistics:	LC_{50} values were calculated using probit analysis (Finney, 1971)

Test procedure: After adding the test solution to the reaction vessels, ten fish were placed in each aquarium. The fish were not fed during the test. The aquaria were not aerated. The fish were observed for mortality and toxic signs (changes in appearance and abnormal behaviour in comparison to the control group) within 1 hour after start of exposure and 4, 24, 48, 72 and 96 h after start of exposure.

Dead fish were removed from the test vessels. After the observations made at 24h intervals, temperature, oxygen content and pH were measured in each test vessel.

Findings:

Analytical results:

The analytical results were expressed as mg formulation/L. This was done, to achieve an easier comparability with the nominal concentrations of the test samples, which were provided in the same dimension. The results of the test samples were therefore quantified by direct comparison against calibration solutions, which were prepared by using the same GLP-certified test item (the formulation BAS 555 01 F) as used in the aquatic ecotoxicology studies. Since the same substance/product is used as test and reference item, this procedure directly provides results based on formulation, without further calculations (conversion between concentrations based on formulation and active ingredient).

The analytically detected concentrations for BAS 555 01 F are shown in Table B.9.3.1-1. The measured concentrations ranged from 82.0% - 98.8% of nominal at test initiation and from 88.5% - 97.5% of nominal at test termination. Therefore, the biological results are based on nominal concentrations.

Table B.9.3.1-1: Analytically determined concentrations of the test substance BAS 555 01 F during exposure in the aquaria.

Nominal concentration (mg form./L)	Measured concentration at 0h		Measured concentration at 96h		Mean measured concentration[a]	
	concentration (mg form./L)	% of nominal	concentration (mg form./L)	% of nominal	concentration (mg form./L)	% of nominal
0	<LOQ	-	<LOQ	-	-	-
1	0.82	82.0	0.87	86.5	0.84	84.2
2.2	2.05	93.0	1.93	87.7	1.99	90.3
5	4.80	96.0	4.45	89.0	4.63	92.4
10	9.81	98.1	9.54	95.4	9.67	96.7
22	21.74	98.8	21.45	97.5	21.59	98.1

[a]*geometric mean value; LOQ = limit of quantification*

Biological results: The cumulative mortality throughout the test is shown in Table B.9.3.1-2. After 96 hours, BAS 555 01 F caused no mortality up to a concentration of 5 mg form./L. 100% mortality were observed at the highest test rate of 22 mg form./L. The 24, 48, 72 and 96h LC_{50} values (based on nominal concentrations) were calculated to be 14.8, 14.8, 13.2 and 10 mg form./L, respectively (95% confidence limits could not be determined).

Table B.9.3.1-2: Cumulative mortality of *Oncorhynchus mykiss* exposed to BAS 555 01 F.

Nominal concentration (mg form./L)	Number of fish	1h	4h	24h	48h	72h	96h
0	10	0	0	0	0	0	0
1	10	0	0	0	0	0	0
2.2	10	0	0	0	0	0	0
5	10	0	0	0	0	0	0
10	10	0	0	0	0	1	5
22	10	0	10	10	10	10	10

The only toxic symptom observed was apathy, which occurred at the test concentrations of 10 and 22 mg BAS 555 01 F/L throughout the whole exposure period. For the test concentrations of up to 5 mg BAS 555 01 F/L, no toxic signs were observed.

Conclusions:
In a static acute toxicity study of BAS 555 01 F the LC_{50} (96 h) based on nominal concentrations on the rainbow trout was about 10 mg form./L. The NOEC (96 h) was determined to be 5 mg form./L (nominal).

RMS comments:
The validity criteria of the most recent version of OECD Test Guideline 203 are met:

- The mortality in the control did not exceed 10% (measured: 0%)
- The dissolved oxygen concentration was at least 60% of the air saturation value throughout the test (measured: 8.3-11.2 mg/L, which corresponds to approximately 75-100% of the air saturation value at 12°C)

Consequently, this study is considered acceptable for use in the risk assessment.

The analytical method used (CP-371) could be fully validated according to the current EU Guidance SANCO/3029/99 rev. 4. The LOQ was set at 0.000101 mg/L for *cis*-metconazole and 0.001054 mg/L for *trans*-metconazole (please refer to Vol. 3 (CA), Section B.5.1.2.6 – study no. 11, for further details). The fortification range covers the tested concentrations and the endpoints.

The following endpoints, based on nominal concentrations, will be considered in the risk assessment:
LC_{50} (*Oncorhynchus mykiss*, 96h) = 10 mg form./L (equivalent to 0.85 mg a.s./L)
NOEC (*Oncorhynchus mykiss*, 96h) = 5 mg form/L (equivalent to 0.43 mg a.s./L)

Report:	**CP10.2.1/02. Oliveiri C.E., Boeri R.L. and Ward T.J. (2000a) Acute toxicity of AC 900768 (Metconazole) in a 90 g/L SL formulation (RLF 12307) to *Daphnia magna* under static test conditions**

Report No.: MK-560-011
Guidelines: OECD 202; EEC Method C2; EPA 72-2
GLP: Yes

Previous evaluation: None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application

Materials and Methods:
Test substance: BAS 555 01 F (Batch No.: R1811-181, contains 92.3 g/L of the active ingredient metconazole)
Test species: *Daphnia magna*
Number of organisms, age: 2 x 10 organisms/concentration, less than 24h old
Type of test: 48h static toxicity test
Applied and measured concentrations:
Nominal test concentrations: 0 (control), 3.8, 7.5, 15, 30, 60 and 120 mg formulation/L (corresponding to 0, 0.34, 0.66, 1.3, 2.6, 5.3, and 11 mg a.s./L)
Mean measured concentrations: 0 (control), 2.97, 6.04, 12.9, 26.1, 49.5 and 102 mg formulation/L (corresponding to 0, 0.262, 0.533, 1.14, 2.30, 4.37 and 9.00 mg a.s./L)
Test conditions : glass vessels, test volume 200 mL (dilution water: carbon filtered deionized water)

temperature: 19.7-20.6°C
pH: 7.1-7.3
oxygen content: 8.4-9.1 mg/L
total hardness: 176 mg/L as $CaCO_3$ at test initiation
photoperiod: 16:8 h light:dark

Analytical methods: gas chromatography (GC-NPD), Method FAMS 058-01

Statistics: Descriptive statistics, probit analysis and binominal/nonlinear interpolation methods

Test procedure: The numbers of immobilised *D. magna* and the occurrence of sublethal effects were determined visually and recorded initially and after 24 and 48 h after the start of exposure. Samples of the test solution were taken immediately after introduction of the test materials and at the end of the test.

Findings:

Analytical results: Analytical verification of the active substance concentrations was conducted for each test concentration at the beginning and at the end of the test. Based on the measured active substance concentrations, the concentration of the test item was calculated (taking into account the active substance concentration in the formulation and the formulation density of 1.047 g/mL). The calculated test item concentrations at test initiation ranged from 80.9% to 90% of nominal. At test termination the calculated values ranged from 65.8% to 95% of nominal. Therefore the following biological results are based on mean measured concentrations of the test item.

Table B.9.3.1-3: Calculated concentration of BAS 555 01 F in the test media collected during the acute toxicity test with *Daphnia magna*.

Nominal concentration of test item (mg form./L)	Calculated concentration of test item (mg form./L)[1]					
	0 hour	% of nominal	48 hours [2]	% of nominal	Mean	% of nominal
0 (control)	<0.0113 [3]	-	<0.0113	-	<0.0113	-
			<0.0113	-		
3.8	3.15	82.9	2.50	65.8	2.97	78.2
			3.07	80.8		
7.5	6.07	80.9	5.29	70.5	6.04	80.5
			6.72	89.6		
15	13.5	90.0	10.9	72.7	12.9	86.0
			13.6	90.7		
30	26.4	88.0	22.8	76.0	26.1	87.0
			28.5	95.0		
60	50.0	83.3	44.1	73.5	49.5	82.5
			53.8	89.7		
120	100	83.3	92.7	77.3	102	85.0
			114	95.0		

[1]The concentration of test item was calculated based on the measured concentration of active substance (in mg a.s./L), the concentration of active substance in the test item (92.3 g a.s./L) and the density of the test item (1.047 g/mL); [2]48 hour samples were analysed twice due to low initial results. The mean of the 48 hour values is used in the calculation of the concentration mean; [3]0.0113 mg form./L is the limit of quantification in water

Biological results: The immobilization of *Daphnia magna* for the different concentrations tested is shown in Table B.9.3.1-4. No immobility occurred at concentrations up to 6.04 mg test item/L. At concentrations of 49.5 mg test item/L and higher all daphnids were immobile after 24 and 48 hours. No other sub-lethal effects were observed during the test.

The 24 and 48h EC_{50} values were calculated to be 12.3 and 9.28 mg test item/L, respectively (95% confidence limits 9.95-15.1 mg test item/L and 6.04-12.9 mg test item/L).

Table B.9.3.1-4: Immobilisation of *Daphnia magna* exposed to a range of concentrations of BAS 555 01 F.

Mean measured concentration of test item (mg form./L)	Number of *Daphnia magna*	Cumulative total immobilized		% immobilized	
		24h	48h	24h	48 h
0 (control)	20	0	0	0	0
2.97	20	0	0	0	0
6.04	20	0	0	0	0
12.9	20	15	19	75	95
26.1	20	17	19	85	95
49.5	20	20	20	100	100
102	20	20	20	100	100

Conclusion:
In a 48 hours static acute toxicity study with *Daphnia magna* the EC_{50} of BAS 555 01 F was determined to be 9.28 mg/L, the NOEC was 6.04 mg/L (mean measured).

RMS Comments:
The validity criteria of the most recent version of OECD Test Guideline 202 are met:

- The immobilisation in the control did not exceed 10% (measured: 0%)
- The dissolved oxygen concentration at the end of the test was > 3 mg/L (measured: 8.4 mg/L)

Consequently, this study is considered acceptable for use in the risk assessment.

The analytical method used (FAMS 058-01) could be fully validated according to the current EU Guidance SANCO/3029/99 rev. 4 for 0.05-0.5 mg/L metconazole. For higher concentrations, the method is not fully validated, but based on the available information, there are indications that concentrations > 0.5 mg/L are also covered. Therefore, the method can be considered "fit for purpose". An LOQ was set at 0.05 mg a.s./L for metconazole (please refer to Vol. 3 (CA), Section B.5.1.2.6 – study no. 2-5, for further details).

The following endpoints, based on mean measured concentrations, will be considered in the risk assessment:

EC_{50} (*Daphnia magna*, 48h) = 9.28 mg form./L (95% confidence limits = 6.04-12.9 mg form./L) (equivalent to 0.82 mg a.s./L)

NOEC (*Daphnia magna*, 48h) = 6.04 mg form./L (equivalent to 0.53 mg a.s./L)

Report:	**CP10.2.1/03. Oliveiri C.E., Boeri R.L. and Ward T.J. (2000b) Effects of AC 900768 (Metconazole) in a 90 g/L SL formulation (RLF 12307) on growth of the green alga, *Selenastrum capricornutum***

Report No.:	MK-560-012
Guidelines:	OECD 201; EEC Guideline No. L 383 A Method C3
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application

Material and Methods:

Test substance:	BAS 555 01 F (Batch No.: R1811-181, contains 92.3 g/L of the active ingredient metconazole)
Test species:	*Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum*)
Number of organisms:	3 replicates per concentration (6 in the control); 10^4 cells/mL at initiation
Type of test:	72 h static toxicity test
Applied and measured concentrations:	
Nominal test concentrations:	0 (control), 0.50, 1.0, 2.0, 4.0 and 8.0 mg formulation/L (corresponding to 0, 0.044, 0.088, 0.18, 0.35 and 0.71 mg a.s./L)
Mean measured concentrations:	0 (control), 0.466, 0.901, 1.82, 3.72 and 6.91 mg formulation/L (corresponding to 0, 0.0410, 0.0795, 0.160, 0.328 and 0.610 mg a.s./L)
Test conditions:	Sterile synthetic test medium adjusted to pH 7.5 Erlenmeyer flasks; test volume: 100 mL temperature: 23.3-23.5°C pH: 7.4-7.5 at test initiation, 7.5-10.1 at test termination Photoperiod: constant illumination at about 7700 lux Continuous shaking at 100 rpm
Analytical methods:	gas chromatography (GC)
Statistics:	Descriptive statistics; nonlinear regression estimation procedure (according to Bruce and Versteeg, 1992) for EC_{50} calculation; two-tailed Bonferroni's test for NOEC calculation
Test procedure:	At 24h intervals after the start of incubation, the number of algal cells in each test vessel was determined by microscopic counts using a haemocytometer.

Findings:

Analytical results: Analytical verification of test item concentrations was conducted in each concentration at test initiation and at test termination. Based on the measured active substance concentrations, the concentration of the test item was calculated (taking into account the active substance concentration in the formulation and the formulation density of 1.047 g/mL). The calculated test item concentrations ranged from 87.0% to 98.6% of nominal at test initiation. At test termination the calculated values ranged from 85.8% to 91.3% of nominal. The following biological results are based on mean measured concentrations of the test item.

Table B.9.3.1-5: Calculated concentration of BAS 555 01 F in the test media collected during the toxicity test with *Pseudokirchneriella subcapitata.*

Nominal concentration of test item (mg form./L)	Calculated concentration of test item (mg form./L)[1]					
	0 hour	% of nominal	72 hours [2]	% of nominal	Mean	% of nominal
0 (control)	<0.0113 [3]	-	<0.0113	-	<0.0113	-
0.50	0.493	98.6	0.438	87.6	0.466	93.2
1.0	0.928	92.8	0.874	87.4	0.901	90.1
2.0	1.85	92.5	1.78	89.0	1.82	91.0
4.0	3.78	94.5	3.65	91.3	3.72	93.0
8.0	6.96	87.0	6.86	85.8	6.91	86.4

[1]*The concentration of test item was calculated based on the measured concentration of active substance (in mg a.s./L), the concentration of active substance in the test item (92.3 g a.s./L) and the density of the test item (1.047 g/mL);* [2]*Samples were centrifuged prior to analysis to remove algal cells;* [3]*0.0113 mg form./L is the limit of quantification in water*

Biological results: No morphological effects were observed at any concentration tested. The effects on algal growth are summarized in Table B.9.3.1-6 and Table B.9.3.1-7.

The 24, 48 and 72h E_bC_{50} values (based on area under the growth curve) were calculated to be 3.34, 3.59 and 3.94 mg test item/L, respectively (95% confidence limits 2.80-3.97, 3.27-3.94 and 3.69-4.21 mg/L). The 24, 48 and 72h E_rC_{50} values (based on growth rate) were calculated to be 4.02, 6.06 and > 6.91 mg test item/L, respectively (95% confidence limits 3.43-4.72 mg/L, 5.86-6.27 mg/L and could not be determined). The NOEC was 1.82 mg test item/L.

Table B.9.3.1-6: Reduction in biomass (area under the growth curve) for *Pseudokirchneriella subcapitata* exposed to a range of concentrations of BAS 555 01 F.

Mean measured concentration (mg form./L)	Mean cell concentration (cells mL^{-1} 10^3)			Calculated mean area under the growth curve (A) x 10^3			Percent inhibition		
	24h	48h	72h	0-24h	0-48h	0-72h	0-24h	0-48h	0-72h
Control	37	274	1443	324	3816	24180	-	-	-
0.466	34	365	1462	288	3636	24120	11	5	0
0.901	33	295	1492	276	3972	25176	15	-4	-4
1.82	34	237	1350	288	3300	22104	11	14	9
3.72	20	144	831	120	1848	13308 *	63	52	45
6.91	13	39	277	36	420	3972 *	89	89	84

** Statistically significant difference compared to the control (Bonferroni-test; α = 0.05)*

Table B.9.3.1-7: Reduction in average specific growth rate for *Pseudokirchneriella subcapitata* exposed to a range of concentrations of BAS 555 01 F.

Mean measured concentration (mg form./L)	Mean cell concentration (cells mL^{-1} 10^3)			Calculated average specific growth rate (μ)			Percent of control		
	24h	48h	72h	0-24h	0-48h	0-72h	0-24h	0-48h	0-72h
Control	37	274	1443	0.055	0.069	0.069	-	-	-
0.466	34	365	1462	0.051	0.068	0.069	93	99	100
0.901	33	295	1492	0.050	0.071	0.070	91	103	101
1.82	34	237	1350	0.051	0.066	0.068	93	96	99
3.72	20	144	831	0.029	0.056	0.061 *	53	81	88
6.91	13	39	277	0.011	0.028	0.046 *	20	41	67

** Statistically significant difference compared to the control (Bonferroni-test; α = 0.05)*

Conclusions:
In a 72-hour algae test with *Pseudokirchneriella subcapitata* the E_rC_{50} of BAS 555 01 F was determined to be > 6.91 mg/L, the E_bC_{50} was 3.94 mg/L based on mean measured concentrations.

RMS comments:
In the study report, the average specific growth rate is expressed in terms of h^{-1}. In order to be able to compare the performance of the control to the validity criteria of OECD Test Guideline 201, the average specific growth rate for the control was recalculated in terms of day^{-1} by the RMS. Further, as the study report does not mention the coefficient of variation for the average specific growth rate and section-by-section growth rate in the control, these values were also calculated by the RMS. Based on the calculated values, the validity criteria of OECD Test Guideline 201 are met:

- The biomass in the control increased exponentially by a factor of at least 16 within the test period. This corresponds to a specific growth rate of at least 0.92 day^{-1} (measured: 1.66 day^{-1} for the period 0-72h)
- The coefficient of variation of average specific growth rate during the whole test period in replicate control cultures did not exceed 7% (measured: 1.1%)
- The mean coefficient of variation for section-by-section growth rates in the control cultures did not exceed 35% (measured: 17.8%).

Consequently, this study is considered acceptable for use in the risk assessment.

The analytical method used (FAMS 058-01) could be fully validated according to the current EU Guidance SANCO/3029/99 rev. 4 for 0.05-0.5 mg/L metconazole. For higher concentrations, the method is not fully validated, but based on the available information, there are indications that concentrations > 0.5 mg/L are also covered.. Therefore, the method can be considered "fit for purpose". An LOQ was set at 0.05 mg a.s./L for metconazole (please refer to Vol. 3 (CA), Section B.5.1.2.6 – study no. 2-5, for further details).

In accordance with the new data requirements (Commission Regulation EU No 283/2013), the EC_{10} and EC_{20} values should be calculated. Where these values cannot be estimated, an explanation should be provided. This was not addressed in the study report. However, following a request from the RMS, the applicant performed the additional calculations to determine the EC_{10} and EC_{20}. These calculations are reported in Horn C. & Schneider C. (2016; KCA8.2/01). Statistical recalculation was performed with ToxRatProfessional Version 2.10. However, Horn C. & Schneider C. (2016) calculated EC_x values only for the period 0-24h, while the endpoints for the period 0-72h should be considered in the risk assessment. Therefore, RMS calculated EC_{10} and EC_{20} for the period 0-72h. Calculations were performed using the drc-package in the R statistical environment, Version 3.3.0 (R Development Core Team, 2016). Dose-response curve models were selected through goodness-of-fit metrics via the 'mselect' command, which compares the log likelihood value, Akaike's information criterion (AIC), the estimated residual standard error and the p-value from a lack-of-fit test for different models. The model with the best fit for the data was Weibull type 1 (4 parameters) for both biomass and growth rate.

The following endpoint, based on mean measured concentrations, are considered relevant for the risk assessment:

E_rC_{50} (*Pseudokirchnerialla subcapitata*, 72h) > 6.91 mg form./L (95% confidence limits not determined) (equivalent to 0.609 mg a.s./L)
E_rC_{20} (*Pseudokirchnerialla subcapitata*, 72h) = 4.56 mg form./L (95% confidence limits = 4.46-4.66 mg form./L) (equivalent to 0.402 mg a.s./L)
E_rC_{10} (*Pseudokirchnerialla subcapitata*, 72h) = 3.31 mg form./L (95% confidence limits = 3.27-3.36 mg form./L) (equivalent to 0.292 mg a.s./L)

E_bC_{50} (*Pseudokirchnerialla subcapitata*, 72h) = 3.94 mg form./L (95% confidence limits = 3.69-4.21 mg form./L) (equivalent to 0.347 mg a.s./L)
E_bC_{20} (*Pseudokirchnerialla subcapitata*, 72h) = 2.90 mg form./L (95% confidence limits = 2.52-3.27 mg form./L) (equivalent to 0.256 mg a.s./L)
E_bC_{10} (*Pseudokirchnerialla subcapitata*, 72h) = 2.14 mg form./L (95% confidence limits = 1.98-2.31 mg form./L) (equivalent to 0.189 mg a.s./L)
NOEC (*Pseudokirchnerialla subcapitata*, 72h) = 1.82 mg form./L (equivalent to 0.160 mg a.s./L)

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

The endpoints obtained in the acute studies with the formulated product BAS 555 01 F on *Onkorhynchus mykiss*, *Daphnia magna* and algae are a factor of 2.5 to 3.5 below the endpoint for the active substance (see Table B.9.4.1-1). However, based on the argumentation presented in Section B.9.4.1 and the calculations in Table B.9.4.1-2 andTable B.9.4.1-3, it is demonstrated that the formulation does not cause significant unexpected (additional) toxicity to aquatic organisms. No synergism is expected to occur due to co-formulants in case of fish and algae. For *Daphnia* the formulation shows slightly increased toxicity compared to the results of the active substance due to the toxicity of the main formulant "ethoxylated C9-11 alcohols". However, the main formulant "ethoxylated C9-11 alcohols" is readily biodegradable and will not occur together with the active substance under long-term conditions in the aquatic environment. Therefore, the studies conducted with the active substance can be used to assess the chronic risk resulting from BAS 555 01 F applications. No chronic toxicity studies with the product BAS 555 01 F is required.

B.9.3.3. Further testing on aquatic organisms

Microcosm or mesocosm studies
No microcosm or mesocosm studies have been performed with the formulated product BAS 555 01 F and the active substance metconazole. As the risk assessment based on the available laboratory data indicates an acceptable risk to aquatic organisms (aquatic invertebrates, algae and aquatic plants; for fish the risk is acceptable for most FOCUS exposure scenarios), such studies are not required.

Residue data in fish
The log P_{OW} of metconazole was determined to be 3.85 (see Volume 3, Section B.2). Two bioconcentration studies (BCF studies) are available, which are summarized in Volume 3 (AS) Section B.9.2.2.3.

In the first study (CA8.2.2.3/01; [redacted] 1996) the apparent steady state, after exposure of fish to metconazole at nominal exposure levels of 0.04 and 0.4 mg/L, was reached after 2 days. After termination of the exposure, radioactivity levels in fish tissues decreased rapidly with a half-life of *ca.* 0.41-0.45 days. Kinetic bioconcentration factors based on total radioactivity (TRR) were relatively low in whole fish (114 to 119), which indicated an intensive metabolic clearance of metconazole in bluegill sunfish. The accumulated residues were rapidly eliminated from whole fish with a depuration rate of 1.5-1.7 per day after the fish were withdrawn from exposure. The time to reach 50% depuration was 0.41-0.45 days.

For the second study (CA8.2.2.3/02; [redacted] M., 2002) the apparent steady state, after exposure of fish to metconazole at nominal exposure levels of 0.04 and 0.4 mg/L, was reached after 2 days of exposure. The kinetic bioconcentration factors (BCF_k) for whole fish based on total radioactivity (TRR) were 114 (low concentration) and 106 (high concentration). The half-lives for elimination varied between 0.53 and 0.58 days. The time for elimination of 90% of the activity varied between 1.8 and 1.9 days. The predominant residue in fish was the unmetabolized parent metconazole, which accounted for 39% - 67% TRR in low dose fish, 78% - 91% TRR in high dose fish.

Based on these two BCF studies, despite the relatively high lipophilicity of metconazole, it can be concluded that the risk of bioaccumulation is low, due to the low accumulation and rapid excretion of metconazole from fish. Thus, residues of metconazole following the use of BAS 555 01 F in fish are of low concern and no accumulation in the food chain is to be expected.

Additionally, a biomagnification study on juvenile rainbow trout from peer-reviewed literature is available (CA8.2.8/01; Konwick B.J. *et al.*, 2006). Refer to Volume 3 (AS), Section B.9.2.8 for a summary. In this study *Oncorhynchus mykiss* were exposed to dietary concentrations of a mixture of chiral triazole fungicides including metconazole and α-hexachlorocyclohexane (α-HCH) during an 8-day uptake period followed by a depuration period of 16 days. Fish were fed a spiked diet containing 28.14 µg metconazole/g wet weight (mean measured) at a feeding rate of 1.5% of the fish body weight. In parallel, a control group which received a diet of untreated food was kept under the same conditions. Steady state was reached within 1 day, consistent with the low $t_{1/2}$ of 1.1 days and also consistent with the BCF studies described above. No test item was detected in fish on any collection day due to the fast elimination. Exposure to the test item did not appear to influence the health of the rainbow trout. There was no significant difference in lipid percentage or liver somatic index between treatment and control fish and there were no signs of stress (e.g. colouration change, behaviour) or mortality in either treatment. Body weights and whole fish and liver growth rates of the exposed fish were not significant over the course of the study. The biotransformation rate of metconazole accounted for the major proportion (89.5%) of the test item elimination. The obtained steady-state biomagnification factor (BMFss) was 0.019. Since the BMF_{ss} is clearly below 1, no risk for biomagnification occurs, i.e. metconazole does not accumulate via the food chain.

Accumulation in aquatic non-target organisms

Bioaccumulation of the active substance metconazole under natural conditions is not expected to occur (see "Residue data in fish" above). Additional studies are not required or necessary to determine bioaccumulation in aquatic non-target organisms.

B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISMS

The risk assessment for effects on aquatic organisms is updated according to the new **EFSA Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (2013)**[12], which has been noted in the meeting of the Standing Committee on Plants, Animals, Food and Feed on 11 July 2014. This document will be referred to as 'the EFSA Guidance Document for aquatic organisms (2013)'.

B.9.4.1. Summary of toxicity

Toxicity of the active substance and the formulation BAS 555 01 F

Aquatic acute and chronic toxicity studies were conducted with fish, aquatic invertebrates and algae for the active substance metconazole and the representative formulation BAS 555 01 F. A summary of the relevant toxicity data for metconazole and the representative formulation BAS 555 01 F is provided in Table B.9.4.1-1.

Table B.9.4.1-1: Summary of aquatic toxicity data on metconazole (BAS 555 F), tested as technical active substance or in the representative formulation BAS 555 01 F.

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
Fish					
Rainbow trout (*Oncorhynchus mykiss*)	BAS 555 F (metconazole) 83:17 *cis:trans*	72 hours, acute (semi-static)	LC_{50}	**2.1 mg a.s./L**	CA8.2.1/01 [redacted] 1990
Rainbow trout (*Oncorhynchus mykiss*)	BAS 555 F (metconazole) 95% *cis*	96 hours, acute (semi-static)	LC_{50}	4.0 mg a.s./L [N]	CA8.2.1/04 [redacted] 1991b
Rainbow trout (*Oncorhynchus mykiss*)	BAS 555 01 F	96 hours, acute (static)	LC_{50}	**10 mg form./L [N] (= 0.85 mg a.s./L)**	CP10.2.1/01 [redacted] 2003
Fathead minnow (*Pimephales promelas*)	BAS 555 F (metconazole) 83:17 *cis:trans*	96 hours, acute (semi-static)	LC_{50}	3.9 mg a.s./L	CA8.2.1/02 [redacted] 1991a
Common carp (*Cyprinus carpio*)	BAS 555 F (metconazole) 85:15 *cis:trans*	96 hours, acute (flow-through)	LC_{50}	3.99 mg a.s./L	CA8.2.1/03 [redacted] 1996a
Sheepshead minnow (*Cyprinodon variegatus*)	BAS 555 F (metconazole) 85:15 *cis:trans*	96 hours, acute (static)	LC_{50}	6.3 mg a.s./L	CA8.2.1/05 [redacted] 2005
Bluegill sunfish (*Lepomis macrochirus*)	BAS 555 F (metconazole) 80:20 *cis:trans*	96 hours, acute (static)	LC_{50}	4.9 mg a.s./L [N]	CA8.2.1/06 [redacted] 2008a
Zebrafish (*Danio rerio*)	BAS 555 F (metconazole) 80:20 *cis:trans*	96 hours, acute (static)	LC_{50}	6.8 mg a.s./L [N]	CA8.2.1/07 [redacted] 2008b

[12] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013; 11(7):3290. 268 pp.

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
Threespine stickleback (*Gasterosteus aculeatus*)	BAS 555 F (metconazole) 85:15 *cis:trans*	96 hours, acute (static)	LC_{50}	4.2 mg a.s./L [N]	CA8.2.1/08 [redacted] 2010
Rainbow trout (*Oncorhynchus mykiss*)	BAS 555 F (metconazole) 85:15 *cis:trans*	28 days, chronic (flow-through)	NOEC EC_{20} EC_{10}	1.14 mg a.s./L 1.40 mg a.s./L 1.26 mg a.s./L	CA8.2.2.1/01 [redacted] 1996b
Rainbow trout (*Oncorhynchus mykiss*)	BAS 555 F (metconazole) 85:15 *cis:trans*	95 days, chronic (flow-through)	NOEC EC_{20} EC_{10}	0.00291 mg a.s./L 0.00504 mg a.s./L **0.00398 mg a.s./L**	CA8.2.2.1/02 [redacted] 2001
Sheepshead minnow (*Cyprinodon variegatus)*	BAS 555 F (metconazole) 85:15 *cis:trans*	33 day, chronic (flow-through)	NOEC EC_{20} EC_{10}	0.011 mg a.s./L ND ND	CA8.2.2.1/03 [redacted] 2009
Fathead minnow (*Pimephales promelas*)	BAS 555 F (metconazole) 95% *cis*	35 day, chronic (flow-through)	NOEC EC_{20} EC_{10}	0.011 mg a.s./L 0.027 mg a.s./L 0.021 mg a.s./L	CA8.2.2.1/04 [redacted] 1992
Fathead minnow (*Pimephales promelas*)	BAS 555 F (metconazole) 85:15 *cis:trans*	5 months, chronic (flow-through)	NOEC EC_{20} EC_{10}	0.00358 mg a.s./L 0.0097 mg a.s./L 0.0077 mg a.s./L	CA8.2.2.2/01 [redacted] 2008
Aquatic invertebrates					
Water flea (*Daphnia magna*)	BAS 555 F (metconazole) 83:17 *cis:trans*	48 hours, acute (static)	EC_{50}	4.2 mg a.s./L [I]	CA8.2.4.1/01 Toy R., 1990
Water flea (*Daphnia magna*)	BAS 555 F (metconazole) 95% *cis*	48 hours, acute (static)	EC_{50}	**3.6 mg a.s./L** [N]	CA8.2.4.1/02 Toy R., 1991b
Water flea (*Daphnia magna*)	BAS 555 01 F	48 hours, acute (static)	EC_{50}	**9.28 mg form./L (=0.82 mg a.s./L)**	CP10.2.1/02 Oliveiri C.E. *et al.*, 2000a
Water flea (*Daphnia magna*)	BAS 555 F (metconazole) 85:15 *cis:trans*	21 days, chronic (semi-static)	NOEC EC_{20} EC_{10}	0.16 mg a.s./L 0.172 mg a.s./L **0.149 mg a.s./L**	CA8.2.5.1/01 Jatzek H.J., 2002
Water flea (*Daphnia magna*)	BAS 555 F (metconazole) 95% *cis*	21 days, chronic (semi-static)	NOEC EC_{20} EC_{10}	0.21 mg a.s./L 0.276 mg a.s./L 0.215 mg a.s./L	CA8.2.5.1/02 Toy R., 1991c

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
Sediment dwelling aquatic invertebrates					
Midge (*Chironomus riparius*)	BAS 555 F (metconazole) 85:15 *cis:trans*	28 day, chronic, spiked water (static)	NOEC	**2.12 mg a.s./L** [I] **(8.23 mg a.s./kg)**	CA8.2.5.3/01 England D.C. *et al.*, 1997
			EC_{20}	2.58 mg a.s./L[I]	
			EC_{10}	2.21 mg a.s./L [I]	
Algae					
Green microalgae (*Pseudo-kirchneriella subcapitata*)	BAS 555 F (metconazole) 83:17 *cis:trans*	72 hours, chronic (static)	72h E_bC_{50}	1.7 mg a.s./L [I]	CA8.2.6.1/01 Toy R., 1990
			72h E_bC_{20}	0.44 mg a.s./L [I]	
			72h E_bC_{10}	0.19 mg a.s./L [I]	
			72h ErC_{50}	**2.2 mg a.s./L** [I]	
			72h E_rC_{20}	1.22 mg a.s./L [I]	
			72h E_rC_{10}	0.90 mg a.s./L [I]	
			NOEC	0.38 mg a.s./L [I]	
Green microalgae (*Pseudo-kirchneriella subcapitata*)	BAS 555 01 F	72 hours, chronic (static)	72h E_bC_{50}	3.94 mg form./L (= 0.347mg a.s./L)	CP10.2.1/03 Oliveiri C.E., 2000b
			72h E_bC_{20}	2.90 mg form./L (= 0.256 mg a.s./L)	
			72h E_bC_{10}	2.14 mg form./L (= 0.189 mg a.s./L)	
			72h E_rC_{50}	**> 6.91 mg form./L (> 0.609 mg a.s./L)**	
			72h E_rC_{20}	4.56 mg form./L (= 0.402 mg a.s./L)	
			72h E_rC_{10}	3.31 mg form./L (= 0.292 mg a.s./L)	
Aquatic plants					

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value[M]	Reference
Lemna gibba	BAS 555 F (metconazole)	7 days, chronic (static)	E_yC_{50}	0.077 mg a.s./L [I]	CA8.2.7/01 Brzozowska K., 2014
			E_yC_{20}	0.008 mg a.s./L [I]	
			E_yC_{10}	0.003 mg a.s./L [I]	
			ErC_{50}	**0.527 mg a.s./L [I]**	
			E_rC_{20}	0.022 mg a.s./L [I]	
			E_rC_{10}	0.004 mg a.s./L [I]	
			NOEC	0.003 mg a.s./L [I]	
Bioconcentration					
Lepomis macrochirus	Metconazole	28 days uptake, 14 days depuration	BCF_{SS}(TRR)	105.1[1)]	CA8.2.2.3/01 [redacted] 1996
Lepomis macrochirus	Metconazole	28 days uptake, 14 days depuration	BCF_{SS}(TRR) BCF_{SS}(parent)	101.0 [1)] 59.0 [1)]	CA8.2.2.3/02 [redacted] M., 2002
Oncorhynchus mykiss	Metconazole	8 days uptake, 16 day depuration; Dietary exposure	BMF_{SS}	0.019	CA8.2.8/01 Konwick B.J. *et al.*, 2006

Notes: ***bold*** *– values used for risk assessment*
[M] *– based on mean measured concentrations unless otherwise stated*
[N] *– based on nominal concentration(s)*
[I] *– based on initial measured concentration(s)*
[1)] *– value corrected to 5% lipid content*
ND – could not be determined

Comparison of active substance and formulation toxicity

For fish, aquatic invertebrates and algae, a comparison was made between the measured active substance toxicity and the measured formulation toxicity, expressed in terms of active substance (see Table B.9.4.1-2). Ecotoxicity studies are biological test systems which are characterized by a certain natural biological variability when repeating a study. Hence, a threshold has to be defined for when an increased/decreased formulation toxicity effect can no longer be seen as only due to biological variability. The EFSA Guidance Document for aquatic organisms proposes a factor of 5, i.e if the formulation toxicity (expressed in terms of active substance) is less than a factor 5 below the active substance toxicity, there is no indication that the formulation is more toxic than the active substance. Based on the values presented in Table B.9.4.1-2, the formulation toxicity is a factor of 2.4 to 5.3 below the active substance toxicity. Consequently, BAS 555 01 F seems to be slightly more toxic compared to the active substance metconazole.

Table B.9.4.1-2: Comparison of the toxicity of the formulated product BAS 555 01 F and the active substance metconazole

Test species	Endpoint & Test duration	Measured formulation toxicity of BAS 555 01 F ($EC_{x_{PPP}}$) [mg product/L]	Formulation toxicity, expressed in terms of a.s. ($EC_{x_{PPP\text{-}a.s.}}$) [mg a.s./L] [1)]	Measured toxicity of metconazole ($EC_{x\ a.s.}$) [mg a.s./L]	$EC_{x_{a.s.}}$/ $EC_{x_{PPP\text{-}a.s.}}$)
O. mykiss	LC_{50}, 96 h	10	0.86	2.1	2.4
D. magna	EC_{50}, 48 h	9.28	0.80	4.2 [2)]	5.3
P. subcapitata	E_rC_{50}, 72 h	> 6.91	> 0.595	2.2	3.7

[1)] The measured formulation toxicity was expressed in terms of active substance takinginto account the nominal content of the active substance (i.e. 90 g metconazole/L) and the product density of 1.046 g/cm³

[2)] The highest of both available EC_{50} values for D. magna for metconazole was used, as this value results in the largest difference between active substance and formulation endpoints.

Further investigation should therefore take into account potential toxicity of formulants as well. According to the EFSA Guidance Document for aquatic organisms (2013), measured and calculated toxicity of the formulated product should be compared to determine synergistic, additive or antagonistic effects of active ingredients and/or co-formulants within a formulation (please refer to Section 10.3 for the EFSA Guidance Document for aquatic organisms for details). To determine the respective formulation effect, EFSA proposed to calculate the model deviation ratio (MDR), which divides the calculated formulation toxicity ($LC_{50}/EC_{50\ mix\text{-}CA}$) by the measured formulation toxicity ($LC_{50}/EC_{50\ PPP}$). As a threshold for when an increased/decreased formulation toxicity effect can no longer be seen as only additive, EFSA also proposes a factor of 5 (i.e. if the MDR is between 0.2 and 5 the observed and calculated formulation toxicities are considered in agreement).

BAS 555 01 F contains as major ingredient the adjuvant "ethoxylated C9-11 alcohols" (~ 600 g/L; CAS No.: 68439-46-3; please refer to Volume 4, Section C.1.3.2 for details) with a moderate toxicity range of 1-10 mg/L for EC_{50} (fish, daphnia, algae) according to the safety data sheet (MSDS, DocID 2015/1041983). MDR calculations considering the main formulant "ethoxylated C9-11 alcohols" were conducted, and are shown in Table B.9.4.1-4. Since there are no exact endpoints for "ethoxylated C9-11 alcohols" available, the MDR in Table B.9.4.1-3 should be seen rather as an estimation for potential increased toxicity without stressing too much the numeric value. The MDR values in Table B.9.4.1-3 indicate that, the slightly higher toxicity of BAS 555 01 F compared to the active substance metconazole is mainly caused by the presence of the formulant "ethoxylated C9-11 alcohols". If the formulant "ethoxylated C9-11 alcohols" is also considered in the calculations, BAS 555 01 F does not cause an increased or synergistic toxicity.

Table B.9.4.1-3: Comparison of the toxicity of the formulated product BAS 555 01 F, and the calculated mixture toxicity of the active substance and the formulant "ethoxylated C9-11 alcohols"

Test species	Endpoint & Test duration	Measured toxicity of metconazole ($EC_{x\ a.s.}$) [mg a.s./L]	Measured toxicity of "ethoxylated C9-11 alcohols" ($EC_{x\ formulant}$) [mg a.s./L] [1)]	Calculated formulation toxicity ($EC_{xmix\text{-}CA}$) [mg product/L] [2)]	Measured toxicity of BAS 555 01 F (EC_{xPPP}) [mg product/L]	MDR ($EC_{xmix\text{-}CA}$ / EC_{xPPP})
O. mykiss	LC_{50}, 96 h	2.1	3.162	4..497	10	0.45
D. magna	EC_{50}, 48 h	4.2	3.162	4.953	9.28	0.53
P. subcapitata	E_rC_{50}, 72 h	2.2	3.162	4.535	> 6.91	0.66

MDR = model deviation ratio

[1)]Endpoints for "ethoxylated C9-11 alcohols" are provided in the respective MSDS (DocID 2015/1041983). Since all endpoints are reported as ranges from 1 – 10 mg/L the geometric mean was calculated for MDR assessment

[2)] The theoretical formulation toxicity was calculated using Equation 13 from the EFSA guidance document on aquatic organisms. The contribution of the active substance and the co-formulant to the toxicity of the formulation (ECx_i in Equation 13) was calculated the based on the measured toxicity data of metconazole and "ethoxylated C9-11 alcohols", the nominal contents of the active substance (i.e. 90 g metconazole/L) and the formulant (i.e. 600 g "ethoxylated C9-11 alcohols"/L) and the product density of 1.046 g/cm³.

The slightly higher acute toxicity of BAS 555 01 F, compared to the active substance metconazole, is assessed through a formulation drift risk assessment. For the chronic risk from the formulation, the applicant argued that this would be covered by the risk assessment for the active substance for a number of reasons. First of all, the co-formulants causing the higher acute toxicity ("ethoxylated C9-11 alcohols") are readily degradable (i.e. the increased aquatic toxicity of BAS 555 01 F is likely to occur only due to short-term drift events after application when the intact formulation is present in the water body). Further, additional entry pathways (runoff or drainage) are not possible for the intact formulation and also long-term exposure of aquatic organisms to BAS 555 01 F cannot occur. RMS agrees that due to the rapid degradation of the co-formulants, long-term exposure of aquatic organisms to BAS 555 01 F is unlikely. However, chronic effects mays still occur from short-term exposure. According to the data requirements for plant protection products (Regulation (EU) No. 284/2013, section 10.2.2), chronic toxicity studies should be conducted with the formulation where it is not possible to extrapolate from data obtained in the corresponding studies on the active substance. A trigger of 10 is proposed, i.e. if the plant protection product is more acutely toxic than the active substance as manufactured by a factor of 10, then chronic toxicity studies with the formulation are required. As this is not the case, such studies are not considered required, and RMS agrees that the chronic risk due to application of the formulation BAS 555 01 F is covered by the risk assessment for the active substance.

Selection of endpoints for the risk assessment

For metconazole, some of the toxicity studies with aquatic organisms used test material with a different isomer ratio (95% *cis*) than the currently used isomer ratio of 85:15 (*cis:trans*). Toxicity results for the respective species are all in the same range, independent of the test material isomeric ratio. This is in line with the results for other groups of non-target organisms for which studies with both isomer ratios of metconazole are available (e.g. birds and mammals; please refer to the other Sections in this Volume for an overview of the different endpoints). Based on all available data, there are no indications that there are differences in toxicity between metconazole 85:15 *cis:trans* and 95% *cis*. Therefore, all available studies for aquatic organisms are considered relevant and are taken into account in the risk assessment.

Based on the results from the water-sediment study by Knight L. (2015c; refer to Volume 3 (CA), Section B.8.2.2.3 for a summary), no change in isomeric composition of metconazole is expected in water and sediment. Therefore, a risk assessment based on the available toxicity studies can be considered representative.

In general, when more than one reliable endpoint is available (for the same species, or for different species of the same group), the lowest endpoint for each species group (fish, aquatic invertebrates or algae) is used in the first tier of the risk assessment. For algae and aquatic plants, endpoints for both growth rate and biomass/yield are reported in Table B.9.4.1-1. Following the new EFSA Guidance Document for aquatic organisms (2013), growth rate is the preferred endpoint to be used in the risk assessment since it is more robust considering varying test conditions. Biomass endpoints should not be used, as direct use of the biomass concentrations without logarithmic transformation cannot be applied to an analysis of results from a system in exponential growth (ECHA, 2008)[13]. Yield endpoints are only included for cases where specific regulatory requirements in some countries may need to be fulfilled.

The EFSA Guidance Document for aquatic organisms (2013) recommends that the risk assessment for chronic risk for all groups of aquatic organisms should use the EC_x approach in preference to the NOEC/LOEC approach. However, there is still some uncertainty about the choice of the effect percentile in the EC_x. Further, as existing study methods were not designed to estimate EC_x, these values might not always be available. Therefore, for practicality reasons, the Guidance Document proposes to use EC_{10} in the chronic RA scheme (except for algae and plants) for the time being, until new knowledge on the choice of EC_x comes available. When the EC_{10} is not available, the NOEC can still be used. In Pesticides Peer Review Meeting 133, it was agreed that for aquatic organisms the approach as recommended in the EFSA Guidance Document (2013) should be followed (see EFSA, 2015[14]). For metconazole, EC_{10} values are available for the chronic studies with fish, aquatic invertebrates and for the studies with algae and aquatic plants. For algae and aquatic plants, EC_{50} values are used in the risk assessment scheme. Consequently, only for fish and aquatic invertebrates, EC_{10} values from the respective studies will be considered in the risk assessment.

According to the revised data requirements under Regulation (EC) No 1107/2009 (Commission Regulations (EU) 283/2013 and 284/2013 for the active ingredient and the plant protection products, respectively), the risk to amphibians shall be addressed. Nevertheless, unlike fish and other aquatic organism, toxicity tests for amphibian species are not requested. In the EU there is no guidance or validated regulatory protocols yet available neither on the type of regulatory testing necessary nor how to conduct a risk assessment for amphibian. In the case of metconazole, there are no studies in the literature on the toxicity of this substance on amphibians.

According to the new EFSA Guidance Document for aquatic organisms (2013) amphibians should be included in the aquatic risk assessment. In absence of GLP studies the assessment should be based on any existing relevant information (testing of amphibian is not recommended at first instance due to animal welfare reasons). With regard to the aquatic risk assessment several data analyses indicate that the risk assessment for aquatic organisms (and fish in particular) covers the risk assessment for aquatic

[13] ECHA (European Chemicals Agency), 2008. Guidance on information requirements and chemical safety assessment. Chapter R.7b: Endpoint specific guidance. Version 1.1. Helsinki, Finland: European Chemicals Agency. 234 p.

[14] EFSA, 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.

phases of amphibians (Aldrich 2009[15]; Fryday and Thompson 2012[16]; Weltje et al. 2013[17]). A common conclusion of these data evaluations is that other aquatic endpoints (generally available for pesticides) cover the potential toxicity to amphibians in water. Consequently, it is considered that the risk assessment performed for fish as the most sensitive group of aquatic organisms should also cover the risk to amphibians.

Taking into account all of the above, the endpoints shown in Table B.9.4.1-4 will be used in the Tier 1 risk assessment for aquatic organisms (see section 0). The other available endpoints will be further considered in the Tier 2 risk assessment (see Section B.9.4.3.1).

Table B.9.4.1-4: Endpoints for the active substance metconazole and the formulation BAS 555 01 F used in the Tier 1 risk assessment for aquatic organisms

Substance	Time span	Species group	Test organism	Selected endpoint for use in risk assessment
Metconazole	Acute	Fish	*Oncorhynchus mykiss*	LC_{50} = 2.1 mg a.s./L
		Aq. Invertebrates	*Daphnia magna*	EC_{50} = 3.6 mg a.s./L
	Chronic	Fish	*Oncorhynchus mykiss*	EC_{10} = 0.00398 mg a.s./L
		Aq. Invertebrates	*Dapnia magna*	EC_{10} = 0.149 mg a.s./L
			Chironomus riparius	NOEC = 8.23 mg a.s./kg dry sediment
		Algae	*Pseudokirchneriella subcapitata*	E_rC_{50} = 2.2 mg a.s./L
		Aq. plants	*Lemna gibba*	E_rC_{50} = 0.527 mg a.s./L
BAS 555 01 F	Acute	Fish	*Oncorhynchus mykiss*	LC_{50} = 10 mg form./L (= 0.85 mg a.s./L)
		Aq. Invertebrates	*Daphnia magna*	EC_{50} = 9.28 mg form./L (= 0.82 mg a.s./L)
	Chronic	Algae	*Pseudokirchneriella subcapitata*	E_rC_{50} > 6.91 mg form./L (> 0.609 mg a.s./L)

Toxicity of the metabolites of metconazole

According to Vol. 3 Section B.8 (Fate & Behaviour in the Environment), the major metabolites of metconazole formed in aquatic systems are M13 (= CL359139; M555F013) and 1,2,4-triazole (=M555F020). As aquatic organisms may be exposed to these major metabolites, the risk following such exposure needs to be assessed.

Laboratory toxicity studies with representative freshwater species have been submitted for the metabolite 1,2,4-triazole. The endpoints obtained from these studies are summarized in Table B.9.4.1-5.

For metabolite M13, no toxicity studies have been submitted. This metabolite was found in the water-sediment study close to a level of 9% of the AR (Please refer to Volume 3 (AS) Section B.8.2.2). In order to pose a higher risk to aquatic organisms than the parent, metabolite M13 would have to be 10

[15] Aldrich A.P. (2009). Empfindlichkeit von Amphibien gegenüber Pflanzenschutzmitteln. *AGRAR Forschung* 16:466–471

[16] Fryday S. & Thompson H. (2012). Toxicity of pesticides to aquatic and terrestrial life stages of amphibians and occurrence, habitat use and exposure of amphibian species in agricultural. Food and Environment research agency, UK.

[17] Weltje L., Simpson P., Gross M., Crane M., Wheeler J.R. (2013). Comparative acute and chronic sensitivity of fish and amphibians: a critical review of data. *Environmental Toxicology and Chemistry*, Vol. 32(5):984-994.

times more toxic than metconazole. As discussed in the previous EFSA Conclusion for metconazole (2006)[18], this is considered as unlikely since the structure of M13 similar to metconazole and the only difference is the formation of a carboxylic acid group. The carboxylic acid group would make it more water soluble and easier for organisms to excrete. Further, PEC calculations resulted in only low predicted concentrations of 7.257 and 5.806 µg/L for applications in cereals and winter oilseed rape, respectively (maximum FOCUS Step 1 PEC_{SW} values, see Section 0). Therefore, the risk from the metabolite M13 is expected to be lower compared to metconazole, and thus considered to be covered by the risk assessment for metconazole.

Table B.9.4.1-5: Summary of aquatic toxicity data on 1,2,4-triazole, a major metabolite of metconazole (BAS 555 F).

Species	Test substance	Timescale (test type)	Endpoint	Toxicity value [M]	Reference
Metabolite: DCVA					
Fish					
Rainbow trout (*Oncorhynchus mykiss*)	1,2,4-triazole	96 hours, acute (static)	LC_{50}	**600 mg/L**	CA 8.2.1/09 [redacted] 1983
Rainbow trout (*Oncorhynchus mykiss*)	1,2,4-triazole	28 days, chronic (semi-static)	NOEC	**3.20 mg/L** [N]	CA8.2.2.1/05 [redacted] 2002
Aquatic invertebrates					
Water flea *(Daphnia magna)*	1,2,4-triazole	48 hours, acute (static)	EC_{50}	**> 100 mg/L** [N]	CA 8.2.4.1/03 Bell G., 1995
Algae					
Green microalgae *(Pseudo-kirchnerialla subcapitata)*	1,2,4-triazole	96 hours, chronic (static)	72h E_bC_{50} 72h E_bC_{20} 72h E_bC_{10} 72h ErC_{50} 72h E_rC_{20} 72h E_rC_{10} NOEC	13 mg/L 7.18 mg/L 5.95 mg/L **> 31 mg/L** 11.33 mg/L 8.31 mg/L 3.1 mg a.s./L	KCA 8.2.6.1/03 Palmer S.J. *et al.*, 2001

Notes: ***bold*** *– values used for risk assessment*
[M] *– based on mean measured concentrations unless otherwise stated*
[N] *– based on nominal concentration(s)*

[18] EFSA Scientific Report (2006) 64, 1-71. Conclusion regarding the peer review of the pesticide risk assessment of the active substance metconazole.

B.9.4.2. Summary of exposure

The critical use pattern of BAS 555 01 F considered in the risk assessment below is presented in Table B.9.4.2-1

Table B.9.4.2-1: Use pattern of BAS 555 01 F for use as foliar spray, considered in the risk assessment.

Crop	Application time (BBCH growth stage)	Max. number of applications	Interval between applications [d]	Application rate per treatment	
				Metconazole [kg a.s./ha]	BAS 555 01 F [L/ha]
Cereals (winter/spring)	30 - 69	1 - 2	21	0.090	1.0
Winter oilseed rape	13 – 20 (autumn) 21 – 71 (spring) [1)]	1 - 2 [2)]	min. 90 [2)]	0.072	0.8
	21 – 71 (spring)	1 - 2	14	0.072	0.8

[1)] According to the GAP a first application in autumn (between BBCH 13 and BBCH 20) is followed by a second application in spring (between BBCH 21 and BBCH 71)

[2)] According to the GAP a minimum interval of 90 days should be considered. However, for calculation of the PECSW values used throughout this volume, an interval of 150 days was used, which is assumed to account for the interval between the first application in autumn and the second application in spring.

Aquatic organisms may be exposed to BAS 555 01 F through spray drift, and to the active substance metconazole and its metabolites through spray drift, run-off and drainage from the application site into adjacent water bodies. Exposure of aquatic organisms from these pathways was estimated by calculating Predicted Environmental Concentrations in surface water (PEC_{SW}) and sediment (PEC_{SED}) according to the recommendations of the FOCUS working group on surface water scenarios using the FOCUS surface water models.

Exposure to the formulation BAS 555 01 F

Aquatic organisms may be exposed to the formulation BAS 555 01 F through spray drift. Other routes of exposure such as runoff or drainage are not considered relevant for the formulation as such (only relevant for the active substance and its metabolites). Further, as the formulation will rapidly dissolve in its different components (active substance and co-formulants) in surface water, it is considered sufficient to take into account only the exposure through spray drift from 1 application.

The maximum instantaneous $PEC_{sw,\,ini}$ values from entry through spray-drift that occur immediately after a single application was calculated using the following equation:

$$PEC_{SW}\,(\mu g/L) = \frac{\%Drift_{90th\,\%ile} \times Application\ rate\ (g/ha)}{Waterdepth\ (cm) \times 10}$$

For the proposed use in winter and spring cereals, and in winter oilseed rape (autumn/spring application), a drift rate of 1.93%, which is the 90th percentile without mitigation measures, was calculated using the drift calculator in the FOCUS Surface water tool SWASH (v1). The application rate 1.0 L/ha (equivalent to 1.046 kg/ha, considering a density of 1.046 g/cm³) for cereals was used as a worst case approach, covering application rate of 0.8 L/ha in winter oilseed rape. The depth of the static water body was assumed to be 30 cm. The resulting **maximum instantaneous $PEC_{sw,ini,drift}$ value is 6.729 µg BAS 555 01 F/L** for application in cereals and winter oilseed rape.

Exposure to metconazole (BAS 555 F) and metabolites

Calculations were performed considering the pathways spray drift, drainage and runoff. 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. Furthermore, a tiered approach was applied at Step 3 and 4 level for the PEC calculations. In Tier 1 the default input parameters according to FOCUS were used for the calculations. In Tier 2, the default foliar DT_{50} value of 10 days was refined by a DT_{50} of 2 and 8.7 days for application in cereals and winter oilseed rape, respectively. These refined DT_{50} values were determined based on experimental data. For Tier 3, the interception values implemented in FOCUS MACRO for the Tier 2 drainage scenarios were refined to be in agreement with the values recommended by FOCUS for the respective BBCH growth stages specified in the GAP.

For all crops, only the worst-case PEC value, either resulting from calculations for single or multiple applications, are used for the risk assessment. A summary of the worst-case $PEC_{sw, max}$ and $PEC_{sed, max}$ values for metconazole and its major metabolites used for the aquatic risk assessment are provided below. The FOCUS Step 1 and Step 2 PEC values for the proposed use in winter and spring cereals and winter oilseed rape are shown in Table B.9.4.2-2. The FOCUS Step 3 and Step 4 PEC values for the proposed use in winter cereals are shown in Table B.9.4.2-3, for the use in spring cereals in Table B.9.4.2-4, and for the use in winter oilseed rape in Table B.9.4.2-5 (autumn application) and Table B.9.4.2-6 (spring application). Full details of the PEC calculations are presented in the section on fate and behaviour (see Volume 3 (PPP), Section B.8.2.6). Overall the calculated PEC values submitted by the applicant were considered acceptable.

Table B.9.4.2-2: Worst-case FOCUS Step 1 and 2 $PEC_{sw, max}$ and $PEC_{sed, max}$ values for metconazole and its metabolites following application of BAS 555 01 F in winter cereals and winter oilseed rape

Test substance	$PEC_{sw, max}$ [µg/L]		$PEC_{sed, max}$ [µg/kg dry sediment]	
	Step 1	Step 2 - EU North / South	Step 1	Step 2 - EU North / South
Winter cereals [1)]				
Metconazole	26.367	7.855	271.474	82.437
M13 (M555F013)	7.257	2.207	0.725	0.220
1,2,4-triazole (M555F020)	1.056	0.288	0.940	0.257
Winter oilseed rape [2)]				
Metconazole	21.094	4.352	217.179	45.420
M13 (M555F013)	5.806	1.225	0.580	0.122
1,2,4-triazole (M555F020)	0.845	0.143	0.752	0.127

[1)] At FOCUS Step 1 and 2 only the crop "winter cereals" was considered for PEC calculations, as winter cereals represent the worst-case application scenario covering use in spring cereals (for details please refer to Volume 3 (PPP) Section B.8.2.6).

[2)] At FOCUS Step 1 and 2 only autumn application was considered for PEC calculations, as autumn application is the worst-case application scenario covering spring application (for details please refer to Volume 3 (PPP) Section B.8.2.6).

Table B.9.4.2-3: Worst-case FOCUS Step 3 and 4 $PEC_{sw,max}$ values for metconazole in different water bodies following applications of BAS 555 01 F in winter cereals

FOCUS Scenarios		Type of PEC_{sw}	FOCUS Step 3 - edge of field	FOCUS Step 4 - PEC_{sw} [µg/L]		
			PEC_{sw} [µg/L]	5 m D	10 m D	10 m D + R
tier 1 (default foliar DT_{50} value of 10 days)						
D1	ditch	max	1.020	1.020	1.020	1.020
		twa (7 d)	0.971	0.970	0.970	0.970
	stream	max	0.639	0.639	0.639	0.639
		twa (7 d)	0.606	0.606	0.606	0.606
D2	ditch	max	2.065	2.065	2.065	2.065
		twa (7 d)	1.101	1.101	1.101	1.101
	stream	max	1.291	1.291	1.291	1.291
		twa (7 d)	0.695	0.633	0.633	0.633
D3	ditch	max	0.570	0.154	0.082	0.082
		twa (7 d)	0.092	0.025	0.013	0.013
D4	pond	max	0.080	0.079	0.077	0.077
		twa (7 d)	0.078	0.077	0.076	0.076
	stream	max	0.475	0.213	0.213	0.213
		twa (7 d)	0.078	0.078	0.078	0.078
D5	pond	max	0.071	0.070	0.069	0.069
		twa (7 d)	0.069	0.069	0.068	0.068
	stream	max	0.460	0.166	0.119	0.119
		twa (7 d)	0.040	0.040	0.040	0.040
D6	ditch	max	0.572	0.295	0.295	0.295
		twa (7 d)	0.215	0.066	0.066	0.066
R1	pond	max	0.119	0.116	0.111	0.053
		twa (7 d)	0.112	0.110	0.104	0.050
	stream	max	0.664	0.664	0.664	0.302
		twa (7 d)	0.079	0.079	0.079	0.036
R3	stream	max	0.709	0.709	0.709	0.319
		twa (7 d)	0.099	0.099	0.099	0.044
R4	stream	max	0.581	0.581	0.581	0.264
		twa (7 d)	0.181	0.181	0.181	0.082
tier 2 (refined DT_{50} of 2 days)						
D1	ditch	max	0.838 0.814 *	0.475	0.475	0.475
		twa (7 d)	0.722 0.699 *	0.446	0.446	0.446
	stream	max	0.505	0.298	0.298	0.298
		twa (7 d)	0.278	0.278	0.278	0.278
D2	ditch	max	1.635 0.659 *	1.635 0.589 *	1.635 0.589 *	1.635
		twa (7 d)	0.889 0.561 *	0.878 0.296 *	0.878 0.296 *	0.878
	stream	max	1.023 0.535 *	1.023	1.023	1.023
		twa (7 d)	0.625 0.435*	0.503	0.503	0.503
D3	ditch	max	0.570	0.154	0.082	0.082
		twa (7 d)	0.092	0.025	0.013	0.013
D4	pond	max	0.039	0.038	0.036	0.036
		twa (7 d)	0.037	0.036	0.035	0.035
	stream	max	0.475	0.174	0.095	0.095
		twa (7 d)	0.034	0.034	0.034	0.034
D5	pond	max	0.060	0.059	0.058	0.058

FOCUS Scenarios		Type of PEC_{sw}	FOCUS Step 3 - edge of field	FOCUS Step 4 - PEC_{sw} [µg/L]		
			PEC_{sw} [µg/L]	5 m D	10 m D	10 m D + R
		twa (7 d)	0.058	0.058	0.057	0.057
	stream	max	0.460	0.166	0.101	0.101
		twa (7 d)	0.034	0.034	0.034	0.034
D6	ditch	max	0.572	0.158	0.158	0.158
		twa (7 d)	0.215	0.055	0.034	0.034
R1	pond	max	0.066	0.063	0.057	0.031
		twa (7 d)	0.062	0.059	0.054	0.029
	stream	max	0.374	0.264	0.264	0.120
		twa (7 d)	0.033	0.033	0.033	0.015
R3	stream	max	0.527	0.319	0.319	0.144
		twa (7 d)	0.058	0.044	0.044	0.020
R4	stream	max	0.377	0.169	0.169	0.076
		twa (7 d)	0.050	0.050	0.050	0.023

D = Drift mitigation using no-spray buffer zones, R = runoff mitigation by vegetated filter strips.
** Tier 3 refined interception values based on tier 2 PEC values.*

Table B.9.4.2-4: Worst-case FOCUS Step 3 and 4 $PEC_{sw, max}$ values for metconazole in different water bodies following applications of BAS 555 01 F in spring cereals

FOCUS Scenarios		Type of PEC_{sw}	FOCUS Step 3 - edge of field	FOCUS Step 4 - PEC_{sw} [µg/L]		
			PEC_{sw} [µg/L]	5 m D	10 m D	10 m D + R
tier 1 (default foliar DT_{50} value of 10 days)						
D1	ditch	max	1.227	1.227	1.227	1.227
		twa (7 d)	1.187	1.187	1.187	1.187
	stream	max	0.767	0.767	0.767	0.767
		twa (7 d)	0.740	0.740	0.740	0.740
D3	ditch	max	0.570	0.155	0.082	0.082
		twa (7 d)	0.093	0.025	0.013	0.013
D4	pond	max	0.105	0.104	0.103	0.103
		twa (7 d)	0.104	0.103	0.101	0.101
	stream	max	0.466	0.260	0.260	0.260
		twa (7 d)	0.103	0.103	0.103	0.103
D5	pond	max	0.062	0.061	0.060	0.060
		twa (7 d)	0.060	0.060	0.059	0.059
	stream	max	0.479	0.175	0.104	0.104
		twa (7 d)	0.032	0.032	0.032	0.032
R4	stream	max	0.617	0.617	0.617	0.281
		twa (7 d)	0.194	0.194	0.194	0.088
tier 2 (refined DT_{50} of 2 days)						
D1	ditch	max	0.919 0.811 *	0.775 0.241 *	0.775 0.193 *	0.775
		twa (7 d)	0.796 0.695 *	0.743 0.209 *	0.743 0.179 *	0.743
	stream	max	0.506	0.485	0.485	0.485
		twa (7 d)	0.463	0.463	0.463	0.463
D3	ditch	max	0.570	0.155	0.082	0.082
		twa (7 d)	0.093	0.025	0.013	0.013
D4	pond	max	0.057	0.056	0.054	0.054
		twa (7 d)	0.056	0.055	0.054	0.054
	stream	max	0.466	0.170	0.137	0.137
		twa (7 d)	0.053	0.053	0.053	0.053
D5	pond	max	0.042	0.041	0.040	0.040
		twa (7 d)	0.041	0.040	0.039	0.039
	stream	max	0.479	0.175	0.093	0.093
		twa (7 d)	0.022	0.022	0.022	0.022
R4	stream	max	0.377	0.260	0.260	0.117
		twa (7 d)	0.075	0.075	0.075	0.034

D = Drift mitigation using no-spray buffer zones, R = runoff mitigation by vegetated filter strips.

** Tier 3 refined interception values based on tier 2 PEC values.*

Table B.9.4.2-5: Worst-case FOCUS Step 3 and 4 $PEC_{sw, max}$ values for metconazole in different water bodies following applications of BAS 555 01 F in winter oilseed rape (autumn application)

FOCUS Scenarios		Type of PEC_{sw}	Step 3 - edge of field	FOCUS Step 4 - PEC_{sw} [µg/L]		
			PEC_{sw} [µg/L]	5 m D	10 m D	10 m D + R
tier 1 (default foliar DT_{50} value of 10 days)						
D2	ditch	max	1.527	1.527	1.527	1.527
		twa (7 d)	0.638	0.635	0.635	0.635
	stream	max	0.984	0.984	0.984	0.984
		twa (7 d)	0.422	0.381	0.381	0.381
D3	ditch	max	0.458	0.124	0.066	0.066
		twa (7 d)	0.120	0.032	0.017	0.017
D4	pond	max	0.057	0.056	0.055	0.055
		twa (7 d)	0.056	0.055	0.054	0.054
	stream	max	0.394	0.198	0.198	0.198
		twa (7 d)	0.056	0.056	0.056	0.056
D5	pond	max	0.042	0.041	0.040	0.040
		twa (7 d)	0.041	0.040	0.039	0.039
	stream	max	0.425	0.155	0.104	0.104
		twa (7 d)	0.023	0.018	0.018	0.018
R1	pond	max	0.060	0.059	0.056	0.026
		twa (7 d)	0.057	0.056	0.053	0.025
	stream	max	0.400	0.400	0.400	0.179
		twa (7 d)	0.047	0.047	0.047	0.021
R3	stream	max	0.556	0.556	0.556	0.253
		twa (7 d)	0.097	0.097	0.097	0.044
tier 2 (refined DT_{50} of 8.7 days)						
D2	ditch	max	1.527 0.868 *	1.527 0.868 *	1.527 0.868 *	1.527
		twa (7 d)	0.638 0.522 *	0.635 0.410 *	0.635 0.410 *	0.635
	stream	max	0.984	0.984	0.984	0.984
		twa (7 d)	0.422	0.380	0.380	0.380
D3	ditch	max	0.458	0.124	0.066	0.066
		twa (7 d)	0.120	0.032	0.017	0.017
D4	pond	max	0.057	0.056	0.055	0.055
		twa (7 d)	0.056	0.055	0.054	0.054
	stream	max	0.394	0.198	0.198	0.198
		twa (7 d)	0.056	0.056	0.056	0.056
D5	pond	max	0.042	0.041	0.040	0.040
		twa (7 d)	0.041	0.040	0.039	0.039
	stream	max	0.425	0.155	0.104	0.104
		twa (7 d)	0.023	0.018	0.018	0.018
R1	pond	max	0.059	0.057	0.055	0.026
		twa (7 d)	0.056	0.055	0.052	0.025
	stream	max	0.397	0.397	0.397	0.177
		twa (7 d)	0.046	0.046	0.046	0.021
R3	stream	max	0.553	0.553	0.553	0.252
		twa (7 d)	0.097	0.097	0.097	0.043

D = Drift mitigation using no-spray buffer zones, R = runoff mitigation by vegetated filter strips.

** Tier 3 refined interception values based on tier 2 PEC values.*

Table B.9.4.2-6: Worst-case FOCUS Step 3 and 4 $PEC_{sw, max}$ values for metconazole in different water bodies following applications of BAS 555 01 F in winter oilseed rape (spring application)

FOCUS Scenarios		Type of PEC_{sw}	Step 3 - edge of field	FOCUS Step 4 - PEC_{sw} [µg/L]		
			PEC_{sw} [µg/L]	5 m D	10 m D	10 m D + R
tier 1 (default foliar DT_{50} value of 10 days)						
D2	ditch	max	1.605	1.605	1.605	1.605
		twa (7 d)	0.854	0.854	0.854	0.854
	stream	max	1.000	1.000	1.000	1.000
		twa (7 d)	0.494	0.494	0.494	0.494
D3	ditch	max	0.455	0.123	0.065	0.065
		twa (7 d)	0.062	0.017	0.009	0.009
D4	pond	max	0.050	0.049	0.048	0.048
		twa (7 d)	0.049	0.048	0.047	0.047
	stream	max	0.350	0.147	0.147	0.147
		twa (7 d)	0.048	0.048	0.048	0.048
D5	pond	max	0.048	0.048	0.047	0.047
		twa (7 d)	0.047	0.046	0.046	0.046
	stream	max	0.314	0.111	0.084	0.084
		twa (7 d)	0.026	0.026	0.026	0.026
R1	pond	max	0.077	0.075	0.072	0.033
		twa (7 d)	0.073	0.071	0.069	0.032
	stream	max	0.471	0.471	0.471	0.214
		twa (7 d)	0.058	0.058	0.058	0.026
R3	stream	max	0.425	0.387	0.387	0.177
		twa (7 d)	0.055	0.055	0.055	0.025
tier 2 (refined DT_{50} of 8.7 days)						
D2	ditch	max	1.587 0.808 *	1.587 0.808 *	1.587 0.808 *	1.587
		twa (7 d)	0.845 0.444 *	0.845 0.444 *	0.845 0.444 *	0.845
	stream	max	0.988	0.988	0.988	0.988
		twa (7 d)	0.488	0.488	0.488	0.488
D3	ditch	max	0.455	0.123	0.065	0.065
		twa (7 d)	0.062	0.017	0.009	0.009
D4	pond	max	0.047	0.047	0.045	0.045
		twa (7 d)	0.046	0.046	0.045	0.045
	stream	max	0.350	0.140	0.140	0.140
		twa (7 d)	0.046	0.046	0.046	0.046
D5	pond	max	0.047	0.046	0.046	0.046
		twa (7 d)	0.046	0.045	0.045	0.045
	stream	max	0.314	0.111	0.082	0.082
		twa (7 d)	0.025	0.025	0.025	0.025
R1	pond	max	0.072	0.071	0.068	0.032
		twa (7 d)	0.069	0.067	0.065	0.030
	stream	max	0.439	0.439	0.439	0.199
		twa (7 d)	0.054	0.054	0.054	0.024
R3	stream	max	0.425	0.363	0.363	0.166
		twa (7 d)	0.051	0.051	0.051	0.023

D = Drift mitigation using no-spray buffer zones, R = runoff mitigation by vegetated filter strips.

** Tier 3 refined interception values based on tier 2 PEC values.*

B.9.4.3. Risk assessment for exposure via surface water

The risk assessment is conducted for the formulation BAS 555 01 F, the active substance metconazole and the metabolite 1,2,4-triazole. For the formulation BAS 555 01 F, only an acute risk assessment is performed. As discussed in Section B.9.4.1, the chronic risk due to application of BAS 555 01 F is considered covered by the risk assessment for the active substance metconazole. The risk assessment for metconazole is also expected to cover the risk from the metabolite M13 (= CL359139; M555F013). This metabolite has a similar structure compared to the active substance, and thus it is expected that the toxicity is comparable. Further, the PEC_{SW} values for this metabolite are low (maximum FOCUS Step 1 PEC_{SW} of 7.257 and 5.806 µg/L for application in cereals and winter oilseed rape, respectively) (see also Section B.9.4.1).

B.9.4.3.1. Determination of the Regulatory Acceptable Concentrations (RAC)

According to the EFSA Guidance Document for aquatic organisms (2013), a Regulatory Acceptable Concentration (RAC) is calculated for each of the relevant groups of aquatic organisms, by dividing the toxicity endpoint by the relevant assessment factor (AF).

For the acute risk assessment for fish and aquatic invertebrates, the $RAC_{sw,ac}$ is calculated with the following equation:

$$RAC_{sw,ac} = \frac{EC_{50} \,/\, LC_{50}}{100}$$

For the chronic risk assessment for fish and aquatic invertebrates, the $RAC_{sw,ch}$ is calculated with the following equation:

$$RAC_{sw,ch} = \frac{EC_{10} \,/\, NOEC}{10}$$

The $RAC_{sw,ch}$ for algae and aquatic plants is calculated by the following equation:

$$RAC_{sw,ch} = \frac{E_r C_{50}}{10}$$

Tier 1 RAC values

For the first tier risk assessment, RAC values are determined based on the lowest available endpoints for each group of organisms. These endpoints are summarized for metconazole and the formulation BAS 555 01 F in Table B.9.4.1-4, and for the metabolite 1,2,4-triazole in Table B.9.4.1-5 above. The Tier 1 RAC values derived from these endpoints are shown in Table B.9.4.3.1-1.

For the active substance metconazole, the overall Tier 1 RAC for acute risk (lowest $RAC_{sw,ac}$) is 21 µg a.s./L, and the overall Tier 1 RAC for chronic risk (lowest $RAC_{sw,ch}$) is 0.398 µg a.s./L for surface water and 823 µg a.s./kg for sediment. From the Tier 1 data, it is clear that the risk assessment will be driven by the chronic risk to fish, since the chronic RAC for fish is at least two orders of magnitude lower compared to the other groups of organisms. As a risk assessment based in this low chronic RAC value would not result in an acceptable risk for all relevant exposure scenarios, all available data for fish is used to derive a Tier 2 RAC value (see below).

For the formulation BAS 555 01 F, the overall Tier 1 RAC for acute risk (lowest $RAC_{sw,ac}$) is 92.8 µg form./L, and the overall Tier 1 RAC for chronic risk (lowest $RAC_{sw,ch}$) is > 691 µg form./L for surface water.

For the metabolite 1,2,4-triazole, the overall Tier 1 RAC for acute risk (lowest $RAC_{sw,ac}$) is > 1000 µg/L, and the overall Tier 1 RAC for chronic risk (lowest $RAC_{sw,ch}$) is 320 µg/L for surface water.

Table B.9.4.3.1-1: Tier 1 RAC values for metconazole, the formulation BAS 555 01 F and metabolite 1,2,4-triazole for surface water and sediment for the different groups of aquatic organisms

	Species group	Endpoint	Assessment factor	RAC
Metconazole				
Acute effect assessment	Fish	LC_{50} = 2.1 mg a.s./L	100	21 µg a.s./L
	Aquatic invertebrates	EC_{50} = 3.6 mg a.s./L	100	36 µg a.s./L
			Overall acute RAC	**21 µg a.s./L**
Chronic effect assessment	Fish	EC_{10} = 0.00398 mg a.s./L	10	0.398 µg a.s./L
	Daphnia magna	EC_{10} = 0.149 mg a.s./L	10	14.9 µg a.s./L
	Chironomus riparius	NOEC = 8.23 mg a.s./kg	10	823 µg a.s./kg
	Algae	E_rC_{50} = 2.2 mg a.s./L	10	220 µg a.s./L
	Aquatic plants	E_rC_{50} = 0.527 mg a.s./L	10	52.7 µg a.s./L
			Overall chronic RAC (surface water)	**0.398 µg a.s./L**
			Overall chronic RAC (sediment)	**823 µg a.s./kg**
BAS 555 01 F				
Acute effect assessment	Fish	LC_{50} = 10 mg form./L	100	100 µg form./L
	Aquatic invertebrates	EC_{50} = 9.28 mg form/L	100	92.8 µg form./L
			Overall acute RAC	**92.8 µg form./L**
Chronic effect assessment	Algae	E_rC_{50} > 6.91 mg form./L	10	> 691 µg form./L
			Overall chronic RAC (surface water)	**> 691 µg form./L**
1,2,4-triazole				
Acute effect assessment	Fish	LC_{50} = 600 mg/L	100	6000 µg/L
	Aquatic invertebrates	EC_{50} > 100 mg/L	100	> 1000 µg/L
			Overall acute RAC	**> 1000 µg/L**
Chronic effect assessment	Fish	NOEC = 3.20 mg/L	10	320 µg/L
	Algae	E_rC_{50} > 31 mg/L	10	> 3100 µg/L
			Overall chronic RAC (surface water)	**320 µg/L**

Notes: RAC = Regulatory Acceptable Concentration

Tier 2 RAC values

According to the EFSA Guidance Document for aquatic organisms (2013), a Tier 2 effect assessment can be performed if, besides the basic data requirements, additional laboratory toxicity tests are provided. This Tier 2 assessment can be based on standard laboratory studies with additional species,

that can be used in either the Geomean assessment factor (AF) approach or in the species sensitivity distribution (SSD) approach. Another possibility is that laboratory studies with a refined exposure pattern are submitted that are subsequently used in a refined exposure laboratory test AF approach.

For metconazole, both acute and chronic standard laboratory toxicity studies with additional fish species have been submitted. The endpoints obtained from all available studies with metconazole on fish are summarized in Table B.9.4.3.1-2. For the acute risk assessment for fish, sufficient data is available to apply the SSD approach. For the chronic risk assessment, the applicant proposed two approaches for using the additional data: a reduction in the standard assessment factor in the risk assessment, and the use of the Geomean approach. Both suggested approaches are discussed below.

Table B.9.4.3.1-2: Summary of toxicity endpoints of metconazole and fish

Test species	Test system	Endpoint [mg a.s./L]			Reference
		LC_{50}	NOEC	EC_{10}	
Oncorhynchus mykiss	semi-static - 72 h	2.1	0.53	--	CA8.2.1/01 [redacted] 1990
Oncorhynchus mykiss	semi-static - 96 h	4.0 [2)]	0.75	--	CA8.2.1/04 [redacted] 1991b
Pimephales promelas	semi-static - 96 h	3.9	1.8	--	CA8.2.1/02 [redacted] 1991a
Danio rerio [1)]	static - 96 h	6.8	2.2	--	CA8.2.1/07 [redacted] 2008b
Lepomis macrochirus [1)]	static - 96 h	4.9	2.2	--	CA8.2.1/06 [redacted] 2008a
Cyprinus carpio	flow-through 96 h	3.99	0.814	--	CA8.2.1/03 [redacted] 1996a
Gasterosteus aculeatus [1)]	static - 96 h	4.2	3.2	--	CA8.2.1/08 [redacted] 2010
Cyprinodon variegatus [1)]	Static - 96 h	6.3	4.6	--	CA8.2.1/05 [redacted] 2005
Species sensitivity distribution HC_5		**2.216**	--	--	
Oncorhynchus mykiss	flow-through 95 d (ELS)	--	0.00291	0.00398	CA8.2.2.1/02 [redacted] 2001
Pimephales promelas	flow-through 35 d (ELS) [1)]	--	0.011	0.021	CA8.2.2.1/04 [redacted] 1992
	flow-through 5 months (FLC)	--	0.00358	0.0077	CA8.2.2.2/01 [redacted] W., 2008
Cyprinodon variegatus [1)]	Flow-through 33d (ELS)	--	0.011	--	CA8.2.2.1/03 [redacted] 2009

[1)] New studies conducted after Annex I inclusion; [2)] This endpoint is not used to calculate the SSD and HC5, as only the lowest endpoint for Oncorhynchus mykiss is considered in these calculations.

Species sensitivity distribution (SSD) approach for the acute risk assessment for fish

Species may vary markedly in their sensitivity to plant protection products (PPPs). This variation in direct toxicity can be described by constructing an SSD. SSDs are used to calculate the concentration at which a 5% of species are expected to suffer direct toxic effects. This concentration, the hazardous concentration, is expressed as the HC_5 value and represents the value that affects 5% of the species tested. When compared with the first tier effect assessment on the basis of standard test species, SSDs have the advantage of making more use of the available laboratory toxicity data for a larger array of species. They describe the range of sensitivity rather than focusing on a single value, they enable

estimates to be made of the proportion of the species affected at different concentrations, and they can be shown together with confidence limits showing the sampling uncertainty due to the limited number of species tested. According to the EFSA Guidance Document on aquatic organisms (2013), it is recommended to apply a SSD approach if data on a sufficient number of species is available for the respective group of aquatic organisms. For fish, the SSD approach can be applied if toxicity data for at least 5 different species are available.

For metconazole, acute toxicity studies for 6 additional fish species are available, besides the standard laboratory acute toxicity studies with *Oncorhynchus mykiss* (see Table B.9.4.3.1-2). All these studies were considered acceptable for use in the risk assessment. It is noted that different exposure regimes were used in these studies (i.e. in total four static studies, three semi-static studies and one flow-through study are available). The available LC_{50} values obtained are however not consistently lower for one exposure regime compared to the two others. Further, in all studies, the concentration in the test water was analytically confirmed and, where necessary, endpoints were calculated based on mean measured concentrations. Therefore, it is considered acceptable to combine the endpoints from studies in which different exposure regimes were used in the SSD calculations. SSD analysis was performed with the software tool ETC 2.0 (RIVM, 2004)[19]. For these calculations, the lowest of the two endpoints available for *Oncorhynchus mykiss* (2.1 mg a.s./L) was used. The results of the SSD analysis are shown below.

In a first step, the toxicity data (LC_{50}) were subjected to three different goodness-of-fit tests (Anderson-Darling, Kolmogorov-Smirnov and the Cramer von Mises), where the normality was checked. The respective analysis of the LC_{50} values confirms normal distribution of the data (see Table B.9.4.3.1-3).

Table B.9.4.3.1-3: Analysis of normality of the LC_{50} data considered in the SSD calculations for fish.

Parameters of the normal distribution						
Name	**Value**		**Description**			
mean	0.637		mean of the log toxicity values			
s.d.	0.168		sample standard deviation			
n	7		sample size			
Goodness-of-fit tests						
Sign. level	**Tests for normality n = 7**					
	Anderson-Darling		**Kolmogorov-Smirnov**		**Cramer von Mises**	
0.05	0.752	Accepted	0.895	Accepted	0.126	Accepted
Statistic	0.402		0.738		0.0460	

[19] Van Vlaardingen PLA, Traas TP, Wintersen AM and Aldenberg T (2004). ETX 2.0, a program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. RIVM report 601501028/2004.

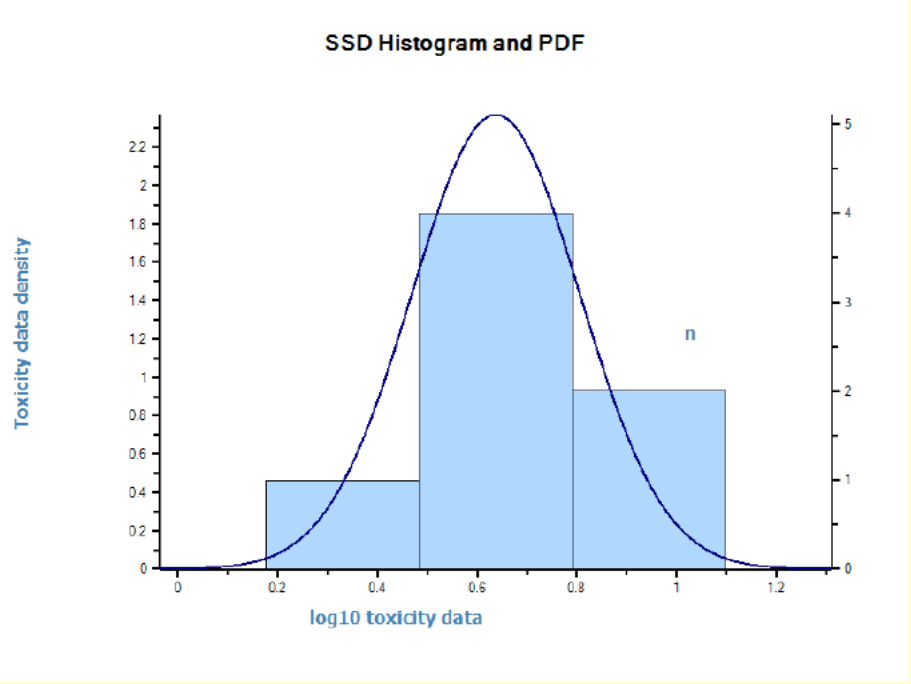


Figure B.9.4.3.1-1: Species sensitivity distribution histogram (based on LC_{50} values for 7 aquatic vertebrate species)

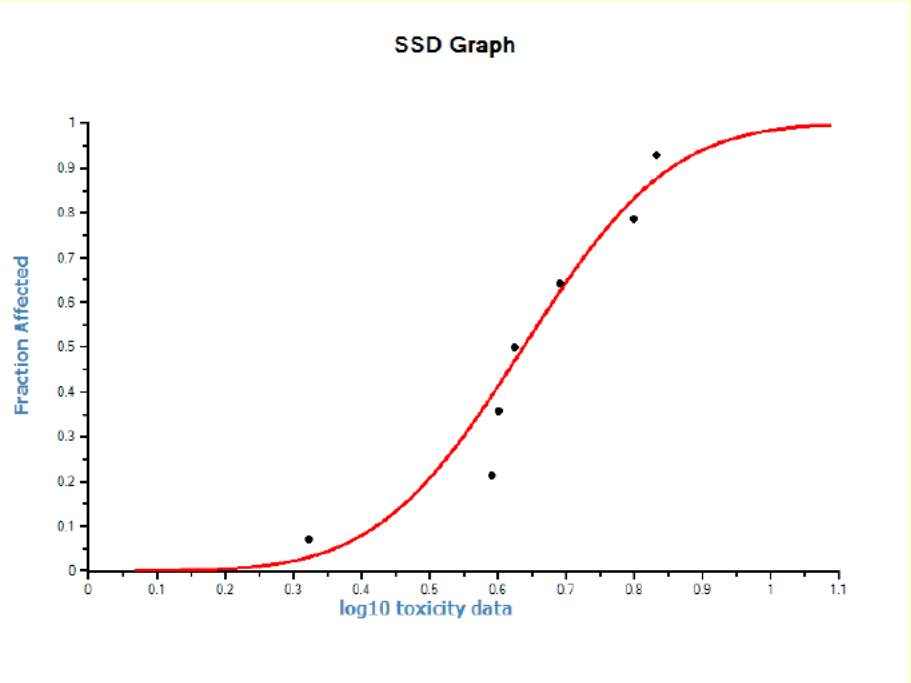


Figure B.9.4.3.1-2: Species sensitivity distribution graph (based on LC_{50} values for 7 aquatic vertebrate species)

The median HC_5 (hazardous concentration to 5% of the tested species that is predicted with 50% certainty) and also the lower limit HC_5 values ($LLHC_5$; hazardous concentration to 5% of the tested species that is predicted with 95% certainty) were derived from the SSD curve, and are shown in Table B.9.4.3.1-4. The median HC_5 for aquatic vertebrates was calculated as 2.216 mg a.s./L.

Table B.9.4.3.1-4: HC_5 results based on acute LC_{50} data for 7 aquatic vertebrate species

Name	Value	log10(value)	Description
LL HC_5	1.161	0.0647	lower estimate of the HC_5
HC_5	**2.216**	0.3455	median estimate of the HC_5
UL HC_5	3.035	0.4821	upper estimate of the HC_5
sprHC_5	2.615	0.4174	spread of the HC_5 estimate

According to the recommendations of the EFSA Guidance Document on aquatic organisms (2013), an assessment factor (AF) of 9 should be applied on the median HC_5 from an SSD constructed with LC_{50} values for fish or other aquatic vertebrates to derive an SSD-RAC when latency of effects is not to be expected. In the case of metconazole, latency of effects is likely to be not an issue, based on the standard ELS studies with *O. mykiss*, *P. promelas* and *C. varietagus* (see also the text in B.9.4.3.2 on the use of $PEC_{SW,twa}$ in the chronic risk assessment for fish). Application of an AF of 9 on the HC_5 of 2.216 mg a.s./L results in an **SSD-RAC_{ac} of 246.2 µg a.s./L**.

The Geometric mean-AF approach for the chronic risk assessment for fish

As described above, the SSD approach can be applied for fish if toxicity data for at least 5 different species are available. In case additional toxicity data is available, but their number is too low to apply the SSD approach, the EFSA Guidance Document for aquatic organisms (2013) proposes to use the geometric mean of the available toxicity values within a taxonomic group. In the EFSA Guidance Document for aquatic organisms (2013), it is stated that the geometric mean can be used for both acute and reproductive endpoints. However, in the more recent EFSA PPR Panel Opinion on the effect assessment for pesticides on sediment organisms (2015)[20], it is stated that the use of the geometric mean approach is currently not recommended for the chronic effects assessment. This is because the validity of the geometric mean approach could not be investigated due to a limited amount of chronic toxicity data available and the diversity of sub-lethal endpoints (e.g. growth, biomass, emergence, reproduction) used in chronic toxicity testing, even for species within the same taxonomic group. During a meeting in preparation for a corrigendum of the EFSA Guidance Document for aquatic organisms, held in September 2016[21], the experts agreed to harmonise the text in the EFSA Guidance Document for aquatic organisms to what is proposed in the PPR Panel Opinion for sediment organisms. Overall, it can thus be concluded that, until further scientifically underpinned, the geometric mean approach should not be used in the the chronic risk assessment.

Nevertheless, the available chronic toxicity studies for fish were assessed to see whether the conditions for calculating a geometric mean, as currently included in the EFSA Guidance Document for aquatic organisms (2013), were met. Although EC_{10} values are the preferred endpoint for use in the risk assessment (see above), the NOEC values are considered here, as EC_{10} values could not be calculated for some of the available studies. As in the available studies the NOEC is always lower than the EC_{10}, these endpoints are more conservative. The EFSA Guidance document states that only endpoints that are comparable can be pooled to calculate the geometric mean (e.g. endpoints like body weight reduction and endpoints for reproduction should not be mixed). When combining data from multiple species for chronic endpoints, additional care should be taken. Aside from the uncertainty that may arise from the use of NOECs (as opposed to L(E)C_{50}s for acute data or other EC_x values), there is considerably more

[20] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2015. Scientific Opinion on the effect assessment for pesticides on sediment organisms in edge-of-field surface water. EFSA Journal, 13(7):4176, 145 pp. doi:10.2903/j.efsa.2015.4176.

[21] Minutes available online: https://www.efsa.europa.eu/en/events/event/160914b

potential for pooling data that is not directly comparable biologically. This may result from endpoints that are ostensibly comparable being assessed at different life stages, after different exposure windows, or which may reflect different toxic responses.

For metconazole, fish early-life stage (ELS) toxicity tests are available with three different species (see Table B.9.4.3.1-2). Despite differences in study duration, which are due to species differences in developmental speed, the study design is highly comparable as they were performed according to the same OECD Test Guideline. The 95-day ELS study under flow-through conditions with *Oncorhynchus mykiss* (CA8.2.2.1/02; [redacted] 2001) yielded the lowest endpoint (NOEC = 0.00291 mg a.s./L). This endpoint was based on a significant effect on body weight, body length and survival at the next higher test concentration of 0.00924 mg a.s./L. From the 35-day ELS study under flow-through conditions with *Pimephales promelas* (CA8.2.2.1/04; [redacted] 1992) a NOEC of 0.011 mg a.s./L was derived. This NOEC was based on a significant effect on larval dry weight at the next higher dose of 0.031 mg a.s./L. A NOEC of 0.011 mg a.s./L was also obtained from the 33-day ELS study under flow-through conditions with *Cyprinodon variegatus* (CA8.2.2.1/03; [redacted] 2009). This NOEC was also based on a significant effect on larval growth parameters (length and dry weight) at the higher test concentration of 0.024 mg a.s./L. As the endpoints from the available ELS studies are all based on the same parameters, the conditions from the EFSA Guidance Document for aquatic organisms (2013) are considered to be met. It could thus be considered acceptable to pool these endpoints to calculate a geometric mean value.

In addition to the ELS studies, a fish full life cycle (FLC) study with *Pimephales promelas* is also available (CA8.2.2.2/01; [redacted] 2008). In this study, no effects on body weight were observed in larvae of the F1 generation up to a concentration of 0.01043 mg a.s./L. Small effects on body length were observed at 0.01043 mg a.s./L. The NOEC for growth was set at 0.00358 mg a.s./L based on a marked reduction in body weight and body length of female fish in the F2 generation in the highest concentration group at 0.01043 mg a.s./L. Effects on reproductive parameters (fertility and egg production) were also observed at this test concentration. The study design of an FLC study is different from an ELS study (e.g. the main difference is a longer duration of the FLC study, which continues over 2 generations). Therefore, according to the recommendations from the EFSA Guidance Document, the endpoint from this study should not be pooled with the endpoints from the ELS studies. However, the overall NOEC of 0.00358 mg a.s./L from the FLC study is based on the same parameters (growth) as the NOECs derived from the ELS studies. In addition, since the NOEC from the FLC study is lower than most endpoints from the ELS studies, including this value in the geometric mean calculations would result in a more conservative geometric mean value. Therefore, it could be considered acceptable to also include the NOEC from the FLC study in the calculation of geometric mean endpoint.

Based on the NOEC values from the three available ELS studies with *O. mykiss*, *P. promelas* and *C. variegatus*, and the FLC study with *P. promelas* (as listed in Table B.9.4.3.1-2), a geometric mean NOEC value of 0.00596 mg a.s./L can be calculated. The NOEC for the most sensitive species is less than a factor 10 below the geometric mean of all tested species, and therefore potentially acceptable. According to the EFSA Guidance Document for aquatic organisms (2013), the first tier assessment factor (AF) should be applied to this geometric mean value to derive a RAC. Applying the AF of 10 for the chronic risk assessment would result in a **Geomean-RAC$_{SW,ch}$ value of 0.596 µg a.s./L**.

As stated above, the use of the geometric mean approach is currently not recommended for the chronic effects assessment. Therefore, the value calculated above **will only be considered as supportive information** in the Weight of Evidence approach outlined below.

Reduction of the standard assessment factor in the chronic risk assessment for fish

The applicant submitted the following argumentation to justify a reduction of the standard assessment factor (AF) in the chronic risk assessment for fish (*text in italic*):

To further support the risk assessment in addition to the mandatory acute and long-term fish studies, further tests were performed to investigate differences in sensitivity among different fish species and reduce uncertainty related to the standard risk assessment. Seven species have been tested with Oncorhynchus mykiss being the most sensitive test species (see Table B.9.4.3.1-2).

Differences in sensitivity among the seven species are not more than about a factor of 3 to 4 in both the acute and the chronic studies (which may actually just indicate a rather small difference in sensitivity in addition to the 'normal' variance in test results). O. mykiss is the most sensitive species tested both in acute and chronic tests. The Commission regulation (EU) 546/2011 trigger values of 100 and 10 for acute and long term studies, respectively, address uncertainties with respect to:

1. *inter-species differences in sensitivity,*
2. *laboratory to field extrapolation,*
3. *acute to chronic exposure (only for acute data).*

The low inter-species differences in sensitivity regarding exposure to metconazole enables to consider a reduction of the standard assessment factor in the risk assessment.

The long-term data with fish show that chronic exposure is required to cause the observed low endpoints. The long-term NOEC value was obtained in a 95-day fish ELS study under flow-through conditions with constant exposure. The observed effects (weight and body-length were impaired at a concentration of 10 µg a.s./L) are most likely caused by chronic exposure rather than short term pulses. Also in the FLC study with fathead minnow length and weight of fish was reduced in the F2 generation at 10 µg a.s./L (highest concentration tested). Effects on reproductive parameters (fertility and egg production) were observed at this test concentration, too. The reduced fertility was caused by 3 pairs of fish having one non-fertilized clutch each at the beginning of the counting period. Two of them had fertilized clutches thereafter; one pair of fish did not produce further eggs. These effects may thus be considered as transient or of minor relevance. Egg production of the fish was affected only at the end of the counting period (after day 114) in the highest test concentration of 10 µg a.s./L. This suggests that long term exposure is needed to induce negative effects on egg production. All other treatments up to and including 3.58 µg a.s./L had a (slightly) increased number of eggs. Also the recently obtained study with C. variegatus, a marine species, confirms the sensitivity range with a NOEC of 11 µg/L (mean measured). Here the endpoint is based on general growth parameters (length and dry weight) as well.

In conclusion, the outcome of the various studies with metconazole justifies the reduction of the chronic assessment factor from 10 to 5. By applying this assessment factor to the most sensitive endpoint $NOEC_{(ELS)}$ *= 2.91 µg/L, a* $RAC_{chronic}$ *= 0.582 µg/L is derived for the refined risk assessment.*

The former guidance document for the risk assessment for aquatic organisms (SANCO/3268/2001 rev. 4 (final); Guidance Document on Aquatic Ecotoxicology in the context of the Directive 91/414/EEC) states that 'if a considerable number of additional species was tested in valid studies, then it is possible that the AFs that are applied to the lowest toxicity value could be lowered by up to an order of magnitude'. It is however not further specified how much additional data would be needed to allow for lowering the AF. The new EFSA Guidance Document for aquatic organisms (2013) acknowledges that when more data are available and the risk assessment is still based on the lowest toxicity value without adjusting the AF, the average level of protection may exceed the level implied by the provisions of the

Regulation for authorization of plant protection products. It further makes reference to the EFSA PPR Panel Opinion (EFSA PPP Panel, 2006)[22] on the approaches to deal with additional toxicity data, in which methods for lowering the AF are described. Although lowering the AF is currently not listed in the EFSA Guidance Document on aquatic organisms (2013) as an option to refine the risk assessment, it was agreed during a meeting in preparation for a corrigendum of the EFSA Guidance Document for aquatic organisms (held in September 2016[23]) that when the Geomean approach cannot be applied, a Weight of Evidence (WoE) approach can be used. In this case, the lowest available endpoint should be used, but the AF could be reduced following the considerations of the PPR Panel (EFSA, 2006). Such a WoE approach is also recommended in the EFSA PPR Panel Opinion on sediment organisms (EFSA PPR Panel, 2015)[24].

According to EFSA PPR Panel (2006), the overall AF can be interpreted as follows: $AF_{overall} = AF_{species} \times AF_{other}$ where $AF_{species}$ is the AF to allow for uncertainty due to variation in sensitivity among species (e.g. all fish) and AF_{other} is intended for other sources of uncertainty. The contribution of both elements is not defined, but in EFSA PPR Panel (2015) it is considered reasonable to maintain as a default approach the assumption from SANCO/3268/2001 rev. 4 (final) that for acute toxicity data, the $AF_{species}$ and AF_{other} have a more or less equal weight (so 10 for both $AF_{species}$ and $AF_{other} = 10 \times 10 = 100$ for the $AF_{overall}$). Consequently, depending on the number of acute toxicity data for different test species availabl, the $AF_{overall}$ to be applied to the $L(E)C_{50}$ of the most sensitive test species may vary from a value larger than 10 up to 100. The more additional toxicity data available, the lower the $AF_{overall}$ might be. EFSA PPR Panel (2015) assumes that in the chronic risk assessment, the $AF_{species}$ and AF_{other} do not have an equal weight since, amongst others, the uncertainty of the acute to chronic extrapolation is already addressed. Furthermore, the AF should be larger than the AF of 3 used in the chronic SSD approach. Therefore, EFSA PPR Panel (2015) proposes that the $AF_{overall}$ to be applied to the chronic toxicity value of the most sensitive species may vary from 4 up to 10. The more additional chronic toxicity data are available, the lower the AF might be.

As stated in the argumentation from the applicant above, acute toxicity data for seven different fish species are available. Further, for three of these species also chronic toxicity studies have been submitted. The available data indicates that the difference in sensitivity between species is limited (no more than a factor 3 to 4 difference among the endpoints). Further, both in the acute and chronic studies, *Oncorhynchus mykiss* was the most sensitive among the tested species. Overall, RMS considers that there is sufficient data available to be used in a WoE approach. The proposal from the applicant to lower the AF from 10 to 5 is considered reasonable. By applying the assessment factor of 5 to the most sensitive chronic endpoint (NOEC = 2.91 µg a.s./L, from the ELS study with *O. mykiss*), a Tier 2 WoE-$RAC_{SW,ch}$ of 0.582 µg a.s./L is obtained. This RAC value is comparable to the one calculated based on the Geomean approach (see above).

As stated above, the EC_{10} is considered the preferred endpoint for use in the risk assessment over the NOEC. Applying the assessment factor of 5 to the lowest available chronic EC_{10} value for fish of 0.00398 mg a.s./L (see Table B.9.4.1-4), results in a **Tier 2 WoE-$RAC_{SW,ch}$ of 0.796 µg a.s./L**.

[22] EFSA (European Food Safety Authority), 2006. Opinion of the Scientific Panel on Plant health, Plant Protection Products and their Residues on a request from the EFSA related to the assessment of the acute and chronic risk to aquatic organisms with regard to the possibility of lowering the assessment factor if additional species were tested. *The EFSA Journal*, 301, 45 pp. doi:10.2903/j.efsa.2006.301.

[23] Minutes available online: https://www.efsa.europa.eu/en/events/event/160914b

[24] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2015. Scientific Opinion on the effect assessment for pesticides on sediment organisms in edge-of-field surface water. EFSA Journal, 13(7):4176, 145 pp. doi:10.2903/j.efsa.2015.4176.

B.9.4.3.2. Risk assessment for the active substance metconazole

In the acute risk assessment, the $RAC_{SW,ac}$ (RAC in surface water (SW) for adverse effects of pesticide exposure occurring within a relatively short period after exposure) is always compared with the $PEC_{SW,max}$ derived from the predicted exposure profile. In the chronic risk assessment, the $RAC_{SW,ch}$ (RAC in surface water for adverse effects of pesticide exposure that develop slowly and/or have a long-lasting course and that are caused by short- or long-term exposure) is in the first instance compared with the $PEC_{SW,max}$, and under certain conditions with a $PEC_{SW,twa}$. If the RAC exceeds the relevant PEC_{sw} value, the risk can be considered low. If the RAC is lower than the PEC_{sw} value, further consideration is necessary (e.g. by considering PEC_{sw} values from the next FOCUS Step, or by refining the endpoints used to derive the RAC).

In Table B.9.4.3.2-1 to Table B.9.4.3.2-4, the RAC values for surface water and sediment for the different groups of aquatic organisms are compared to the FOCUS Step 1 to Step 3 PEC_{SW} and PEC_{SED} values for metconazole for the proposed uses in winter and spring cereals and winter oilseed rape. This comparison is done based on the Tier 1 RAC value for all groups of aquatic organisms. When a Tier 2 RAC value is available (i.e. for the acute and chronic risk to fish, see Section B.9.4.3.1 for details), an additional comparison is made based on this Tier 2 RAC. For the FOCUS Step 3 PEC_{SW} values, only the values from the highest available Tier are used (Tier 2 or Tier 3 values, see Section 0 for details).

Following this comparison, the acute risk to fish and aquatic invertebrates, the chronic risk to aquatic invertebrates, algae, aquatic plants and sediment dwelling organisms can be considered acceptable based on FOCUS Step 1 or Step 2 PEC values for all proposed uses. The chronic risk to fish is however not acceptable at FOCUS Step 2.

Based on the Tier 1 $RAC_{SW,ch}$ for fish of 0.398 µg a.s./L, the chronic risk to fish is acceptable at FOCUS Step 3 for some scenarios, but not all. For the use in cereals, the risk is not acceptable for the D1 ditch, D1 stream, D3 ditch, D4 stream and D5 stream scenario for both winter and spring cereals, and for the D2 ditch, D2 stream, D6 ditch and R3 stream scenario for winter cereals. For the use in oilseed rape, an acceptable use is not demonstrated for the D2 ditch, D2 stream, D3 ditch, D5 stream and R3 stream for the autumn application and for the D2 ditch, D2 stream, D3 ditch, R1 stream and R3 stream for the spring application.

Taking into account the Tier 2 WoE-$RAC_{SW,ch}$ for fish of 0.796 µg a.s./L, the risk is acceptable at FOCUS Step 3 for all scenarios, except for the D1 ditch scenario in cereals (both winter and spring) and for the D2 ditch and D2 stream scenarios in oilseed rape (both autumn and spring application). Further consideration is thus necessary for these scenarios.

In Table B.9.4.3.2-5 to Table B.9.4.3.2-8, the Tier 1 and Tier 2 RAC value for surface water for the chronic risk to fish is compared to the maximum FOCUS Step 4 PEC_{SW} values (Tier 2 or Tier 3) for those scenarios for which the risk was not acceptable at FOCUS Step 3. Based on the Tier 1 RAC, the risk is acceptable for all scenarios in winter cereals with a 5 m no spray buffer zone, except for the D1 ditch, D2 ditch and D2 stream scenarios. For the latter scenarios, more stringent risk mitigation measures, up to a 10 m no spray buffer zone and vegetated buffer strip, did not result in an acceptable risk. For spring cereals, the risk is acceptable for all scenarios if a 5 m no spray buffer zone is applied, except for the D1 sream scenario. For this scenario, more stringent risk mitigation measures, up to a 10 m no spray buffer zone and vegetated buffer strip, did also not result in an acceptable risk. For the use in winter oilseed rape (both autumn and spring application), no acceptable risk was demonstrated for

the D2 ditch and D2 stream scenarios, even with a 10 m no spray buffer zone and vegetated buffer strip. For the autumn application, the risk was acceptable for the D3 ditch and D5 stream scenario with a 5 m no spray buffer zone and for the R3 stream scenario with a 10m no spray buffer zone and vegetated buffer strip. For the spring application, the risk was acceptable with a 5 m no spray buffer zone for the D3 ditch and R3 stream scenario, and with a 10 m no spray buffer zone and vegetated buffer strip for the R1 stream scenario.

Based on the Tier 2 RAC, the risk was acceptable for all scenarios for winter and spring cereals, provided that a 5 m no spray buffer zone was applied. For the use in oilseed rape (both autumn and spring applications), the risk was acceptable for all scenarios with a 5 m no spray buffer zone, except for the D2 ditch and D2 stream scenario. For the latter scenarios, more stringent mitigation measures, up to a 10 m no spray buffer zone and vegetated buffer strip, did not result in an acceptable risk.

Table B.9.4.3.2-1: Comparison of the relevant RAC values and the maximum PEC_{SW} and PEC_{SED} for metconazole at FOCUS Steps 1 to 3 following application of BAS 555 01 F in winter cereals (at 1-2 x 90 g a.s./ha).

Scenario	fish acute		fish chronic		Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant	Sed. dweller prolonged
	Oncorhynchus mykiss	*SSD approach*	*Oncorhynchus mykiss*	*WoE approach*	*Daphnia magna*	*Daphnia magna*	*Pseudokircherniella subcapitata*	*Lemna gibba*	*Chironomus riparius*
Level of assessment	Tier 1	Tier 2	Tier 1	Tier 2	Tier 1	Tier 1	Tier 1	Tier 1	Tier 1
RAC	21 µg/L	246.2 µg/L	0.398 µg/L	0.796 µg/L	36 µg/L	14.9 µg/L	220 µg/L	52.7 µg/L	823 µg/kg dry sediment
FOCUS Step 1 PEC values	**26.367**	26.367	**26.367**	**26.367**	26.367	**26.367**	26.367	26.367	271.474
FOCUS Step 2 PEC values									
North Europe	7.855	7.855	**7.855**	**7.855**	7.855	7.855	7.855	7.855	82.437
South Europe	7.855	7.855	**7.855**	**7.855**	7.855	7.855	7.855	7.855	82.437
FOCUS Step 3 PEC values – Tier 2 (values marked with * are Tier 3 values)									
D1 / ditch	-	-	**0.814 ***	**0.814 ***	-	-	-	-	-
D1 / stream	-	-	**0.505**	0.505	-	-	-	-	-
D2 / ditch	-	-	**0.659 ***	0.659 *	-	-	-	-	-
D2 / stream	-	-	**0.535 ***	0.535 *	-	-	-	-	-
D3 / ditch	-	-	**0.570**	0.570	-	-	-	-	-
D4 / pond	-	-	0.039	0.039	-	-	-	-	-
D4 / stream	-	-	**0.475**	0.475	-	-	-	-	-
D5 / pond	-	-	0.060	0.060	-	-	-	-	-
D5 / stream	-	-	**0.460**	0.460	-	-	-	-	-
D6 / ditch	-	-	**0.572**	0.572	-	-	-	-	-
R1 / pond	-	-	0.066	0.066	-	-	-	-	-
R1 / stream	-	-	0.374	0.374	-	-	-	-	-
R3 / stream	-	-	**0.527**	0.527	-	-	-	-	-
R4 / stream	-	-	0.377	0.377	-	-	-	-	-

Notes: PEC values in bold indicate that the $PEC_{SW/SED}$ exceeds the RAC, and thus that further consideration is necessary

Table B.9.4.3.2-2: Comparison of the relevant RAC values and the maximum PEC_{SW} and PEC_{SED} for metconazole at FOCUS Steps 1 to 3 following application of BAS 555 01 F in spring cereals (at 1-2 x 90 g a.s./ha).

Scenario	fish acute		fish chronic		Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant	Sed. dweller prolonged
	Oncorhynchus mykiss	*SSD approach*	*Oncorhynchus mykiss*	*WoE approach*	*Daphnia magna*	*Daphnia magna*	*Pseudokircherniella subcapitata*	*Lemna gibba*	*Chironomus riparius*
Level of assessment	Tier 1	Tier 2	Tier 1	Tier 2	Tier 1	Tier 1	Tier 1	Tier 1	Tier 1
RAC	21 µg/L	246.2 µg/L	0.398 µg/L	0.796 µg/L	36 µg/L	14.9 µg/L	220 µg/L	52.7 µg/L	823 µg/kg dry sediment
FOCUS Step 1 PEC values	**26.367**	26.367	**26.367**	**26.367**	26.367	**26.367**	26.367	26.367	271.474
FOCUS Step 2 PEC values									
North Europe	7.855	7.855	**7.855**	**7.855**	7.855	7.855	7.855	7.855	82.437
South Europe	7.855	7.855	**7.855**	**7.855**	7.855	7.855	7.855	7.855	82.437
FOCUS Step 3 PEC values – Tier 2 (values marked with * are Tier 3 values)									
D1 / ditch	-	-	**0.811 ***	**0.811 ***	-	-	-	-	-
D1 / stream	-	-	**0.506**	0.506	-	-	-	-	-
D3 / ditch	-	-	**0.570**	0.570	-	-	-	-	-
D4 / pond	-	-	0.057	0.057	-	-	-	-	-
D4 / stream	-	-	**0.466**	0.466	-	-	-	-	-
D5 / pond	-	-	0.042	0.042	-	-	-	-	-
D5 / stream	-	-	**0.479**	0.479	-	-	-	-	-
R4 / stream	-	-	0.377	0.377	-	-	-	-	-

Notes: PEC values in bold indicate that the $PEC_{SW/SED}$ exceeds the RAC, and thus that further consideration is necessary

Table B.9.4.3.2-3: Comparison of the relevant RAC values and the maximum PEC_{SW} and PEC_{SED} for metconazole at FOCUS Steps 1 to 3 following application of BAS 555 01 F in winter oilseed rape (at 1-2 x 72 g a.s./ha) – autumn application.

Scenario	fish acute		fish chronic		Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant	Sed. dweller prolonged
	Oncorhynchus mykiss	*SSD approach*	*Oncorhynchus mykiss*	*WoE approach*	*Daphnia magna*	*Daphnia magna*	*Pseudokircherniella subcapitata*	*Lemna gibba*	*Chironomus riparius*
Level of assessment	Tier 1	Tier 2	Tier 1	Tier 2	Tier 1	Tier 1	Tier 1	Tier 1	Tier 1
RAC	21 µg/L	246.2 µg/L	0.398 µg/L	0.796 µg/L	36 µg/L	14.9 µg/L	220 µg/L	52.7 µg/L	823 µg/kg dry sediment
FOCUS Step 1 PEC values	**21.094**	21.094	**21.094**	**21.094**	21.094	**21.094**	21.094	21.094	217.179
FOCUS Step 2 PEC values									
North Europe	4.352	4.352	**4.352**	**4.352**	4.352	4.352	4.352	4.352	45.420
South Europe	4.352	4.352	**4.352**	**4.352**	4.352	4.352	4.352	4.352	45.420
FOCUS Step 3 PEC values – Tier 2 (values marked with * are Tier 3 values)									
D2 / ditch	-	-	**0.868 ***	**0.868 ***	-	-	-	-	-
D2 / stream	-	-	**0.984**	**0.984**	-	-	-	-	-
D3 / ditch	-	-	**0.458**	0.458	-	-	-	-	-
D4 / pond	-	-	0.057	0.057	-	-	-	-	-
D4 / stream	-	-	0.394	0.394	-	-	-	-	-
D5 / pond	-	-	0.042	0.042	-	-	-	-	-
D5 / stream	-	-	**0.425**	0.425	-	-	-	-	-
R1 / pond	-	-	0.059	0.059	-	-	-	-	-
R1 / stream	-	-	0.397	0.397	-	-	-	-	-
R3 / stream	-	-	**0.553**	0.553	-	-	-	-	-

Notes: PEC values in bold indicate that the $PEC_{SW/SED}$ exceeds the RAC, and thus that further consideration is necessary

Table B.9.4.3.2-4: Comparison of the relevant RAC values and the maximum PEC_{SW} and PEC_{SED} for metconazole at FOCUS Steps 1 to 3 following application of BAS 555 01 F in winter oilseed rape (at 1-2 x 72 g a.s./ha) – spring application.

Scenario	fish acute		fish chronic		Aquatic invertebrates	Aquatic invertebrates prolonged	Algae	Higher plant	Sed. dweller prolonged
	Oncorhynchus mykiss	*SSD approach*	*Oncorhynchus mykiss*	*WoE approach*	*Daphnia magna*	*Daphnia magna*	*Pseudokircherniella subcapitata*	*Lemna gibba*	*Chironomus riparius*
Level of assessment	Tier 1	Tier 2	Tier 1	Tier 2	Tier 1	Tier 1	Tier 1	Tier 1	Tier 1
RAC	21 µg/L	246.2 µg/L	0.398 µg/L	0.796 µg/L	36 µg/L	14.9 µg/L	220 µg/L	52.7 µg/L	823 µg/kg dry sediment
FOCUS Step 1 PEC values	**21.094**	21.094	**21.094**	**21.094**	21.094	**21.094**	21.094	21.094	217.179
FOCUS Step 2 PEC values									
North Europe	4.352	4.352	**4.352**	**4.352**	4.352	4.352	4.352	4.352	45.420
South Europe	4.352	4.352	**4.352**	**4.352**	4.352	4.352	4.352	4.352	45.420
FOCUS Step 3 PEC values – Tier 2 (values marked with * are Tier 3 values)									
D2 / ditch	-	-	**0.808 ***	**0.808 ***	-	-	-	-	-
D2 / stream	-	-	**0.988**	**0.988**	-	-	-	-	-
D3 / ditch	-	-	**0.455**	0.455	-	-	-	-	-
D4 / pond	-	-	0.047	0.047	-	-	-	-	-
D4 / stream	-	-	0.350	0.350	-	-	-	-	-
D5 / pond	-	-	0.047	0.047	-	-	-	-	-
D5 / stream	-	-	0.314	0.314	-	-	-	-	-
R1 / pond	-	-	0.072	0.072	-	-	-	-	-
R1 / stream	-	-	**0.439**	0.439	-	-	-	-	-
R3 / stream	-	-	**0.425**	0.425	-	-	-	-	-

Notes: PEC values in bold indicate that the $PEC_{SW/SED}$ exceeds the RAC, and thus that further consideration is necessary

Table B.9.4.3.2-5: Comparison of the relevant RAC values for the chronic risk to fish and the maximum PECsw for metconazole at FOCUS Step 4 following application of BAS 555 01 F according to the proposed use in winter cereals (at 1-2 x 90 g a.s./ha).

Scenario	fish chronic					
	Oncorhynchus mykiss			*WoE approach*		
Level of assessment	Tier 1			Tier 2		
RAC	0.398 µg/L			0.796 µg/L		
Mitigation options	**5 m D**	**10 m D**	**10 m D+R**	**5 m D**	**10 m D**	**10 m D+R**
FOCUS Step 4 PEC values – Tier 2 (values marked with * are Tier 3 values)						
D1 / ditch	**0.475**	**0.475**	**0.475**	0.475	0.475	0.475
D1 / stream	0.298	0.298	0.298	0.298	0.298	0.298
D2 / ditch	**0.589 ***	**0.589 ***	**0.589 ***	0.589 *	0.589 *	0.589 *
D2 / stream	**0.535 ***	**0.535 ***	**0.535 ***	0.535 *	0.535 *	0.535 *
D3 / ditch	0.154	0.082	0.082	0.154	0.082	0.082
D4 / stream	0.174	0.095	0.095	0.174	0.095	0.095
D5 / stream	0.166	0.101	0.101	0.166	0.101	0.101
D6 / ditch	0.158	0.158	0.158	0.158	0.158	0.158
R3 / stream	0.319	0.319	0.144	0.319	0.319	0.144

Table B.9.4.3.2-6: Comparison of the relevant RAC values for the chronic risk to fish and the maximum PECsw for metconazole at FOCUS Step 4 following application of BAS 555 01 F according to the proposed use in spring cereals (at 1-2 x 90 g a.s./ha).

Scenario	fish chronic					
	Oncorhynchus mykiss			*WoE approach*		
Level of assessment	Tier 1			Tier 2		
RAC	0.398 µg/L			0.796 µg/L		
Mitigation options	**5 m D**	**10 m D**	**10 m D+R**	**5 m D**	**10 m D**	**10 m D+R**
FOCUS Step 4 PEC values – Tier 2 (values marked with * are Tier 3 values)						
D1 / ditch	0.241*	0.193*	-	0.241*	0.193*	-
D1 / stream	**0.485**	**0.485**	**0.485**	0.485	0.485	0.485
D3 / ditch	0.155	0.082	0.082	0.155	0.082	0.082
D4 / stream	0.170	0.137	0.137	0.170	0.137	0.137
D5 / stream	0.175	0.093	0.093	0.175	0.093	0.093

Table B.9.4.3.2-7: Comparison of the relevant RAC values for the chronic risk to fish and the maximum PEC_{SW} for metconazole at FOCUS Step 4 following application of BAS 555 01 F according to the proposed use in winter oilseed rape (at 1-2 x 72 g a.s./ha) – autumn application.

Scenario	**fish chronic**					
	Oncorhynchus mykiss			*WoE approach*		
Level of assessment	Tier 1			Tier 2		
RAC	0.398 µg/L			0.796 µg/L		
Mitigation options	**5 m D**	**10 m D**	**10 m D+R**	**5 m D**	**10 m D**	**10 m D+R**
FOCUS Step 4 PEC values – Tier 2 (values marked with * are Tier 3 values)						
D2 / ditch	**0.868 ***	**0.868 ***	-	**0.868 ***	**0.868 ***	-
D2 / stream	**0.984**	**0.984**	**0.984**	**0.984**	**0.984**	**0.984**
D3 / ditch	0.124	0.066	0.066	0.124	0.066	0.066
D5 / stream	0.155	0.104	0.104	0.155	0.104	0.104
R3 / stream	**0.553**	**0.553**	0.252	0.553	0.553	0.252

Table B.9.4.3.2-8: Comparison of the relevant RAC values for the chronic risk to fish and the maximum PEC_{SW} for metconazole at FOCUS Step 4 following application of BAS 555 01 F according to the proposed use in winter oilseed rape (at 1-2 x 72 g a.s./ha) – spring application.

Scenario	**fish chronic**					
	Oncorhynchus mykiss			*WoE approach*		
Level of assessment	Tier 1			Tier 2		
RAC	0.398 µg/L			0.796 µg/L		
Mitigation options	**5 m D**	**10 m D**	**10 m D+R**	**5 m D**	**10 m D**	**10 m D+R**
FOCUS Step 4 PEC values – Tier 2 (values marked with * are Tier 3 values)						
D2 / ditch	**0.808 ***	**0.808 ***	-	**0.808 ***	**0.808 ***	-
D2 / stream	**0.988**	**0.988**	**0.988**	**0.988**	**0.988**	**0.988**
D3 / ditch	0.123	0.065	0.065	0.123	0.065	0.065
R1 / stream	**0.439**	**0.439**	0.199	0.439	0.439	0.199
R3 / stream	0.363	0.363	0.166	0.363	0.363	0.166

*Notes: D = Drift mitigation by no-spray buffer zones; N = Drift mitigation by drift reducing nozzles; PEC values in bold indicate that the $PEC_{SW/SED}$ exceeds the RAC, and thus that further consideration is necessary; PEC values marked with * are Tier 3 values*

Use of $PEC_{SW,twa}$ in the chronic Tier 1 risk assessment for fish

The risk assessment presented in the tables above is based on worst-case initial PEC values. However, the EFSA Guidance Document for aquatic organisms (2013) foresees that under certain conditions a time-weighted average PEC ($PEC_{SW,twa}$) may be used in the chronic risk assessment. The applicant submitted an argumentation to demonstrate that in case of the chronic risk assessment for fish, it is warranted to use the $PEC_{SW,twa}$. This argumentation is presented below (*text in italic*):

The long-term data with fish show that chronic exposure is required to cause the observed low endpoints. The long-term NOEC value was obtained in a 95-day fish ELS study under flow-through conditions with constant exposure. The observed effects (weight and body-length were impaired at a concentration of 10 µg a.s./L) are most likely caused by chronic exposure rather than short term pulses. Also in the FLC study with fathead minnow length and weight of fish was reduced in the F2 generation at 10 µg a.s./L (highest concentration tested). Effects on reproductive parameters (fertility and egg production) were observed at this test concentration, too. The reduced fertility was caused by 3 pairs of fish having one non-fertilized clutch each at the beginning of the counting period. Two of them had fertilized clutches thereafter; one pair of fish did not produce further eggs. These effects may thus be considered as transient or of minor relevance. Egg production of the fish was affected only at the end of the counting period (after day 114) in the highest test concentration of 10 µg a.s./L. This suggests that long term exposure is needed to induce negative effects on egg production. All other treatments up to and including 3.58 µg a.s./L had a (slightly) increased number of eggs. Also the recently obtained study with C. variegatus, a marine species, confirms the sensitivity range with a NOEC of 11 µg/L (mean measured). Here the endpoint is based on general growth parameters (length and dry weight) as well.

The available data indicate that prolonged exposure rather than short term pulses is necessary to induce effects in fish. Since constant exposure is not expected in the field, the comparison of $PEC_{sw,max}$ values with effect concentrations obtained under flow-through conditions might be overly conservative and more realistic time-weighted average values (PEC_{twa}) may be used in combination with the NOEC to calculate TER values.

Further, the applicant checked the suitability of using $PEC_{SW,twa}$ in the chronic risk assessment for fish against the decision scheme presented in section 4.5.2 of the EFSA Aquatic Guidaince Document (2013). Each step of this decision scheme is discussed in detail below:

1. *Chronic Assessment. Is $PEC_{sw;max}$ (of highest available tier) > $RAC_{sw;ch}$ (of highest available tier)?*

   The $PEC_{SW,max}$ values presented in Table B.9.4.3.2-1 to Table B.9.4.3.2-5 above indicate that in a number of cases this value exceeds the Geomean-$RAC_{SW,ch}$ of 0.596 µg a.s./L for fish, especially if no risk mitigation measures are considered.

   Yes: Go to 2
   No: Low chronic risk

2. *Is the $RAC_{sw;ch}$ derived from a test with algae, or from a long-term (≥ 7 days) test with another water organism and the following conditions apply: (i) loss of the a.s. from water is more than 20 % of nominal at the end of the exposure period and (ii) the toxicity estimate (e.g. EC_{10} or NOEC) is expressed in terms of nominal/initially measured concentration of the a.s.?*

The Geomean-$RAC_{SW,ch}$ of 0.596 µg a.s./L was calculated based on the endpoints derived from the studies by [redacted] (2001; CA8.2.2.1/02), [redacted] (2009; CA8.2.2.1/03), [redacted] (1992; CA8.2.2.1/04) and [redacted] (2008; CA8.2.2.2/01). In these studies, the concentration of the active substance was not maintained within 80% of nominal for all test concentrations throughout the test. However, the endpoints (NOEC) was all expressed in terms of mean measured concentrations.

Yes: $PEC_{sw;twa}$ not appropriate (low risk not demonstrated)
No: Go to 3

3. *Is the $RAC_{sw;ch}$ based on treatment-related responses of the relevant test species early in the chronic test (e.g. during the initial 96-hours observed mortality/immobility in tests with animals, or 50 % reduction in growth rate in tests with macrophytes, in the treatment level above the one from which the $RAC_{sw;ch}$ is derived) or is the acute to chronic ratio (acute $L(E)C_{50}$/chronic NOEC or acute $L(E)C_{50}$/chronic EC_{10}) based on immobility or mortality < 10?*

The NOEC of 0.00291 mg a.s./L from the ELS study with Rainbow trout ([redacted] 2001; CA8.2.2.1/02) is based on growth parameters and the first effects on survival can only be seen at a very late stage (day 62, after swim-up). The NOEC is thus not based on treatment-related responses of fish early in the chronic test. The same holds true for the other chronic fish studies of which the endpoints are used to derive the Geomean-$RAC_{SW,ch}$ ([redacted] 2009; [redacted] 1992; [redacted]., 2008).
The acute to chronic ratio for rainbow trout, based on an acute LC_{50} = 2.1 mg a.s./L and a chronic NOEC = 0.00291 mg a.s./L, is 722 and thus > 10. For *Pimephales promelas* (LC_{50} = 3.8 mg a.s./L and NOEC = 0.11 mg a.s./L) and *Cyprinodon variegatus* (LC_{50} = 6.3 mg a.s./L and NOEC = 0.11 mg a.s./L), the acute to chronic ratio is 35 and 57, respectively.

Yes: $PEC_{sw;twa}$ not appropriate (low risk not demonstrated)
No: Go to 4

4. *Is it demonstrated by the notifier that, for the organisms and the PPP under evaluation and/or PPP with a similar toxic mode of action (read-across information), the following phenomena are not likely: (i) latency of effects due to short-term exposure; (ii) the co-occurrence of exposure and specific sensitive life stages that last a short time only?*

No specific studies have been performed to investigate a possible latency of effects. The applicant argued that latency of effects due to short-term exposure can be excluded based on the results of the chronic fish studies, since unspecific growth effects at study termination are the most sensitive parameters and the BCF / BMF studies do not indicate a potential for accumulation of substance in fish. RMS agrees that based on the available studies, there are no indications that short-term exposure will lead to delayed effects.

Yes: Go to 5 [used of $PEC_{SW,7d\text{-}twa}$ justified]
No: $PEC_{sw;twa}$ not appropriate (low risk not demonstrated)

Overall, the results of the decision scheme from the EFSA Guidance Document for aquatic organisms (2013) indicate that the criteria for using the $PEC_{SW,twa}$ are fulfilled. However, according to the EFSA Guidance Document for aquatic organisms (2013), the use of the time-weighted average (TWA)

concentration approach in the risk assessment for plant protection products (PPPs) is based on the observation that effects of PPPs on aquatic organisms may be similar when exposed for a short time to a greater concentration or for a longer time to a smaller concentration, a phenomenon referred to as reciprocity. Reciprocity relates to Haber's law, which assumes that toxicity depends on the product of concentration and time. Given that linear reciprocity is a prerequisite of the TWA approach, it has to be clearly demonstrated that this concept is applicable to the toxicity of metconazole to fish.

The applicant did not provide any argumentation to demonstrate reciprocity. Therefore, RMS checked this criterion by plotting the effects on survival observed in the ELS study with *Pimephales promelas* by [redacted] (1992; CA8.2.2.1/04) against exposure time*concentration. Only the data for those concentrations where a statistically significant effect on survival was found after 35 days were considered for this analysis (i.e. 0.093, 0.28, 0.83 and 2.5 mg a.s./L). The study report contains data on mortality after 1,2, 3, 4, 5, 6, 7, 14, 21, 28 and 35 days of exposure. As the first larvae only emerged after 3 days, the data for the first two days were also not taken into account. Data points where the mortality did not increase compared the previous assessment day were not included in the plot as well. Table B.9.4.3.2-9 shows the observed mortality rates for the different test concentrations. To express the observed effect on mortality relative to the control, the mortality rate was corrected for control mortality using the Schneider-Orelli formula. In Figure B.9.4.3.2-1, the corrected mortality rate was plotted against exposure time*concentration for the data for day 3 to 35. Linear regression analysis on the data for these days shows a relatively bad fit ($R^2 = 0.2902$). Consequently, linear reciprocity is not demonstrated based on the data from the ELS study with *Pimephales promelas*. Survival data over a longer time period is also available from the prolonged toxicity study by [redacted] (1996b; CA8.2.2.1/01) and the ELS study by [redacted] (2001; CA8.2.2.1/02), both performed with *Oncorhynchus mykiss*. A detailed analysis regarding reciprocity based on the data from these studies is not reported here. However, similar results as for *Pimephales promelas* were obtained. Therefore, RMS considers that **$PEC_{SW,twa}$ values should not be used in the chronic risk assessment for fish**.

It should further be noted that during Pesticides Peer Review Meeting 133 on general recurring issues in ecotoxicology, it was concluded that until futher guidance on reciprocity and latency of effects are available, then the use of TWA approaches are unlikely to be sufficiently robust to be used in regulatory risk assessment (Please refer to the EFSA Technical report on the outcome of this meeting (EFSA,2015)[25] for further details).

[25] EFSA, 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.

Table B.9.4.3.2-9: Observed mortality (%), Corrected mortality (%) and Exposure time * Concentration values for the data measured on day 3, 4, 5, 6, 7, 14, 21, 28 and 35 in the study by [redacted] (1992) (CA8.2.2.1/04)

Day	Mortality (%)					Corrected mortality (%) [1]				Exposure time * concentration			
	C	T3	T4	T5	T6	T3	T4	T5	T6	T3	T4	T5	T6
3	0	1.1	0	0	0	1.1	0	0	0	0.28	0.84	2.49	7.5
4	0	2.3	3.5	4.5	4.5	2.2	3.4	4.5	4.5	0.37	1.12	3.32	10
5	4.7	12.4	6.9	11.4	18.0	8.0	2.3	7.0	13.9	0.47	1.4	4.15	12.5
6	7.1	12.4[$]	8.1	12.5	24.7	5.7[$]	1.1	5.9	19.0	0.56[$]	1.68	4.98	15
7	7.1	12.6[$]	8.1[$]	12.5[$]	27.0	5.7[$]	1.1[$]	5.9[$]	21.4	0.65[$]	1.96[$]	5.81[$]	17.5
14	9.4	19.1	47.1	87.5	73.0	10.7	41.6	86.2	70.2	1.30	3.92	11.62	35
21	10.6	32.6	90.8	100	100	24.6	89.7	100	100	1.95	5.88	17.43	52.5
28	10.6	58.4	94.3	100[$]	100[$]	53.5	93.6	100[$]	100[$]	2.60	7.84	23.24[$]	70[$]
35	11.8	61.8	96.6	100[$]	100[$]	56.7	96.1	100[$]	100[$]	3.26	9.8	29.05[$]	87.5[$]

C: Control; T3: treatment level 0.093 mg a.s./L, T4: treatment level 0.28 mg a.s./L; T5: treatment level 0.83 mg a.s./L; T6: treatment level 2.5 mg a.s./L; [1]Mortality was corrected using the Schneider-Orelli formula; [$]Data point not included in the reciprocity assessment

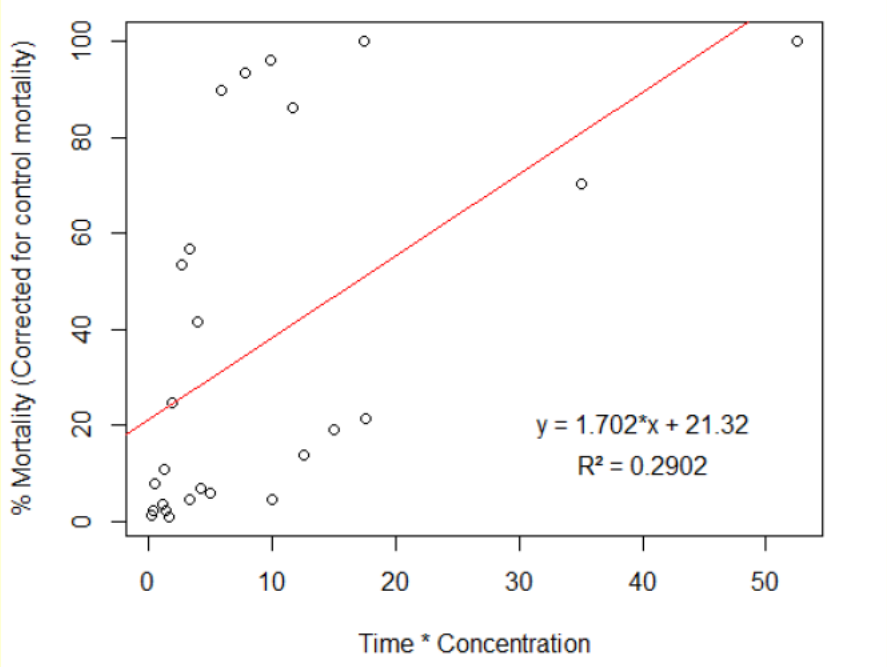


Figure B.9.4.3.2-1: Effect of Time * Concentration on the % mortality, based on data for day 3, 4, 5, 6, 7, 14, 21, 28 and 35 from the study by [redacted] (1992) (CA8.2.2.1/04)

Conclusion

With the exception of the chronic risk to fish, the acute and chronic risk to aquatic organisms (in surface water and sediment) is acceptable based on FOCUS Step 1 or Step 2 values for the proposed uses of BAS 555 01 F in winter and spring cereals and in winter oilseed rape. Based on the Tier 2 RAC, the chronic risk to fish for the proposed use in winter and spring cereals was acceptable at FOCUS Step 3 for most of the FOCUS scenarios, and at FOCUS Step 4 with a 5 m no spray buffer zone for the D1 ditch scenario. For the proposed use in winter oilseed rape, the risk was acceptable at FOCUS Step 3 for most of the FOCUS scenarios, except for the D2 ditch and D2 stream scenario. For the latter scenarios, no acceptable risk could be demonstrated, even when a 10 m no spray buffer zone and vegetate buffer strip was applied at FOCUS Step 4.

Further risk mitigation measures for the D2 ditch and D2 stream scenario for the proposed use in winter oilseed rape could be dealt with at Member State level.

B.9.4.3.3. Risk assessment for the formulation BAS 555 01 F

As discussed above, only an acute risk assessment is performed for the formulation BAS 555 01 F. The slightly higher toxicity of the formulation compared to the active substance is caused by the co-formulants "ethoxylated C9-11 alcohols" (see Section B.9.4.1). Because these co-formulants are readily biodegradable, the increased toxicity of BAS 555 01 F can only occur due to short-term drift events after application when the intact formulation is present in the water body. Long-term exposure to BAS 555 01 to aquatic organisms cannot occur. Therefore, the chronic risk due to application of BAS 555 01 F is considered covered by the risk assessment for the active substance metconazole.

In the acute risk assessment, the $RAC_{SW,ac}$ (RAC in surface water (SW) for adverse effects of pesticide exposure occurring within a relatively short period after exposure) is always compared with the $PEC_{SW,max}$ derived from the predicted exposure profile. If the RAC exceeds the relevant PEC_{sw} value, the risk can be considered low. If the RAC is lower than the PEC_{sw} value, further consideration is necessary (e.g. by considering PEC_{sw} values from the next FOCUS Step, or by refining the endpoints used to derive the RAC).

In Section 0, the maximum instantaneous $PEC_{sw,ini,drift}$ for the use in winter and spring cereals was calculated to be 6.729 µg BAS 555 01 F/L. As the application rate in cereals (1.0 L/ha) exceeds the application rate in winter oilseed rape (0.8 L/ha), this PEC value is also protective for the use in winter oilseed rape. In Table B.9.4.3.3-1, the RAC values for surface water for BAS 555 01 F for the different groups of aquatic organisms (as summarized in Table B.9.4.3.1-1) are compared to this maximum instantaneous $PEC_{sw,ini,drift}$. As the RAC values for the three groups of aquatic organisms largely exceed the calculated PEC value, **the acute risk to aquatic organisms following application of BAS 555 01 F according to the proposed uses in winter and spring cereals and oilseed rape is considered acceptable.**

Table B.9.4.3.3-1: Comparison of the relevant RAC values and the maximum instantaneous $PEC_{SW,ini,drift}$ for BAS 555 01 F following application of BAS 555 01 F in winter and spring cereals (at 1-2 x 1.0 L/ha) and winter oilseed rape (at 1-2 x 0.8 L/ha).

Scenario	fish acute	Aquatic invertebrates	Algae
	Oncorhynchus mykiss	*Daphnia magna*	*Pseudokircherniella subcapitata*
Level of assessment	Tier 1	Tier 1	Tier 1
RAC	100 µg/L	92.8 µg/L	> 691 µg/L
Maximum instantaneous $PEC_{sw,ini,drift}$ (µg/L)	6.729	6.729	6.729

Notes: PEC values in bold indicate that the $PEC_{SW/SED}$ exceeds the RAC, and thus that further consideration is necessary

B.9.4.3.4. Risk assessment for the metabolites of metconazole

According to Vol. 3 Section B.8 (Fate & Behaviour in the Environment), the major metabolites of metconazole formed in aquatic systems are M13 (= CL359139; M555F013) and 1,2,4-triazole (= M555F020). As aquatic organisms may be exposed to these major metabolites, the risk following such exposure needs to be assessed. As discussed in Section B.9.4.1, metabolite M13 has a similar structure compared to the active substance, and thus it is expected that the toxicity is comparable. As further the the PEC_{SW} values for this metabolite are low (maximum FOCUS Step 1 PEC_{SW} of 7.257 and 5.806 µg/L for application in cereals and winter oilseed rape, respectively), the risk from metabolite M13 is considered covered by the risk assessment for the active substance metconazole. For metabolite 1,2,4-triazole, the risk assessment is performed below.

In the acute risk assessment, the $RAC_{SW,ac}$ (RAC in surface water (SW) for adverse effects of pesticide exposure occurring within a relatively short period after exposure) is always compared with the $PEC_{SW,max}$ derived from the predicted exposure profile. In the chronic risk assessment, the $RAC_{SW,ch}$ (RAC in surface water for adverse effects of pesticide exposure that develop slowly and/or have a long-lasting course and that are caused by short- or long-term exposure) is in the first instance compared with the $PEC_{SW,max}$, and under certain conditions with a $PEC_{SW,twa}$. If the RAC exceeds the relevant PEC_{sw} value, the risk can be considered low. If the RAC is lower than the PEC_{sw} value, further consideration is necessary (e.g. by considering PEC_{sw} values from the next FOCUS Step, or by refining the endpoints used to derive the RAC).

In Table B.9.4.3.4-1 and, the RAC values for surface water for the different groups of aquatic organisms are compared to the FOCUS Step 1 to Step 2 values for 1,2,4-triazole for the proposed uses in winter and spring cereals and winter oilseed rape. As the RAC values for the three groups of aquatic organisms largely exceed the calculated PEC value, **the risk to aquatic organisms from exposure to 1,2,4-triazole following application of BAS 555 01 F according to the proposed uses in winter and spring cereals and oilseed rape is considered acceptable.**

Table B.9.4.3.4-1: Comparison of the relevant RAC values and the maximum PEC_{SW} for 1,2,4-triazole at FOCUS Steps 1 to 2 following application of BAS 555 01 F in winter and spring cereals (at 1-2 x 90 g a.s./ha).

Scenario	fish acute	fish chronic	Aquatic invertebrates	Algae
	Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Daphnia magna*	*Pseudokirchneriella subcapitata*
Level of assessment	Tier 1	Tier 1	Tier 1	Tier 1
RAC	6000 µg/L	320 µg/L	> 1000 µg/L	> 3100 µg/L
FOCUS Step 1 PEC values (µg/L)				
	1.056	1.056	1.056	1.056
FOCUS Step 2 PEC values (µg/L)				
North Europe	0.288	0.288	0.288	0.288
South Europe	0.288	0.288	0.288	0.288

Notes: PEC values in bold indicate that the $PEC_{SW/SED}$ exceeds the RAC, and thus that further consideration is necessary

Table B.9.4.3.4-2: Comparison of the relevant RAC values and the maximum PEC_{SW} for 1,2,4-triazole at FOCUS Steps 1 to 2 following application of BAS 555 01 F in winter oilseed rape (at 1-2 x 72 g a.s./ha).

Scenario	fish acute	fish chronic	Aquatic invertebrates	Algae
	Oncorhynchus mykiss	*Oncorhynchus mykiss*	*Daphnia magna*	*Pseudokircherniella subcapitata*
Level of assessment	Tier 1	Tier 1	Tier 1	Tier 1
RAC	6000 µg/L	320 µg/L	> 1000 µg/L	> 3100 µg/L
FOCUS Step 1 PEC values (µg/L)	0.845	0.845	0.845	0.845
FOCUS Step 2 PEC values (µg/L)				
North Europe	0.146	0.146	0.146	0.146
South Europe	0.146	0.146	0.146	0.146

Notes: PEC values in bold indicate that the $PEC_{SW/SED}$ exceeds the RAC, and thus that further consideration is necessary

B.9.4.4. Risk assessment for exposure via groundwater

In Volume 3 (PPP) Section B.8.2.4, the calculated 80th percentile average annual leachate concentrations of metconazole and its metabolite 1,2,4-triazole were below 0.1 µg L^{-1} in all tested scenarios. Therefore, it was concluded that the leaching of unacceptable amounts of substances to groundwater after application of metconazole to winter and spring cereals and winter oilseed rape is highly unlikely. As a consequence, a risk assessment for groundwater was not considered necessary.

B.9.5. EFFECTS ON ARTHROPODS

B.9.5.1. Effects on bees

B.9.5.1.1. Acute toxicity to bees

The representative formulation for Annex I renewal of metconazole (BAS 555 01 F) is different from the one used for the initial Annex I inclusion. Therefore, new acute oral and contact toxicity studies with BAS 555 01 F on honeybees have been submitted. A summary of the available studies is provided below.

Report:	**CP10.3.1.1/01. Schmitzer S. & Weber B. (2002) Effects of BAS 555 01 F (acute contact and oral) on honey bees (*Apis mellifera* L.) in the laboratory**
Report No.:	2002/1012936
Guidelines:	OECD 213, OECD 214, Recent recommendations of the ICPBR group (Avignon France 1999)
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and methods:	
Test substance:	BAS 555 01 F (Batch No.: R2066-079, contains 89.2 g/L of the active ingredient metconazole)
Test species:	honeybees (*Apis mellifera carnica* L.); worker bees
Number of organisms, age:	3 replicates containing 10 bees for each test concentration and the controls, bees approx.. 4-6 weeks old
Type of test:	acute oral and contact toxicity test (48 h)
Applied concentrations:	
Oral test:	nominal dosages: 5.0, 10.0, 20.0, 30.0 and 40.0 µg a.s./bee, resulting in an actual uptake of 5.9, 10.9, 22.7, 34.3 and 42.7 µg a.s./bee; water control (tap water/syrup), and reference item (Perfekthion EC, 400 g/L dimethoate; measured doses of 0.04, 0.09, 0.17 and 0.34 µg dimethoate/bee);
Contact test:	nominal dosages: 5.0, 10.0, 20.0, 30.0 and 40.0 µg a.s./bee; water control (CO2/tap water+Adhäsit), and reference item (Perfekthion EC, 400 g/L dimethoate; nominal doses of 0.10, 0.15, 0.20 and 0.30 µg dimethoate/bee)
Exposure route:	
Oral test:	20-24 mg of BAS 555 01 F contaminated food (test item solution and syrup were mixed together in a way that the final syrum solution was 50%) were offered in syringes which were weighed before and after introduction into the cages (duration of uptake did not exceed 2 hours)
Contact test:	one single 5 µL droplet of BAS 555 01 F in solvent (solvent = tap water + Adhäsit) placed on the ventral bee thorax using a Burkard applicator. For the controls one 5 µL droplet of tap water with 1% Adhäsit was used. A single 5µL droplet was chosen in deviation to the guideline

	recommendation of 1 µL, since a higher volume ensure a more reliable dispersion of the test item. Experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are expected.
Feeding:	commercial ready-to-use syrum (Apiinvert; 30% sacharose, 31% glucose, 39% fructose) *ad libitum*; was given directly after treatment in syringes; no replacements of the food during the exposure phase
Test conditions:	Temperature: 25°C Relative humidity: 52-66%. Lighting: bees were placed in darkness during exposure (except during observations)
Test procedure:	Assessment of bee mortality and behavioural effects were done after 4, 24 and 48 hours.
Statistics:	Descriptive statistics. Probit analysis for the calculation of the LD_{50} values.

Findings:

Oral toxicity test:

The results of the oral toxicity test are summarized in Table B.9.5.1.1-1. No mortality was observed after 48 hours in the control. In the test item treatment the mortality ranged between 16.7% and 83.3% after 48 hours and was in a dose related pattern. The LD_{50} was determined to be 17.8 µg a.s./bee. Behavioural impairments such as uncoordinated movements and apathy of isolated bees were observed during the experiment.

Table B.9.5.1.1-1: Toxicity of BAS 555 01 F to honeybees (*Apis mellifera*) in an oral toxicity test

Treatment [µg a.s./bee]	Uptake of test item [µg a.s./bee]	Mortality [%]		Corrected mortality [%]	
		24 h	48 h	24 h	48 h
Water/syrup control	--	0.0	0.0	--	--
40.0	42.7	83.3	83.3	83.3	83.3
30.0	34.3	53.3	56.7	53.3	56.7
20.0	22.7	56.7	56.7	56.7	56.7
10.0	10.9	43.3	43.3	43.3	43.3
5.0	5.9	16.7	16.7	16.7	16.7
		Endpoint [µg a.s./bee]			
LD_{50} (95% CL) [1)]		17.8 (13.4 - 23.6)			

[1)] *Calculated with Probit analysis, based on mortality data after 48 hours of exposure.*

Contact toxicity test:

The results of the contact toxicity test are summarized in Table B.9.5.1.1-2. No mortality was observed after 48 hours in the control. In the test item treatment the mortality ranged between 3.3% and 93.3% after 48 hours in a dose related pattern. Behavioral impairments such as uncoordinated movements and apathy of isolated bees were observed in this contact test.

Table B.9.5.1.1-2: Toxicity of BAS 555 01 F to honeybees (*Apis mellifera*) in a contact toxicity test

Treatment [µg a.s./bee]	Mortality [%]		Corrected mortality [%]	
	24 h	48 h	24 h	48 h
Control	0.0	0.0	--	--
40.0	83.3	93.3	83.3	93.3
30.0	63.3	73.3	63.3	73.3
20.0	20.0	53.3	20.0	53.3
10.0	3.3	6.7	3.3	6.7
5.0	3.3	3.3	3.3	3.3
	Endpoint [µg a.s./bee]			
LD_{50} (95% CL) [1)]	19.6 (16.9 - 22.7)			

[1)] *Calculated with Probit analysis, based on mortality data after 48 hours of exposure*

Conclusions:
In an acute toxicity test (48 h) with BAS 555 01 F on honeybees (*Apis mellifera* L), the acute oral LD_{50} value (48 h) was calculated to be 17.8 µg a.s./bee and the acute contact LD_{50} value (48 h) was calculated to be 19.6 µg a.s./bee.

RMS comments:
The validity criteria of OECD Test Guideline 213 and 214 are met:

- The average mortality for the total number of controls did not exceed 10% at the end of the test (measured: 0%)
- The LD_{50} of the toxic standard meets the specified range for dimethoate (0.10 – 0.35 µg/bee for the oral and 0.10 – 0.30 µg/bee for the contact test) (measured: 0.10 µg/bee for the oral test and 0.14 µg/bee for the contact test)

Therefore, this study is considered acceptable for use in the risk assessment.

The following endpoints will be used in the risk assessment:

$LD_{50, oral}$ (*Apis mellifera*, 48h) = 17.8 µg a.s./bee (corresponding to 208.7 µg product/bee)
$LD_{50, contact}$ (*Apis mellifera*, 48h) = 19.6 µg a.s./bee (corresponding to 229.8 µg product/bee)

Report:	**CP10.3.1.1/02. Kling A. (2007) Assessment of side effects of BAS 555 01 F to the honey bee, *Apis mellifera* L. in the laboratory**

Report No.: 2007/1050640
Guidelines: OECD 213, OECD 214
GLP: Yes

Previous evaluation: None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application

Materials and methods:

Test substance: BAS 555 01 F (Batch No.: 200001, contains 88.9 g/L of the active ingredient metconazole)
Test species: honeybees (*Apis mellifera carnica* L.); young adult worker bees
Number of organisms, age: 5 replicates containing 10 bees for each test concentration and the controls
Type of test: acute oral and contact toxicity test (48 h)

Applied concentrations:	
Oral test:	nominal dosages: 32.77, 65.54, 131.07, 262.16 and 524.31 µg product/bee (corresponding to 2.82, 5.64, 11.28, 22.56 and 45.11 µg a.s./bee), resulting in an actual uptake of 36.99, 70.15, 122.18, 244.83, and 455.44 µg product/bee (corresponding to 3.18, 6.04, 10.51, 21.07 and 39.19 µg a.s./bee); water control (50% (w/v) aqueous sucrose solution), and reference item (Perfekthion EC, 400 g/L dimethoate; nominal doses of 0.08, 0.10, 0.14, 0.21 µg dimethoate/bee);
Contact test:	nominal dosages: 32.77, 65.54, 131.07, 262.16 and 524.31 µg product/bee (corresponding to 2.82, 5.64, 11.28, 22.56 and 45.11 µg a.s./bee); water control (tap water), and reference item (Perfekthion EC, 400 g/L dimethoate; nominal doses of 0.10, 0.14, 0.20 and 0.30 µg dimethoate/bee)
Exposure route:	
Oral test:	A quantity of 250 µL of sugar solution (control), test item and reference item solution was offered to each cage of 10 bees. After a period of about 6 hours the test item solutions were taken up by the bees and the feeders were replaced. The amount of test item solution consumed (mean value of 10 bees) was determined by weighing the feeders before and after feeding.
Contact test:	BAS555 01 F was dissolved and diluted in tap water. Bees were anaesthetised with CO2. 2 µL of tap water (control), 2 µL of test item or reference item solution were applied to the dorsal side of the thorax of each bee.
Feeding:	50% aqueous sucrose solution *ad libitum*
Test conditions:	Temperature and relative humidity were not correctly measured due to a defect thermohygrograph. The test was however performed in a climate control unit adjusted to a temperature of 25°C and humidity of 50-70%. As no alarm signal was given by the climate chamber during the test, it can be assumed that these values were maintained.
	Lighting: bees were placed in darkness during exposure (except during observations)
Test procedure:	Assessment of bee mortality and behavioural effects were done after 4, 24 and 48 hours.
Statistics:	Descriptive statistics. Probit analysis for the calculation of the LD_{50} values.

Findings:

<u>Oral toxicity test:</u>

The results of the oral toxicity test are summarized in Table B.9.5.1.1-3. After 48 hours of oral exposure, no mortality was observed in the control. In the test item treatment groups, consumption of 10.51, 21.07 and 39.19 µg a.s./bee resulted in 6.0, 16.0 and 20.0% mortality, respectively. No behavioral abnormalities were observed after 48 hours. After 48 hours, the LD_{50} for the reference item was calculated to be 0.15 µg dimethoate/bee.

Table B.9.5.1.1-3: Toxicity of BAS 555 01 F to *Apis mellifera* L. (honeybee) in an oral toxicity test

Treatment [µg BAS 555 01 F/bee]	Actual uptake of BAS 555 01 F [µg BAS 555 01 F/bee]	Treatment [µg a.s./bee]	Actual uptake of metconazole [µg a.s./bee]	Mean mortality [%]	
				24 h	48 h
Control	--	--	--	0.0	0.0
32.77	36.99	2.82	3.18	0.0	0.0
65.54	70.15	5.64	6.04	0.0	0.0
131.07	122.18	11.28	10.51	6.0	6.0
262.16	244.83	22.56	21.07	16.0	16.0
524.31	455.44	45.11	39.19	20.0	20.0
Endpoint					
LD_{50} (48 h) [1)]	**µg BAS 555 01 F/bee**		**µg a.s./bee**		
	> 455.44		> 39.19		

[1)] *Calculated with Probit analysis, based on mortality data after 48 hours of exposure*

Contact toxicity test:

The results of the oral toxicity test are summarized in Table B.9.5.1.1-4. After 48 hours of contact exposure, 6.0% mortality was observed in the water control. In the test item treatment groups, 16.0% and 66.0% mortality (10.6% and 63.8% corrected mortality) was observed after contact exposure to 262.16 and 524.31 µg BAS 555 01 F/bee, respectively. At the highest two dose levels at the assessment 4 hours after application some bees were recorded as affected, moribund and with coordination problems. It can however be assumed that these bees died until the final assessment. No remarkable behavioural abnormalities were observed at the dose levels of 32.77, 65.54 and 131.07 µg BAS 555 01 F/bee. After 48 hours, the LD_{50} for the reference item was calculated to be 0.22 µg dimethoate/bee.

Table B.9.5.1.1-4: Toxicity of BAS 555 01 F to *Apis mellifera* L. (honeybee) in a contact toxicity test

Treatment	BAS 555 01 F	Mean mortality [%]		Corrected mortality [1)]	
[µg a.s./bee]	[µg/bee]	24 h	48 h	24 h	48 h
Control	Control	6.0	6.0	--	--
36.99	32.77	0.0	0.0	-6.4	-6.4
70.15	65.54	0.0	0.0	-6.4	-6.4
122.18	131.07	0.0	0.0	-6.4	-6.4
244.83	262.16	12.0	16.0	6.4	10.6
455.44	524.31	60.0	66.0	57.4	63.8
Endpoint (nominal)					
LD_{50} (48 h) (95% CL) [2)]	**µg a.s./bee**		**µg BAS 555 01 F/bee**		
	38.24 (33.22 – 44.36)		444.46 (386.07 – 515.52)		

[1)] *Corrected mortality (according to Schneider-Orelli 1947);* [2)] *Calculated with Probit analysis, based on mortality data after 48 hours of exposure.*

Conclusions:

In an acute toxicity test with BAS 555 01 F on honeybees, the oral LD_{50} value (48 h) was determined to be > 455.44 µg BAS 555 01 F/bee (equivalent to > 39.19 µg a.s./bee) and the contact LD_{50} value (48 h) was determined to be 444.46 µg BAS 555 01 F/bee (equivalent to 38.24 µg a.s./bee).

RMS comments:

The validity criteria of OECD Test Guideline 213 and 214 are met:

- The average mortality for the total number of controls did not exceed 10% at the end of the test (measured: 0% in the oral test and 6% in the contact test)

- The LD_{50} of the toxic standard meets the specified range for dimethoate (0.10 – 0.35 µg/bee for the oral and 0.10 – 0.30 µg/bee for the contact test) (measured: 0.15 µg/bee for the oral test and 0.22 µg/bee for the contact test)

Therefore, this study is considered acceptable for use in the risk assessment.

The following endpoints will be used in the risk assessment:

$LD_{50, oral}$ (*Apis mellifera*, 48h) > 455.44 µg product/bee (corresponding to > 39.19 µg a.s./bee)

$LD_{50, contact}$ (*Apis mellifera*, 48h) = 444.46 µg product/bee (corresponding to 38.24 µg a.s./bee)

B.9.5.1.2. Chronic toxicity to bees

The acute toxicity of metconazole and BAS 555 01 F are shown to be comparable (less than a factor of 5 difference), and so toxicity of metconazole is not considered to be affected by formulation. Therefore the chronic toxicity of BAS 555 01 F is considered to be appropriately represented by the chronic toxicity of metconazole (see Volume 3 (AS), Section B.9.3.1.2) and so chronic studies with the product are not required.

B.9.5.1.3. Effects on honeybee development and other honeybee life stages

The acute toxicity of metconazole and BAS 555 01 F are shown to be comparable (less than a factor of 5 difference), and so toxicity of metconazole is not considered to be affected by formulation. Therefore the effects on honeybee development of BAS 555 01 F is considered to be appropriately represented by the effects on honeybee development of metconazole (see Volume 3 (AS), Section B.9.3.1.3) and so studies on the effects on honeybee development and other honeybee life stages with the product are not required.

B.9.5.1.4. Sub-lethal effects

Further tests investigating sub-lethal effects, such as behavioural and reproductive effects, on bee colonies are not required since the risk assessments based on acute and chronic data and on semi-field studies indicate an acceptable risk to bees, and there are no indications of sub-lethal effects based on either the mode of action or existing tests.

B.9.5.1.5. Cage and tunnel tests

No cage and tunnel tests with the representative formulation BAS 555 01 F are available. Instead, a new semi-field study with the formulation BAS 555 00 F (an EC formulation containing 60 g/L of the active substance metconazole) has been submitted. Although performed with another formulation, this semi-field study is considered representative for the risk assessment for BAS 555 01 F (please refer to Section B.9.6.1.2 for a detailed argumentation regarding the representativeness of this study). This study is summarized below.

Report:	**CP10.3.1.5/01. Franke M. (2013)** **Effects of BAS 555 00 F on the honeybee Apis mellifera L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development**
Report No.:	2012/1111494
Guidelines:	EPPO PP 1/170 (4) (2010), OECD 75 (2007), current recommendations of the German AG Bienenschutz (2011)
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application

Objective:

The purpose of this study was to determine the potential effects of BAS 555 00 F on honeybees (*Apis mellifera*) after application on flowering *Phacelia tanacetifolia* under semi-field conditions. Observations included mortality, foraging activity, behaviour, general and detailed brood development of the bee colonies before and after application.

Materials and methods:

Test substance:	BAS 555 00 F (Batch No.: 0003255328, contains 60.1 g/L of the active ingredient metconazole)
Test species:	honeybees (*Apis mellifera carnica* P.), small healthy and queen-right colonies from a commercial beekeeper in Leipzig/Rehbach, Germany, maintained according to normal beekeeping practice. Each colony consisted of 11 combs, with 4-8 brood combs with all brood stages present and 6-10 combs with food.
Number of organisms:	one colony per tunnel and four tunnels (replicates) per treatment group
Type of test:	Honeybee semi-field test (tunnel test) in *Phacelia tanacetifolia*
Test plots:	The test site was located in Cunnersdorf, Germany; The plots were placed on meadow land not in use for agricultural production during the past 5 years. No other pesticides besides the test item were applied for at least 3 years before and after sowing of *Phacelia*. Tunnel size: 18 m length x 6 m width x 2.5 m height. Effective crop area of 93.5 m^2.
Test plants:	*Phacelia tanacetifolia*, at BBCH stage 63 (30% of flowers open) for all treatments at the time of placing the bees in the tunnels
Applied concentrations:	Control (tap water); 1500 mL BAS 555 00 F (corresponding to 90 g a.s./ha); The reference item (Insegar 25 WG, containing 250 g fenoxycarb/kg) was applied at a rate of 1200 g product/ha (equivalent to 300 g a.s./ha); All substances were applied with a volume of 400 L water/ha during daily bee flight.
Test conditions:	Natural field conditions. Weather conditions were good during application: sunny (20 – 50% clouds), temperature 23.4°C – 24.3°C, slight wind (0.3 – 0.5 m/s) and no precipitation. The weather was variable with slight rain on DAT 1 (17.0 mm), DAT 2 (22.5 mm), DAT 5 (3.0 mm) and DAT 6 (3.5 mm) but warm for the remaining exposure period.

Test design:
Four days before application bees were introduced into the tunnels. At the time of application, the *Phacelia* was at BBCH crop stage 63-65 (full flowering). Applications were carried out during bee-flight activity. Dead bee traps were attached to the entrance of the hives and linen sheets were spread on the ground for mortality assessment in the crop.
Post-application exposure period was 7 days (the hives were removed out of the tunnels in the early morning of DAT 8). After the exposure phase the colonies were removed from the tunnels and placed to a non-treated monitoring site, without flowering main crops or intensive agriculture nearby and the further brood development was assessed until DAT (day after application) 28.
Assessments of mortality (of adult bees and pupae) and bee behaviour were carried out 3 days before application, on the day of application and on the following 28 days after application. Foraging activity of the bees was assessed 3 days before application, on the day of application and on the following 7 days after application. Additionally, the condition of the colonies (food stores, brood status and colony strength) was assessed on DAT -1, DAT 4, DAT 9, DAT 14, DAT 21 and DAT 27 equal to BFD 0 (**b**rood **f**ixing **d**ay, marking of eggs for further detailed brood assessment), BFD 5, BFD 10, BFD 15, BFD 22 and BFD 28. Detailed brood status assessments were carried out on DAT -1, 4, 9, 14 and 21 equal to BFD 0, BFD 5, BFD 10, BFD 15 and BFD 22.

Statistics: Descriptive statistics. Pre-treatment data: Tukey-test ($\alpha = 0.05$); post-treatment data: Student-t test ($\alpha = 0.05$) or Welch-t test (mortality and brood termination rate/brood indices: one-sided greater, $\alpha = 0.05$).

Findings:
Mortality
Adult honeybees:
The results for adult mortality are summarized in Table B.9.5.1.5-1. The mortality of the honeybees on the days before application was on a low and similar level in the control, test item and reference item treatments, indicating comparable and well adapted colonies. No statistically significant differences were observed between the three treatment groups during pre-exposure, DAT -3 to DAT 0 nor at overall comparison before application (Tukey-test, two sided, $\alpha = 0.05$).

Neither on the day of application nor on any other day was an increased mortality in the test item treatment compared to the control observed. The overall daily mean mortality during the exposure and post-exposure period in the test item treatment was on a comparable level with the control treatment, 11.4 and 11.1 dead bees/colony/day, respectively. No statistically significant effects on mortality were observed in the test item treatment group compared to the control neither directly after application during the exposure between DAT 0 and DAT 7, nor during post-exposure from DAT 7 to DAT 28, nor at the overall comparisons (Student-t test or Welch-t test, one-sided greater, $\alpha = 0.05$).

The exposure of the honeybees to the reference item did not result in an increased number of dead adult bees.

Table B.9.5.1.5-1: Summary of adult honeybee mortality after application of BAS 555 00 F during daily bee flight under semi-field conditions

Assessment day	Mortality [no. of dead adult honeybees]					
	Control		BAS 555 00 F		Reference item	
	Mean [1)]	± SD	Mean [1)]	± SD	Mean [1)]	± SD
DAT -3	6.0	4.9	6.8	1.9	10.8	0.5
DAT -2	19.5	8.3	18.3	3.9	20.0	7.3
DAT -1	18.5	10.6	17.5	7.5	24.5	5.8
DAT $0_{b\,a}$	11.8	8.4	10.5	4.4	7.5	3.4
Daily mean DAT -3 to $0_{b.a.}$	**13.9**	**9.3**	**13.3**	**6.6**	**15.7**	**8.3**
DAT $0_{a\,a}$ + 2h	3.3	1.5	3.0	3.2	3.8	1.7
DAT $0_{a\,a}$ + 6h	2.3	1.2	5.0	4.1	2.8	2.9
Sum DAT $0_{a.a.}$	**5.5**	**2.4**	**8.0**	**7.1**	**6.5**	**4.5**
DAT 1 morning	12.3	5.3	22.0	7.4	13.8	5.6
DAT 1 midday	4.5	1.9	5.3	2.2	6.3	1.0
DAT 1 afternoon	4.0	2.8	2.8	1.3	3.5	2.6
Sum DAT 1	**20.8**	**8.3**	**30.0**	**6.4**	**23.5**	**5.4**
DAT 2	20.5	5.7	22.3	8.7	24.8	5.1
DAT 3	21.8	7.5	19.8	1.7	27.5	10.3
DAT 4	20.8	12.9	19.3	6.9	18.3	7.7
DAT 5	15.8	6.9	15.0	2.2	16.8	4.6
DAT 6	14.3	7.8	13.0	2.9	18.0	5.3
DAT 7	8.8	1.9	14.0	5.7	10.8	2.8
Daily mean DAT $0_{a.a.}$ to 7	**16.0**	**8.8**	**17.7**	**8.1**	**18.3**	**8.6**
Daily mean DAT 8 to 28	**9.6**	**6.6**	**8.6**	**6.1**	**8.6**	**6.6**
Daily mean DAT $0_{a.a.}$ to 28	**11.4**	**7.8**	**11.1**	**7.9**	**11.3**	**8.4**

[1)] Mean of four replicates (tunnels); b.a.= before application, a.a. = after application, DAT = days after treatment.

Honeybee larvae/pupae:

The results for larval/pupal mortality are summarized in Table B.9.5.1.5-2. No increased mortality of honeybee larvae or pupae was observed on the days before application in the control, test item and reference item treatments, indicating comparable, healthy and well adapted colonies. On DAT 0 after application (DAT $0_{a\,a}$), and between DAT $0_{a\,a}$ and DAT 28, no relevant numbers of dead larvae or pupae were found in the control and in the test item treatment, respectively. In the reference item, a distinctly increased number of pupae were found dead between DAT 8 and DAT 19.

Table B.9.5.1.5-2: Summary of pupal mortality after application of BAS 555 00 F during daily bee flight under semi-field conditions

Mortality of pupae/colony/day during:	Mean mortality (± SD) per treatment group [1)]					
	Control		BAS 555 00 F		Reference item	
	mean	± SD	mean	± SD	mean	± SD
pre-application phase	0.0	0.0	0.2	0.5	0.1	0.3
exposure phase in the tunnels	0.1	0.2	0.1	0.6	1.3	2.3
phase outside the tunnels [2)]	0.3	1.2	0.0	0.1	16.0	22.9
overall after application	0.2	1.0	0.0	0.3	11.9	20.6

[1)] Mean of four replicates (tunnels); [2)] Dead bees found in dead bee traps, only.

Foraging activity:

The results for foraging activity are summarized in Table B.9.5.1.5-3. The overall mean foraging activity during the assessment time prior to application was on a high and comparable level, with 8.4, 8.5 and 8.7 bees/m²/day in the control, the test item and the reference item treatment, respectively, indicating that the bees had adapted to the new environmental conditions. Shortly before application, the foraging activity was 10 – 13 (mean of 11.5 bees/m²), 10 – 15 (mean of 12 bees/m²) and 8 – 14 bees/m² (mean of 11.3 bees/m²) in the control, the test item and in the reference item treatment groups, respectively. Thus, the bees were sufficiently exposed during the application.

Neither on the day of application nor on all following assessment days was the foraging activity in the test item and reference item treatment reduced compared to the control group. Furthermore, it was on the same level compared to the control and pre-application assessment. Consequently, the overall daily mean foraging activity was very similar in the control (10.1 bees/m²/day) and the test item treatment group (10.8 bees/m²/day); this proved to be not statistically significantly different (Student-t test, one-sided smaller, $\alpha = 0.05$). Similarly, the reference item saw no statistically significant decrease in foraging activity when compared to the control, with an overall daily mean foraging activity of 10.5 bees/m²/day (Student-t test, one-sided smaller, $\alpha = 0.05$). Due to rainfall on DAT 1 and DAT 2 (17.0 mm and 22.5 mm, respectively), flight activity was reduced in all treatment groups, but was fully recovered by DAT 3; thereafter, flight activity remained at a pre-application level.

Table B.9.5.1.5-3: Foraging activity after application of BAS 555 00 F during daily bee flight under semi-field conditions

Assessment day	Foraging activity [honeybees/m²]					
	Control		BAS 555 00 F		Reference item	
	Mean [1)]	± SD	Mean [1)]	± SD	Mean [1)]	± SD
DAT -3	6.3	0.8	6.3	1.6	6.8	1.8
DAT -2	5.5	0.8	5.2	1.1	6.0	1.7
DAT -1	10.4	1.5	10.5	1.8	10.8	2.2
DAT $0_{b\,a}$	11.5	0.9	12.0	2.0	11.3	2.3
Daily mean DAT -3 to $0_{b.a.}$	**8.4**	**0.7**	**8.5**	**1.6**	**8.7**	**1.9**
DAT $0_{a\,a}$ + 2h	10.8	0.6	12.3	2.2	12.4	2.9
DAT $0_{a\,a}$ + 6h	10.6	1.5	10.6	0.4	10.2	1.0
Mean DAT $0_{a.a.}$	**12.0**	**0.2**	**12.2**	**1.6**	**11.8**	**2.0**
DAT 1 morning	3.6	0.3	3.5	1.0	4.1	1.7
DAT 1 midday	4.5	1.4	4.6	1.1	4.8	1.9
DAT 1 afternoon	5.3	1.1	5.1	1.2	5.2	1.8
Mean DAT 1	**4.4**	**0.9**	**4.4**	**1.1**	**4.7**	**1.7**
DAT 2	4.6	1.3	4.9	1.0	4.8	1.4
DAT 3	13.3	1.1	14.5	1.9	13.7	2.8
DAT 4	12.9	1.0	13.2	1.2	13.3	2.0
DAT 5	11.3	0.7	12.4	1.3	12.1	1.3
DAT 6	11.0	0.5	12.3	1.1	11.7	1.4
DAT 7	11.0	0.7	12.4	1.4	11.8	1.1
Daily mean DAT $0_{a.a.}$ to 7	**10.1**	**0.5**	**10.8**	**1.0**	**10.5**	**1.0**

[1)] Mean of four replicates (tunnels); b.a. = before application, a.a. = after application, DAT = days after treatment.

Bee behaviour:
The exposure of honeybees to BAS 555 00 F did not cause behavioural abnormalities, e.g. intoxication symptoms or different behaviour when compared to the behaviour in the control group. The bees were calm and actively foraging nectar and pollen on the treated *Phacelia tanacetifolia* field.

Colony strength:
The results for colony strength are shown in Table B.9.5.1.5-4. During colony assessment conducted one day before application (DAT -1), the estimated average number of bees per colony was similar in the control, test item and reference item treatment groups, respectively. During the post-application period, there was a similar development of the colony strength in the control and the test item treatment group, both increasing by +78% and +83%, respectively, if compared to DAT -1. The colony strength of the reference item treatment group was slightly lower in comparison to the control, where the average number of bees/colony increased by +54% in comparison to DAT -1.

Table B.9.5.1.5-4: Summary of honeybee colony strength after application of BAS 555 00 F during daily bee flight under semi-field conditions

Assessment day	Colony strength [estimated average number of bees/colony]								
	Control			BAS 555 00 F			Reference item		
	Mean [1)]	± SD	% [2)]	Mean [1)]	± SD	% [2)]	Mean [1)]	± SD	% [2)]
DAT -1 (BFD 0)	4894	268	--	5006	195	--	4275	331	--
DAT 4 (BFD 5)	5091	626	+4	5709	310	+14	5063	932	+18
DAT 9 (BFD 10)	5850	431	+20	6581	1330	+31	6497	1274	+52
DAT 14 (BFD 15)	6328	498	+29	7791	515	+56	6413	1296	+50
DAT 21 (BFD 22)	6581	613	+34	7116	576	+42	5231	854	+22
DAT 27 (BFD 28)	8691	665	+78	9169	490	+83	6581	726	+54

[1)] Mean of four replicates (tunnels); [2)] Relative change in comparison to DAT -1 calculated from the respective mean values; DAT = days after treatment; BFD = brood area fixing day.

General brood assessments – Brood area
On DAT -1, honeybee queens were healthy, actively laying eggs and the brood amount was generally on a comparable level in all test colonies. Overall, the total mean brood nest area (sum of comb area occupied by eggs, larvae and capped cells) in the control and in the test item treatment group increased during the course of the study in a comparable way. Regarding the mean comb area of a respective brood stage, the test item colonies contained eggs, larvae or pupae on a similar level compared with the control during the study period.
The total mean brood nest size in the reference item group was on a lower level on the assessments performed between DAT 4 and DAT 27, in comparison to the control. The effect of the reference item on brood development showed that the test system was sensitive to detect possible effects on brood development.

Detailed brood assessments in marked cells
Brood termination rate (%):
In the course of the study the mean brood termination rates in test item treatment were lower if compared to the control and not statistically significantly different from the control at any assessment day (STUDENT-t test, one sided greater, $\alpha = 0.05$). 22 days after BFD, the mean brood termination rate was

23.9% and 14.9% in the control and the test item treatment, respectively. Thus, no test item related effect on the brood development was detected.

Overall, the mean brood termination rates in the test item treatment was lower than the control and not statistically significantly different from the control at any assessment day (Student-t test, one-sided greater, $\alpha = 0.05$). The high termination rate of 65.6% in the reference item indicated the suitability of the test system to detect potential effects of the test item on brood development.

Table B.9.5.1.5-5: Summary of honeybee brood termination rates

Assessment day	Mean brood termination rate [%]					
	Control		BAS 555 00 F		Reference item	
	Mean [1)]	± SD	Mean [1)]	± SD	Mean [1)]	± SD
BFD 5	18.3	20.5	10.9	6.8	56.7	45.7
BFD 10	22.3	24.5	13.1	6.3	64.7	42.4
BFD 15	23.9	25.0	14.7	8.2	65.6	41.1
BFD 22	23.9	25.1	14.9	8.2	65.6	41.1

[1)] Mean of four replicates; BFD = Brood fixing day.

Brood index:
During the study and according to the termination rates, the brood indices in the test item group were slightly higher than the control values, indicating a successful and similar development of initially marked eggs. No statistically significant differences were observed at any assessment day (Student-t test, one-sided smaller, $\alpha = 0.05$). In the reference item treatment, an effect of decreased brood indices was observed during the course of the study, which was not statistically significantly different from the control (Student-t test, one-sided smaller, $\alpha = 0.05$).

Table B.9.5.1.5-6: Summary of brood indices

Assessment day	Mean brood index [n]					
	Control		Test item		Reference item	
	Mean [1)]	± SD	Mean [1)]	± SD	Mean [1)]	± SD
BFD 0	1.0	0.0	1.0	0.0	1.0	0.0
BFD 5	2.4	0.6	2.7	0.2	1.3	1.4
BFD 10	3.1	1.0	3.5	0.3	1.4	1.7
BFD 15	3.0	1.0	3.4	0.3	1.4	1.6
BFD 22	3.8	1.3	4.3	0.4	1.7	2.1

[1)] Mean of four replicates; BFD = Brood fixing day.

Brood compensation index:
The brood compensation index showed a continuous brood development in both the test item and in the control group. Similar to the brood indices, the brood compensation indices were very similar to the control values and slightly higher than the brood indices, indicating that the brood stage losses were at least partially compensated by the queen laying new eggs in emptied cells.
Despite a great loss of brood stages in the reference item group between BFD 0 and BFD 10, several of the emptied cells were refilled with eggs resulting in higher brood compensation indices but which were still lower than the corresponding indices of the control and test item on these assessment days.

Table B.9.5.1.5-7: Summary of brood-compensation indices

Assessment day	Mean brood compensation index [n]					
	Control		Test item		Reference item	
	Mean [1)]	± SD	Mean [1)]	± SD	Mean [1)]	± SD
BFD 0	1.0	0.0	1.0	0.0	1.0	0.0
BFD 5	2.4	0.6	2.7	0.2	1.3	1.3
BFD 10	3.2	0.8	3.5	0.2	1.6	1.6
BFD 16	3.3	0.7	3.5	0.3	2.1	1.5
BFD 22	4.2	0.8	4.4	0.3	3.4	1.0

[1)] *Mean of four replicates; BFD = Brood fixing day.*

Conclusions:
BAS 555 00 F was applied in a single application at a rate of 1500 mL product/ha (equivalent to 90 g metconazole/ha) to flowering *Phacelia tanacetifolia* under semi-field conditions during active honeybee foraging conditions. Following the application, no adverse test item related effects on honeybee mortality, foraging activity, behaviour, colony development, and colony strength or bee brood development were observed. Overall, based on the results of this study it can be concluded, that BAS 555 00 F applied at a rate of 1500 mL product/ha will not adversely affect honeybees or honeybee colonies.

RMS comments:
This study was performed according to the recommendations of OECD Test Guideline 75 and EPPO PP 1/170. The mortality in the control was consistently low, indicating that the colonies were healthy and adapted to the tunnel conditions. Further, there was an effect of the reference item on honeybee brood mortality, indicating that the test system was sensitive to detect possible effects on brood development. Overall, this study is considered acceptable for use in the risk assessment.

B.9.5.1.6. Field tests with honeybees

Field tests with honeybees are not required since the risk assessments based on acute and chronic data and on semi-field studies indicate an acceptable risk to bees.

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

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

The representative formulation for Annex I renewal of metconazole (BAS 555 01 F) is different from the one used for the initial Annex I inclusion. Therefore, new standard Tier 1 laboratory studies with the standard non-target arthropod species *Typhlodromus pyri* and *Aphidius rhopalosiphi* have been submitted. In addition, new standard laboratory studies with two additional species, *Chrysoperla carnea* and *Aleochara bilineata* have been submitted. All newly submitted studies were performed with the representative formulation BAS 555 01 F. A summary of these studies is provided below.

Report:	**CP10.3.2.1/01. Schwiening S. & Buetzler R. (2002) Effects of BAS 555 01 F on the predatory mite *Typhlodromus pyri* in the laboratory - Dose response test**
Report No.:	2002/1012750
Guidelines:	Blümel *et al.* (2000)[26]
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and Methods:	
Test substance:	BAS 555 01 F (Batch No.: R2066-079, contains 89.2 g/L of the active ingredient metconazole)
Test species:	*Typhlodromus pyri* (predatory mite), protonymphs, less than 24h old
Number of organisms:	3 replicate arenas for the test item treatments, the reference item treatment and the control; each with 20 protonymphs per replicate
Food:	a mixture of pine (*Pinus nigra*) and birch (*Betula sp.*) pollen (3:1) *ad libitum*
Type of test:	laboratory test (exposure to fresh residues on glass plates)
Applied concentrations:	water control (purified water), 31, 56, 100, 180 and 324 mL product/ha (equivalent to 2.77, 5.00, 8.92, 16.06 and 28.90 g a.s./ha). Applications were made in a volume equivalent to 200 L/ha.
	Positive control: BAS 152 11 I (400 g/L dimethoate), applied at a rate of 6 mL/ha, in the equivalent of 200 L/ha.
Test conditions:	temperature: 24-26°C relative humidity: 60-91% light regime: 16:8 hours light:dark, 635-795 lux

Study design and Methods:

The test arenas were similar to those used for the 'open method' described by Blümel *et al.* (2000). They comprised glass plates formed from two microscope slide cover slips (each 24 mm x 60 mm in area), fixed by gluing small cover slides (20 mm x 20 mm) to both side-ends. The main cover slips were fixed

[26] Blümel S., *et al.* (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products. In: Candolfi, M.P., *et al.* (eds) Guidelines to evaluate the side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/WPRS. Pub.

so that they were touching, but there was effectively a narrow channel between them. When the plate was laid on water-saturated tissue paper, water was drawn into this channel by means of capillary action. The treatment solution was applied and allowed to dry. A thin line of sticky insect trap glue was drawn around the glass plate to provide a barrier to prevent mites leaving the arena. The plates were placed on wet tissue paper laid over a water-saturated synthetic foam block. Mites were transferred to the test arenas using a fine brush. Mortality was assessed at day 2 and day 7 after test initiation. (DAT). Further three assessments were carried out with a maximum interval of 3 days up to day 14. At each assessment, the number of mites in each replicate were recorded as alive, dead (no sign of movement) or escaped. The number of eggs laid and the number of live and dead juvenile stages per female were counted at day 7, day 10, day 13 and day 14 after test initiation in the control and all treatment groups with a 7-day corrected mortality < 50%.

A Probit regression analysis was performed on the 7-day mortality data in order to determine the LR_{50} for the test item. The 7-day mortality data were analyzed using Dunnett's Test, reproduction data was analyzed using a Bonferroni t-test ($\alpha = 0.05$).

Findings:
The results are summarized in Table B.9.5.2.1-1. After 7 days of exposure, the test item produced dose-related mortality rates of between 10% and 100%. The mean mortality in the control amounted to 16.7%. Based on this, corrected mortalities for the different dose rates were calculated to be between -8.0% and 100%. Statistically significant differences compared to the control were observed in test item concentrations of 100 mL/ha and higher (Dunnett test, $\alpha = 0.05$). The LR_{50} value was determined to be 95 mL/ha BAS 555 01 F (95% CL = 70 - 130 mL/ha BAS 555 01 F). The reference item produced 54.0% corrected mortality of exposed mites after 7 days.

The reproductive capacity was assessed in the second week of the test for the control and the 31 mL/ha and 56 mL/ha treatment group. 12.7 eggs/female were produced in the control as well as 10.8 and 10.4 in the treatment groups. Thus, an effect on reproduction of 15.0% and 18.1% could be calculated, which was not statistically significantly different from the control (Bonferroni t-test, $\alpha = 0.05$).

Table B.9.5.2.1-1: Effects on predatory mites (*Typhlodromus pyri*) exposed to BAS 555 01 F in a laboratory trial

Treatment	Rate [1] [mL/ha]	Mortality [2] [%]	Corrected mortality [3] [%]	Reproduction [4] [eggs/ female]	Effects on reproduction [%]
Control	--	16.7	--	12.7	--
BAS 555 01 F	31	10.0	-8.0	10.8	15.0
BAS 555 01 F	56	41.7	30.0	10.4	18.1
BAS 555 01 F	100	66.7 *	60.0	--	--
BAS 555 01 F	180	71.7 *	66.0	--	--
BAS 555 01 F	324	100.0 *	100.0	--	--
Endpoints [mL BAS 555 01 F/ha]					
LR_{50} (95% CL)[5]	95 (70 - 130)				

[1] *Application rate in 200 L water/ha.*
[2] *Mortality after 7 days of exposure to BAS 555 01 F on glass surface.*
[3] *Corrected mortality according to Schneider-Orelli (1947). Negative values indicate a higher survival than in the control treatment.*
[4] *Reproduction: mean number of eggs per female from day 7 to day 14 determined at 4 assessments.*
[5] *Median lethal rate calculated by probit analysis (with 95% Confidence Limits).*
**= statistically significant differences compared to the control (Dunnett test, $\alpha = 0.05$).*

Conclusions:
The LR_{50} obtained under worst-case laboratory conditions for BAS 555 01 F on *Typhlodromus pyri* was 95 mL/ha BAS 555 01 F in 200 L water/ha. The reproduction of *T. pyri* was not affected up to an application rate of 56 mL/ha BAS 555 01 F in 200 mL water/ha.

RMS comments:
The validity criteria of the test guideline are met:

- The mortality rate in the control did not exceed 20% on day 7 (measured: 16.7%)
- The cumulative mean number of eggs per female in the control was ≥ 4 eggs/female (measured: 12.7 eggs/female)
- The cumulative mean mortality (control corrected) of protonymphs on day 7 exposed to the reference substance was ≥ 50% (measured: 54.0%)

It is noted that the reference item was only applied at a rate of 6 mL/ha, while the test guideline recommends an application rate between 9 and 15 mL/ha. However, as the validity criterion for the reference item is met, this deviation is considered only minor. It is further noted that only 3 replicates of 20 protonymphs were used per treatment in the test, while the test guideline recommends a minimum of 5 replicates (i.e. 100 nymphs). However, as the other validity criteria are met, this is not considered to invalidate the study results. Consequently, this study is considered acceptable for use in the risk assessment.

Endpoints for mortality:
LR_{50} (*Typhlodromus pyri*, 7 days) = 95 mL product/ha (equivalent to 8.47 g a.s./ha)
NOER (*Typhlodromus pyri*, 7 days) = 56 mL product/ha (equivalent to 5.0 g a.s./ha)

Endpoints for reproduction:
NOER (*Typhlodromus pyri*, 7 days) = 56 mL product/ha (equivalent to 5.0 g a.s./ha)

Report:	**CP10.3.2.1/02. Drexler A. (2002)** **Effects of BAS 555 01 F on the parasitoid *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) in a laboratory trial - Dose-response**
Report No.:	2008/1010707
Guidelines:	Mead-Briggs *et al.* (2000)[27]
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and Methods:	
Test substance:	BAS 555 01 F (Batch No.: R2066-079, contains 89.2 g/L of the active ingredient metconazole)
Test species:	*Aphidius rhopalosiphi* (parasitic wasp), adults, less than 48h old
Number of organisms:	3 replicates/treatment, each with 10 adult wasps (with a minimum of 5 females) per replicate
Food:	1:3 solution of honey in water
Type of test:	laboratory test (exposure to fresh residues on glass plates)
Applied concentrations:	water control (deionized water), 70, 170, 420, 1000 and 2400 mL product/ha (equivalent to 6.2, 15.2, 37.5, 89.2 and 214.1 g a.s./ha). Applications were made in a volume equivalent to 200 L/ha. Positive control: BAS 152 11 I (400 g/L dimethoate), applied at a rate of 0.25 mL/ha, in the equivalent of 200 L/ha.
Test conditions:	temperature: 18.6-22.7°C relative humidity: 54.7-79.6% light regime: 16:8 hours light:dark, 766-1198 lux during exposure and 3290-5020 lux during reproduction assessment.

Study design and Methods:

The bioassay was based on the laboratory test guideline of Mead-Briggs *et al.* (2000). For the mortality assessment, test units comprised of two glass plates (approximately 10 x 10 cm) fitted with the treated surface inwards onto an square metal frame. Four holes (10 mm in diameter) drilled through each of the side walls of the frame provided ventilation and were covered with fine-gauge stainless steel mesh. One hole was left uncovered as an access hole for the introduction of the parasitoids and was sealed with a cotton wool bung. To prevent build-up of any pesticide vapours and to maintain the environmental conditions in the arenas, air was forced through the units using a small pump linked with plastic tubing to one of the holes in the wall. Observations were made at approximately 2, 24 and 48 h after introduction of the adult wasps in the test units. Numbers of wasps were recorded as alive, affected, moribund and dead. At 48 h, any *moribund* wasps were included in with the *dead* insects for calculations of the percentage mortality.

[27] Mead-Briggs, M.A. *et al.* (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, Aphidius rhopalosiphi (DeStefani-Perez) (Hymenoptera: Braconidae). In: Candolfi, M.P., *et al.* (eds) Guidelines to evaluate the side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/WPRS. Pub.

To determine any sub-lethal treatment effects on the surviving wasps, females from the highest two treatment rates of the test item in which corrected mortality effects are less or equal to 40% were used for a subsequent reproduction assessment. The test unit for the reproduction assessment was constructed as follows: A plastic cylinder was placed over a pot of approximately 25 untreated wheat seedlings. When used for the experiment, the wheat seedlings were 9 days old and had been infested with > 100 *Rhopalosiphum padi* aphids. The cylinder was sealed by nylon netting. Female wasps were individually and carefully transferred to a test unit for a period of 24h. After 24h the wasps were removed from the wheat seedlings and the parasitized aphids were left to develop on the seedlings. After 11 days the parasitized aphids (mummies) were counted.

The mortality data were analysed using Fisher's Exact Test ($\alpha = 0.05$), with the individual test item treatments being compared to the control. A log-log analysis was performed on the 48-h mortality data in order to determine the LR_{50} for the test item. The reproduction data were analysed using a Bonferroni t-test ($\alpha = 0.05$).

Findings:
The results are summarized in Table B.9.5.2.1-2. After 48 hours, the mortality in the test item treatments ranged between 3.3% and 100% in comparison to 0% in the control. Based on these results the corrected mortalities were 3.3%, 10.0%, 40.0%, 100% and 100% at rates of 70, 170, 420, 1000 and 2400 mL/ha BAS 555 01 F, respectively. Statistically significant differences compared to the control were observed in the three highest test item rates (Fisher's Exact Test, $\alpha = 0.05$). The LR_{50} value was determined to be: LR_{50} = 461 mL BAS 555 01 F/ha. The reference item caused 100% mortality of exposed wasps 48 hours after application.

In the fecundity assessments, the average number of parasitized aphids was 3.08 and 2.62 mummies/female in the tested test item treatments of 170 mL/ha and 420 mL/ha compared to 7.0 in the control, resulting in effects on reproduction of 56.0% and 62.6%. These effects were statistically significantly different when compared to the control (Bonferroni t-test, $\alpha = 0.05$).

Table B.9.5.2.1-2: Effects on parasitoids (*Aphidius rhopalosiphi*) exposed to BAS 555 01 F in a laboratory trial

Treatment	Rate [mL/ha] [1)]	Mortality [%] [2)]	Corrected mortality [%] [3)]	Reproduction [mummies/ female]	Effects on reproduction [%]
Control	--	0.0	--	7.0	--
BAS 555 01 F	70	3.3	3.3	--	--
BAS 555 01 F	170	10.0	10.0	3.08 **	56.0
BAS 555 01 F	420	40.0 *	40.0	2.62 **	62.6
BAS 555 01 F	1000	100.0 *	100.0	--	--
BAS 555 01 F	2400	100.0 *	100.0	--	--
Endpoints [mL/ha]					
LR_{50} (95% CL)[4)]	461 (410 - 512)				

[1)] Application rate in 200 L water/ha.
[2)] Mortality: after 48 hours of exposure to BAS 555 01 F on glass surface.
[3)] Corrected mortality according to Schneider-Orelli (1947).
[4)] Median lethal rate calculated by log-log analysis (with 95% Confidence Limits).
** =statistically significant differences compared to the control (Fisher's Exact Test, $\alpha = 0.05$).*
*** = statistically significant differences compared to the control (Bonferroni t-test, $\alpha = 0.05$).*

Conclusions:
The LR_{50} obtained under worst-case laboratory conditions for BAS 555 01 F on parasitic wasp *Aphidius rhopalosiphi* was 461 mL/ha BAS 555 01 F in 200 L/ha water. Unacceptable effects on reproduction occurred at the tested application rates of 170 mL/ha and 420 mL/ha BAS 555 01 F in 200 mL/ha water.

RMS comments:
The following validity criteria of the test guideline are met:

- Mortality in the control was <13 % after 48 hours exposure (measured: 0.0%).
- Wasps in the control treatment produced ≥ 5 mummies per female, with no more than two wasps producing zero values (measured: 7.0 mummies/female)
- Mortality in the reference item after 48 hours was as expected (measured: 100%)

It is noted that only three replicates containing 10 wasps were used per treatment, while the test guideline recommends that at least four such replicates should be used. However, as the other validity criteria are met, this is not considered to invalidate the study results. Consequently, this study is considered acceptable for use in the risk assessment.

Endpoints for mortality:
LR_{50} (*Aphidius rhopalosiphi*, 48h) = 461 mL product/ha (equivalent to 41.1 g a.s./ha)
NOER (*Aphidius rhopalosiphi*, 48h) = 170 mL product/ha (equivalent to 15.2 g a.s./ha)

Endpoints for reproduction:
NOER (*Aphidius rhopalosiphi*, 48h) < 170 mL product/ha (equivalent to 15.2 g a.s./ha)

Report:	**CP10.3.2.1/03. Drexler A. (2002b)** **Effect of BAS 555 01 F on the green lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae) in a laboratory trial - Dose response**
Report No.:	2002/1008629
Guidelines:	Vogt *et al.* (2000)[28]
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and Methods:	
Test substance:	BAS 555 01 F (Batch No.: R2066-079, contains 89.2 g/L of the active ingredient metconazole)
Test species:	*Chrysoperla carnea* (lacewing), larvae, 2-3 days old
Number of organisms:	35 replicates/treatment, each with 1 larva per replicate
Food:	larvae: UV-sterilised eggs of *Sitotroga cerealella* adults: specified food mixture (composition not reported)
Type of test:	laboratory test (exposure to fresh residues on glass plates)

[28] Vogt H., *et al* (2000). Laboratory method to test effects of plant protection products on larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae). In: Candolfi, M.P., *et al.* (eds) Guidelines to evaluate the side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/WPRS. Pub.

Applied concentrations:	water control (deionized water), 70, 170, 420, 1000 and 2400 mL product/ha (equivalent to 6.2, 15.2, 37.5, 89.2 and 214.1 g a.s./ha). Applications were made in a volume equivalent to 200 L/ha.
	Positive control: BAS 152 11 I (400 g/L dimethoate), applied at a rate 30 mL/ha in the equivalent of 200L/ha water.
Test conditions :	temperature: 23.3-26.6°C
	relative humidity: 53.3-82.0%
	light regime: 16:8 hours light:dark, 1700-4500 lux (mortality assessment) and 1900-6300 lux (reproduction assessment)

Study design and Methods:
Mortality assessment: A test unit consisted of a glass plate with the test item sprayed on the surface. After the test item had dried the glass plate was placed in a Plexiglas tray and was covered with a plastic sheet with holes on top. Each hole had a diameter of 7.6-7.8 cm. Glass rings (7.5 cm diameter) of 1.5 cm height were covered with a thin layer of Fluon and placed in each of the holes. Thus, small arenas for individual placing of the larvae are formed. One larvae was introduced into each unit. Mortality of the exposed individuals was assessed regularly. During the mortality assessment food (eggs of *S. cerealella*) was added regularly. At least once a week old food items were removed from the test units.

Four to five days after pupation, the pupae were carefully transferred into untreated preserving jars (separated per treatment) and food and water were provided for the emerging adults. The number of lacewings that emerged successfully was also recorded regularly.

Reproduction assessment: Reproduction assessments were carried out using insects from the control and all test item treatments. The adults in the individual treatments used for the assessments emerged within 7 days. As the adult lacewings emerged, they were transferred to glass containers (preserving jars with at least 1L volume), which were covered with cotton gauze to avoid escaping of adult lacewings. This cotton gauze also served as an oviposition site. The number of eggs laid over a 24 hour period were counted twice a week. Therefore, the adult lacewings were shifted into new jars covered with new gauze before and after each evaluation. Two egg samples, covering an egg-laying period of 24 hours, were taken within one week to assess the number of eggs/female. The eggs received from the gauze were stored afterwards in a separate box until hatching of the larvae was completed. The number of undeveloped eggs and larvae that diet during hatching was counted to calculate the hatching rate. During the reproduction assessment, food and water was changed regularly.

The mortality data were analysed using Fisher's Exact Test ($\alpha = 0.05$).

Findings:
The results are summarized in Table B.9.5.2.1-3. In the water treated control a mortality of 5.7% was observed. In the test item treatments mortality ranged between 2.9% and 51.4%. This resulted in a corrected mortality between -3.0% and 48.5%. Statistically significant differences compared to the control were observed in the highest treatment group (Fisher's Exact Test, $\alpha = 0.05$). The LR_{50} value was derived to be > 2400 mL/ha BAS 555 01 F. The reference item produced 97.1% mortality of exposed lacewings. This results in a corrected mortality of 97.0%.

Table B.9.5.2.1-3: Effects on lacewings (*Chrysoperla carnea*) exposed to BAS 555 01 F in a laboratory trial

Treatment	Rate [1] [mL/ha]	Mortality [2] [%]	Corrected mortality [3] [%]	Mean Fertility [eggs/female/day]	Effects on fertility [4] [%]	Mean hatching rate [%]	Effects on hatching rate [4] [%]
Control	0.0	5.7	--	34.6	--	82.5	--
BAS 555 01 F	70	2.9	-3.0	32.0	7.5	84.0	-1.8
BAS 555 01 F	170	5.7	0.0	31.7	8.4	84.7	-2.7
BAS 555 01 F	420	2.9	-3.0	36.8	-6.4	87.9	-6.5
BAS 555 01 F	1000	14.3	9.1	36.8	-6.4	81.3	1.5
BAS 555 01 F	2400	51.4 *	48.5	35.6	-2.9	85.3	-3.4
Endpoint [mL BAS 555 01 F/ha]							
LR_{50}	> 2400						

[1] Application rate-in terms of mL product per 200 L water/ha.

[2] Mortality after exposure to residues of BAS 555 01 F on treated glass plates.

[3] Mortality corrected according to Schneider-Orelli (1947). Negative value means a decreased mortality compared to the control.

[4] Negative value means an increased reproduction compared to the control.

** = statistically significant differences compared to the control (Fisher's Exact Test, $\alpha = 0.05$)*

The number of eggs per female per day ranged from 31.7 to 36.8 in the BAS 555 01 F treatment groups compared to 34.6 eggs in the control. In the control treatment 82.5% of the eggs hatched. The hatching rate in the test item groups was 81.3% to 87.9%. As all groups exceed the mean number of 15 eggs/female, no treatment related effects on reproduction could be shown. No effects on the hatching rate of the F1-generation can be observed, as all hatching rates are well above a mean value of 70%.

Conclusions:

The LR_{50} was derived to be > 2400 mL/ha BAS 555 01 F. No effects on reproduction of the lacewings were observed when BAS 555 01 F was applied up to a rate of 2400 mL/ha in 200 L/ha water.

RMS comments:

The validity criteria of the test guideline were met:

- The cumulative mortality in the control was ≤ 20% (measured: 5.7%)
- The fecundity in the control was ≥ 15 eggs/female/day (measured: 34.6 eggs/female/day)
- The mean hatching rate of the eggs in the control was ≥ 70% (measured: 82.5%)
- The mortality in the reference item treatment was ≥ 50% (measured: corrected mortality of 97.0%)

Consequently, this study is considered acceptable for use in the risk assessment.

Endpoints for mortality:

LR_{50} (*Chrysoperla carnea*, 13-19 days) > 2400 mL product/ha (equivalent to 214.1 g a.s./ha)

NOER (*Chrysoperla carnea*, 13-19 days) = 1000 mL/ha (equivalent to 89.2 g a.s./ha)

Endpoints for reproduction:

NOER (*Chrysoperla carnea*, 13-19 days) = 2400 mL/ha (equivalent to 214.1 g a.s./ha)

Report:	**CP10.3.2.1/04. Buehler A. (2003) Effect of BAS 555 01 F on the rove beetle *Aleochara bilineata* GYLL. (Coleoptera: Staphylinidae) in a laboratory trial**
Report No.:	2003/1006374
Guidelines:	Grimm *et al.* (2000)[29]
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and Methods:	
Test substance:	BAS 555 01 F (Batch No.: R2066-079, contains 89.2 g/L of the active ingredient metconazole)
Test species:	*Aleochara bilineata* (rove beetle), adults (1-7 days old)
Number of organisms:	4 replicate units, each containing 10 males and 10 females per treatment
Food:	mosquito larvae
Host organism:	onion fly pupae (*Delia antiqua*)
Type of test:	laboratory test (exposure to fresh residues on quartz sand)
Applied concentrations:	water control (deionised water), 2000 mL product/ha (equivalent to 178.4 g a.s./ha). Applications were made in a volume equivalent to 400 L/ha.
	Positive control: BAS 152 11 I (400 g/L dimethoate), applied at a rate 1200 mL/ha in the equivalent of 400L/ha water.
Test conditions:	temperature: 18.4-23.6°C (exposure phase); 18.4-24.5°C (emergence phase)
	relative humidity: 50.3-84.2% (exposure phase); 52.0-82.3% (emergence phase)
	light regime: 16:8 hours light:dark, 231-683 lux (exposure phase)

Study design and Methods:

The exposure units consisted of plastic boxes (18.3 x 13.6 x 6 cm), covered with plastic lids. The inner area of the lids was cut out, leaving an edge of approximately 1 cm. The removed part of the lid was replaced by gauze. The exposure units were filled with 700 cm³ dry quartz sand. The sand was moistened with 70 mL tap water before application. Exposure of the beetles was reached via spray treatment of the soil surface. 10 pairs of beetles were introduced to each test unit immediately after application. Approximately 1 hour after application the beetles were fed with mosquito larvae, and further every working day. At day 7, 14 and 21 after the start of exposure (DAT7, DAT 14 and DAT 21), approximately 500 *Delia antiqua* pupae per replicate were added. The pupae were carefully mixed and distributed homogenously in the test substrate. At 2-3 day intevals, the water content of the substrate in the exposure units was checked and adjusted where necessary by addition of deionised water.

The adult beetles were exposed to the test item for 28 days. After 28 days all surviving adult beetles were removed from the test units. The sand with the parasitized fly pupae was kept under test conditions during one further week (until DAT 35) for drying of the substrate. On DAT 35, the pupae were sieved

[29] Grimm C., *et al* (2000). A test for evaluating the chronic effects of plant protection products on the rove beetle *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae) under laboratory and extended laboratory conditions. In: Candolfi, M.P., *et al.* (eds) Guidelines to evaluate the side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/WPRS. Pub.

out of the substrate and were placed in hatching units. The pupae of each replicate were transferred into a separate emergence container.

In the hatching units the parasitized pupae were placed in a funnel which was placed on a glass beaker. The bottom of the funnel was perforated with holes through which the emerged beetles fell into the glass beaker below. The pupae remained in the funnel. Assessment of reproduction was carried out by counting the number of beetles emerged from the parasitized onion fly pupae. Emergence was monitored until the control treatment fell below a rate of two beetles per replicate per day (in this case until day 63 after application).

The reproduction data was analysed using Tukey's multiple comparison ($\alpha = 0.05$).

Findings:
The results are summarized in Table B.9.5.2.1-4. In the test item treatment a mean number of 897.3 beetles hatched compared to 891.0 beetles in the control. This results in an effect on reproduction of -0.7%. The number of beetles hatched in the test item group was not statistically significantly different compared to the control (Tukey's multiple comparison, $\alpha = 0.05$). The reference item produced an effect on reproduction of 94.7% compared to the control.

Table B.9.5.2.1-4: Effects on rove beetles (*Aleochara bilineata*) exposed to BAS 555 01 F in a laboratory trial

Treatment	Application rate [1] [mL/ha]	Mean number of emerged beetles	Effects on reproduction [%] [2]
Control	0	891.0	--
BAS 555 01 F	2000	897.3	-0.7
	Endpoint [mL BAS 555 01 F/ha]		
ER_{50}	> 2000		

[1] *Application rate in 400 L/ha water*
[2] *Negative value means a decreased mortality compared to the control.*

Conclusions:
In a worst-case laboratory test with *Aleochara bilineata*, BAS 555 01 F caused no unacceptable effects on reproduction if applied at a rate of 2000 mL/ha. The ER_{50} was derived to be > 2000 mL/ha BAS 555 01 F.

RMS comments:
The validity criteria of the test guideline were met:

- Mean number of emerged beetles in the control group was > 400 beetles per replicate (measured: 891 beetles)
- The effect on reproduction in the reference item compared to the control was ≥ 50% (measured: 94.7%)

It is noted that deviations of the recommended temperature and relative humidity occurred during the study. The short deviations are however not considered to influence the results of the study, as the humidity conditions for the beetles were sufficient due to the continuous moistening of the quartz sand. Further, the normal development of the control animals indicates no influence regarding the higher temperature. Consequently, this study is considered acceptable for use in the risk assessment.

Endpionts for reproduction:
ER_{50} (*Aleochara bilineata*, 28 days) > 2000 mL product/ha (equivalent to 178.4 g a.s./ha)

B.9.5.2.2. Extended laboratory testing, aged residue studies with non-target arthropods

The representative formulation for Annex I renewal of metconazole (BAS 555 01 F) is different from the one used for the initial Annex I inclusion. Therefore, new extended laboratory studies with BAS 555 01 F, with the non-target arthropod species *Typhlodromus pyri* and *Aphidius rhopalosiphi* have been submitted. A summary of these studies is provided below.

Report:	**CP10.3.2.2/01. Eden A. (2008) Effects of BAS 555 01 F on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) in an extended laboratory trial - Dose response**
Report No.:	2003/1014069
Guidelines:	Blümel *et al.* (2000)[30]
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and Methods:	
Test substance:	BAS 555 01 F (Batch No.: R2066-079, contains 89.2 g/L of the active ingredient metconazole)
Test species:	*Typhlodromus pyri* (predatory mite), protonymphs, less than 24h old
Number of organisms:	3 replicate arenas for the test item and reference item treatment, 5 replicates in the control; each with 20 protonymphs per replicate
Food:	pollen (*Pinus* sp.)
Type of test:	extended laboratory test (exposure to fresh residues on bean leaf disks)
Applied concentrations:	water control (tap water), 125, 250, 500, 1000 and 2000 mL product/ha (equivalent to 11.2, 22.3, 17,8, 89.2 and 178.4 g a.s./ha). Applications were made in a volume equivalent to 200 L/ha. Positive control: BAS 152 11 I (400 g/L dimethoate), applied at a rate of 40 mL product/ha (= 16.0 g a.s./ha), in the equivalent of 200 L/ha.
Test conditions:	temperature: 20.9-26.2°C relative humidity: 53.9-80.1% light regime: 16:8 hours light:dark, 944-1814 lux

Study design and Methods:

For each test unit a fresh primary leaf was detached from a bean plant (*Phaseolus vulgaris*). Each leaf was cut to a disc of ~ 5 cm in diameter and then applied with the test item, tap water or the reference item. The treated leaf disc was laid on a cotton-pad, with its sprayed underside facing upwards. The whole leaf disc was in close contact with the humid cotton-pad. The cotton-pad with the leaf disc was placed in the centre of a petri-dish (diameter 9 cm) which was filled with tap water. The treated underside of the leaf disk served as test arena for 20 mites.

[30] Blümel S., *et al.* (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products. In: Candolfi, M.P., *et al.* (eds) Guidelines to evaluate the side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/WPRS. Pub.

The test covered a period of 2 weeks and recorded the mortality as well as the reproduction of predatory mites. The assessment mortality was carried out 3 and 7 days after application. Animals that have survived, dead ones and animals that escaped from the leaves were recorded. For further calculations, only the overall mortality up to day 7 after application was used. Escapers were counted as dead individuals. During the second week the oviposition rate was estimated for the control and all test item groups displaying less than 50% corrected mortality, using the remaining adult mites. These assessments were conducted on days 7, 10, 13 and 14. The number of males, females and offspring was recorded. Reproduction was expressed as the cumulative number of eggs per female.

The reproduction data were analysed using a t-Test ($\alpha = 0.05$). A Log-Log analysis was performed in order to determine the LR_{50} for the test item.

Findings:
The results of this study are summarized in Table B.9.5.2.2-1. After 7 days of exposure mortality rates between 26.7% and 100.0% could be detected in the test item treatments. Control mortality was 11.0%, which resulted in corrected mortality rates ranging from 17.6% to 100.0%. The LR_{50} value was determined to be: LR_{50} = 188 mL BAS 555 01 F/ha (95% CL = 170 - 208 mL BAS 555 01 F/ha). The reference item produced a corrected mortality of 100% of exposed mites after 7 days.

The mean number of eggs per female was 4.9 in the control and 4.4 in the tested test item group. This resulted in an effect on reproduction of 9.5%. The mean number of eggs per female was not statistically significantly reduced at the lowest test item rate (t-Test, $\alpha = 0.05$).

Table B.9.5.2.2-1: Effects on predatory mites (*Typhlodromus pyri*) exposed to BAS 555 01 F in an extended laboratory trial

Treatment	Rate[1)] [mL/ha]	Mortality[2)] [%]	Corrected mortality[3)] [%]	Reproduction[4)] [eggs/female]	Effect on reproduction [%]
Control	--	11.0	--	4.9	--
BAS 555 01 F	125	26.7	17.6	4.4	9.5
BAS 555 01 F	250	83.3	81.3	--	--
BAS 555 01 F	500	100.0	100.0	--	--
BAS 555 01 F	1000	100.0	100.0	--	--
BAS 555 01 F	2000	100.0	100.0	--	--
Endpoint [mL BAS 555 01 F/ha]					
LR_{50} (95% CL)[5)]	188 (170 - 208)				

[1)] Application rate in 200 L water/ha.
[2)] Mortality after 7 days of exposure to BAS 555 01 F on the underside of detached bean leaves.
[3)] Corrected mortality according to Schneider-Orelli (1947).
[4)] Reproduction: mean number of eggs per female from day 7 to 14.
[5)] Median lethal rate calculated by log-log analysis (with 95% Confidence Limits).

Conclusions:
The LR_{50} of BAS 555 01 F, obtained under extended laboratory conditions, on the predatory mite *Typhlodromus pyri* was determined to be LR_{50} = 188 mL/ha. BAS 555 01 F caused no unacceptable effects on reproduction of *Typhlodromus pyri* at an application rate of 125 mL/ha BAS 555 01 F in 200 L water/ha.

RMS comments:
The validity criteria of the test guideline are met:

- The mortality rate in the control did not exceed 20% on day 7 (measured: 11.0%)

- The cumulative mean number of eggs per female in the control (from day 7 to day 14) was ≥ 4 eggs/female (measured: 4.9)
- The cumulative mean mortality (control corrected) of protonymphs on day 7 exposed to the reference substance was ≥ 50% (measured: 100%)

It is noted that the lower end of the range of the temperature and relative humidity during the test was below the values recommended by the test guideline. However, the observed deviations were only short and are therefore not considered to influence the results of the test. It is further noted that only 3 replicates of 20 protonymphs were used per treatment in the test, while the test guideline recommends a minimum of 5 replicates (i.e. 100 nymphs). However, as the other validity criteria are met, this is not considered to invalidate the study results. Consequently, this study is considered acceptable for use in the risk assessment.

Endpoints for mortality:
LR_{50} (*Typhlodromus pyri*, 7 days) = 188 mL product/ha (equivalent to 16.8 g a.s./ha)

Endpoints for reproduction:
NOER (*Typhlodromus pyri*, 7 days) = 125 mL product/ha (equivalent to 11.2 g a.s./ha)

Report:	**CP10.3.2.2/02. Hanewald N. & Petrik-Steisslinger D. (2007) Evaluation of the duration of effects of BAS 555 01 F on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) - Aged residue trial**

Report No.: 2007/1018768
Guidelines: Blümel *et al.* (2000)[31]
GLP: Yes

Previous evaluation: None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application

Objective:
The purpose of this study was to determine the duration and extend of the effects of dried residues of BAS 555 01 F applied to bean seedlings on the predatory mite *Typhlodromus pyri* in the laboratory.

Materials and Methods:

Test substance:	BAS 555 01 F (Batch No.: 200001, contains 88.9 g/L of the active ingredient metconazole)
Test species:	*Typhlodromus pyri* (predatory mite), protonymphs, less than 24h old
Number of organisms:	In each bioassay the control as well as the test item treatment consisted of 5 replicates. The reference item treatment consisted of 3 replicates. Each replicate contained 20 protonymphs.
Food:	pollen (*Pinus* sp.)

[31] Blümel S., *et al.* (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products. In: Candolfi, M.P., *et al.* (eds) Guidelines to evaluate the side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/WPRS. Pub.

Type of test:	extended laboratory test (exposure to fresh and aged residues on treated bean leaves)
Applied concentrations:	control (water), 1000 and 2000 mL product/ha (equivalent to 88.9 and 177.8 g a.s./ha). Applications were made in a volume equivalent to 400 L water/ha.
	Reference item: BAS 152 11 I (400 g/L dimethoate), applied at a rate of 80 mL product/ha (= 32.0 g a.s./ha), in the equivalent of 400 L/ha.
Test conditions (exposure conditions):	
	temperature: 24.5-25.8°C relative humidity: 63.7-86.0% light regime: 16:8 hours light:dark, 1146-1887 lux

Study design and Methods:
The test plants were beans (*Phaseolus vulgaris*, var. St. Andreas), which were grown in pots of 11 cm diameter for 22 days under outdoor conditions until the BBCH growth stage of 12. Application of the test item was carried out on whole plants in the laboratory using a laboratory spray track. In total, three bioassays were performed, which started at 0 DAT, 3 DAT and 7 DAT (DAT = Days After Treatment). The first bioassay (0 DAT) started ca. 1 hour after drying of the spray residues. Remaining plants assigned for subsequent bioassays were stored rain protected under outdoor conditions for the aging of the spray residues.

For each bioassay the leaves were cut from the treated bean plants. Each leaf disc was placed with its underside down on a wet cotton-pad. The mites were thus exposed to the treated upper side of the bean leaves. The whole leaf was in close contact with the wet cotton to prevent an escape of the mites and to ensure the water supply of the mites. The cotton-pad and the bean leaf were laid in the centre of a petri dish (diameter 9 cm) which was filled with tap water. The treated upper side of the leaf served as test arena for 20 mites.

The assessment of mortality was conducted on day 1 and day 7 of each bioassay. Animals that have survived, dead ones and animals that escaped from the leaves were recorded. For further calculations, only the overall mortality up to day 7 after application was used. Escapers were counted as dead individuals. For the first bioassay (0 DAT), the oviposition rate was estimated during the second week using the remaining adult mites. These assessments were conducted on days 7, 10, 13 and 14. The number of males, females and offspring was recorded. Reproduction was expressed as the cumulative number of eggs per female. Because the results of the 0 DAT bioassay indicated that the test item had no adverse effects, all other bioassays were terminated (3 DAT and 7 DAT) and therefore not reported.

The mortality and reproductino data were analysed using ANOVA and Bonferroni t-tests ($\alpha = 0.05$).

Findings:
After 7 days of exposure the mortality in the initial bioassay (0 DAT) was 9% in the control, 25% in the 1000 mL/ha and 22% in the 2000 mL/ha BAS 555 01 F treatment group. This resulted in corrected mortalities of 17.6% and 14.3%, respectively. The mean number of eggs per female was 10.31 in the control, 9.53 in the 1000 mL/ha and 10.78 in the 2000 mL/ha BAS 555 01 F treatment group. No statistically significant differences in mortality and reproduction were observed in any of the test item treatments (Bonferroni t-test, $\alpha = 0.05$). The results of the first bioassay (0 DAT) are summarized in Table B.9.5.2.2-2. The reference item produced a corrected mortality of 90.8% of exposed mites after 7 days.

Since the results of the 0 DAT bioassay indicated that the test item had no unacceptable effects the bioassays started on 3 DAT and 7 DAT were terminated and not reported.

Table B.9.5.2.2-2:Effects on predatory mites (*Typhlodromus pyri*) exposed to BAS 555 01 F in an extended laboratory trial.

Treatment	Rate [1] [mL/ha]	Mortality [2] [%]	Corrected mortality [3] [%]	Reproduction [4] [eggs/ female]	Effect on reproduction [5] [%]
Bioassay DAT 0					
Control	--	9.0	--	10.31	--
BAS 555 01 F	1000	25.0	17.6	9.53	7.6
BAS 555 01 F	2000	22.0	14.3	10.78	-4.6

[1] *Application rate in 400 L water/ha.*
[2] *Mortality after 7 day exposure to BAS 555 01 F on the upper side detached bean leaves.*
[3] *Corrected mortality according to Schneider-Orelli (1947).*
[4] *Reproduction: mean number of eggs per female from day 7 to 14.*
[5] *Negative values indicate an increase in reproduction compared to the control.*

Conclusions:
Exposure to freshly dried residues (0 DAT) of BAS 555 01 F, applied to bean plants at rates of 1000 and 2000 mL/ha, caused no unacceptable effects on the survival and reproduction of the predatory mite *Typhlodromus pyri*. The LR_{50} was determined to be > 2000 mL BAS 555 01 F/ha.

RMS comments:
The validity criteria of the test guideline are met:

- The mortality rate in the control did not exceed 20% on day 7 in any of the bioassays (measured: between 9.0%)
- The cumulative mean number of eggs per female in the control (from day 7 to day 14) was ≥ 4 eggs/female in all bioassays (measured: 10.31 eggs/female)
- The cumulative mean mortality (control corrected) of protonymphs on day 7 exposed to the reference substance was ≥ 50% (measured: 90.8%)
- Consequently, this study is considered acceptable for use in the risk assessment.

Endpoints for mortality:
LR_{50} (*Typhlodromus pyri*, 7 days) > 2000 mL product/ha (equivalent to 177.8 g a.s./ha)
NOER (*Typhlodromus pyri*, 7 days) = 2000 mL product/ha (equivalent to 177.8 g a.s./ha)

Endpoints for reproduction:
NOER (*Typhlodromus pyri*, 7 days) = 2000 mL product/ha (equivalent to 177.8 g a.s./ha)

Report:	**CP10.3.2.2/03. Moll M. & Buetzler R. (2003) Effects of BAS 555 01 F on the parasitoid *Aphidius rhopalosiphi*, extended laboratory study - Dose response test**

Report No.: 2003/1006386
Guidelines: Mead-Briggs *et al.* (2000)[32], Mead-Briggs *et al.* (2002)[33]
GLP: Yes

Previous evaluation: None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application

Objective:
The aim of this study was to determine the effects of fresh dry residues of BAS 555 01 F on adults of the parasitoid *Aphidius rhoplosiphi* under extended laboratory test conditions (exposure to fresh dry residues on treated barley seedlings)

Materials and Methods:

Test substance:	BAS 555 01 F (Batch No.: R2066-079, contains 89.2 g/L of the active ingredient metconazole)
Test species:	*Aphidius rhopalosiphi* (parasitic wasp), adults, less than 48h old
Number of organisms:	mortality assessment: 6 replicates/treatment, each with 5 adult wasps per replicate (i.e. a total of 30 wasps per treatment);
	reproduction assessment: 20 replicates of 1 female per treatment.
Food:	10% fructose solution, sprayed on the plants before treatment
Type of test:	extended laboratory test (exposure to fresh residues on barley seedlings)
Applied concentrations:	water control (deionised water), 500, 800, 1000, 1600 and 2000 mL product/ha (equivalent to 44.6, 71.4, 89.2, 142.7 and 178.4 g a.s./ha). Applications were made in a volume equivalent to 400 L/ha.
	Positive control: BAS 152 11 I (400 g/L dimethoate), applied at a rate of 10.0 mL/ha, in the equivalent of 400 L/ha.
Test conditions:	temperature: 19-24°C
	relative humidity: 60-90% during acclimatization, exposure and parasitization period, 63-75% during the post-parasitization period
	light regime: 16:8 hours light:dark, 1100-2000 lux (exposure period); 900-1200 lux (parasitization period), 7000-17400 lux (post-parasitization period)

Study design and Methods:
Mortality and repellence assessments: Adult parasitoids were exposed to fresh treatment residues applied to barley plants (*Hordeum vulgare* 'Theresa'). The test units consisted of pots (13 cm in diameter) containing 8-10 seedlings per pot. The plants were used for the bioassay when they had 2 fully expanded leaves (BBCH 11-12). Before application, they were trimmed with scissors to an even height

[32] Mead-Briggs, M.A. *et al.* (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, Aphidius rhopalosiphi (DeStefani-Perez) (Hymenoptera: Braconidae). In: Candolfi, M.P., *et al.* (eds) Guidelines to evaluate the side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. IOBC/WPRS. Pub.

[33] Mead-Briggs, M.A. *et al.* (2002). An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera, Braconidae). Draf guideline (Current improvements by the ring-test group)

of 10 cm and a fructose solution was sprayed on the plants. After treatment, the plants were enclosed within a clear acrylic cylinder (20 cm high and 10 cm in diameter), the top of which was covered with fine mesh gauze.

After drying of the test units (10-20 minutes after application), five wasps were transferred to each test arena. The condition of the wasps was recorded at approximately 2, 24 and 48 h after their introduction. Numbers of wasps were recorded as *alive*, *affected*, *moribund* or *dead*. To determine whether fresh residues of the test product were repellent, observations on the position of the individual wasps were made (plant, cylinder or sand) at five occasions with 30 minute intervals starting 30 minutes after the introduction of all wasps.

Reproductive assessments: Reproduction assessments were initiated at 48h using surviving females from the control treatment and for each treatment group displaying less than 50% corrected mortality. Female wasps were confined individually over pots containing approximately 17-25 barley seedlings. These were untreated and had been infested with host aphids (*Rhopalosiphum padi*, 200-300 individuals of all developmental stages). The wasps were confined over the pots using clear acrylic cylinders (30 cm high and 10 cm in diameter) which were closed with a fine gauze for ventilation. The adult wasps were removed after 24h, with a note made of where females were not found or were dead, and the aphid-infested plants were then kept under similar conditions for a further 11-12 days, before the number of mummies that developed on each plant was recorded.

The mortality data were analysed using a Mann-Whitney-U-Test, the data for settling behaviour using Fishers Exact Test and reproduction data using a Bonferroni t-test ($\alpha = 0.05$).

Findings:

The results are summarized in Table B.9.5.2.2-3. After 48 h of exposure no mortality could be detected in the control and test item treatments. No statistically significant differences compared to the control were observed in all test item treatments (Mann-Whitney-U-Test, $\alpha = 0.05$). The LR_{50} could not be calculated because no effects of BAS 555 01 F were observed. It was estimated > 2000 mL/ha BAS 555 01 F. The reference item caused 100% corrected mortality.

Table B.9.5.2.2-3: Effects on parasitoids (*Aphidius rhopalosiphi*) exposed to BAS 555 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	0	0.0	--	62.8	--
BAS 555 01 F	500	0.0	0.0	50.0	20.4
BAS 555 01 F	800	0.0	0.0	57.2	8.9
BAS 555 01 F	1000	0.0	0.0	41.2	34.4
BAS 555 01 F	1600	0.0	0.0	58.5	6.8
BAS 555 01 F	2000	0.0	0.0	55.9	11.0
Endpoint [mL/ha BAS 555 01 F]					
LR_{50}	> 2000				

[1] *Application rate in 400 L water/ha.*

[2] *Mortality: after 48 hours of exposure to BAS 555 01 F on barley seedlings.*

[3] *Corrected mortality according to Schneider-Orelli (1947).*

[4] *Reproduction: mean number of parasitized aphids/surviving female.*

[5] *Calculated with the exact raw data.*

The mean number of infested parasitized mummies per female was 62.8 in the control and ranged from 41.2 to 58.5 in the test item groups. This resulted in effects on reproduction between 6.8% and 34.4%. The mean number of parasitized mummies was not significantly reduced in any of the test item groups compared to the control (Bonferroni t-Test, $\alpha = 0.05$). No repellent effect was observed in any of the test item rates.

Conclusions:
The LR_{50} value was determined to be > 2000 mL/ha BAS 555 01 F. In an extended laboratory study with *Aphidius rhopalosiphi* the test item caused no unacceptable effects on survival and reproduction if applied up to and including a rate of 2000 mL/ha BAS 555 01 F in 400 L water/ha.

RMS comments:
The following validity criteria of the test guideline are met:

- Mortality in the control was <13 % after 48 hours exposure (measured: 0.0%).
- Wasps in the control treatment produced ≥ 5 mummies per female, with no more than two wasps producing zero values (measured: 62.8 mummies/female, with only one parasitoid producing zero values)
- Mortality in the reference item after 48 hours was ≥ 50% (measured: 100%)

Consequently, this study is considered acceptable for use in the risk assessment.

Endpoints for mortality:
LR_{50} (*Aphidius rhopalosiphi*, 48h) > 2000 mL product/ha (equivalent to 178.4 g a.s./ha)
NOER (*Aphidius rhopalisiphi*, 48h) = 2000 mL/ha (equivalent to 178.4 g a.s./ha)

Endpoints for reproduction:
NOER (*Aphidius rhopalisiphi*, 48h) = 2000 mL/ha (equivalent to 178.4 g a.s./ha)

B.9.5.2.3. Semi-field studies with non-target arthropods

Semi-field studies with non-target arthropods are not required since the risk assessments based on laboratory and extended laboratory studies indicate an acceptable risk to non-target arthropods (other than bees).

B.9.5.2.4. Field studies with non-target arthropods

Field studies with non-target arthropods are not required since the risk assessments based on laboratory and extended laboratory studies indicate an acceptable risk to non-target arthropods (other than bees).

B.9.6. RISK ASSESSMENT FOR ARTHROPODS

B.9.6.1. Risk assessment for bees

The risk assessment for effects on bees is conducted in accordance with Regulation (EC) No. 1107/2009 and the **Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC** (SANCO/10329/2002) and the **revised EPPO scheme** (2010)[34].

Following the data requirements according to Regulation (EU) No. 283/2013, data on the chronic risk to adult honeybees and honeybee larvae are available. Further, data on the acute risk to bumble bees have been submitted. However, in the currently notified SANCO Guidance Document, these data are not considered in the risk assessment scheme. For the chronic risk assessment for honeybees, the approach outlined in the revised EPPO scheme could be partly used. Further, a new guidance document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees) has been published in 2013 by EFSA[35], in which risk assessment schemes for the chronic risk to adult honeybees and honeybee larvae, and for the risk to bumblebees are described. This Guidance Document is however not yet noted by the Standing Committee on Plants, Animals, Food and Feed. Nevertheless, as agreed in Pesticides Peer Review Expert Meeting 133 and recommended by EFSA (2015)[36], a risk assessment for chronic risk to honeybees according to the new EFSA Guidance Document is included below. For bumble bees, a risk assessment according to the new EFSA Guidance Document is included below as well, as this is the only risk assessment scheme for bumble bees currently available. By doing so, all available data on bees is taken into account in a risk assessment.

B.9.6.1.1. Toxicity

Acute contact and oral toxicity studies were conducted with adult honeybees (*Apis mellifera*) for metconazole and the representative formulation BAS 555 01 F. For BAS 555 01 F, two acute toxicity studies are available. As a conservative approach, the lowest available endpoints of these two studies will be used in the risk assessment.

Additional studies investigating the chronic toxicity to adults of *Apis mellifera*, the acute and chronic toxicity to larvae of *Apis mellifera* and the acute oral and contact toxicity to adult bumblebees (*Bombus terrestris*) have also been submitted, in accordance with the new data requirements (Regulation (EU) No 283/2013). Endpoints from these studies will be used in the risk assessment. A summary of the available toxicity endpoints for bees and the endpoints used in the risk assessment are shown in Table B.9.6.1.1-1.

[34] EPPO/OEPP (2010). Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(2)). Bulletin OEPP/EPPO Bulletin 40: 323-331.
[35] European Food Safety Authority (2013). Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2013; 11(7):3295. doi:10.2903/j.efsa.2013.3295
[36] EFSA, 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.

Table B.9.6.1.1-1: Summary of bee toxicity data on metconazole

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Honeybees					
Honeybee (*Apis mellifera*)	BAS 555 F (metconazole) 83:17 *cis:trans*	96h acute contact and oral toxicity test	96 h LD_{50} (contact) 72 h LD_{50} (oral)	**> 100 µg a.s./bee** **85 µg a.s./bee**	CA8.3.1.1/01 Harrison E.G. & Hillaby J.M. (1991)
Honeybee (*Apis mellifera*)	BAS 555 01 F	48h acute contact and oral toxicity test	LD_{50} (contact) LD_{50} (oral)	**229.8 µg product/bee (= 19.6 µg a.s./bee)** **208.7 µg product/bee (= 17.8 µg a.s./bee)**	CP10.3.1.1/01 Schmitzer S. & Weber B., 2002
Honeybee (*Apis mellifera*)	BAS 555 01 F	48h acute contact and oral toxicity test	LD_{50} (contact) LD_{50} (oral)	444.46 µg product/bee (= 38.24 µg a.s./bee) > 455.44 µg product/bee (> 39.19 µg a.s./bee)	CP10.3.1.1/02 Kling A., 2007
Honeybee (*Apis mellifera*)	BAS 555 F (metconazole)	10 day chronic adult oral toxicity test	LC_{50} NOEC LDD_{50} NOED	2.938 mg a.s./kg food 0.257 mg a.s./kg food **50.0 µg a.s./bee/day** 5.5 µg a.s./bee/day	CA8.3.1.2/01 Kleebaum K., 2014 CA8.3.1.2/02 Kleebaum K., 2015b
Honeybee (*Apis mellifera*)	BAS 555 F (metconazole)	72 h acute larval toxicity test, single exposure	72h LC_{50} NOEC 72h LD_{50} NOED	> 2.926 g a.s./kg food 1.463 g a.s./kg food > 99.2 µg a.s./larva 49.6 µg a.s./larva	CA8.3.1.3/01 Kleebaum K., 2015
Honeybee (*Apis mellifera*)	BAS 555 F (metconazole)	22 day chronic larval toxicity test, repeated exposure	8 day LC_{50} 8 day NOEC 8 day LD_{50} 8 day NOED 22 day EC_{50} 22 day NOEC 22 day ED_{50} 22 day NOED	> 649 mg a.s./kg food 162 mg a.s./kg food > 100 µg a.s./larva 25 µg a.s./larva 299 mg a.s./kg food 81 mg a.s./kg food 46.1 µg a.s./larva **12.5 µg a.s./larva**	CA8.3.1.3/02 Kleebaum K., 2017
Bumblebees					
Bumble bee (*Bombus terrestris*)	BAS 555 F (metconazole)	96h acute contact and oral toxicity test	LD_{50} (contact) LD_{50} (oral)	**> 100 µg a.s./bee** **111.1 µg a.s./bee**	CA8.3.1.1/02 Haupt S., 2015a CA8.3.1.1/03 Haupt S., 2015b

bold **-** *values used in the risk assessment*

It should be noted that for the toxicity to honeybee larvae, both an acute and chronic endpoint are available. The former was obtained from a study according to OECD TG 237 (2013) in which larvae were exposed only once to metconazole contaminated food. The latter was obtained from a study according to OECD TG 239 (2016) in which larvae were repeatedly exposed to metconazole (from day 3 until day 6 of the study). According to the risk assessment described in the EPPO scheme and the EFSA Guidance Document for bees, the chronic risk to honeybee larvae should be assessed. Therefore, only the endpoint from the chronic repeated exposure study is used in the risk assessment.

B.9.6.1.2. Risk assessment for honeybees according to SANCO/10329/2002 and EPPO (2010)

Note: In this section, a first tier risk assessment for honeybees according to Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002) is presented. This Guidance Document is the currently noted guidance document for honeybees. The risk assessment presented here is thus the current legal and regulatory basis for registrations.

In addition, the EPPO revised scheme (2010) was used to address the chronic risk to adult honeybees and honeybee larvae. It should be noted that this risk assessment scheme has been criticised as not being suitable for addressing the Specific Protection Goals which are outlined in the Scientific Opinion of the EFSA PPR Panel (2012)[37]. Nevertheless, it is considered useful to include an assessment according to this EPPO scheme below.

Exposure

BAS 555 01 F is applied by foliar application. Consequently, honeybee exposure may occur either through direct over-spray (contact exposure) or via residues on plants while bees are foraging for food (contact or oral exposure). The critical use patterns relevant for the proposed uses of BAS 555 01 F are given in Table B.9.6.1.2-1.

Table B.9.6.1.2-1: Use pattern of BAS 555 01 F for use as foliar spray

Crop	Application time (BBCH growth stage)	Max. number of applications	Interval between applications [d]	Application rate per treatment	
				Metconazole [kg a.s./ha]	BAS 555 01 F [L/ha]
Cereals (winter/spring)	30 - 69	1 - 2	21	0.090	1.0
Winter oilseed rape	13 – 20 (autumn) 21 – 71 (spring) [1)]	1 - 2	90	0.072	0.8
	21 – 71 (spring)	1 - 2	14	0.072	0.8

[1)] According to the GAP a first application in autumn (between BBCH 13 and BBCH 20) is followed by a second application in spring (between BBCH 21 and BBCH 71)

In the available plant metabolism studies performed in wheat, oilseed rape and peas, the **parent metconazole** was the major residue constituent in the vegetative plant parts (straw or foliage). In seeds, however, the **metabolite triazolylalanine** was the main component (see Volume 3 (AS), Section B.7.2.1). For wheat, the parent metconazole remained the major component in straw (up to 32% of the TRR). A large amount of the radioactive residues was further characterized as monohydroxylated metabolites. Each of these metabolites was however only found at low levels (below 10% TRR). In

[37] EFSA Panel on Plant Protection Products and their Residues (2012). Scientific opinion on the science behind the development of a risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2012;10(5):2668, 275 pp., doi:10.2903/j.efsa.2012.2668

straw, a slight isomer conversion from *cis* to *trans* was observed. In wheat grain, the parent was extensively metabolised into mainly triazolylalanine (which accounted for 33% of the TRR) and to a lesser extent into triazolylacetic acid (which accounted for 9% of the TRR). For oilseed rape, the major compound in foliage was the unchanged parent, which accounted for 55-93% or 60-96% of the TRR at the various sampling intervals, depending on the radioactive label used. At harvest, the parent compound metconazole and the metabolite triazolylalanine were the predominant compounds recovered in oilseed rape seeds and accounted each for 24% and 40% of the TRR, respectively. Minor components including monohydroxylated derivates of the parent compound and its glucose conjugates were also characterized in foliage, pods and seeds, which were all found at low levels (below 10% TRR). For peas, the unchanged parent compound metaconazole was the most prominent component in foliage (67-96 %TRR) and straw (54-68 %TRR), whereas triazolylalanine was the main component in pea seeds (up to 85%TRR); metconazole was present at lower levels in seed (2.2-21 %TRR).

Overall, as no major metabolites of metconazole are present in the vegetative plant parts, exposure of bees to metabolites can be considered minimal, and the risk low. Consequently, the risk assessment will only be performed for the active substance metconazole and the formulation BAS 555 01 F.

Risk assessment

First Tier risk assessment

The **acute risk to honeybees** from the use of metconazole was assessed using the maximum single application rate (expressed as g/ha) and the LD_{50} value (expressed as µg/bee) to calculate hazard quotients according to following equation:

$$Hazard\ Quotient\ (HQ) = \frac{maximum\ single\ application\ rate\ (g/ha)}{LD_{50}\ (\mu g/bee)}$$

Hazard quotients were calculated for oral exposure (HQ_O) and contact exposure (HQ_C) for metconazole and BAS 555 01 F. A Hazard Quotient of less than 50 indicates a low risk to bees in the field (EPPO, 2010).

Table B.9.6.1.2-2 and Table B.9.6.1.2-3 show the calculated HQ values for metconazole and BAS 555 01 F, respectively. The acute oral and contact HQ values for both metconazole and BAS 555 01 F are below the trigger of 50, indicating an acceptable acute risk to honeybees for all proposed uses.

Table B.9.6.1.2-2: Acute risk to adult honeybees from oral and contact exposure to metconazole following the proposed uses of BAS 555 01 F – HQ values for metconazole.

Exposure route	Crop	Application rate (g a.s./ha)	LD_{50} (µg a.s./bee)	HQ	Trigger value
Oral	Winter and spring cereals	90	85	1.06	50
	Winter oilseed rape	72		0.85	
Contact	Winter and spring cereals	90	> 100	< 0.90	50
	Winter oilseed rape	72		< 0.72	

Note: HQ values shown in bold exceed the trigger.

Table B.9.6.1.2-3: Acute risk to adult honeybees from oral and contact exposure to metconazole following the proposed uses of BAS 555 01 F – HQ values for BAS 555 01 F.

Exposure route	Crop	Application rate (g product./ha) [1)]	LD_{50} (µg product/bee)	HQ	Trigger value
Oral	Winter and spring cereals	1046	208.7	5.01	50
	Winter oilseed rape	836.8		4.01	
Contact	Winter and spring cereals	1046	229.8	4.55	50
	Winter oilseed rape	836.8		3.64	

Note: HQ values shown in bold exceed the trigger; [1)] maximum application rate in mL/ha multiplied by the product density of 1.046 g/cm³.

For **the chronic risk assessment for adult honeybees and honeybee larvae**, the revised EPPO scheme (2010) suggests to calculate the ratio between the NOEL (oral) and the exposure. For adult bees, the exposure is assessed through the amount of residues that may be ingested by a bee in one day. The ratio between the NOEL (= NOED in µg a.s./bee/day) and the exposure (also in µg a.s./bee/day) is then calculated as follows:

$$TER_{chronic,adult} = \frac{NOED_{oral}[\mu g\ a.s./bee/day]}{Amount\ of\ residues\ ingested\ by\ a\ bee\ in\ one\ day\ [\mu g\ a.s./bee/day]}$$

The available chronic endpoint for larvae is expressed over the total developmental period. Threfore, the exposure for larvae is assessed through the amount of residues that may be ingested by the larvae over that period. For larvae, the ratio between the NOEL (in µg a.s./larva) and the exposure (also in µg a.s./larva) is calculated by the following equation:

$$TER_{chronic,larvae} = \frac{NOEL_{oral}[\mu g\ a.s./larva]}{Amount\ of\ residues\ ingested\ by\ a\ larva\ [\mu g\ a.s./larva]}$$

To calculate the residue intake, data on the residues of metconazole in nectar and pollen is needed, in combination with data on the consumption of nectar and pollen. For metconazole, one residue trial in which residues in pollen were measured has been submitted (CA6.10.1/01 Plier S., 2014a; see Volume 3 (CA) Section B.7.7.1 for a summary). In all trials in that study, residue values of < 0.01 mg/kg were found. Appendix F of the EFSA Guidance Document for bees (2013) makes reference to one study in which the residues of metconazole in nectar were measured. For this study, a RUD value of 3.7 mg/kg is reported (i.e. application of 1 kg a.s./ha in rape resulted in a residue of 3.7 mg a.s./kg in nectar). It is assumed that this RUD value is the maximum measured value. Specific residue data for metconazole in pollen are not available in this dataset.

Recently, a review paper on pesticide residue data for pollen and nectar has been published (Kyriakopoulou *et al.*, 2017), in which RUD values for residues of metconazole in pollen and nectar are reported (see Table 28 on page 75 of that report). These residue data for metconazole were obtained from one study comprising of 5 field trials in which a formulation containing metconazole and pyraclostrobin was applied. The RUD values from this study are in general higher compared to the other available data for metconazole. Given the limited number of studies available, it is therefore considered reasonable to use the values reported in Kyriakopoulou *et al.* (2017) in the risk assessment. As the chronic risk is assessed, it is considered acceptable to use the median residue values for metconazole, being a residue of 8.897 mg/kg in pollen and 1.423 mg/kg in nectar, following a foliar application of 1 kg a.s./ha. For the proposed uses in winter and spring cereals and winter oilseed rape, an application rate of 90 and 72 g a.s./ha, respectively, is intended. For the use in cereals, this application rate results in an expected residue of 0.801 mg a.s./kg in pollen and 0.128 mg a.s./kg in nectar. These

residue values cover the expected values for the proposed use in winter oilseed rape, and will be used in the assessment below.

Data for consumption of nectar and pollen by adult honeybees and honeybee larvae are given in the EFSA PPR Opinion on bees (2012)[38]. For adult honeybees, only nectar consumption is relevant. The maximum amount of sugar in nectar an adult bee consumes per day is given as 128 mg/bee/day. Based on a worst-case sugar content in nectar of 15%, this is equivalent to a nectar consumption of 853 mg/bee/day. For honeybee larvae, both nectar and pollen consumption are relevant. The maximum amount of sugar in nectar a larva consumes is given as 59.4 mg/5 days, which corresponds to a nectar consumption of 369 mg/5 days (based on a worst-case sugar content in nectar of 15%). The maximum amount of pollen consumed by a larva is 2 mg/5 days.

To calculate the residue intake of metconazole by adult honeybees and honeybee larvae, the consumed amounts of pollen and nectar are multiplied with relevant measured residue values in nectar and pollen (see Table B.9.6.1.2-4). The calculated chronic TER value is given in Table B.9.6.1.2-5. This TER is compared to the trigger of 1 as proposed in the revised EPPO scheme (2010). As both the chronic TER for larvae and adult bees exceed the trigger of 1, the risk can be considered acceptable.

Table B.9.6.1.2-4: Total residue intake for adult honeybees following exposure to metconazole (for an application of 1 mg a.s./kg).

Honeybee stage	Adult	Larva
Residue in pollen	0.801 mg a.s./kg (= 0.000801 µg a.s./ mg)	0.801 mg a.s./kg (= 0.000801 µg a.s./mg)
Pollen consumption	0	2 mg/larva/5 days
Residue intake through pollen	0 µg a.s./bee	0.0016 µg a.s./larva/5 days
Residue in nectar	0.128 mg a.s./kg (= 0.000128 µg a.s./mg	0.128 mg a.s./kg (= 0.000128 µg a.s./mg)
Nectar consumption	853 mg/bee/day	369 mg/larva/5 days
Residue intake through nectar	0.109 µg a.s./bee/day	0.0472 µg a.s./larva/5days
Total residue intake	**0.109 µg a.s./bee/day**	**0.0488 µg a.s./larva/5 days**

Table B.9.6.1.2-5: Chronic risk to adult bees and larvae from oral and contact exposure to metconazole following the use of BAS 555 01 F in winter and spring cereals and winter oilseed rape.

Honeybee stage	Exposure route	NOED	Worst case residue intake	TER_{ch}	Trigger value
Adult	Oral	5.5 µg a.s./bee/day	0.109 µg a.s./bee/day	50.5	1
Larvae	Oral	12.5 µg a.s./larva	0.0488 µg a.s./larva	256.1	1

Note: TER values shown in bold are below the trigger.

Risk assessment based on Higher Tier studies

In addition to the laboratory tests, a semi-field study (tunnel test) was conducted with BAS 555 00 F (Franke M., 2013; CP10.3.1.5/01). BAS 555 00 F is an EC formulation containing 60 g/L of the active substance metconazole. This formulation differs in composition from the representative formulation BAS 555 01 F (refer to Volume 4, Section C.1.3.2 for details). To assess whether this study is suitable to address the risk to honeybees, the toxicity of BAS 555 00 F and BAS 555 01 F to honeybees in

[38] EFSA Panel on Plant Protection Products and their Residues (2012). Scientific opinion on the science behind the development of a risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2012;10(5):2668, 275 pp., doi:10.2903/j.efsa.2012.2668.

laboratory studies was compared. Based on the endpoints available for both formulations, which are summarized in Table B.9.6.1.2-6, the are no clear indications that one of the fomulations is more toxic than the other. In addition, the variability of endpoints were always within a factor of 5, which is considered to be within the biological variability (as for example stated for honeybees in the EFSA Guidance Document for bees, 2013). Only in one case the endpoint for BAS 555 00 F is more than a factor 5 below the endpoint of BAS 555 01 F. This is however caused by the endpoint for BAS 555 00 F being a "higher than" endpoint. Based on the above, the toxicity of BAS 555 01 F and BAS 555 00 F seems to be predominantly driven by the active ingredient and is considered to be comparable with respect to effects on honeybees. In consequence, the semi-field study conducted with BAS 555 00 F is considered suitable to address the risk of BAS 555 01 F to honeybees.

Table B.9.6.1.2-6: Comparison of the toxicity of the formulations BAS 555 00 F and BAS 555 01 F to honeybees

Test species / system	Test item	LR_{50} or LD_{50} (95% confidence limits)		Reference	Toicity ratio based on a.s.
		µg a.s./bee	µg product/bee		
A. mellifera / Acute oral toxicity, laboratory	BAS 555 00 F	$LD_{50\ oral}$ (72h) > 11.22 µg/bee	$LD_{50\ oral}$ (72h) > 187 µg/bee	Engelhard E.K. *et al.*, 1995 [a]	0.29-0.6
	BAS 555 00 F	$LD_{50\ contact}$ (48h) = 8.38 µg/bee	$LD_{50\ oral}$ (48h) > 139.7 µg/bee	Schmitzer, 1999 [a]	0.21-0.47
	BAS 555 01 F	$LD_{50\ oral}$ (48h) = 17.8 µg/bee (13.4-23.6)	$LD_{50\ oral}$ (48h) = 208.7 µg/bee	CP10.3.1.1/01 Schmitzer S. & Weber B., 2002	-
	BAS 555 01 F	$LD_{50\ oral}$ (48h) > 39.19 µg/bee	$LD_{50\ oral}$ (48h) > 455.44 µg/bee	CP10.3.1.1/025 Kling A., 2007	-
A. mellifera / Acute contact toxicity, laboratory	BAS 555 00 F	$LD_{50\ contact}$ (72h) > 12 µg/bee (0.06-0.11)	$LD_{50\ contact}$ (72h) > 200 µg/bee	Engelhard E.K. *et al.*, 1995 [a]	0.31-0.61
	BAS 555 00 F	$LD_{50\ contact}$ (48h) > 6 µg/bee	$LD_{50\ contact}$ (48h) > 100 µg/bee	Schmitzer, 1999 [a]	0.16-0.31
	BAS 555 01 F	$LD_{50\ contact}$ (48h) = 19.6 µg/bee (19.6-22.7)	$LD_{50\ contact}$ (48h) = 229.8 µg/bee	CP10.3.1.1/01 Schmitzer S. & Weber B., 2002	-
	BAS 555 01 F	$LD_{50\ contact}$ (48h) = 38.24 µg/bee (32.22-44.36)	$LD_{50\ contact}$ (48h) = 444.46 µg/bee (386.7-515.52)	CP10.3.1.1/025 Kling A., 2007	-

[a] *Study summarized in the original DAR for metconazole (RMS Belgium, January 2004)*

In the semi-field study by Franke M. (2013), BAS 555 00 F was applied at a rate of 1500 mg formulation/ha (90 g metconazole/ha), which covers the intended application rate in winter and spring cereals and winter oilseed rape. The product was applied during active foraging of the honeybees onto flowering *Phacelia tanacetifolia* enclosed within a tunnel. The application of BAS 555 00 F caused no unacceptable effects on adult and pupal honeybee mortality, foraging activity, behaviour, colony strength and brood development. This further supports the results from the Tier 1 risk assessment above, which indicates an acceptable acute and chronic risk to honeybees.

Conclusion:

The acute and chronic risk to honeybees from the active substance metconazole and the formulation BAS 555 01 F is acceptable at Tier 1 for the proposed uses in winter and spring cereals and winter oilseed rape.

B.9.6.1.3. Risk assessment for honeybees according to EFSA (2013)

Note: The EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)(2013) is currently not yet noted by the Standing Committee on Plants, Animals, Food and Feed. However, chronic toxicity data for adult honeybees and acute data for honeybee larvae are available, in line with the data requirements according to Regulation (EU) No. 283/2013. As these data are not considered in the currently notified Guidance Document (SANCO/10329/2002), a Screening step/Tier 1 risk assessment according to EFSA (2013) is presented in this section, so that all available data on bees is taken into account in a risk assessment.

Exposure

BAS 555 01 F is applied by foliar application. For spray applications, honeybees are considered to be exposed through a number of different exposure routes:

- Exposure via contact from spray deposits when bees are either foraging the treated crop, weeds in the field, plants in the field margin and the adjacent crop.
- Oral exposure through the consumption of nectar and pollen from the treated crop, weeds in the field, plants in the field margin, the adjacent crop, succeeding crop/permanent crop the following year.
- Oral exposure through the consumption of contaminated water (guttation water, surface water, water in puddles).

The critical use patterns relevant for the proposed uses of BAS 555 01 F are given in Table B.9.6.1.2-1, in Section B.9.6.1.2.

Risk assessment

For each of the exposure routes, a risk assessment is performed considering the acute risk to adult worker bees, the chronic risk to adult honeybees resulting from repeated exposure and the risk to honeybee larvae. The following scenarios are considered in the risk assessment:

- Risk from foraging the treated crop
- Risk from foraging an adjacent crop
- Risk from foraging on weeds in the treated field
- Risk from foraging in the field margin
- Risk from foraging the following year on a permanent crop or on a succeeding crop for annual crops

The risk assessment starts with a screening step which is based on the worst case scenario (which for honeybees is the treated crop scenario). If the screening step scenario fails, then all other scenarios have to be assessed in the first tier unless it is justified that a specific scenario is not relevant because exposure is expected to be negligible.

Further, a risk assessment for the consumption of contaminated water was also performed. As no studies are available that address the possible accumulative effect of metconazole in honeybees, a risk assessment for accumulative effects could not be performed.

Contact exposure assessment – screening step

According to the EFSA Guidance Document on bees, the hazard quotient (HQ) for contact exposure is calculated by the following equation at the screening step:

$$HQ = \frac{AR}{LD_{50\ contact}}$$

Where AR = application rate in g a.s./ha
$LD_{50,contact}$ is expressed in µg a.s./bee

The screening step assessment is based on exposure in the field. If the HQ is lower than the trigger of 42 (for downward spray) or 85 (for sideward spray), the risk is considered acceptable. If the HQ exceeds the trigger, a potential risk is identified and also all other scenarios need to be considered.

The calculated HQ values for the screening step are shown in Table B.9.6.1.3-1. As the HQ values are below the trigger for all of the proposed uses, the acute risk from contact exposure is considered acceptable.

Table B.9.6.1.3-1: Acute contact exposure of adult honeybees to metconazole following the proposed uses of BAS 555 01 F – screening step.

Test substance	Crop	Application rate (g/ha)	LD_{50} (µg/bee)	HQ	Trigger value
Metconazole	Winter and spring cereals	90	> 100	< 0.90	42
	Winter oilseed rape	72	> 100	< 0.72	42
BAS 555 01 F	Winter and spring cereals	1046 [1)]	229.8	4.55	42
	Winter oilseed rape	836.8 [1)]	229.8	3.64	42

[1)] maximum application rate in mL/ha multiplied by the product density of 1.046 g/cm³; HQ values shown in bold exceed the trigger.

Oral exposure assessment – screening step

According to the EFSA Guidance Document on bees, the following formulae should be used at the screening step to determine the Exposure Toxicity Ratio (ETR) for acute adult oral exposure, chronic adult oral exposure and chronic exposure to larvae, for products applied by spray application. The application rate (for a single application) is multiplied by a shortcut value, which was derived considering information on feed (nectar and pollen) consumption and worst-case pesticide residue levels of feed items. The relevant shortcut values were derived from Table 3 of the EFSA Guidance Document. For the proposed uses in winter and spring cereals and winter oilseed rape, the shortcut values for downward application are used.

The *ETR for the acute adult oral exposure* is calculated by the following equation:

$$ETR_{acute\ adult\ oral} = \frac{AR \times SV}{LD_{50\ oral}}$$

Where: AR = application rate in kg a.s./ha
$LD_{50,oral}$ is expressed as µg a.s./bee

If this ETR > 0.2, a potential risk is identified, and a first tier risk assessment should be performed. If the ETR is below this trigger, the risk is acceptable.

The *ETR for the chronic adult oral exposure* is calculated by the following equation:

$$ETR_{chronic\ adult\ oral} = \frac{AR \times SV}{LDD_{50}}$$

Where: AR = application rate in kg a.s./ha
LDD_{50} is expressed as µg a.s./bee per day

If this ETR > 0.03, a potential risk is identified, and a first tier risk assessment should be performed. If the ETR is below this trigger, the risk is acceptable.

The *ETR for larvae* is calculated by the following equation:

$$ETR_{larvae} = \frac{AR \times SV}{NOED}$$

Where: AR = application rate in kg a.s./ha
NOED is expressed as µg a.s./larva/development period

If this ETR > 0.2, a potential risk is identified, and a first tier risk assessment should be performed. If the ETR is below this trigger, the risk is acceptable.

According to the EFSA Guidance Document, an ETR for effects on the development of the hypopharyngeal glands (HPG) should also be calculated. As there is currently no validated methodology for the assessment of sublethal effects, no endpoint for the effects on the hypopharyngeal glands of honeybees is available for metconazole. Therefore, the risk assessment for honeybees based on HPG was not performed.

Table B.9.6.1.3-2 shows the calculated ETR values for the oral exposure assessment for all proposed uses. The ETR values for acute oral exposure to adult bees, the chronic oral exposure to adult bees and for the chronic oral exposure to larvae are below the trigger, indicating the risk can be considered acceptable.

Table B.9.6.1.3-2: Acute and chronic oral exposure of adult honeybees and honeybee larvae to metconazole following the proposed uses of BAS 555 01 F – screening step.

Type of assessment	Test substance	Crop	Application rate (kg a.s./ha)	SV	Endpoint	ETR	Trigger value
Acute oral exposure adult bees	Metconazole	Winter and spring cereals	0.090	7.6	85 µg a.s./bee	0.008	0.2
		Winter oilseed rape	0.072	7.6		0.006	0.2
	BAS 555 01 F	Winter and spring cereals	1.046 [1)]	7.6	208.7 µg product/bee	0.038	0.2
		Winter oilseed rape	0.8368 [1)]	7.6		0.030	0.2
Chronic oral exposure adult bees	Metconazole	Winter and spring cereals	0.090	7.6	50.0 µg a.s./bee/day	0.014	0.03
		Winter oilseed rape	0.072	7.6		0.011	0.03
Chronic oral exposure larvae	Metconazole	Winter and spring cereals	0.090	4.4	12.5 µg a.s./ larva	0.032	0.2
		Winter oilseed rape	0.072	4.4		0.025	0.2

[1)] maximum application rate in L/ha multiplied by the product density of 1.046 g/cm³; SV: Shortcut value; ***bold*** *values exceed the trigger, indicating a potential risk.*

Risk assessment based on Higher Tier studies

In addition to the laboratory tests, a semi-field study (tunnel test) was conducted with BAS 555 00 F (Franke M., 2013; CP10.3.1.5/01). BAS 555 00 F is an EC formulation containing 60 g/L of the active substance metconazole. This formulation differs in composition from the representative formulation BAS 555 01 F (refer to Volume 4, Section C.1.3.2 for details). Based on a comparison of the toxicity to honeybees of BAS 555 00 F and BAS 555 01 F in laboratory studies, the results from the available study are considered representative for BAS 555 01 F (refer to Section B.9.6.1.2 for details).

In the semi-field study by Franke M. (2013), BAS 555 00 F was applied at a rate of 1500 mg formulation/ha (90 g metconazole/ha), which covers the intended application rate in winter and spring cereals and winter oilseed rape. The product was applied during active foraging of the honeybees onto flowering *Phacelia tanacetifolia* enclosed within a tunnel. The application of BAS 555 00 F caused no unacceptable effects on adult and pupal honeybee mortality, foraging activity, behaviour, colony strength and brood development. This further supports the results from the Screening step risk assessment above, which indicates an acceptable acute and chronic risk to honeybees.

Risk assessment from exposure to contaminated water

Honeybees are potentially exposed to residues of metconazole in surface water bodies and puddles present in the field. Further, after application to the plant, metconazole is taken up via the leaf and then translocated via the transportation flow. Due to this systemic and translaminar activity, there is a potential exposure to pollinators via residues in guttation droplets.

For exposure to contaminated water, the following equations are used to calculate ETR values for the acute risk:

$$ETR_{acute} = \frac{W \times PEC}{LD_{50}}$$

Where: W = 11.4 µL/bee per day, the water uptake for adult bees.
PEC : the concentration of the active substance in the water (in µg/µL)
LD_{50} = the oral LD_{50} in µg/bee

For the chronic risk to adult bees, the ETR is calculated as follows:

$$ETR_{chronic} = \frac{W \times PEC}{LDD_{50}}$$

Where: W = 11.4 µL/bee per day, the water uptake for adult bees.
PEC : the concentration of the active substance in the water (in µg/µL)
LDD_{50} = the oral LDD_{50} in µg/bee per day based on an exposure of 10 days.

For the chronic risk to honeybee larvae, the ETR is calculated as follows:

$$ETR_{chronic} = \frac{W \times PEC}{NOED}$$

Where: W = 111 µL for larvae (amount of water consumed over 5 days)
PEC : the concentration of the active substance in the water (in µg/µL)
NOED = NOED in µg/bee, based on an exposure of 5 days.

For the risk following consumption of guttation water, the PEC used for the acute risk is assumed to be 100% of the water solubility in the first tier. For the chronic risk to adult honeybees and honeybee larvae, the concentration in the guttation water is assumed to be 54% and 72% of the water solubility, respectively. The PEC values were calculated based on a water solubility of metconazole of 30.4 mg/L (see Volume 3 (AS), Section B.2.5). The calculated ETR values for the risk following exposure to guttation water are shown in Table B.9.6.1.3-3. As the ETR values are below the relevant trigger, the risk from exposure to guttation water is considered acceptable.

Table B.9.6.1.3-3: Risk to adult honeybees and honeybee larvae following the consumption of guttation water contaminated with metconazole following application of BAS 555 01 F in winter and spring cereals, and winter oilseed rape.

Type of assessment	Water consumption (µL)	PEC (µg/µL) [1)]	Endpoint	ETR	Trigger
Acute oral exposure adult bees	11.4	0.0304	85 µg a.s./bee	0.004	0.2
Chronic oral exposure adult bees	11.4	0.0164	50.0 µg a.s./bee/day	0.0037	0.03
Chronic oral exposure larvae	111	0.0219	12.5 µg a.s./ larvae	0.194	0.2

*[1)] based on a maximum water solubility of 30.4 mg/L for metconazole; **bold** values exceed the trigger, indicating a potential risk*

For the assessment of risk from exposure to surface water, it is suggested to use the initial PEC in surface water as calculated by FOCUS. For the present assessment, the available FOCUS Step 1 PEC_{SW} values will be used. The FOCUS Step 1 $PEC_{SW,max}$ for the proposed use in winter and spring cereals (i.e. 1-2 x 90 g a.s./ha) was calculated as 26.367 µg a.s./L, and covers the $PEC_{SW,max}$ for the proposed use in winter oilseed rape (see Section 0). As FOCUS Step 1 PEC_{SW} values are very worst case, a risk assessment based on these values is considered sufficiently protective. The calculated ETR values for the risk following exposure to surface water are shown in Table B.9.6.1.3-4. As the ETR values are well below the relevant trigger, the risk from exposure to surface water is considered acceptable.

Table B.9.6.1.3-4: Risk to adult honeybees and honeybee larvae following the consumption of surface water contaminated with metconazole following application of BAS 555 01 F in winter and spring cereals, and winter oilseed rape.

Type of assessment	Crop	Water consumption (µL)	PEC (µg/µL)	Endpoint	ETR	Trigger
Acute oral exposure adult bees	All proposed uses	11.4	26.367×10^{-6}	85 µg a.s./bee	3.54×10^{-6}	0.2
Chronic oral exposure adult bees	All proposed uses	11.4	26.367×10^{-6}	50.0 µg a.s./bee/day	6.01×10^{-6}	0.03
Chronic oral exposure larvae	All proposed uses	111	26.367×10^{-6}	12.5 µg a.s./ larvae	2.34×10^{-4}	0.2

bold *values exceed the trigger, indicating a potential risk*

Conclusion:

The acute and chronic risk to honeybees from the active substance metconazole and the formulation BAS 555 01 F is acceptable at the screening step for the proposed uses in winter and spring cereals and winter oilseed rape. The risk from consumption of contaminated water is considered acceptable.

B.9.6.1.4. Risk assessment for bumble bees according to EFSA (2013)

Note: The EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees)(2013) is currently not yet noted by the Standing Committee on Plants, Animals, Food and Feed. However, toxicity data for bumble bees are available. As these data are not considered in the currently notified Guidance Document (SANCO/10329/2002), a Tier 1 risk assessment according to EFSA (2013) is presented in this section, so that all available data on bees is taken into account in a risk assessment. As toxicity data for bumblebees are currently not required under legal aspects of the Uniform Principles, this assessment is included for information only, and will not be included in the List of Endpoints.

Exposure

BAS 555 01 F is applied by foliar application. For spray applications, bumblebees are considered to be exposed through a number of different exposure routes:

- Exposure via contact from spray deposits when bees are either foraging the treated crop, weeds in the field, plants in the field margin and the adjacent crop.
- Oral exposure through the consumption of nectar and pollen from the treated crop, weeds in the field, plants in the field margin, the adjacent crop, succeeding crop/permanent crop the following year.
- Oral exposure through the consumption of contaminated water (guttation water, surface water, water in puddles).

Regarding the exposure to contaminated water, it is currently not possible to quantify the level of exposure for non-*Apis* bees. Moreover, the very high level of water fluxes in honeybees at the colony level should be sufficiently protective for bumblebees. Therefore, the risk from exposure to contaminated water for bumblebees is covered by the assessment for honeybees (see Section B.9.6.1.3).

The critical use patterns relevant for the proposed uses of BAS 555 01 F are given in Table B.9.6.1.2-1, in Section B.9.6.1.2.

Risk assessment

For each of the exposure routes, a risk assessment is performed considering the acute risk to adult bumblebees, the chronic risk to adult bumblebees resulting from repeated exposure and the risk to bumblebee larvae. However, for metconazole only data on the acute toxicity of the active substance to bumblebees is available. Therefore, only the acute risk to adult bumblebees will be assessed here.

The following scenarios are considered in the risk assessment:

- Risk from foraging the treated crop
- Risk from foraging an adjacent crop
- Risk from foraging on weeds in the treated field
- Risk from foraging in the field margin
- Risk from foraging the following year on a permanent crop or on a succeeding crop for annual crops

The risk assessment starts with a screening step which is based on the worst case scenario (which for bumblebee adults is the treated crop scenario). If the screening step scenario fails, then all other scenarios

have to be assessed in the first tier unless it is justified that a specific scenario is not relevant because exposure is expected to be negligible.

Contact exposure assessment – screening step

According to the EFSA Guidance Document on bees, the hazard quotient (HQ) for contact exposure is calculated by the following equation at the screening step:

$$HQ = \frac{AR}{LD_{50\ contact}}$$

Where AR = application rate in g a.s./ha
$LD_{50,contact}$ is expressed in µg a.s./bee

The screening step assessment is based on exposure in the field. If the HQ is lower than the trigger of 7 (for downward spray) or 14 (for sideward spray), the risk is considered acceptable. If the HQ exceeds the trigger, a potential risk is identified and also all other scenarios need to be considered.

The calculated HQ values for the screening step are shown in Table B.9.6.1.4-1. As the HQ values are below the trigger for all of the proposed uses, the acute risk from contact exposure is considered acceptable.

Table B.9.6.1.4-1: Acute contact exposure of adult bumblebees to metconazole following the proposed uses of BAS 555 01 F – screening step.

Test substance	Crop	Application rate (g/ha)	LD_{50} (µg/bee)	HQ	Trigger value
Metconazole	Winter and spring cereals	90	> 100	< 0.90	7
	Winter oilseed rape	72	> 100	< 0.72	7

HQ values shown in bold exceed the trigger.

Oral exposure assessment – screening step

According to the EFSA Guidance Document on bees, the following formula should be used at the screening step to determine the Exposure Toxicity Ratio (ETR) for acute adult oral exposure for products applied by spray application. The application rate (for a single application) is multiplied by a shortcut value, which was derived considering information on feed (nectar and pollen) consumption and worst-case pesticide residue levels of feed items. The relevant shortcut values were derived from Table 3 of the EFSA Guidance Document. For the proposed uses in winter and spring cereals and winter oilseed rape, the shortcut values for downward application are used.

The *ETR for the acute adult oral exposure* is calculated by the following equation:

$$ETR_{acute\ adult\ oral} = \frac{AR \times SV}{LD_{50\ oral}}$$

Where: AR = application rate in kg a.s./ha
$LD_{50,oral}$ is expressed as µg a.s./bee

If this ETR > 0.036, a potential risk is identified, and a first tier risk assessment should be performed. If the ETR is below this trigger, the risk is acceptable.

Table B.9.6.1.4-2 shows the calculated ETR values for the oral exposure assessment for all proposed uses. The ETR values for acute oral exposure to adult bumblebees are below the trigger, indicating the risk can be considered acceptable.

Table B.9.6.1.4-2: Acute oral exposure of adult bumble bees to metconazole following the proposed uses of BAS 555 01 F – screening step.

Type of assessment	Test substance	Crop	Application rate (kg a.s./ha)	SV	Endpoint	ETR	Trigger value
Acute oral exposure adult bees	Metconazole	Winter and spring cereals	0.090	11.2	111.1 µg a.s./bee	0.009	0.036
		Winter oilseed rape	0.072	11.2		0.007	0.036

*[1)] maximum application rate in L/ha multiplied by the product density of 0.991 g/cm³; SV: Shortcut value; **bold** values exceed the trigger, indicating a potential risk.*

Conclusion:
The acute risk to adult bumble bees from the active substance metconazole and the formulation BAS 555 01 F is acceptable at the screening step for the proposed uses in winter and spring cereals and winter oilseed rape.

B.9.6.2. Risk assessment for arthropods other than bees

The risk assessment for effects on non-target terrestrial arthropods is conducted according to the **Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC** (SANCO/10329/2002). Reference is made to the **ESCORT II Guidance Document** (Candolfi *et al*., 2001)[39].

B.9.6.2.1. Toxicity

A number of laboratory and extended laboratory studies were conducted with several terrestrial arthropod species for the formulation BAS 555 01 F, which is the representative formulation for Annex I renewal of the active substance metconazole. Standard laboratory (Tier I) tests have been carried out with *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Chrysoperla carnea* and *Aleochara bilineata*, and extended laboratory (Tier II) tests were done with *Typhlodromus pyri* and *Aphidius rhopalosiphi*. These species are tested, in accordance with ESCORT 2 and ESCORT 3, as representative non-target arthropods since they have been found to be particularly sensitive species, and therefore can be considered as indicators of potential effects to the most sensitive arthropods in the field. The results from the available laboratory and extended laboratory studies are summarized in Table B.9.6.2.1-1.

Table B.9.6.2.1-1: Summary of arthropod toxicity data on metconazole in laboratory studies

Species	Test substance	Study type	Application rate [mL product /ha]	Corrected mortality [%]	Sublethal effects [%] 1)	Reference
Standard laboratory studies						
Typhlodromus pyri	BAS 555 01 F	7 days, laboratory test, artificial substrate, 2D exposure to nymphs	31 56 100 180 324 LR_{50} = 95 mL product/ha (=8.47 g a.s./ha) ER_{50} > 56 mL product/ha (> 5.0 g a.s./ha)	-8.0 30 60 66 100	15.0 18.1 -- -- --	CP10.3.2.1/01 Schwiening S. & Buetzler R., 2002
Aphidius rhopalosiphi	BAS 555 01 F	48h, laboratory test, artificial substrate, 2D exposure to adults	70 170 420 1000 2400 LR_{50} = 461 mL/ha (= 41.1 g a.s./ha) ER_{50} < 170 mL product/ha (< 15.2 g a.s./ha)	3.3 10 40 100 100	-- 56.0 62.6 -- --	CP10.3.2.1/02 Drexler A., 2002a

[39] *Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet M-C, Lewis G, Oomen PA, Schmuck R & Vogt H (2001). Guidance Document on Regulatory Testing and Risk Assessment Procedures for Plant Protection Products with Non-Target Arthropods. SETAC, Pensacola, USA. ISBN 1-880611-52-x. Results of the ESCORT II Workshop, Wageningen 21-23 March 2000.*

Species	Test substance	Study type	Application rate [mL product /ha]	Corrected mortality [%]	Sublethal effects [%] [1)]	Reference
Chrysoperla carnea	BAS 555 01 F	13-19 days, laboratory test, artificial substrate, 2D exposure to larvae	70 170 420 1000 2400 LR_{50} > 2400 mL product/ha (> 214.1 g a.s./ha) ER_{50} > 2400 mL product/ha (> 214.1 g a.s./ha)	-3.0 0.0 -3.0 9.1 48.5	-1.8 -2.7 -6.5 1.5 -3.4	CP10.3.2.1/03 Drexler A., 2002b
Aleochara bilineata	BAS 555 01 F	28 days, laboratory test, artificial substrate, 2D exposure to adults	2000 ER_{50} > 2000 mL product/ha (> 178.4 g a.s./ha)	n.d	-0.7	CP10.3.2.1/04 Buehler A., 2003
Extended laboratory studies						
Typhlodromus pyri	BAS 555 01 F	7 days, extended laboratory test, natural substrate, 2D exposure to nymphs	125 250 500 1000 2000 LR_{50} = 188 mL product/ha (= 16.8 g a.s./ha) ER_{50} > 125 mL product/ha (> 11.2 g a.s./ha)	17.6 81.3 100.0 100.0 100.0	9.5 -- -- -- --	CP10.3.2.2/01 Eden A., 2003
Typhlodromus pyri	BAS 555 01 F	7 days, Aged-residue design, natural substrate, 3D exposure to nymphs	**0 DAT:** 1000 2000 LR_{50} > 2000 L product/ha (> 177.8 g a.s./ha) ER_{50} > 2000 L product/ha (> 177.8 g a.s./ha) **3 DAT and 7 DAT:** 1000 2000	 17.6 14.3 n.d. [2)] n.d. [2)]	 7.6 -4.6 n.d. [2)] n.d. [2)]	CP10.3.2.2/02 Hanewald N. & Petrik-Steisslinger D., 2007
Aphidius rhopalosiphi	BAS 555 01 F	48h, extended laboratory test, natural substrate, 3D exposure to adults	500 800 1000 1600 2000 LR_{50} > 2000 mL product/ha (> 178.4 g a.s./ha) ER_{50} > 2000 mL product/ha (> 178.4 g a.s./ha)	0.0 0.0 0.0 0.0 0.0	20.4 8.9 34.4 6.8 11.0	CP10.3.2.2/03 Moll M. & Buetzler R., 2003

[1)] Positive values indicate a decrease in reproduction; negative values indicate an increase in reproduction.
[2)] Bioassays terminated and results not reported as no unacceptable effects were found in the 0 DAT bioassay
n.d. = not determined.

B.9.6.2.2. Exposure

Non-target arthropods living in the crop may be exposed to the formulation BAS 555 01 F by direct overspray during spray operations, or through contact with residues on plant and soil or in food items. The in-field and off-field exposure of non-target terrestrial arthropods to the formulation BAS 555 01 F following the proposed uses in winter and spring cereals and in winter oilseed rape were estimated as recommended in the ESCORT II Guidance Document. The critical use patterns relevant for the uses of BAS 555 01 F are given in Table B.9.6.2.2-1.

Table B.9.6.2.2-1: Use pattern of BAS 555 01 F for use as foliar spray

Crop	Application time (BBCH growth stage)	Max. number of applications	Interval between applications [d]	Application rate per treatment	
				Metconazole [kg a.s./ha]	BAS 555 01 F [L/ha]
Cereals (winter/spring)	30 - 69	1 - 2	21	0.090	1.0
Winter oilseed rape	13 – 20 (autumn) 21 – 71 (spring) [1)]	1 - 2	90	0.072	0.8
	21 – 71 (spring)	1 - 2	14	0.072	0.8

[1)] According to the GAP a first application in autumn (between BBCH 13 and BBCH 20) is followed by a second application in spring (between BBCH 21 and BBCH 71)

In the available plant metabolism studies performed in wheat, oilseed rape and peas, the **parent metconazole** was the major residue constituent in the vegetative plant parts (straw or foliage). In seeds, however, the **metabolite triazolylalanine** was the main component (see Volume 3 (AS), Section B.7.2.1). For wheat, the parent metconazole remained the major component in straw (up to 32% of the TRR). A large amount of the radioactive residues was further characterized as monohydroxylated metabolites. Each of these metabolites was however only found at low levels (below 10% TRR). In straw, a slight isomer conversion from *cis* to *trans* was observed. In wheat grain, the parent was extensively metabolised into mainly triazolylalanine (which accounted for 33% of the TRR) and to a lesser extent into triazolylacetic acid (which accounted for 9% of the TRR). For oilseed rape, the major compound in foliage was the unchanged parent, which accounted for 55-93% or 60-96% of the TRR at the various sampling intervals, depending on the radioactive label used. At harvest, the parent compound metconazole and the metabolite triazolylalanine were the predominant compounds recovered in oilseed rape seeds and accounted each for 24% and 40% of the TRR, respectively. Minor components including monohydroxylated derivates of the parent compound and its glucose conjugates were also characterized in foliage, pods and seeds, which were all found at low levels (below 10% TRR). For peas, the unchanged parent compound metaconazole was the most prominent component in foliage (67-96 %TRR) and straw (54-68 %TRR), whereas triazolylalanine was the main component in pea seeds (up to 85%TRR); metconazole was present at lower levels in seed (2.2-21 %TRR).

Overall, as no major metabolites of metconazole are present in the vegetative plant parts, exposure of non-target arthropods to metabolites can be considered minimal, and the risk low. Arthropods feeding on seeds will however potentially be exposed to the metabolite triazolylalanine. However, triazolylalanine is also a naturally occurring non-toxic component, formed as a biosynthetic product in higher plants (Ikegami et al., 1990). Therefore, the risk to non-target arthropods feeding on seeds can also be considered low. Consequently, the risk assessment will only be performed for the active substance metconazole and the formulation BAS 555 01 F..

In-field exposure:
Within ESCORT II, the in-field exposure for non-target terrestrial arthropods is calculated as a Predicted Environmental Residue (PER) using the equation presented below:

$$PER_{in\text{-}field} = \text{application rate (g/ha)} \times MAF$$

The Multiple Application Factor (MAF) is a generic value which is used to take into account the potential build-up of applied substances between applications, based on the number of applications, the application interval and the DT_{50} value. For the current risk assessment, the standard MAF values from Appendix V of the ESCORT II Guidance Document are used (see Table B.9.6.2.2-2).

The maximum predicted environmental residues (PER), occurring within the field after application of the formulation BAS 555 01 F at the maximum proposed application rate, are presented in Table B.9.6.2.2-2. The worst case PER value of 153 g a.s./ha will be used in the risk assessment.

Table B.9.6.2.2-2: In-field PER values for metconazole following application of the formulation BAS 555 01 F in winter and spring cereals and winter oilseed rape.

Crop	Application rate (g a.s./ha)	Number of applications	MAF	PER (g a.s./ha)
Winter and spring cereals	90	2	1.7	153
Winter oilseed rape	72	2	1.7	122.4

Off-field exposure:
The assessment of risk for areas immediately adjacent to the crop is considered important since these areas represent a natural reservoir for immigration, emigration and reproduction of arthropod populations and provide increased species diversity. Exposure of non-target arthropods living in off-field areas to metconazole following application of the formulation BAS 555 01 F will mainly be due to spray drift from field applications. Off-field PER values were calculated from in-field foliar PER values in conjunction with drift values published by the BBA (2000)[40] as shown in the following equation:

$$PER_{off\text{-}field} = \text{maximum } PER_{in\text{-}field} \times \frac{drift\ \ factor}{vegetation\ \ distribution\ \ factor}$$

Drift factor: According to recommendations of the FOCUS surface water group, the overall 90th percentile probability is assumed to be a reasonable worst case scenario for drift estimation from a single application of a plant protection product. For two applications, 82nd percentile values are considered a reasonable worst case scenario for drift estimation.

For the intended application of BAS 555 01 F in cereals and oilseed rape (2 applications), the drift factor is derived from the category 'field crops', with a drift value of 2.38% at 1 m.

Vegetation distribution factor: The model used to estimate spray drift was developed for drift onto a two-dimensional water surface, and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas. Therefore, for 2-dimensional studies (glass plate or leaf disc),

[40] *90th percentile drift according to BBA (2000). Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000). Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschützmitteln herangezogen werden.*

a vegetation distribution or dilution factor is incorporated into the equation when calculating $PER_{off\text{-}field}$ values. From the ESCORT II Guidance Document a dilution factor of 10 is recommended. For 3-dimensional studies (spray treatment is applied onto whole plants), the vegetation distribution factor of 10 is not appropriate, as any dilution over the 3-dimensional vegetation surface is already accounted for in the study design.

The maximum predicted environmental residues (PER) of metconazole, occurring in the field margin (off-field) after application of the formulation BAS 555 01 F at the maximum proposed application rate, are presented in Table B.9.6.2.2-3. The worst case off-field PER values of 0.36 g a.s./ha for 2D exposure and 3.64 g a.s./ha for 3D exposure will be used in the risk assessment.

Table B.9.6.2.2-3: Off-field foliar PER values for metconazole following the application of the formulation BAS 555 01 F in winter and spring cereals and winter oilseed rape.

Use Pattern	Application rate (g a.s./ha)	Distance from edge of the crop (m)	MAF	Drift factor % drift/100	Off-field PER (g a.s. /ha)	
					2D	3D
Winter and spring cereals	90	1	1.7	0.0238	**0.36**	**3.64**
Winter oilseed rape	72	1	1.7	0.0238	0.29	2.91

B.9.6.2.3. Risk assessment for in-field exposure

The risk to non-target terrestrial arthropods exposed in-field to metconazole following the use of the formulation BAS 555 01 F was assessed according to the ESCORT II Guidance Document.

First tier risk assessment

The potential risk to in-field non-target terrestrial arthropods was assessed by calculating the hazard quotient (HQ), based on the Predicted Environmental Residue $PER_{in\text{-}field}$ and the toxicity value expressed as lethal rate LR_{50}, according to the following equation:

$$in-field\ HQ = \frac{PER_{in-field}\ (g\ a.s./ha)}{LR_{50}\ (g\ a.s./ha)}$$

Hazard quotients in-field were calculated for metconazole following the use of BAS 555 01 F in winter and spring cereals, and winter oilseed rape, based on the worst case in-field PER values. These HQ values are shown in Table B.9.6.2.3-1 for the standard test species *Typhlodromus pyri* and *Aphidius rhopalosiphi*. According to the ESCORT II Guidance Document, the HQ trigger value for laboratory toxicity data (Tier I) is 2.

Table B.9.6.2.3-1: In-field risk to non-target terrestrial arthropods based on laboratory studies (Tier I) from exposure to metconazole following application of the formulation BAS 555 01 F in winter and spring cereals and winter oilseed rape.

Test substance	Test species	LR_{50} (g a.s./ha)	In-field PER (g a.s./ha)	HQ	Trigger value
BAS 555 01 F	*Typhlodromus pyri*	8.47	153	18.06	2
	Aphidius rhopalosiphi	41.1		3.72	

Based on the Tier 1 data, the in-field HQ values for the two standard test species *A. rhopalosiphi* and *T. pyri*, and the in-field soil HQ value for *T. pyri*, exceed the trigger of 2. This indicates a potential in-field risk of metconazole to non-target arthropods following the proposed uses of BAS 555 01 F. Further consideration is thus required.

Tier 2 risk assessment

According to ESCORT II, higher tier studies with *T. pyri* and *A. rhopalosiphi* , as well as testing of two additional species, are required if the HQ values based on Tier 1 studies exceed the trigger of 2. Extended laboratory studies on natural substrate have been submitted for *T. pyri*, *A. rhopalosiphi*. Further, standard laboratory studies with the additional species *Chrysoperla carnea* and *Aleochara bilineata* have been submitted. The results of these studies are summarized in Table B.9.6.2.3-2.

For extended laboratory studies, both lethal (mortality) as well as sublethal (reproduction) parameters should be used when evaluating the data. A trigger value for lethal or sublethal effects of 50% after exposure of the test organisms to residues of the plant protections product was suggested by ESCORT II. This means that the risk can be considered acceptable if the effect on mortality and/or reproduction will be below 50% at a rate equal to the $PER_{in\text{-}field}$.

Table B.9.6.2.3-2: In-field Tier II risk assessment for non-target arthropods

Study	Species	Endpoints [mL/ha]	$PER_{in\text{-}field}$ [g a.s./ha]	Trigger
CP10.3.2.2/01 Eden A., 2003	*Typhlodromus pyri*, extended laboratory test, natural substrate, 2D exposure	LR_{50} = 16.8 g a.s./ha No unacceptable effects on reproduction up to 11.2 g a.s./ha. (ER_{50} > 11.2 g a.s./ha)	153	Endpoint < PER → further testing necessary
CP10.3.2.2/02 Hanewald N. & Petrik-Steisslinger D., 2007	*Typhlodromus pyri*, aged residue design, natural substrate, 3D exposure	No unacceptable effects on total mortality and reproduction on DAT 0 for up to 177.8 g a.s./ha	153	Endpoint > PER → acceptable risk
CP10.3.2.2/03 Moll M. & Buetzler R., 2003	*Aphidius rhopalosiphi*, extended laboratory test, natural substrate, 3D exposure	LR_{50} > 178.4 g a.s./ha No unacceptable effects on reproduction up to 178.4 g a.s./ha	153	Endpoint > PER → acceptable risk
CP10.3.2.1/03 Drexler A., 2002b	*Chrysoperla carnea*, laboratory test, artificial substrate, 2D exposure	LR_{50} > 214.1 g a.s./ha No unacceptable effects on reproduction up to 214.1 g a.s./ha (ER_{50} > 214.1 g a.s./ha)	153	Endpoint > PER → acceptable risk
CP10.3.2.1/04 Buehler A., 2003	*Aleochara bilineata*, laboratory test, artificial substrate, 2D exposure	No unacceptable effects on reproduction up to 178.4 g a.s./ha (ER_{50} > 178.4 g a.s./ha)	153	Endpoint > PER → acceptable risk

PER = predicted environmental rate.

Typhlodromus pyri:
An extended laboratory study was carried out by exposing *T. pyri* to air-dried residues on leaves of bean plants (application on detached leaves, 2D exposure) (Eden A., 2003). Exposure of *T. pyri* on natural substrate leads to a clear reduction of effects compared to the exposure on inert substrate (i.e. glass). The study resulted in a LR_{50} of 16.8 g a.s./ha, reducing the acute toxicity by a factor of 1.97, compared to the exposure on inert substrate. No unacceptable effects on reproduction were observed at the application rates of up to and including 11.2 g a.s./ha. The rates at which no unacceptable effects on survival and reproduction were observed did not exceed the $PER_{in\text{-}field}$ of 153 g a.s./ha. Therefore, an additional test with this sensitive indicator species for a higher tier risk assessment was carried out.

The main criterion for the acceptability of effects on arthropods living in-field is defined as the potential for recovery of any affected population, i.e. demonstrating that residual toxicity declines sufficiently rapidly to allow recovery within one year. This is usually done by aged-residue trials exposing the arthropods to residues, which have been aged for increasing time periods. Thus, aged residue trials are used to show the acceptability of the effects by assessing the decline in residual toxicity of a product. In this case, *T. pyri* was used as bio-indicator because it proved to be the most sensitive species. According to ESCORT 2, the most sensitive test species identified by dose-response studies using natural substrate is selected for aged residue studies. It is anticipated that for less sensitive non-target arthropods the potential re-colonization will be faster.

The aged residue study available for *T. pyri* (Hanewald N. & Petrik-Steisslinger D., 2007) presents a more realistic exposure scenario compared to the extended laboratory study (Eden A., 2003). In the aged residue study, the application was done on whole bean plants, representing a 3-dimensional structure, whereas the application in the extended laboratory study was done on detached leaves, representing a 2-dimensional structure. In the aged residue study, two application rates (88.9 and 177.8 g a.s./ha) were tested. At a rate of 88.9 g a.s./ha, the initial bioassay (0 DAT) resulted in a corrected mortality of 17.6%; for 177.8 g a.s./ha the corrected mortality was 14.3%. Effects on reproduction amounted to 7.6% and -4.6%, respectively. Thus, even at DAT 0, no unacceptable effects are observed after exposure to up to and including 177.8 g a.s./ha, which is higher than the $PER_{in\text{-}field}$ of 153 g a.s./ha. Therefore, the risk to *T. pyri* resulting from exposure to metconazole following the intended use of BAS 555 01 F considering in-field habitats is considered acceptable.

Aphidius rhopalosiphi:
An extended laboratory study was performed by exposing *A. rhopalosiphi* to fresh dried residues on barley seedlings (application to whole plants, 3D exposure) (Moll M. & Buetzler R., 2003). No unacceptable effects on mortality and reproduction were observed up to and including 178.4 g a.s./ha, the highest rate tested. This rate exceeds the $PER_{in\text{-}field}$ of 153 g a.s./ha. Therefore, the risk to *A. rhopalosiphi* resulting from exposure to metconazole following the intended use of BAS 555 01 F considering in-field habitats is considered acceptable.

Chrysoperla carnea:
A standard laboratory study was performed by exposing *C. carnea* to fresh dried residues on glass plates (Drexler A., 2002b). No unacceptable effects on mortality and reproduction were observed up to and including 214.1 g a.s./ha, the highest rate tested. This rate exceeds the $PER_{in\text{-}field}$ of 153 g a.s./ha. Thus, the risk to *C. carnea* resulting from exposure to metconazole following the intended use of BAS 555 01 F considering in-field habitats is considered acceptable.

Aleochara bilineata:
A standard laboratory study was performed by exposing *A. bilineata* to fresh dried residues in quartz sand (Buehler A., 2003). No unacceptable effects on reproduction were observed up to and including 178.4 g a.s./ha, the highest rate tested. This rate exceeds the $PER_{in\text{-}field}$ of 153 g a.s./ha. Therefore, the risk to *A. bilineata* resulting from exposure to metconazole following the intended use of BAS 555 01 F considering in-field habitats is considered acceptable.

Conclusion

The in-field risk of metconazole to non-target terrestrial arthropods was not acceptable based on first tier laboratory studies with the standard test species *Typhlodromus pyri* and *Aphidius rhopalosiphi*. However, based on the available Tier 2 studies with the standard species and studies with two additional species, the in-field risk is considered acceptable for the proposed uses of BAS 555 01 F in winter and spring cereals and winter oilseed rape.

B.9.6.2.4. Risk assessment for off-field exposure

The risk to non-target terrestrial arthropods exposed off-field to metconazole following the use of the formulation BAS 555 01 F was assessed according to the ESCORT II Guidance Document.

First tier risk assessment

The potential risk of the formulation BAS 555 01 F to off-field non-target terrestrial arthropods was assessed by calculating the hazard quotient (HQ), based on the foliar Predicted Environmental Residue $PER_{off\text{-}field}$ and the toxicity value expressed as lethal rate LR_{50}, according to the following equation:

$$off-field\ HQ = \frac{PER_{off-field}(g\ a.s./ha)}{LR_{50}(g\ a.s./ha)} \times correction\ factor$$

Correction factor: The ESCORT II Guidance Document recommends that a correction factor of 10 is applied for laboratory (Tier I) data, to account for the uncertainty with the extrapolation from *Typhlodromus pyri* and *Aphidius rhopalosiphi* as indicator species, to the species diversity expected in off-crop areas.

Hazard quotients off-field were calculated for metconazole following the use of the formulation BAS 555 01 F in winter and spring cereals, and winter oilseed rape, based on the worst case off-field PER values. These HQ values are shown in Table B.9.6.2.4-1 for the standard test species *T. pyri* and *A. rhopalosiphi*. According to the ESCORT II Guidance Document, the HQ trigger value for laboratory toxicity data (Tier I) is 2.

Table B.9.6.2.4-1: Off-field risk to non-target terrestrial arthropods based on laboratory studies (Tier I) from exposure to metconazole following application of the formulation BAS 555 01 F in winter and spring cereals and winter oilseed rape.

Test substance	Test species	LR_{50} (g a.s./ha)	Off-field foliar			Trigger value
			Off-field PER (g a.s./ha)	Correction factor	HQ	
BAS 555 01 F	*Typhlodromus pyri*	8.47	0.36	10	0.430	2
	Aphidius rhopalosiphi	41.1			0.089	

Based on the Tier 1 data, the off-field HQ values for the two standard test species *A. rhopalosiphi* and *T. pyri* are below the trigger of 2. Therefore, the off-field risk of metconazole to non-target arthropods following the proposed uses of BAS 555 01 F can be considered acceptable.

Tier 2 risk assessment

In the Tier 1 assessment, both the off-field HQ values for the two standard test species *A. rhopalosiphi* and *T. pyri* are below the trigger of 2. Therefore, no further testing was necessary. However, as additional studies are available, a Tier 2 risk assessment is included below, for information.

Extended laboratory studies on natural substrate have been submitted for *T. pyri*, *A. rhopalosiphi*. Further, standard laboratory studies with the additional species *Chrysoperla carnea* and *Aleochara bilineata* have been submitted. The results of these studies are summarized in Table B.9.6.2.4-2.

It should be noted that for *T. pyri* also an aged residue study is available (CP10.3.2.2/02; Hanewald L & Petrik-Seisslinger D., 2007). This study is not included in Table B.9.6.2.4-2 because aged residue studies are not considered appropriate to address the off-field risk. In order to allow re-colonisation of the in-field, which is more realistic than real recovery of in-field populations, no effect should be allowed to occur in the off-field.

For extended laboratory studies, both lethal (mortality) as well as sublethal (reproduction) parameters should be used when evaluating the data. A trigger value for lethal or sublethal effects of 50% after exposure of the test organisms to residues of the plant protections product was suggested by ESCORT II. This means that the risk can be considered acceptable if the effect on mortality and/or reproduction will be below 50% at a rate equal to the $PER_{in\text{-}field}$. According to ESCORT 2, a correction factor of 5 should be applied to the $PER_{off\text{-}field}$ in the Tier 2 risk assessment, to account for the uncertainty with the extrapolation from *T. pyri* and *A. rhopalosiphi* as indicator species to the species diversity expected in off-crop areas.

In the extended laboratory tests with *A. rhopalosiphi* and *T. pyri*, and in the standard laboratory tests with *C. carnea* and *A. bilineata*, the application rate at which no effects > 50% on mortality and reproduction were observed exceeded the $PER_{off\text{-}field}$ multiplied by the correction factor (see Table B.9.6.2.4-2). This supports the conclusion of the Tier 1 assessment that the risk resulting from exposure to metconazole following the proposed uses of BAS 555 01 F considering off-field habitats can be considered acceptable.

Table B.9.6.2.4-2: Off-field Tier II risk assessment for non-target arthropods

Study	Species	Endpoints [g a.s./ha]	PER off-field [g a.s./ha]	Correction factor	Trigger
CP10.3.2.2/01 Eden A., 2003	*Typhlodromus pyri*, extended laboratory test, natural substrate, 2D exposure	LR_{50} = 16.8 g a.s./ha No unacceptable effects on reproduction up to 11.2 g a.s./ha. (ER_{50} > 11.2 g a.s./ha)	0.36	5	Endpoint > PER*correction factor → acceptable risk
CP10.3.2.2/03 Moll M. & Buetzler R., 2003	*Aphidius rhopalosiphi*, extended laboratory test, natural substrate, 3D exposure	LR_{50} > 178.4 g a.s./ha No unacceptable effects on reproduction up to 178.4 g a.s./ha 2000 mLproduct/ha	3.64	5	Endpoint > PER*correction factor → acceptable risk
CP10.3.2.1/03 Drexler A., 2002b	*Chrysoperla carnea*, laboratory test, artificial substrate, 2D exposure	LR_{50} > 214.1 g a.s./ha No unacceptable effects on reproduction up to 214.1 g a.s./ha (ER_{50} > 214.1 g a.s./ha)	0.36	5	Endpoint > PER*correction factor → acceptable risk
CP10.3.2.1/04 Buehler A., 2003	*Aleochara bilineata*, laboratory test, artificial substrate, 2D exposure	No unacceptable effects on reproduction up to 178.4 g a.s./ha (ER_{50} > 178.4 g a.s./ha)	0.36	5	Endpoint > PER*correction factor → acceptable risk

Conclusion

The off-field risk to non-target terrestrial arthropods, resulting from exposure to metconazole following the proposed uses of BAS 555 01 F in winter and spring cereals and winter oilseed rape, was acceptable based on first tier laboratory studies with the standard test species *Typhlodromus pyri* and *Aphidius rhopalosiphi*.

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

B.9.7.1. Earthworms

B.9.7.1.1. Earthworms – sub-lethal effects

A new study on the sub-lethal effects of the representative formulation BAS 555 01 F on earthworms has been submitted. In addition, a new laboratory study on the sub-lethal effects of the formulation BAS 555 00 F (an EC formulation containing 60 g/L of the active substance metconazole) on earthworms has been submitted. Although BAS 555 00 F has a different composition compared to BAS 555 01 F, the study with the former formulation is used to compare the laboratory toxicity of the two formulations. This comparison is used in an argumentation to demonstrate that a field study with BAS 555 00 F can be considered representative for the risk assessment for BAS 555 01 F (please refer to Section B.9.8.1 for details). The two available studies are summarized below.

Although no longer a data requiremend according to Regulation (EU) No. 284/2013, a new acute earthworm toxicity study with BAS 555 01 F has been submitted. A summary of this study is included in Section B.9.13.

Report:	**CP10.4.1.1/01. Friedrich S. (2012a)** **Sublethal toxicity of BAS 555 01 F to the earthworm *Eisenia fetida* in artificial soil with 5 % peat**
Report No.:	2012/1182245 (427950)
Guidelines:	OECD Guideline No. 222
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and methods:	
Test substance:	BAS 555 01 F (Batch No.: FRE-000698, contains 90.2 g/L of the active substance metconazole)
Test species:	earthworm (*Eisenia fetida*)
Number of organisms, age, weight:	8 replicates of 10 earthworms for the control and 4 replicates of 10 earthworms for each test concentration, adult worms (approximately 3 months old with clitellum), 270 – 428 mg/earthworm
Type of test:	56 days earthworm reproductive toxicity test
Applied concentrations:	
Nominal concentrations:	control (untreated); 48.7, 82.8, 140.8, 239.3 and 406.8 mg BAS 555 01 F/kg soil dw (nominally equivalent to 4.2, 7.1, 12.1, 20.6 and 35.0 mg a.s./kg soil dw)
Reference item:	Nutdazim 50 FLOW (Carbendazim, SC 500) was tested at test concentrations of 5 and 10 mg product/kg soil dw
Soil type:	artificial soil substrate, containing 5 % sphagnum peat, 20 % kaolin clay, 0.3 % calcium carbonate and 74.7 % industrial quartz sand
Food:	air-dried and finely ground horse manure
Test conditions:	temperature: 18.0 – 21.1 °C

pH: 6.20 – 6.27 (test start), 5.80 – 5.87 (test end)
water content: 24.9 – 25.0 % (equivalent to 58.3 – 58.5 % of WHC) at test start; 24.4 – 24.8 % (equivalent to 57.1 – 58.1 % of WHC) at test end
photoperiod: 16 hours light: 8 hours dark cycle
light intensity: 610 lux

Test procedure:
The test item is dispersible in water. Therefore, test solutions were made by dispersing weighed amounts of the test item in deionised water, immediately prior to application. The test item was dispersed in sufficient deionised water such that the addition of the test solutions to the test substrate resulted in a final water content of 40 – 60 % of WHC. The treated substrate was thoroughly mixed using a laboratory mixer immediately after application.
One day before test start, the dry artificial soil was pre-moistened by adding deionised water to obtain approximately half of the final water content. Earthworms were acclimatised in a separate batch of the artificial soil (mixed with horse manure) for approximately 24 hours before test start.
On the day of the test start, the test item was introduced by dispersing the quantity of test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40 – 60 % of its WHC. Each test vessel was then filled with the treated soil (750 g wet weight corresponding to 600 g dry weight). After a randomising procedure according to the worm fresh weight, selected groups of 10 worms were then randomly assigned to each treatment group. The individually weighed worms (10 worms/vessel) were placed on the surface of the soil. After approximately thirty minutes, the test vessels were closed with perforated transparent lids, which allowed gas exchange between substrate and atmosphere and access of light, but prevented worms from escaping. The test vessels were then set up at random in a controlled-environment test room. One day after application, initially 5 g air-dried and finely ground horse manure was scattered on the soil surface of each test vessel, which was sprinkled with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test. The weekly amount of manure (5 g) depended on the feeding activity.
After four weeks, the adult worms were removed from the test vessels. The number of surviving worms (adult mortality) and their biomass change were determined, behaviour (including feeding activity) and pathological symptoms were recorded. The adult worms were discarded after counting and weighing. Subsequently, the soil of each vessel was mixed carefully with 5 g manure. This was the last feeding occasion during experiment. The test was then continued for another four weeks. The final assessment included counting of surviving juveniles per test vessel, determination of the water content and pH measurements of the artificial soil. Juveniles were counted by manual inspection of the substrate.

Observations:
- At test start:
  - Individual fresh weight (mg/worm)
  - Behaviour of earthworms
  - Determination of physico-chemical parameters (water content, pH) of the artificial soil
- Weekly:
  - Observation of behavioural and pathological symptoms (including the feeding activity)
- 4 weeks after start of exposure:
  - Number of surviving adult earthworms per replicate
  - Observation of behavioural and pathological symptoms
  - Fresh weight of surviving adult earthworms per replicate
- 8 weeks after start of exposure:
  - Number of surviving juveniles per replicate

- Observation of behavioural and pathological symptoms (including morphological alterations)
- Determination of physico-chemical parameters (water content, pH) of the artificial soil

Calculation and Statistics:
The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between the test start and four weeks after treatment) and reproduction (the number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction, mortality and biomass were calculated.
The statistical analysis was performed with the software ToxRat Professional 2.10.06. The EC_x values (number of juveniles) were calculated by Probit analysis using linear max. likelihood regression. Confidence limits (95 %) of the EC_x values were computed by normal approximation. For identifying the NOEC values Fisher's Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Findings:
Mortality: Mortality rates of 0 % - 2.5 % were recorded in the test item treatment groups. 0 % mortality was observed in the control group. No statistically significant mortality compared to the control was observed at any test item concentration. No effects on behaviour (including feeding activity) of the worms were observed during the test.

Biomass: The test item caused no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group at any concentration tested.

Reproduction: Statistically significant effects on the number of juveniles compared to the control group were recorded at 406.8 mg BAS 555 01 F/kg soil dw.

Table B.9.7.1.1-1: Summary of developmental and reproductive effects of the formulation BAS 555 01 F on earthworms (*Eisenia fetida*) in a 56-day reproduction study

BAS 555 01 F (mg/kg soil dw)	control	48.7	82.8	140.8	239.3	406.8
Mean mortality (%) (day 28)	1.3	0.0	2.5	2.5	0.0	2.5
Mean biomass change (%) (day 28)	43.7	41.9	41.2	45.5	44.0	37.2
Mean number of juveniles (day 56)	92.9	97.8	100.0	83.5	86.5	58.0*
Reproduction (%) (day 56)	100	105	108	90	93	62
Endpoints (mg BAS 555 01 F/kg soil dw)						
NOEC (day 28)	406.8					
NOEC (day 56)	239.3					
EC_{50} (day 56)	> 406.8					

Mean mortality: Fisher's Exact Binomial Test with Bonferroni Correction for mortality, α = 0.05, one-sided greater; statistically not significant compared to the control
Mean biomass change: Williams-t-test, α = 0.05, one-sided smaller; statistically not significant compared to the control
Mean number of juveniles/adult: Williams-t-test for reproduction, α = 0.05, one-sided smaller; statistically significant compared to the control at the concentration 406.8 mg BAS 555 01 F/kg soil dw

Results of reference test:
In a separate study the reference item Nutdazim 50 FLOW (Carbendazim, SC 500) had a significant effect on biomass increase and reproduction of *Eisenia fetida*. The reproduction rate was clearly inhibited by 72.7 % and 98.8 % compared to the control at the tested concentrations of 5 and 10 mg product/kg soil dw.

Conclusions:
In a 56-day earthworm reproduction study with BAS 555 01 F, no adverse effects on survival and biomass development could be determined at concentrations up to and including 406.8 mg BAS 555 01 F/kg soil dw. Statistically significant effects on the number of juveniles compared to the control group were determined at the tested concentration of 406.8 mg BAS 555 01 F/kg soil dw. The NOEC for mortality and biomass was determined to be 406.8 mg BAS 555 01 F/kg soil dw, the highest concentration tested. The NOEC for reproduction was determined to be 239.3 mg BAS 555 01 F/kg soil dw.

RMS comments:
The validity criteria of OECD Test Guideline 222 are met:

- Each replicate (containing 10 adults) of the control produced ≥ 30 juveniles by the end of the test (measured: 92.9)
- The coefficient of variation of reproduction in the control was ≤ 30 % (measured: 20.6 %)
- Adult mortality over the initial 4 weeks of the test to be ≤ 10 % (measured: 1.3 %)

Therefore, this study is considered valid and acceptable for use in the risk assessment.

The following endpoints will be used in the risk assessment (endpoints expressed as active substance were calculated from the endpoint expressed in terms of formulation, taking into account a nominal content of 90 g a.s./L and the density of 1.046 g/cm³):

NOEC (*Eisenia fetida*, 28 day) mortality and biomass = 406.8 mg BAS 555 01 F/kg soil dw (equivalent to 35.0 mg a.s./kg soil dw)
NOEC (*Eisenia fetida*, 56 day) reproduction = 239.3 mg BAS 555 01 F/kg soil dw (equivalent to 20.6 mg a.s./kg soil dw)
EC_{50} (*Eisenia fetida*, 56 day) reproduction > 406.8 mg BAS 555 01 F/kg soil dw (equivalent to > 35.0 mg a.s./kg soil dw)

In accordance with the new data requirements (Commission Regulation EU No 284/2013), the EC_{10} and EC_{20} values should be calculated for chronic toxicity studies with earthworms. This was not addressed in the study report. However, as there were treatment related effects higher than 10% only at the highest dose tested, EC_{10} and EC_{20} values cannot reliably be determined from the available data.

Report:	**CP10.4.1.1/02. Friedrich S. (2012b)** **Sublethal toxicity of BAS 555 00 F to the earthworm *Eisenia fetida* in artificial soil with 5 % peat**

Report No.:	2012/1182243 (428918)
Guidelines:	OECD Guideline No. 222
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application

Materials and methods:

Test substance:	BAS 555 00 F (Batch No.: 0003255328, contains 60.1 g/L of the active substance metconazole)
Test species:	earthworm (*Eisenia fetida*)

Number of organisms, age, weight:	8 replicates of 10 earthworms for the control and 4 replicates of 10 earthworms for each test concentration, adult worms (approximately 3 months old with clitellum), 278 – 424 mg/earthworm
Type of test:	56 days earthworm reproductive toxicity test
Applied concentrations:	
Nominal concentrations:	control (untreated); 62.1, 105.6, 179.4, 305.0 and 518.6 mg BAS 555 00 F/kg soil dw (nominally equivalent to 4.2, 7.1, 12.1, 20.6 and 35.0 mg a.s./kg soil dw)
Reference item:	Nutdazim 50 FLOW (Carbendazim, SC 500) was tested at test concentrations of 5 and 10 mg product/kg soil dw
Soil type:	artificial soil substrate, containing 5 % sphagnum peat, 20 % kaolin clay, 0.3 % calcium carbonate and 74.7 % industrial quartz sand
Food:	air-dried and finely ground horse manure
Test conditions:	temperature: 18.5 – 21.4 °C
	pH: 6.16 – 6.22 (test start), 5.74 – 5.95 (test end)
	water content: 24.9 – 25.0 % (equivalent to 58.3 – 58.5 % of WHC) at test start; 24.3 – 24.7 % (equivalent to 56.9 – 57.8 % of WHC) at test end
	photoperiod: 16 hours light: 8 hours dark cycle
	light intensity: 640 lux

Test procedure:

The test item is dispersible in water. Therefore, test solutions were made by dispersing weighed amounts of the test item in deionised water, immediately prior to application. The test item was dispersed in sufficient deionised water such that the addition of the test solutions to the test substrate resulted in a final water content of 40 – 60 % of WHC. The treated substrate was thoroughly mixed using a laboratory mixer immediately after application.

One day before test start, the dry artificial soil was pre-moistened by adding deionised water to obtain approximately half of the final water content. Earthworms were acclimatised in a separate batch of the artificial soil (mixed with horse manure) for approximately 24 hours before test start.

On the day of the test start, the test item was introduced by dispersing the quantity of test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40 – 60 % of its WHC. Each test vessel was then filled with the treated soil (750 g wet weight corresponding to 600 g dry weight). After a randomising procedure according to the worm fresh weight, selected groups of 10 worms were then randomly assigned to each treatment group. The individually weighed worms (10 worms/vessel) were placed on the surface of the soil. After approximately thirty minutes, the test vessels were closed with perforated transparent lids, which allowed gas exchange between substrate and atmosphere and access of light, but prevented worms from escaping. The test vessels were then set up at random in a controlled-environment test room. One day after application, initially 5 g air-dried and finely ground horse manure was scattered on the soil surface of each test vessel, which was sprinkled with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test. The weekly amount of manure (5 g) depended on the feeding activity.

After four weeks, the adult worms were removed from the test vessels. The number of surviving worms (adult mortality) and their biomass change were determined, behaviour (including feeding activity) and pathological symptoms were recorded. The adult worms were discarded after counting and weighing. Subsequently, the soil of each vessel was mixed carefully with 5 g manure. This was the last feeding occasion during experiment. The test was then continued for another four weeks. The final assessment included counting of surviving juveniles per test vessel, determination of the water content and pH measurements of the artificial soil. Juveniles were counted by manual inspection of the substrate.

Observations:

- At test start:
  - Individual fresh weight (mg/worm)
  - Behaviour of earthworms
  - Determination of physico-chemical parameters (water content, pH) of the artificial soil
- Weekly:
  - Observation of behavioural and pathological symptoms (including the feeding activity)
- 4 weeks after start of exposure:
  - Number of surviving adult earthworms per replicate
  - Observation of behavioural and pathological symptoms
  - Fresh weight of surviving adult earthworms per replicate
- 8 weeks after start of exposure:
  - Number of surviving juveniles per replicate
  - Observation of behavioural and pathological symptoms (including morphological alterations)
  - Determination of physico-chemical parameters (water content, pH) of the artificial soil

Calculation and Statistics:

The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between the test start and four weeks after treatment) and reproduction (the number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction and biomass were calculated. The statistical analysis was performed with the software ToxRat Professional 2.10.05.

Fisher's Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Findings:

Mortality: With the exception of 2.5 % mortality at concentration of 105.6 mg BAS 555 00 F/kg soil dw, no mortality was observed in any other test item treatment group and in the control group. No statistically significant mortality compared to the control was observed at any test item concentration. No effects on behaviour (including feeding activity) of the worms were observed during the test.

Biomass: The test item caused no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control treatment.

Reproduction: Statistically significant effects on number of juveniles compared to the control group were recorded at a concentration of 518.6 mg BAS 555 00 F/kg soil dw.

Table B.9.7.1.1-2: Summary of developmental and reproductive effects of the formulation BAS 555 00 F on earthworms (*Eisenia fetida*) in a 56-day reproduction study

BAS 555 00 F (mg/kg soil dw)	control	62.1	105.6	179.4	305.0	518.6
Mean mortality (%) (day 28)	0	0	2.5	0	0	0
Mean biomass change (%) (day 28)	32.6	33.4	34.1	29.7	36.4	32.0
Mean number of juveniles (day 56)	82.0	83.5	77.3	77.8	79.3	68.5*
Reproduction (%) (day 56)	100	102	94	95	97	67
Endpoints (mg BAS 555 00 F/kg soil dw)						
NOEC (day 28)	518.6					
NOEC (day 56)	305.0					
EC_{50} (day 56)	> 518.6					

Mean mortality: Fisher's Exact Binomial Test with Bonferroni Correction for mortality, α = 0.05, one-sided greater; statistically not significant compared to the control
Mean biomass change: Williams-t-test, α = 0.05, one-sided smaller; statistically not significant compared to the control
Mean number of juveniles/adult: Williams-t-test for reproduction, α = 0.05, one-sided smaller; statistically significant compared to the control at the concentration 518.6 mg BAS 555 00 F/kg soil dw

Results of reference test:
In a separate study the reference item Nutdazim 50 FLOW (Carbendazim, SC 500) had a significant effect on biomass increase and reproduction of *Eisenia fetida*. The reproduction rate was clearly inhibited by 72.7 % and 98.8 % compared to the control at the tested concentrations of 5 and 10 mg product/kg soil dw.

Conclusions:
In a 56-day earthworm reproduction study with BAS 555 00 F, no adverse effects on survival and biomass development could be determined at concentrations up to and including 518.6 mg BAS 555 00 F/kg soil dw. Statistically significant effects on the number of juveniles compared to the control group were determined at the tested concentration of 518.6 mg BAS 555 00 F/kg soil dw. The NOEC for mortality and biomass was determined to be 518.6 mg BAS 555 00 F/kg soil dw, the highest concentration tested. The NOEC for reproduction was determined to be 305.0 mg BAS 555 00 F/kg soil dw.

RMS comments:
The validity criteria of OECD Test Guideline 222 are met:

- Each replicate (containing 10 adults) of the control produced ≥ 30 juveniles by the end of the test (measured: 82.0)
- The coefficient of variation of reproduction in the control was ≤ 30 % (measured: 12.7 %)
- Adult mortality over the initial 4 weeks of the test to be ≤ 10 % (measured: 0 %)

Therefore, this study is considered valid and acceptable for use in the risk assessment.

The following endpoints will be used in the risk assessment (endpoints expressed as active substance were calculated from the endpoint expressed in terms of formulation, taking into account a nominal content of 60 g a.s./L and the density of 0.889 g/cm³):

NOEC (*Eisenia fetida*, 28 day) mortality and biomass = 518.6 mg BAS 555 00 F/kg soil dw (equivalent to 35.0 mg a.s./kg soil dw)
NOEC (*Eisenia fetida*, 56 day) reproduction = 305.0 mg BAS 555 00 F/kg soil dw (equivalent to 20.6 mg a.s./kg soil dw)
EC_{50} (*Eisenia fetida*, 56 day) reproduction > 518.6 mg BAS 555 00 F/kg soil dw (equivalent to > 35.0 mg a.s./kg soil dw)

In accordance with the new data requirements (Commission Regulation EU No 284/2013), the EC_{10} and EC_{20} values should be calculated for chronic toxicity studies with earthworms. This was not addressed in the study report. However, as there were treatment related effects higher than 10% only at the highest dose tested, EC_{10} and EC_{20} values cannot reliably be determined from the available data.

B.9.7.1.2. Earthworms – field studies

No earthworm field studies with the representative formulation BAS 555 01 F are available. Instead, a field study with the formulation BAS 555 00 F (an EC formulation containing 60 g/L of the active substance metconazole) has been submitted. Although performed with another formulation, this field study is considered representative for the risk assessment for BAS 555 01 F (please refer to Section B.9.8.1 for a detailed argumentation regarding the representativeness of this study). This study is summarized below.

Report:	**CA10.4.1.2/01. Luehrs U. (2003)** **Field study to evaluate the effects of BAS 555 00 F on earthworms**
Report No.:	2003/1012039
Guidelines:	ISO/CD 11268-3 (1999)
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application

Objective:
The objective of this field study was to investigate possible side-effects of BAS 555 00 F on populations of earthworms in the field. Therefore, a field experiment lasting about one year was performed and the effects of the test item with regard to species composition, biomass and abundance were compared to an untreated control and a reference item.

Materials and methods:

Test substance:	BAS 555 00 F (Batch No.: 162940, contains 58.2 g/L of the active substance metconazole)
Test species:	Naturally occurring population of earthworms comprising all mobile stages (juveniles and adult worms) including endogeic species such as *Aporrectodea caliginosa*, *Aporrectodea limicola*, *Aporrectodea longa*, *Aporrectodea rosea*, *Octolasion tyrtaeum* and *Allolobophora chlorotica* and epigeic species such as *Lumbricis castaneus*, *Lumbricus rubellus* and *Lumbricus terrestris*.
Type of test:	Earthworm field test
Test site:	Arable field site cultivated with summer barley (variety 'Alexis') in Rossdorf, Darmstadt-Dieburg, Germany. Total size: about 2880 m². The selected area did not receive an application of any fertilizer or crop protection product during the study apart from the test item and the reference item, including the control plots.
Applied concentrations:	Untreated control (tap water); BAS 555 00 F: 3 L/ha (equivalent to 180 g a.s./ha); Reference item: DuPont Benlate (50% Benomil), applied at

	8 kg form./ha (equivalent to 4 kg a.s./ha). All treatments were applied at an application volume of 400 L water/ha
Test conditions:	Natural field conditions. During the whole experiment the climatic conditions (e.g. temperature, relative humidity, rain) were recorded. On each earthworm sampling occasion, soil moisture and soil temperature were observed.
	Air temperature and relative humidity during the day of application: 20.6-23.9°C, 42.6-50.9%; precipitation: no rainfall on the application day. Mean soil temperature on the day of application: 15.9-16.0°C.
Soil properties:	sandy loamy silt to silty loam (27.8% sand, 57.6% silt, 14.6% clay), pH 7.5-7.6, 1.07% total organic carbon, max. water holding capacity 22.6%.

Test design:
The test consisted of 3 treatment groups arranged in a randomised complete block design. The study area of 2880 m² was subdivided in 12 experimental plots of 125 m² (10 x 12.5 m), with 2 m distance between the plots and approximately 5m distance to the fields next to the site.
The test consisted of one pre-application sampling (pre-sampling), one application, the surface monitoring and three post-applications samplings. The experimental time schedule was the following:

- pre-sampling on 29.04.2002 (35 days before application)
- application on 03.06.2002
- earthworm surface monitoring on 04.06 – 08.06.2002 (one – four days after application)
- 1st sampling on 01.07.2002 (about 1 month after application)
- 2nd sampling on 30.09.2002 (about 4 months after application)
- 3rd sampling on 21.05.2003 (about 1 year after application)

All treatments were applied with a movable plot sprayer for field application (type “PSG-system 2”; Fa. Schachtner Gerätetchnik, Germany), with an extension tube including 4 spraying nozzles (distance of nozzles of 0.5m).

On August 27 2002, the summer barley was cut and left in the field to mimic the harvest of the crop. The weather conditions in the field were too wet for sowing a new crop in October 2002, so the field was left untreated until the third sampling in May 2003. However, germinating summer barley ensured the soil was covered with plants.

Earthworm surface monitoring consisted of an assessment of alive, moribund and dead earthworms on the soil surface after application. For sampling of earthworms present in the soil (for population sampling), four samples were collected from all plots at each sample collection date. Each of the four samples was collected on a 0.25 m² sample area, at a distance of at least 2 m from each other. Sample places were marked after sampling and were not used for futher sampling.

Earthworm extraction was achieved by using the electrical octet method combined with hand selection. Collected, living earthworms were stored cool in moistened, separate plastic ice-boxes. Within one day after sampling adult earthworms were weighed individually and the species was determined using the glass-tube method for livind earthworms. Juvenile earthworms were determined if possible, but were at least separated into tanylobous and epilobous species and were weighed in groups for each sample.

Statistics: ANOVA, followed by Student-t-test for the mean number and weights of earthworms ($\alpha = 0.05$)

Findings:

Total Earthworm Abundance:

The results for total earthworm abundance are summarized in Table B.9.7.1.2-1. Compared to the control, the total earthworm abundance in the BAS 555 00 F groups was statistically significantly reduced one and four months after the application (to 67.6% and 74.5% of the control values, respectively; see Table B.9.7.1.2-1). One year after application the earthworm abundance in the BAS 555 00 F group reached a value of 81.5% of the control, which was a reduction less than 30% and was not statistically significantly different compared to the control (Student t-test, $\alpha = 0.05$). Therefore recovery was considered sufficient.

The earthworm abundance in the toxic standard treatment was statistically significantly reduced to 39.7% of the control at the second sampling.

Table B.9.7.1.2-1: Summary of total earthworm abundance in a field study with BAS 555 00 F

Treatment group	Pre-sampling 29.04.2002		First sampling 01.07.2002		Second sampling 30.09.2002		Third sampling 21.05.2003	
	Ind./m²	% of control	Ind./m²	% of control	Ind./m²	% of control	Ind./m²	% of control
Untreated control	68.0	100	34.0	100	144.3	100	110.8	100
BAS 555 00 F	87.8	99.6	23.0	67.6*	107.5	74.5*	90.3	81.5
Toxic standard	62.0	91.2	13.5	37.9*	90.0	62.4*	83.8	75.6

*Mean values of four replicates; * significantly different compared to the control. Student-t test, $\alpha = 0.05$.*

Earthworm Biomass:

The results for total earthworm biomass are summarized in Table B.9.7.1.2-2. Earthworm biomass in the groups treated with BAS 555 00 F showed a similar pattern of effect as for abundance. At the first sampling in July 2002, the biomass in the BAS 555 00 F group decreased t0 54.4% of the control values. This decrease was stronger thant the decrease of earthworm abundance, which was reduced to only 67.6% of the control. However, biomass at the first sampling was not statistically significantly different copared to the control. At the second sampling in September 2002, biomass was less affected in the BAS 555 00 F treatment group, with 90.3% of the control values. One year after the application, biomass values in the test item group increased to 94.2% of the control, continuously minimizing the difference to the control and showing no statistical significance throughout the test period (Student t-test, $\alpha = 0.05$). Therefore, the BAS 555 00 F treatment is not considered to show long-term effects on earthworm biomass.

Table B.9.7.1.2-2: Summary of total earthworm biomass in a field study with BAS 555 00 F

Treatment group	Pre-sampling 29.04.2002		First sampling 01.07.2002		Second sampling 30.09.2002		Third sampling 21.05.2003	
	g/m²	% of control	g/m²	% of control	g/m²	% of control	g/m²	% of control
Untreated control	36.2	100	8.4	100	45.2	100	39.3	100
BAS 555 00 F	41.9	115.7	4.6	54.4	40.8	90.3	37.0	94.2
Toxic standard	32.5	89.8	4.1	49.1*	32.3	71.5*	45.5	115.8

*Mean values of four replicates; * significantly different compared to the control. Student-t test, $\alpha = 0.05$.*

In the toxic standard group the biomass was significantly different compared to the control at the first and second sampling (49.1% and 71.5% of control values, respectively). At the third sampling,

approximately 1 year after application, the biomass was no longer statistically significantly different compared to the control.

Earthworm species abundance:
The collected earthworms belonged to six epilobous and three tanylobous species. The most abundant species at test start was *Aporrectodea caliginosa* with 15.8 ind./m² or 58.6% of determined earthworms (mean over all treatment groups), followed by *Allelobophora chlorotica* (6.2 ind./m² or 22.8 of determined earthworms) and *Octolasium tyraeum* (3.5 ind./m² or 13.0% of determined earthworms). The results for the abundance of these three species are shown in Table B.9.7.1.2-3.

The numbers of all species were reduced at the first sampling in all treatment groups due to naturally occurring fluctuations. For the abundance of *Aporrectodea caliginosa* the abundance values were comparable to the control at the first and second sampling. At the third sampling, the abundance of *A. caliginosa* was slightly reduced compared to the control, but the number of individuals sampled in both the control and treatment was considered to be too low to perform a definite assessment. *Allolobophora chlorotica* was found in numbers comparable to the control at all sampling dates. *Octolasium tyrtaeum* was found in numbers slightly lower than in the control at the first and second sampling date and showed a value slightly higher than in the control at the third sampling date. None of the species abundances in the test item treated group was statistically significantly different compared to the control (Student t-test, $\alpha = 0.05$), and the BAS 555 00 F treatment is therefore not considered to show a long term effect on any of these three species.

Table B.9.7.1.2-3: Abundance of *Aporrectodea caliginosa*, *Allolobophora chlorotica* and *Octolasium tyrtaeum* in a field study with BAS 555 00 F

Treatment group	Pre-sampling 29.04.2002		First sampling 01.07.2002		Second sampling 30.09.2002		Third sampling 21.05.2003	
	Ind./m²	% of total	Ind./m²	% of total	Ind./m²	% of total	Ind./m²	% of total
Aporrectodea caliginosa								
Untreated control	18.8	62.0	3.0	38.7	22.8	69.5	4.5	24.3
BAS 555 00 F	17.3	59.5	3.5	45.2	19.5	70.9	2.3	11.4
Toxic standard	11.5	52.9	2.0	50.0	20.8	70.3	8.8	32.7*
Allolobophora chlorotica								
Untreated control	7.5	24.8	2.0	25.8	2.5	7.6	3.3	17.6
BAS 555 00 F	5.0	17.2	3.0	38.7	1.5	5.5	4.8	24.1
Toxic standard	6.0	27.6	0.8	18.8	4.0	13.6	5.0	18.7
Octolasium tyrtaeum								
Untreated control	2.8	9.1	2.5	32.3	5.5	16.8	6.3	33.8
BAS 555 00 F	4.8	16.4	1.3	16.1	3.5	12.7	7.5	38.0
Toxic standard	3.0	13.8	1.0	25.0	3.0	10.2	7.5	28.0

*Mean values of four replicates; * significantly different compared to the control. Student-t test, α = 0.05.*

Juvenile Earthworms:
The abundance values and the ratio of juvenile earthworms are shown in Table B.9.7.1.2-4. At the beginning of the study 55.5% to 64.9% of the total number of earthworms were juveniles (mean over all treatment groups 59.0%). The statistical analysis performed on the total number of juveniles of each sample of the pre-sampling did not show statistically significant differences between the treatment groups and the control (Student t-test, $\alpha = 0.05$).

At the first and second sampling, the total number of juveniles in the test item treated group was lower than in the control. Both values were statistically significantly different compared to the control

(Studtent t-test, α = 0.05). At the third sampling, the total number of juveniles in the test item treated group was still significantly lower than in the control. However, the ratio between the adult and juvenile worms was 22%:78% in the test item group, compared to 17%:83% in the control, which are comparable values. The statistical analysis performed on the ratio of juveniles in each sample did not show any statistically significant differences between test item treatment and control (Student t-test, α = 0.05).

In the toxic standard group, the number of juveniles was statistically significantly lower compared to the control at the first, second and third sampling (Student t-test, α = 0.05).

Table B.9.7.1.2-4: Abundance of juvenile earthworms in a field study with BAS 555 00 F

Treatment groups	Adults		Epilobous juveniles		Tanylobous juveniles		Total juveniles	
	Ind./m²	% of total	Ind./m²	% of total	Ind./m²	% of total	Ind./m²	% of total
Pre-sampling 29.04.2002								
Untreated control	30.3	44.5	19.0	27.9	18.8	27.6	37.8	55.5
BAS 555 00 F	29.0	42.8	20.5	30.3	18.3	26.9	38.8	57.2
Toxic standard	21.8	35.1	22.3	35.9	18.0	29.0	40.3	64.9
First sampling 01.07.2002								
Untreated control	7.8	22.8	20.0	58.8	6.3	18.4	26.3	77.2
BAS 555 00 F	7.8	33.7	11.3	48.9	4.0	17.4	15.3*	66.3
Toxic standard	4.0	29.6	7.0	51.9	2.5	18.5	9.5*	70.4
Second sampling 30.09.2002								
Untreated control	32.8	22.7	99.8	69.2	11.8	8.1	111.5	77.3
BAS 555 00 F	27.5	25.6	64.8	60.2	15.3	14.2	80.0*	74.4
Toxic standard	29.5	32.8	50.3	55.8	10.3	11.4	60.5*	67.2
Third sampling 21.05.2003								
Untreated control	18.5	16.7	76.3	68.8	16.0	14.4	92.3	83.3
BAS 555 00 F	19.8	21.9	63.3	70.1	7.3	8.0	70.5*	78.1
Toxic standard	26.8	31.9	42.8	51.0	14.3	17.0	57.0*	68.1

*Mean values of four replicates; Statistical analysis performed on numbers of juvenile worms; * significantly different compared to the control. Student-t test, α = 0.05.*

Conclusions:

The results of a field study on the effects of BAS 555 00 F showed an initial reduction in earthworm abundance, but recovery to 81.5% of the control was reached within one year. Earthworm biomass showed no statistically significant reduction at any sampling point. Similarly, no significant effects were seen on the abundance of the three main species, *Aporrectodea caliginosa, Allolobophora chlorotica* and *Octolasium tyrtaeum*. The numbers of juveniles were statistically reduced at each sampling date, but at the third sampling (1 year after application) only by 23.6%. However, the ratio between juveniles and adult earthworms was approximately on the same level.

RMS comments:

The study was well performed and reported, and in line with the recommendations of ISO/CD 11268-3. The validity criteria from this Test Guideline were met:

- Earthworm abundance of the test field was on average ≥ 60/m² at test initiation (pre-sampling) (measured: 65.9 individuals/m²)
- Three representative species were present at the field site in a sufficient number (> 10% of total earthworms): the endogeic species *Aporrectodea caliginosa* (58.6% of determined species), *Allolobophora chloritica* (22.8% of determined species) and *Octolasium tyrtaeum* (13% of determined species)

- There was a significant reduction of at least 50% in the earthworms abundance and/or biomass by the reference item (measured: total earthworm abundance was statistically significantly reduced by 60.3% at the 2nd sampling, about 4 months after application, respectively)

Further, an analysis of the reliability of the study according to de Jong *et al.* (2006)[41] was made, which is summarized in the table below. Based on this assessment, the present study is assigned a Reliability Index (Ri) of 2. Consequently, this study is considered acceptable for use in the risk assessment.

Table B.9.7.1.2-5: Reliability assessment of the earthworm field study with BAS 555 00 F according to de Jong *et al.* (2006).

Test item	Assessment	Reliability lower ?
DESCRIPTION		
1. substance	The test substance was properly characterised and reported (e.g. identity of the formulation used, quantity applied, …)	No
2. Test site	The location, field history, soil type, crop and general climatic conditions are reported in sufficient detail	No
3. Application	The mode of application, dosage, application scheme and weather conditions before, during and after applications are sufficiently reported in the study report.	No
4. Test design	The study was performed according to ISO 11268-3. It is noted that this study was conducted before the update of this guideline in 2014, and that it thus could slightly deviate from the most recent version of this guideline.	No
5. Biological system	A sufficient number of individuals, both adult and juveniles, were present, in line with the recommendations of the test guideline. Further, three species were present in numbers exceeding 10% of the total earthworms present. It is however noted that all three dominant species are endogeic species, while anecic species are not present in number exceeding 10%	Yes → Ri2
6. Sampling	The biological sampling method used (electrical extraction) is considered appropriate, and had a sufficiently high efficiency (> 60%). Further, the climatic conditions were monitored, together wot other relevant properties (e.g. additional pesticide treatment). However, there was no analysis of the actual concentration in soil. Further, the pre-treatment sampling was performed 35 days (> 2 weeks) before treatment	Yes → Ri2
RESULTS		
7. Application	No extreme weather conditions were registered. The conditions of the application were as expected. As already noted above, no analysis of the test item in soil was performed.	Yes → Ri2
8. Endpoint	The results were reported in sufficient detail (all raw data is available in the study report) to enable verification of the endpoint: the earthworm species present are listed, together with the aggregations made. There was no significant pre-treatment variation. Further, a sufficiently high number of individuals was recorded in the negative control to enable analysis in most cases. Clear effects were observed in the positive control.	No
9. Elaboration of results	The statistical comparison was performed using a suitable method. Results are presented in sufficient detail (both tables and graphical presentation, for absolute and relative data).	No

[41] De Jong FMW, van Beelen P, Smit CE & Montforts MHMM (2006) Guidance for summarising earthworm field studies. National Institute for Public Health and the Environment, the Netherlands. RIVM report number 601506006/2006.

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

New chronic toxicity studies with either *Hypoaspis aculeifer* or *Folsomia candida* for the representative formulation BAS 555 01 F have been submitted. A summary of the available studies is provided below.

Report:	**CP10.4.2.1/01. Friedrich S. (2012c)** **Effects of BAS 555 01 F on the reproduction of the collembolan *Folsomia candida*.**
Report No.:	2012/1182246 (427951)
Guidelines:	OECD Guideline No. 232, ISO 11267
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and Methods:	
Test substance:	BAS 555 01 F (Batch No.: FRE-000698, contains 90.2 g/L of the active substance metconazole)
Test species:	*Folsomia candida* (springtail)
Number of organisms, age:	8 replicates for the control, 4 replicates per treatment, 10 springtails per replicate, 9 – 12 days old juveniles
Type of test:	28 days reproductive toxicity test
Applied concentrations:	
Nominal concentrations:	untreated control (deionized water); 31.25, 62.5, 125, 250 and 500 mg BAS 555 01 F/kg soil (nominally equivalent to 2.69, 5.38, 10.76, 21.51 and 43.02 mg a.s./kg soil dw)
Reference item:	Boric acid (100 %), the reference item was tested in a separate study
Soil type:	artificial soil substrate, containing 5 % sphagnum peat, 20 % kaolin clay, 0.3 % calcium carbonate and 74.7 % industrial quartz sand
Food:	approximately 2 mg granulated dry yeast at the start of the test and after 14 days
Test conditions:	temperature: 18.4 – 21.4 °C pH: 6.10 – 6.23 (at test start), 5.99 – 6.05 (at test end) water content: 24.9 – 25.0 % (58.3 – 58.5 % of maximum WHC) at test start; 24.4 – 24.8 % (57.1 – 58.1 % of maximum WHC) at test end (maximum water holding capacity: 42.7 % of the dry weight) photoperiod: 16 hours light : 8 hours dark cycle light intensity: 620 lux

Test procedure:
An exactly weighed amount of the test item was dispersed in deionized water to make a stock solution, without addition of solubility mediators, immediately before application. This stock solution was diluted. Each test item dispersion was thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final water content of 40 – 60 % WHC.
Two days before the start of the test, the dry artificial soil was pre-moistened by adding deionized water to obtain approximately half of the final water content. On the day of the test start, the test item was introduced by dispersing the quantity of test item required to obtain the desired test concentration in the

volume of water required to hydrate the soil to 40 – 60 % of its WHC. The control substrate contained the corresponding amount of deionized water only. After thorough mixing, 30 g (wet weight) of the test substrate was placed into each vessel, avoiding compression.
After addition of the test organisms, the test vessels were positioned randomly in a controlled-environment test room, and these positions were re-randomized weekly. The test containers were tightly covered with a glass lid and briefly opened twice a week for aeration. The pH and water content of the test substrate were determined at the start and at the end of the test. The water content was checked weekly by reweighing the additional test vessels. Water loss was compensated if exceeding 2 % of the initial water content.
Four weeks after introducing the test organisms, the parental and juvenile collembolans in the test and control vessels were counted. The test substrate of each replicate was poured into an individual 150 – 200 mL container and the test organisms were floated off the substrate by the addition of water. To improve the contrast between the white collembolans and surrounding water surface, the water was stained dark with ink. After gentle stirring the numbers of parental and juvenile collembolans floating on the surface were determined. Missing parental collembolans are assumed to have died during the test period. Surviving adults and juveniles were counted using a digital image processing system (LemnaTecScanalyzer), an automated counting technique based on a video camera connected to a digital image storage and analysis system. This technique fulfils the requirement of the ISO guideline regarding precision of the counting method (average error < 10 %).

Observations:

- At test start: Determination of physico-chemical parameters of the artificial soil (water content, pH)
- 4 weeks after start of exposure:
    - Number of parental and juvenile collembolans per replicate
    - Determination of physico-chemical parameters of the artificial soil (water content, pH)

Statistics:
The statistical analysis was performed with the software ToxRat Professional 2.105. Fisher's Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the independent test item groups.

Findings:
Mortality:
The test item caused 5.0, 2.5, 7.5, 5.0 and 2.5 % parental mortality at the test concentrations of 31.25, 62.5, 125, 250 and 500 mg BAS 555 01 F/kg soil dw, respectively. 6.3 % parental mortality was observed in the control. No statistically significant effect on parental mortality was found for any concentration tested.

Behaviour:
Differences between the behaviour of the collembolans in the control and the test item treatment groups could not be observed.

Reproduction:
The mean number of juveniles counted 28 days after introduction of the parental collembolans into the test vessels was 837 in the control and 891, 793, 839, 865 and 846 at the test concentrations of 31.25, 62.5, 125, 250 and 500 mg BAS 555 01 F/kg soil dw. No statistically significant effects on the number of juveniles compared to the control group were recorded at any concentration tested.

Table B.9.7.2-1: Summary of mortality and reproductive effects of the formulation BAS 55 01 F on *Folsomia candida* in a 28-day reproductive toxicity test

BAS 555 01 F (mg/kg soil dw)	Control	31.25	62.5	125	250	500
Mortality (%) (day 28)	6.3	5.0	2.5	7.5	5.0	2.5
Mean number of juveniles/replicate (day 28)	837	891	793	839	865	846
Reproduction (% of control)	100	106	95	100	103	101
Endpoints (mg/kg soil dw)						
$NOEC_{mortality / reproduction}$	500					
$LOEC_{mortality / reproduction}$	> 500					
LC_{50}	> 500					
EC_{50}	> 500					

Mortality: Fisher's Exact Binomial Test with Bonferroni Correction for mortality (α = 0.05, one-sided greater): statistically not significant compared to the control
Reproduction: Williams-t-test (α = 0.05, one-sided smaller): statistically not significant compared to the control

Results of reference test:
To verify the sensitivity of the test system, the reference item boric acid is routinely tested at concentrations of 44, 67, 100, 150 and 225 mg/kg soil dw. The collembolans of the reference test were from the same source culture as those used in the definitive test. In the most recent study, the EC_{50} was determined to be 104 mg/kg soil dw. The LC_{50} was determined to be 199 mg/kg soil dw. The NOEC for mortality and for reproduction was determined to be 100 and 44 mg/kg soil dw, respectively.

Conclusions:
In a 28-day Collembola reproduction study with BAS 555 01 F, the LC_{50} and the EC_{50} could not be calculated, but it can be concluded that LC_{50} and EC_{50} are higher than 500 mg BAS 555 01 F/kg soil dw. The NOEC for mortality and reproduction was determined to be 500 mg BAS 555 01 F/kg soil dw, the highest concentration tested.

RMS comments:
The validity criteria of OECD Test Guideline 232 are met:

- The mean adult mortality in the untreated controls did not exceed 20 % at the end of the test (measured: 6.3 %)
- The mean number of juveniles per test vessel in the controls was at least 100 at the end of the test (measured: 837)
- The coefficient of variation calculated for the number of juveniles in the controls was less than 30 % at the end of the test (measured: 12.1 %)

The requirement of the ISO guideline concerning the precision of the counting method (average error < 10 %) was fulfilled, the determined overall error of counting amounted to 3.4 %.
Therefore, the study is considered valid and acceptable for use in the risk assessment.

The following endpoints will be used in the risk assessment (endpoints expressed as active substance were calculated from the endpoint expressed in terms of formulation, taking into account a nominal content of 90 g a.s./L and the density of 1.046 g/cm³):

NOEC (*Folsomia candida*, 28 days) mortality = 500 mg BAS 555 01 F/kg soil dw (equivalent to 43.02 mg a.s./kg soil dw)
NOEC (*Folsomia candida*, 28 days) reproduction = 500 mg BAS 555 01 F/kg soil dw (equivalent to 43.02 mg a.s./kg soil dw)
EC_{50} (*Folsomia candida*, 28 days) reproduction > 500 mg BAS 555 01 F/kg soil dw (equivalent to > 43.02 mg a.s./kg soil dw)

In accordance with the new data requirements (Commission Regulation EU No 284/2013), the EC_{10} and EC_{20} values should be calculated for chronic toxicity studies with earthworms. This was not addressed in the study report. However, as there were no treatment related effects higher than 10%, even at the highest dose tested, EC_{10} and EC_{20} values cannot reliably be determined from the available data.

Report:	**CP10.4.2.1/02. Schulz L. (2012)** **Effects of BAS 555 01 F on the predatory mite *Hypoaspis aculeifer*.**
Report No.:	2012/1182247
Guidelines:	OECD Guideline No. 226
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application

Materials and Methods:

Test substance:	BAS 555 01 F (Batch No.: FRE-000698, contains 90.2 g/L of the active substance metconazole)
Test species:	predatory mite (*Hypoaspis aculeifer*)
Number of organisms, age:	8 replicates for the control, 4 replicates per treatment, 10 predatory mites per replicate, adult females from a synchronized culture with an age difference of 2 days
Type of test:	14 days reproductive toxicity test
Applied concentrations:	
Nominal concentrations:	untreated control (deionized water); 62.5, 125, 250, 500 and 1000 mg BAS 555 01 F/kg soil dw (nominally equivalent to 5.38, 10.76, 21.51, 43.02 and 86.04 g a.s./kg soil dw)
Reference item:	Dimethoate 400 EC (Perfekthion, a.s. dimethoate, nominal content: 400 g/L) was tested in a separate study
Soil type:	artificial soil substrate, containing 5 % sphagnum peat, 20 % kaolin clay, 0.3 % calcium carbonate and 74.7 % industrial quartz sand
Food:	before and during the test, the predatory mites were fed every 2 days with *Tyrophagus putrescentiae* (*ad libitum*)
Test conditions:	temperature: 18.8 – 21.8 °C pH: 5.9 – 6.1 (at test start), 5.5 – 5.8 (at test end) water content: 19.34 – 22.35 % (48.29 – 55.82 % of maximum WHC) at test start; 18.90 – 19.85 % (47.18 – 49.57 % of maximum WHC) at test end (maximum water holding capacity: 40.05 % of the dry weight) photoperiod: 16 hours light : 8 hours dark cycle light intensity: 670 lux

Test procedure:

An exactly weighed amount of the test item was mixed with deionized water to make a stock solution, without addition of solubility mediators, immediately before application. This stock solution was stepwise diluted with deionized water to prepare 4 further test solutions. Afterwards, the test solutions were thoroughly mixed with the artificial soil by means of a hand stirrer. The application was performed in the following order: first the untreated control and thereafter the test item in ascending order. The

treated artificial soil was then used to fill glass vessels after which the predatory mites were introduced on top of the soil.
On day 14 following application of the test item and introduction of the test organisms, surviving mites and juveniles of *Hypoaspis aculeifer* were extracted from each test replicate using a MacFayden high-gradient extractor (heat/light extraction method). Following extraction, all juveniles and adults present in the fixing liquid were counted. Any adult mites not found after extraction were recorded as dead. From these data, the mortality of the adult females and the reproductive output were calculated. The extraction efficiency of the extractor was determined to be 98.5 % in a separate extraction run using vessels containing a known number of juveniles and adult mites kept in untreated test substrate.

Observations: assessment of mortality and reproduction was carried out after the 14-day exposure of the predatory mites

Statistics:
Fisher's Exact Binomial Test with Bonferroni Correction and the Welch-test were used to compare the control with the independent test item groups. An EC_{50} was calculated using Probit analysis.

Findings:
Mortality:
Mortality rates of 2.5 – 10.0 % were recorded in the test item treatment groups. In the control group the mortality rate was 5.0 %. The observed mortality rates for adult mortality in the test item treatment groups compared to the control were not statistically significant.

Behaviour:
Differences between the behaviour and the morphology of the mites in the control and the test item treatment groups could not be observed.

Table B.9.7.2-2: Summary of mortality and reproductive effects of the formulation BAS 555 01 F on predatory mites (*Hypoaspis aculeifer*) in a 14-day reproductive toxicity test

BAS 555 01 F (mg/kg soil dw)	**control**	**62.5**	**125**	**250**	**500**	**1000**
Mortality (%) (day 14)	5.0	10.0	2.5	2.5	2.5	7.5
Mean number of juveniles/replicate (day 14)	275.4	253.3	274.3	285.8	193.3*	28.3*
Reproduction (% of control)	100	92	100	104	70	10
Endpoints (mg BAS 555 01 F/kg soil dw)						
NOEC (mortality)	1000					
NOEC (reproduction)	250					
LOEC (mortality)	> 1000					
LOEC (reproduction)	500					
LC_{50}	> 1000					
EC_{50} (95 % CL, lower – upper)	613.5 (526.2 – 748.7)					
EC_{20} (95 % CL, lower – upper)	444.3 (316.3 – 519.2)					
EC_{10} (95 % CL, lower – upper)	375.4 (229.0 – 454.0)					

Mortality: Fisher's Exact Binomial Test with Bonferroni Correction for mortality ($\alpha = 0.05$, one-sided greater): statistically not significant compared to the control
Reproduction: Welch-t-test ($\alpha = 0.05$, one-sided smaller): statistically significant differences compared to the control at test concentrations 500 and 1000 mg BAS 555 01 F/kg soil dw

Reproduction:
Reproduction rates in the 62.5, 125, 250, 500 and 1000 mg BAS 555 01 F/kg soil dw were 253.3, 274.3, 285.5, 193.3 and 28.3 juveniles, respectively. The mean reproduction in the control reached 275.4 juveniles. BAS 555 01 F showed no statistically significantly adverse effects on reproduction up to and including 250 mg BAS 555 01 F/kg soil dw. However, BAS 555 01 F caused statistically significant effects on reproduction at 500 and 1000 mg BAS 555 01 F/kg soil dw.

Results of reference test:
To verify the sensitivity of the test system, the reference item Dimethoate EC 400 was tested at concentrations of 4.10, 5.12, 6.40, 8.00 and 10.00 mg a.s./kg soil dw. In a separate study, the EC_{50} (reproduction) of the reference item Dimethoate EC 400 was calculated to be 6.87 mg a.s./kg soil dw. The results of the reference test demonstrate the sensitivity of the test system.

Conclusions:
In a 14-day *Hypoaspis aculeifer* reproduction study with BAS 555 01 F, the EC_{50} for reproduction was determined to be 613.5 mg BAS 555 01 F/kg soil dw. The LC_{50} for mortality could not be calculated, but it can be concluded that the LC_{50} is higher than 1000 mg BAS 555 01 F/kg soil dw, the highest concentration tested. The NOEC for mortality was determined to be 1000 mg BAS 555 01 F/kg soil dw. The NOEC for reproduction was determined to be 250 mg BAS 555 01 F/kg soil dw.

RMS comments:
The validity criteria of OECD Test Guideline 226 are met:

- The mean adult female mortality in the controls did not exceed 20 % at the end of the test (measured: 5.0 %)
- The mean measured number of juveniles per replicate in the controls was at least 50 at the end of the test (measured: 275.4)
- The coefficient of variation calculated for the number of juvenile mites per replicate in the control was not higher than 30 % at the end of the test (measured: 13.4 %)

Therefore, the study is considered valid and acceptable for use in the risk assessment.

The following endpoints will be used in the risk assessment (endpoints expressed as active substance were calculated from the endpoint expressed in terms of formulation, taking into account a nominal content of 90 g a.s./L and the density of 1.046 g/cm³):

NOEC (*Hypoaspis aculeifer*, 14 days) mortality = 1000 mg BAS 555 01 F/kg soil dw (equivalent to 86.04 mg a.s./kg soil dw)
NOEC (*Hypoaspis aculeifer*, 14 days) reproduction = 250 mg BAS 555 01 F/kg soil dw (equivalent to 21.51 mg a.s./kg soil dw)
EC_{50} (*Hypoaspis aculeifer*, 14 days) reproduction = 613.5 mg BAS 555 01 F/kg soil dw (equivalent to 52.79 mg a.s./kg soil dw)
EC_{20} (*Hypoaspis aculeifer*, 14 days) reproduction = 444.3 mg BAS 555 01 F/kg soil dw (equivalent to 38.23 mg a.s./kg soil dw)
EC_{10} (*Hypoaspis aculeifer*, 14 days) reproduction = 375.4 mg BAS 555 01 F/kg soil dw (equivalent to 32.30 mg a.s./kg soil dw)

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

B.9.8.1. Risk assessment for earthworms

The risk assessment for effects on earthworms is conducted according to the **Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC** (SANCO/10329/2002 rev. 2).

Toxicity

Acute and chronic toxicity studies were conducted with earthworms for the active substance metconazole and the formulation BAS 555 01 F. In addition, a chronic toxicity study with the formulation BAS 555 00 F has been submitted. The available endpoints for the active substance and the representative formulation are shown in Table B.9.8.1-1.

Based on the results from the aerobic soil metabolism study by Dalkmann P. and Kibat H. (2015; CA7.1.1.1/01) and the soil photolysis study by Knight L.(2015b; CA7.1.1.3/01) (please refer to Volume 3 (CA), Section B.8.1.1 for a summary), no change in isomeric composition of metconazole is expected in soil. Therefore, a risk assessment based on the available toxicity studies can be considered representative.

Two chronic toxicity studies performed with the active substance metconazole are available. In the study by Engelhard E.K. *et al.* (1998; CA8.4.1/02), no effects on mortality, biomass and reproduction were found up to 1.8 mg a.s./kg soil dry weight, the highest dose tested. In the more recent study by Friedrich S. (2014; CA8.4.1/03), higher doses of up to 40 mg a.s./kg soil dry weight were tested. No effects on mortality, biomass and reproduction were found at a concentration of up to 5 mg a.s./kg soil dry weight. Based on the results of both available studies, the endpoints from the study by Friedrich S. (2014) are considered the most relevant endpoints and will therefore be used in the risk assessment.

According to the Fate and Behaviour assessment (see Volume 3, Section B.8), there is one relevant metabolite of metconazole, which requires ecotoxicological assessment in soil: M555F020 (1,2,4-triazole). A chronic toxicity study with earthworms is available for this metabolite, for which the endpoints are summarized in Table B.9.8.1-1. It should however be noted that in the DAR for the active substance epoxiconazole (Germany, March 2006) and the active substance tebuconazole (Denmark, November 2007), an additional chronic toxicity study with earthworms is summarized (Ehlers, 2000; BASF DocID 2000/1021862). In this study by Ehlers (2000), no effects, lethal or sublethal, were observed up to a concentration of 0.071 mg 1,2,4-triazole/kg soil dry weight, the highest concentration tested. The study by Moser Th. & Scheffczyk A. (2004; CA8.4.1/04) tested higher concentrations of 1,2,4-triazole and the results do not contradict the outcome of the study by Ehlers (2000). In the study by Moser Th. & Scheffczyk A. (2004), no effects on mortality, biomass and reproduction were found up to and including a concentration of 1.0 mg 1,2,4-triazole/kg soil dry weight. Therefore, the endpoints from the study by Moser Th. & Scheffczyk A. (2004) are considered the relevant endpoints for use in the risk assessment. This is in line with the conclusions from the assessment for tebuconazole, as only the NOEC of the study by Moser Th. & Scheffczyk A. (2014) is listed in the List of Endpoint in the latest EFSA Conclusion for tebuconazole[42].

[42] EFSA (European Food Safety Authority), 2014. Conclusion on the peer review of the pesticide risk assessment of the active substance tebuconazole. EFSA Journal 2014;12(1):3485, 98 pp. doi:10.2903/j.efsa.2014.3485

Table B.9.8.1-1: Summary of earthworm toxicity data on metconazole, its metabolite 1,2,4-triazole and the formulations BAS 555 01 F and BAS 555 00 F.

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Acute toxicity					
earthworm (*Eisenia foetida foetida*)	WL 136184 (metconazole) 95% *cis*	14 day, acute toxicity test, 10% organic matter	LC_{50} NOEC	**> 1000 mg a.s./kg soil dw** 1000 mg a.s./kg soil dw	CA8.4.1/01 Hillaby J.M. & Harrison E.G. (1991)
earthworm (*Eisenia fetida*)	BAS 555 01 F	14 day, acute toxicity test, 10% organic matter	LC_{50} NOEC	**> 1000 mg form/kg soil dw** (> 85.28 mg a.s./kg soil dw) 1000 mg form/kg soil dw (= 85.28 mg a.s./kg soil dw)	CP10.7/02 Vértesi A. (2003)
Chronic toxicity					
earthworm (*Eisenia fetida*)	AC 900768 (metconazole) 85:15 *cis:trans*	56 day, earthworm reproduction test, 10% organic matter	EC_{50} EC_{20} EC_{10} NOEC	> 1.8 mg a.s./kg soil dw ND ND ≥ 1.8 mg a.s./kg soil dw	CA8.4.1/02 Engelhard E.K. *et al.* (1998)
earthworm (*Eisenia fetida*)	BAS 555 F (metconazole)	56 day, earthworm reproduction test, 10% organic matter	EC_{50} EC_{20} EC_{10} NOEC	> 40 mg a.s./kg soil dw 16 mg a.s./kg soil dw 7.8 mg a.s./kg soil dw **5 mg a.s./kg soil dw**	CA8.4.1/03 Friedrich S. (2014)
earthworm (*Eisenia fetida*)	1,2,4-triazole	56 day, earthworm reproduction test, 10% organic matter	EC_{50} EC_{20} EC_{10} NOEC	5.7 mg/kg soil dw 3.19 mg/kg soil dw 1.81 mg/kg soil dw **1.0 mg/kg soil dw**	CA8.4.1/04 Moser Th. & Scheffczyk A. (2004)
earthworm (*Eisenia fetida*)	BAS 555 01 F	56 day, earthworm reproduction test, 5% organic matter	EC_{50} EC_{20} EC_{10} NOEC	> 406.8 mg form/kg soil dw (> 35.0 mg a.s./kg soil dw) ND ND **239.3 mg form/kg soil dw** **(= 20.6 mg a.s./kg soil dw)**	CP10.4.1.1/01 Friedrich S. (2012a)
earthworm (*Eisenia fetida*)	BAS 555 00 F	56 day, earthworm reproduction test, 5% organic matter	EC_{50} EC_{20} EC_{10} NOEC	> 518.6 mg form/kg soil dw (> 35.0 mg a.s./kg soil dw) ND ND 305.0 mg form/kg soil dw (= 20.6 mg a.s./kg soil dw)	CP10.4.1.1/02 Friedrich S. (2012b)

Notes: ***bold*** *– values used for risk assessment*
ND – could not be determined

In line with Commission Regulations (EU) No. 283/2013 and 284/2013, EC_{10} values are available in addition to the NOEC for some of the chronic earthworm studies. Although the SANCO Guidance Document (SANCO/10329/2002 rev. 2) suggests to use the NOEC in the risk assessment, it was recently agreed at Pesticides Peer Review Expert Meeting 133 to also consider the EC_{10}. Where a reliable EC_{10} value is available, then the lower between this value and the NOEC should be used in the risk assessment. For more details and the rationale behind this decision, reference is made to the technical

report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015)[43].

Exposure

The critical use patterns relevant for the uses of BAS 555 01 F are given in Table B.9.8.1-2. The exposure of soil organisms to metconazole and its soil metabolite 1,2,4-triazole was estimated by calculating the maximum Predicted Environmental Concentrations in soil (PEC_{soil}) (see Volume 3 (PPP), Section B.8.1.3). For multiple applications, the worst-case maximum PEC_{soil} will be the one immediately after the final application. The worst-case use pattern of BAS 555 01 F foresees in two applications to cereals at a rate of 90 g metconazole/ha (equivalent to 1.0 L BAS 555 01 F/ha) and two applications to oilseed rape at a rate of 72 g metconazole/ha (equivalent to 0.8 L BAS 555 01 F/ha).

Table B.9.8.1-2: Use pattern of BAS 555 01 F for use as foliar spray

Crop	Application time (BBCH growth stage)	Max. number of applications	Interval between applications [d]	Application rate per treatment	
				Metconazole [kg a.s./ha]	BAS 555 01 F [L/ha]
Cereals (winter/spring)	30 - 69	1 - 2	21	0.090	1.0
Winter oilseed rape	13 – 20 (autumn) 21 – 71 (spring) [1)]	1 - 2	90	0.072	0.8
	21 – 71 (spring)	1 - 2	14	0.072	0.8

[1)] *According to the GAP a first application in autumn (between BBCH 13 and BBCH 20) is followed by a second application in spring (between BBCH 21 and BBCH 71)*

The maximum PEC_{soil} values were calculated for metconazole and its soil metabolite 1,2,4-triazole for a worst-case application scenario of 2 x 72 g a.s. ha^{-1} to winter oilseed rape (autumn application). For this application scenario, a conservative interception value of 40% for the first application at BBCH 13 to 20 and of 80% for the second application at BBCH 21 to 71 was assumed, resulting in a total yearly soil load of 57.6 g a.s./ha. The calculated PEC_{soil} values cover the use in cereals and winter oilseed rape (spring application). The worst-case PEC_{soil} values for the proposed uses of BAS 555 01 F are presented in Table B.9.8.1-3. As the $PEC_{soil, accu}$ values are higher compared to the $PEC_{soil, max}$, the former values will be used in the risk assessment.

Table B.9.8.1-3: Worst-case Predicted Environmental Concentriation (PECsoil) values for metconazole and its relevant metabolite in soil (1,2,4-triazole) after application of the formulation BAS 555 01 F at 2 x 72 g a.s./ha in winter oilseed rape (autumn application).

Test substance	$PEC_{soil, max}$ [mg/kg dry soil]	$PEC_{soil, plateau}$ [mg/kg dry soil]	$PEC_{soil, accu}$ [mg/kg dry soil]
metconazole	0.058	0.013	0.071
1,2,4-triazole	0.001	< 0.001	0.001

[43] 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.

Toxicity-exposure ratio

Acute and long-term toxicity exposure ratios (TER_A and TER_{LT}) were calculated according to the following equations:

$$TER_A = \frac{LC_{50}}{Maximum\ PEC_{soil}}$$

$$TER_{LT} = \frac{NOEC}{Maximum\ PEC_{soil}}$$

The toxicity of lipophilic substances (i.e. substance with a log P_{OW} > 2) usually depends on the organic carbon content (f_{OC}) of the substrate as this governs adsorption and thus pore water concentration. As the artificial substrate in laboratory tests has a higher f_{OC} than many natural soils, the endpoints for studies with soil organisms for these substances have to be corrected by a soil factor of 2. At Pesticides Peer Review Expert Meeting 133, it was agreed that this correction factor of 2 should be applied even if a lower organic matter content was used in OECD test soils (for example 5% instead of 10%), as it is currently not sufficiently clear whether or not there is a one-to-one relationship between the organic matter content and the toxicity to soil organisms. For further details and the rationale behind this decision, reference is made to the technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015)[44].

The log P_{OW} of metconazole was determined to be 3.85 (see Volume 3, Section B.2). Therefore, the correction factor of 2 should be applied to the metconazole endpoints. For the metabolite 1,2,4-triazole, a study to identify the log P_{OW} value for the metabolite 1,2,4-triazole is not available within the renewal dossier for metconazole. In the revised Addendum for the confirmatory data for the Triazole Derivative Metabolites (RMS UK, May 2016), a log P_{OW} of -0.62 to -0.71 is reported for 1,2,4-traizole. This value however still needs to be confirmed (see Volume 3, Section B.2). Based on the currently available log P_{OW} value, it seems that there is no need to apply the correction factor of 2 to the endpoints for this metabolite.

While acute toxicity data is no longer required according to the new data requirements (Regulation (EU) No. 283/2013 and 284/2013), acute endpoints are available for metconazole and the formulation BAS 555 01 F. TER values for the **acute risk assessment** are shown in Table B.9.8.1-4, for information.

Table B.9.8.1-4: Acute risk (TER_A) to earthworms for metconazole and the formulation BAS 555 01 F, based on worst-case max PEC_{SOIL} values for the proposed uses.

Test species	Test substance	LC_{50} (mg a.s./kg)	$LC_{50,CORR}$ (mg a.s./kg)	Max PEC_{SOIL} (mg a.s./kg)	TER_A
Eisenia fetida	Metconazole	> 1000	> 500	0.071	7042
	BAS 555 01 F	> 85.28	> 42.64	0.071	600.6

The acute TER values for metconazole and the representative formulation BAS 310 55 I are much higher than the Annex VI acute trigger value of 10, indicating an acceptable acute risk to earthworms following the proposed use of BAS 555 01 F in winter and spring cereals, and winter oilseed rape.

[44] 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.

TER values for the **chronic risk assessment** are shown in Table B.9.8.1-5. Based on the laboratory toxicity endpoints and worst-case maximum PEC_{SOIL} values the long-term TER values for earthworms are higher than the annex VI trigger value of 5, indicating an acceptable chronic risk.

Table B.9.8.1-5: Chronic risk (TER_{LT}) to earthworms for metconazole, its relevant metabolite 1,2,4-triazole and the formulation BAS 555 01 F, based on worst-case max PEC_{SOIL} values for the proposed uses.

Test species	Test substance	NOEC (mg a.s./kg)	$NOEC_{CORR}$ (mg a.s./kg)	Max PEC_{SOIL} (mg a.s./kg)	TER_{LT}
Eisenia fetida	Metconazole	5	2.5	0.071	35.21
	BAS 555 01 F	20.6	10.3	0.071	145.1
	1,2,4-triazole	1.0	1.0	0.001	1000

In addition to the laboratory toxicity studies, a field study with BAS 555 00 F on earthworms (CP10.4.1.2/01 Luehrs U., 2003) has been carried out to further determine the potential risk to earthworm populations under natural field conditions. BAS 555 00 F is an EC formulation containing 60 g/L of the active substance metconazole, and differs in composition compared to the representative formulation BAS 555 01 F (refer to Volume 4, Section C1.3.2 for details). For both BAS 555 00 F and BAS 555 01 F, a chronic laboratory earthworm toxicity study is available (CP10.4.1.1/02 Friedrich S. 2012b and CP10.4.1.1/01 Friedrich S. 2012a, respectively). From these studies, the same NOEC (20.6 mg a.s./kg soil dry weight, see Table B.9.8.1-1) was derived for both formulations. Therefore, the toxicity of BAS 555 01 F and BAS 555 00 F seems to be predominantly driven by the active ingredient and is considered to be comparable with respect to effects on earthworms. In consequence, the field study conducted with BAS 555 00 F is considered suitable to address the risk of BAS 555 01 F to earthworms.

The test site in the study by Luehrs U. (2003) was an arable field located in Germany, which was cultivated with summer barley, as a natural habitat of earthworms. BAS 555 00 F was applied once at 3.0 L/ha (equivalent to 180 g metconazole/ha). Monitoring of the earthworm populations was performed about 1, 4 and 12 months after application. The application of 180 g metconazole/ha resulted in an initial reduction in earthworm abundance, but recovery to 81.5% of the control was reached within one year. Earthworm biomass did not show a statistically significant reduction at any sampling point. Similarly, no significant effects were seen on the abundance of the three main species, *Aporrectodea caliginosa, Allolobophora chlorotica* and *Octolasium tyrtaeum*. The numbers of juveniles were statistically reduced at each sampling date, but at the third sampling (1 year after application) only by 23.6%. However, the ratio between juveniles and adult earthworms was approximately on the same level. Overall, it was considered that an application of BAS 555 00 F at a rate of 180 g metconazole/ha did not show unacceptable long-term effects on natural earthworm populations.

The study by Luehrs U. (2003) was assigned a Reliability Index (Ri) of 2 (reliable with restrictions) following a reliability assessment according to de Jong *et al.* (2006). Consequently, this study is considered suitable for use in the risk assessment. The exposure in the field study by Luehrs U. (2003) should be known to assess whether it covers the exposure following the intended uses of BAS 555 01 F. As the actual concentration of metconazole in soil following the application of BAS 555 00 F was not measured, the exposure in the field study was estimated. The BBCH stage of the summer barley at the time of application is not reported in the study report, but since the application was done relatively close to harvest of the crop (application on 03.06.2002 and harvest on 27.08.2002), a crop interception of 80% is assumed (the interception value reported in the FOCUS groundwater assessment guidance document (FOCUS, 2014) for spring cereals BBCH 30-69). Taking into account an interception of 80%, an application of 180 g a.s./ha results in a soil load of 36 g a.s./ha. This covers the total yearly soil load for the proposed use of BAS 555 01 F in cereals and the spring application in winter oilseed rape, as

calculated in Volume 3 (PPP), Section B.8.1.3. However, a higher total yearly soil load of 57.6 g a.s./ha is expected from the autumn application in winter oilseed rape. Consequently, the field study by Luehrs U. (2003) can only be used in a risk assessment for the use in cereals and the spring application in winter oilseed rape.

It is further noted that in line with the EFSA Scientific Opinion on the development of a soil ecoregions concept[45], grassland are recommended as suitable for earthworm trials. Further, as field data for only one location (Germany) and one field is available, it can be argued that this study is not representative for the whole intended area of use. For example, the study site might not be relevant for Southern Europe, in terms of climatic and biological conditions and presence of different species. Nevertheless, for the use in cereals and the spring application in winter oilseed rape, this study supports the conclusion from the Tier 1 risk assessment that the risk to earthworms following application of BAS 555 01 F according to the proposed uses can be considered acceptable. The relevance of the available field study for specific regions in Europe might further be addressed at Member State level.

Conclusion:
The acute and long-term risk to earthworms from exposure to metconazole following the intended uses of BAS 555 01 F in winter and spring cereals, and winter oilseed rape is acceptable.

[45] EFSA Panel on Plant Protection Products and their Residues (PPR) (2010). Scientific Opinion on the development of a soil ecoregions concept using distribution data on invertebrates. *EFSA Journal* 8(10):1820.

B.9.8.2. Risk assessment for non-target soil meso- and macrofauna (other than earthworms)

As for earthworms, the risk assessment for effects on non-target soil meso- and macrofauna is conducted according to the **Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC** (SANCO/10329/2002 rev. 2).

Toxicity

Chronic toxicity studies with non-target soil meso- and macrofauna were conducted with the active substance metconazole, the representative formulation BAS 555 01 F and the relevant metabolite 1,2,4-triazole. The available endpoints are summarized in Table B.9.8.2-1.

Table B.9.8.2-1: Summary of non-target soil meso- and macrofauna (other than earthworms) toxicity data on metconazole, its metabolite 1,2,4-triazole and the formulations BAS 555 01 F.

Organism	Test substance	Timescale (Test type)	Endpoint	Toxicity value	Reference
Folsomia candida	BAS 555 F (metconazole)	28 day, reproduction test, 5% organic matter	EC_{50} EC_{20} EC_{10} NOEC	> 320 mg a.s./kg soil dw 102.40 mg a.s./kg soil dw 46.46 mg a.s./kg soil dw **40 mg a.s./kg soil dw**	CA8.2.4.1/01 Ganssman M., 2013
Folsomia candida	BAS 555 01 F	28 day, reproduction test, 5% organic matter	EC_{50} EC_{20} EC_{10} NOEC	> 500 mg form/kg soil dw (> 43.02 mg a.s./kg soil dw) ND ND **500 mg form/kg soil dw (= 43.02 mg a.s./kg soil dw)**	CP10.4.2.1/01 Friedrich S., 2012c
Folsomia candida	1,2,4-triazole	28 day, reproduction test, 10% organic matter	EC_{20} EC_{10} NOEC	1.74 mg/kg soil dw **1.51 mg/kg soil dw** 1.8 mg/kg soil dw	CA8.4.2.1/03 Moser Th. & Scheffczyk A., 2002
Hypoaspis aculeifer	BAS 555 F (metconazole)	14 day, reproduction test, 5% organic matter	EC_{50} EC_{20} EC_{10} NOEC	138.2 mg a.s./kg soil dw 90.3 mg a.s./kg soil dw 72.3 mg a.s./kg soil dw **62.5 mg a.s./kg soil dw**	CA8.4.2.1/02 Schulz. L., 2014a
Hypoaspis aculeifer	BAS 555 01 F	14 day, reproduction test, 5% organic matter	EC_{50} EC_{20} EC_{10} NOEC	613.5 mg form/kg soil dw (= 52.79 mg a.s./kg soil dw) 444.3 mg form/kg soil dw (= 38.23 mg a.s./kg soil dw) 375.4 mg form/kg soil dw (= 32.30 mg a.s./kg soil dw) **250 mg form/kg soil dw (= 21.51 mg a.s./kg soil dw)**	CP10.4.2.1/02 Schulz L., 2012
Hypoaspis aculeifer	1,2,4-triazole	14 day, reproduction test, 5% organic matter	EC_{20} EC_{10} NOEC	241 mg a.s./kg soil dw 190 mg a.s./kg soil dw **171 mg a.s./kg soil dw**	CA8.4.2.1/04 Schulz L., 2014b

Notes: ***bold*** *– values used for risk assessment*
ND – could not be determined

In line with Commission Regulations (EU) No. 283/2013 and 284/2013, EC_{10} values are available in addition to the NOEC for some of the chronic earthworm studies. Although the SANCO Guidance Document (SANCO/10329/2002 rev. 2) suggests to use the NOEC in the risk assessment, it was recently agreed at Pesticides Peer Review Expert Meeting 133 to also consider the EC_{10}. Where a reliable EC_{10} value is available, then the lower between this value and the NOEC should be used in the risk assessment. For more details and the rationale behind this decision, reference is made to the technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015)[46].

Exposure

The critical use patterns relevant for the uses of BAS 555 01 F are given in Table B.9.8.2-2. The exposure of soil organisms to metconazole and its soil metabolite 1,2,4-triazole was estimated by calculating the maximum Predicted Environmental Concentrations in soil (PEC_{soil}) (see Volume 3 (PPP), Section B.8.1.3). For multiple applications, the worst-case maximum PEC_{soil} will be the one immediately after the final application. The worst-case use pattern of BAS 555 01 F foresees in two applications to cereals at a rate of 90 g metconazole/ha (equivalent to 1.0 L BAS 555 01 F/ha) and two applications to oilseed rape at a rate of 72 g metconazole/ha (equivalent to 0.8 L BAS 555 01 F/ha).

Table B.9.8.2-2: Use pattern of BAS 555 01 F for use as foliar spray

Crop	Application time (BBCH growth stage)	Max. number of applications	Interval between applications [d]	Application rate per treatment	
				Metconazole [kg a.s./ha]	BAS 555 01 F [L/ha]
Cereals (winter/spring)	30 - 69	1 - 2	21	0.090	1.0
Winter oilseed rape	13 – 20 (autumn) 21 – 71 (spring) [1)]	1 - 2	90	0.072	0.8
	21 – 71 (spring)	1 - 2	14	0.072	0.8

[1)] According to the GAP a first application in autumn (between BBCH 13 and BBCH 20) is followed by a second application in spring (between BBCH 21 and BBCH 71)

The maximum PEC_{soil} values were calculated for metconazole and its soil metabolite 1,2,4-triazole for a worst-case application scenario of 2 x 72 g a.s. ha^{-1} to winter oilseed rape (autumn application). For this application scenario, a conservative interception value of 40% for the first application at BBCH 13 to 20 and of 80% for the second application at BBCH 21 to 71 was assumed, resulting in a total yearly soil load of 57.6 g a.s./ha. The calculated PEC_{soil} values cover the use in cereals and winter oilseed rape (spring application). The worst-case PEC_{soil} values for the proposed uses of BAS 555 01 F are presented in Table B.9.8.2-3. As the $PEC_{soil, accu}$ values are higher compared to the $PEC_{soil, max}$, the former values will be used in the risk assessment.

Table B.9.8.2-3: Worst-case Predicted Environmental Concentriation (PECsoil) values for metconazole and its relevant metabolite in soil (1,2,4-triazole) after application of the formulation BAS 555 01 F at 2 x 72 g a.s./ha in winter oilseed rape (autumn application).

Test substance	$PEC_{soil, max}$ [mg/kg dry soil]	$PEC_{soil, plateau}$ [mg/kg dry soil]	$PEC_{soil, accu}$ [mg/kg dry soil]
metconazole	0.058	0.013	0.071
1,2,4-triazole	0.001	< 0.001	0.001

[46] 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.

Toxicity-exposure ratio

As only chronic toxicity endpoints for non-target soil meso-and macrofauna are available, only long-term toxicity exposure ratios (TER_{LT}) were calculated according to the following equation:

$$TER_{LT} = \frac{NOEC\ or\ EC_x}{Maximum\ PEC_{soil}}$$

The toxicity of lipophilic substances (i.e. substance with a log P_{OW} > 2) usually depends on the organic carbon content (f_{OC}) of the substrate as this governs adsorption and thus pore water concentration. As the artificial substrate in laboratory tests has a higher f_{OC} than many natural soils, the endpoints for studies with soil organisms for these substances have to be corrected by a soil factor of 2. At Pesticides Peer Review Expert Meeting 133, it was agreed that this correction factor of 2 should be applied even if a lower organic matter content was used in OECD test soils (for example 5% instead of 10%), as it is currently not sufficiently clear whether or not there is a one-to-one relationship between the organic matter content and the toxicity to soil organisms. For further details and the rationale behind this decision, reference is made to the technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology (EFSA, 2015)[47].

The log P_{OW} of metconazole was determined to be 3.85 (see Volume 3, Section B.2). Therefore, the correction factor of 2 should be applied to the metconazole endpoints. For the metabolite 1,2,4-triazole, a study to identify the log P_{OW} value for the metabolite 1,2,4-triazole is not available within the renewal dossier for metconazole. In the revised Addendum for the confirmatory data for the Triazole Derivative Metabolites (RMS UK, May 2016), a log P_{OW} of -0.62 to -0.71 is reported for 1,2,4-traizole. This value however still needs to be confirmed (see Volume 3, Section B.2). Based on the currently available log P_{OW} value, it seems that there is no need to apply the correction factor of 2 to the endpoints for this metabolite.

The TER values for the chronic risk assessment are shown in Table B.9.8.2-4. Based on the laboratory toxicity endpoints and maximum PEC_{SOIL} values the long-term TER values for *Hypoaspis aculeifer* and *Folsomia candida* are higher than the annex VI trigger value of 5, indicating an acceptable risk.

Table B.9.8.2-4: Chronic risk (TER_{LT}) to non-target soil meso- and macrofauna (other than earthworms) for metconazole, its relevant metabolite 1,2,4-triazole and the formulation BAS 555 01 F, based on worst-case max PEC_{SOIL} values for the proposed uses.

Test species	Test substance	NOEC (mg a.s./kg)	$NOEC_{CORR}$ (mg a.s./kg)	Max PEC_{SOIL} (mg a.s./kg)	TER_{LT}
Folsomia candida	Metconazole	40	20	0.071	281.7
	BAS 555 01 F	43.02	21.51	0.071	303.0
	1,2,4-triazole	1.51 [1)]	1.51	0.001	1510
Hypoaspis aculeifer	Metconazole	62.5	31.25	0.071	440.1
	BAS 555 01 F	21.51	10.76	0.071	151.5
	1,2,4-triazole	171	171	0.001	171000

[1)] This endpoint represents the EC_{10} value instead of the NOEC.

[47] 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.

According to the SANCO Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev. 2), a litterbag study is required for substances with a DT_{90} in soil > 365 days. As metconazole generally has a DT_{90} > 365 days in field dissipation studies (see Volume 3 (AS), Section B.8.1), such a litterbag study was submitted for the initial inclusion of metconazole, and summarized in the original DAR (January 2004). As this study was not considered acceptable following experts' consultation (refer to the EFSA conclusion for metconazole from 2006[48] for details), a new litterbag study was performed, which was submitted for renewal (CP10.7/03 Klein S. & Meister A., 2003). A summary of this study is provided in Section B.9.13. Although a litterbag study is no longer a data requirement according to Regulation (EU) No. 284/2013, it can provide supportive information to the risk assessment.

In the study by Klein S. & Meister A. (2003), the product BAS 555 00 F was applied 2 times, with a 10 day interval, on an arable field site under conventional use (crop: summer barley) in Germany. The first application consisted of 1.5 L BAS 555 00 F/ha (90 g metconazole/ha), and the second application of 3.0 BAS 555 00 F/ha (180 g metconazole/ha). The effects of BAS 555 00 F on the degradation of buried organic wheat straw was compared to a water control at intervals of 29, 98, 158 and 277 days after burial. No adverse long-term effects on functions of soil organisms, i.e. the organic matter decomposition process, was found. As also discussed under Section B.9.6.1.2 and B.9.8.1, studies with BAS 555 00 F can be considered representative for the formulation BAS 555 01 F. Further, the application rate exceeded the worst-case application rate of BAS 555 01 F of 2 x 90 g a.s./ha in cereals. Therefore, the results from the study by Klein S. & Meister A. (2003) support the conclusion from the Tier 1 risk assessment above, that the risk to soil meso- and macrofauna can be considered acceptable.

Conclusion: The long-term risk to non-target meso- and macrofauna (other than earthworms) from exposure to metconazole following the intended uses of BAS 555 01 F in winter and spring cereals, and winter oilseed rape is acceptable.

[48] EFSA Scientific Report (2006) 64, 1-71, Conclusion regarding the peer review of the pesticide risk assessment of the active substance metconazole.

B.9.9. EFFECTS ON SOIL NITROGEN TRANSFORMATION

A new study on effects of BAS 555 01 F on soil nitrogen transformation has been submitted. A summary of the available study is provided below. Although no longer a data requirement according to Regulation (EU) No. 284/2013, a new soil respiration study with BAS 555 01 F has also been submitted. A summary of this study is included in Section B.9.13.

Report:	**CP10.5/01. Koelzer U. (2003a)** **Assessment of side effects of BAS 555 01 F on the activity of soil microflora, nitrogen turnover**
Report No.:	2003/1004137
Guidelines:	OECD Test Guideline 216
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Material and Methods:	
Test substance:	BAS 555 01 F (Batch No.: R2066-079, contains 89.2 g/L of the active ingredient metconazole)
Type of test:	soil nitrification tests (28 days)
Test soil:	A biologically active agricultural soil: loamy sand soil (57.8% sand, 32.6% silt, 9.6% clay), pH 6.31, 1.10% C_{org}, WHC: 34.1%
Test design:	3 replicates per concentration and control
Applied concentrations:	
Nominal:	Control, 1.116 mg BAS 555 01 F/kg dry soil (corresponding to a single application rate of 0.8 L/ha, equivalent to 72 g a.s./ha), 11.16 mg BAS 555 01 F/kg dry soil (corresponding to an application rate of 8.0 L/ha, equivalent to 720 g a.s./ha). Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm³
Reference material:	Dinoterb (purity: 97.0%), tested at 3.33, 6.67 and 13.33 mg/kg soil dry weight in a separate study
Test conditions:	soil moisture: approximately 45% of maximum water holding capacity (WHC_{max}) at test start. The water content of the soil was adjusted one a week when needed.
	pH: 6.37-6.68
	soil samples were incubated at 20 ± 2°C in the dark
Statistics:	Descriptive statistics

Test procedure:

On the day of treatment, the test soil was amended with ground Lucerne meal at a rate of 0.5% of the dry soil weight. Test solutions containing the appropriate amount of BAS 555 01 F in water were applied directly to the test soil. The amount of test solution added to the test soil resulted in a final water content of 45% WHC_{max}. After addition of the test solutions (BAS 555 01 F) and water (control) to the soil, the soil samples were thoroughly mixed. The control samples were treated with water only. Each treatment group contained 3300 g moist soil.

After application, three 1100 g soil sub-samples (one for each replicate in each treatment group) were placed in 1000 mL glass bottles. The bottles were closed loosely with screw caps and weighed for the determination of the starting weight. About every 7 days the amount of moisture loss was determined by reweighing, and water was added to adjust vessels to the starting weight.
At 0, 7, 14 and 28 days after application of the test item, samples of 50 g were taken from the bulk batches for nitrogen transformation measurements. Soil nitrification was determined by measuring the NH_4^+-N and NO_3^--N contents of aqueous soil extracts by means of calibrated ion sensitive electrodes and the expandable Ionanalyser. The concentrations of NH_4^+-N and NO_3-N in the soil were then calculated from the measured values.

Findings:
No adverse effects of BAS 555 01 F on nitrogen transformation in soil were observed in both test item concentrations after 28 days. Only slight deviations from the control between -10.9 and 18.9% were measured after 28 days. The results are summarized in Table B.9.9-1.

In a separate study the reference item Dinoterb produced a clear effect (12.5%, 31.7% and 92.9% stimulation).

Table B.9.9-1: Effects of BAS 555 01 F on soil micro-organisms (nitrogen transformation) on days 7, 14 and 28 of incubation

Soil (days)	Control	1.116 mg BAS 555 01 F per kg dry soil equivalent to 0.8 L/ha		11.16 mg BAS 555 01 F per kg dry soil equivalent to 8.0 L/ha	
	NO_3-N [mg/kg dry soil/day]	NO_3-N [mg/kg dry soil/day]	% Deviation from the control [1)]	NO_3-N [mg/kg dry soil/day]	% Deviation from the control [1)]
Loamy sand soil (7 d)	10.1	10.2	+0.28	9.02	-11.1
Loamy sand soil (14 d)	16.0	15.7	-1.97	14.4	-10.4
Loamy sand soil (28 d)	28.0	25.0	-10.9	22.7	-18.9

[1)] *Based on NO_3-nitrogen production; - = inhibition, + = stimulation*

Conclusions:
BAS 555 01 F caused no short-term and no long-term effects on the soil nitrogen transformation (measured as NO_3-N production) in a field soil tested up to a concentration of 0.72 kg metconazole/ha, equivalent to a field application rate of 8.0 L BAS 555 01 F/ha.

RMS comments:
The coefficients of variation in the control were maximum 7.95%. Thus, the validity criterion of OECD Test Guideline 216 (i.e. the variation between replicate control samples needs to be less than 15%) was met for all sampling dates (0, 7, 14 and 28 days). Consequently, this study is considered acceptable for use in the risk assessment.

BAS 555 01 F had no adverse effect of > 25% on soil nitrogen turnover at concentrations of up to 11.16 mg BAS 555 01 F/kg soil dry weight (equivalent to 0.95 mg a.s./kg soil dry weight), the highest concentration tested.
NOEC (soil nitrogen turnover, 28 days) = 11.16 mg form./kg soil dry weight (equivalent to 0.95 mg a.s./kg soil dry weight).

B.9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

The risk assessment for effects on soil organisms is conducted according to **the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC** (SANCO/10329/2002 rev. 2).

Toxicity

Studies on the effects of the active substance metconazole on soil nitrogen transformation have not been submitted. However, studies with the representative formulation BAS 555 01 F are available instead. According to the Fate and Behaviour assessment (see Volume 3, Section B.8), 1,2,4-triazole (M555F020) is a relevant metabolite of metconazole, which requires an ecotoxicological assessment in soil. A study on the effects of 1,2,4-triazole on soil nitrogen transformation is also available. The endpoints for the representative formulation and relevant metabolites are summarized in Table B.9.10-1.

In addition to the studies on the effect on soil nitrogen transformation, studies on the effect on soil respiration have also been submitted for the formulation BAS 555 01 F and the metabolite 1,2,4-triazole. Although these studies are no longer a data requirement according to Regulation (EU) No. 283/2013 and 284/2013, these studies are considered as supportive information. The available endpoints are also summarized in Table B.9.10-1.

Table B.9.10-1: Summary of data on the effect of metconazole in the formulation BAS 555 01 F and the relevant metabolites of metconazole in soil on soil nitrogen transformation and soil respiration

Test system	Test substance	Test soil	Duration of exposure	NOEC	References
N transformation	BAS 555 01 F	Loamy sand	28 days	11.16 mg form./kg soil d.w. (= **0.95 mg a.s./kg soil d.w.**)	CP10.5/01 Koelzer U., 2003a
C transformation	BAS 555 01 F	Loamy sand	28 days	11.16 mg form./kg soil d.w. (= **0.95 mg a.s./kg soil d.w.**)	CP10.7/01 Koelzer U., 2003b
N transformation	1,2,4-triazole	Sandy loam	28 days	**0.353 mg/kg soil d.w.**	CA8.5/01 Voelkel W., 2000
C transformation	1,2,4-triazole	Sandy loam	28 days	**0.353 mg/kg soil d.w.**	CA8.5/01 Voelkel W., 2000

bold: *values used for risk assessment*

Exposure

The critical use patterns relevant for the uses of BAS 555 01 F are given in Table B.9.10-2. The exposure of soil organisms to metconazole and its soil metabolite 1,2,4-triazole was estimated by calculating the maximum Predicted Environmental Concentrations in soil (PEC_{soil}) (see Volume 3 (PPP), Section B.8.1.3). For multiple applications, the worst-case maximum PEC_{soil} will be the one immediately after the final application. The worst-case use pattern of BAS 555 01 F foresees in two applications to cereals at a rate of 90 g metconazole/ha (equivalent to 1.0 L BAS 555 01 F/ha) and two applications to oilseed rape at a rate of 72 g metconazole/ha (equivalent to 0.8 L BAS 555 01 F/ha).

Table B.9.10-2: Use pattern of BAS 555 01 F for use as foliar spray

Crop	Application time (BBCH growth stage)	Max. number of applications	Interval between applications [d]	Application rate per treatment	
				Metconazole [kg a.s./ha]	BAS 555 01 F [L/ha]
Cereals (winter/spring)	30 - 69	1 - 2	21	0.090	1.0
Winter oilseed rape	13 – 20 (autumn) 21 – 71 (spring) [1)]	1 - 2	90	0.072	0.8
	21 – 71 (spring)	1 - 2	14	0.072	0.8

[1)] According to the GAP a first application in autumn (between BBCH 13 and BBCH 20) is followed by a second application in spring (between BBCH 21 and BBCH 71)

The maximum PEC_{soil} values were calculated for metconazole and its soil metabolite 1,2,4-triazole for a worst-case application scenario of 2 x 72 g a.s. ha^{-1} to winter oilseed rape (autumn application). For this application scenario, a conservative interception value of 40% for the first application at BBCH 13 to 20 and of 80% for the second application at BBCH 21 to 71 was assumed, resulting in a total yearly soil load of 57.6 g a.s./ha. The calculated PEC_{soil} values cover the use in cereals and winter oilseed rape (spring application). The worst-case PEC_{soil} values for the proposed uses of BAS 555 01 F are presented in Table B.9.10-3. As the $PEC_{soil, accu}$ values are higher compared to the $PEC_{soil, max}$, the former values will be used in the risk assessment.

Table B.9.10-3: Worst-case Predicted Environmental Concentriation (PECsoil) values for metconazole and its relevant metabolite in soil (1,2,4-triazole) after application of the formulation BAS 555 01 F at 2 x 72 g a.s./ha in winter oilseed rape (autumn application).

Test substance	$PEC_{soil, max}$ [mg/kg dry soil]	$PEC_{soil, plateau}$ [mg/kg dry soil]	$PEC_{soil, accu}$ [mg/kg dry soil]
metconazole	0.058	0.013	0.071
1,2,4-triazole	0.001	< 0.001	0.001

Risk assessment

The risk to soil nitrogen transformation is based on comparison of the relevant PEC_{soil} values to the test concentrations at which < 25% effects were observed in the soil nitrogen transformation tests. Table B.9.10-4 summarizes the results from the soil nitrogen transformation tests and the relevant worst-case PEC_{soil} values.

Table B.9.10-4: Summary of the soil nitrogen transformation endpoints for metconazole in the formulation BAS 555 01 F and for the relevant metconazole metabolites, and risk assessment based on worst-case PEC_{soil} values for the proposed uses of BAS 555 01 F.

Test substance	Test rate (mg a.s./kg soil d.w.)	% effect on soil nitrogen transformation at 28 days after treatment [1)]	PEC_{SOIL} (mg/kg soil d.w.)	Acceptable risk
Metconazole in BAS 555 01 F	0.95	-18.9	0.071	yes
1,2,4-triazole	0.353	-1.5	0.001	yes

[1)] % effect – negative values indicate a reduction in the treated sample compared to the control

The effect on nitrogen transformation rates is < ± 25% for metconazole in the representative formulation BAS 555 01 F and for the metabolite 1,2,4-triazole, at treatment rates of about 13 tand 353 times the highest PEC_{soil} values, respectvely. Therefore, the risk to soil nitrogen transformation processes can be considered acceptable for the proposed uses of BAS 555 01 F.

Table B.9.10-5 summarizes the results from the soil carbon transformation tests and relevant PEC_{soil} values. Although data on the effects on soil carbon transformation is not longer required according to Regulation (EU) No. 283/2013 and 284/2013, this data is available. Therefore, a risk assessment for soil carbon transformation in included, for information.

Table B.9.10-5: Summary of the soil carbon transformation endpoints for metconazole in the formulation BAS 555 01 F and for the relevant metconazole metabolites, and risk assessment based on worst-case PEC_{soil} values for the proposed uses of BAS 555 01 F.

Test substance	Test rate (mg a.s./kg soil d.w.)	% effect on soil carbon transformation at 28 days after treatment [1)]	PEC_{SOIL} (mg/kg soil d.w.)	Acceptable risk
Metconazole in BAS 555 01 F	0.95	-7.72	0.071	yes
1,2,4-triazole	0.353	8.3	0.001	yes

1) % effect – negative values indicate a reduction in the treated sample compared to the control

The effect on carbon transformation rates is < ± 25% for metconazole in the representative formulation BAS 555 01 F and for the metabolite 1,2,4-triazole, at treatment rates of about 13 tand 353 times the highest PEC_{soil} values, respectvely. Therefore, the risk to soil carbon transformation processes can be considered acceptable for the proposed uses of BAS 555 01 F.

Conclusion:
The risk to soil non-target micro-organisms from exposure to metconazole after application of BAS 555 01 F according to the proposed uses in winter and spring cereals, and winter oilseed rape is acceptable.

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

B.9.11.1. Summary of screening data

No screening studies using the formulated product have been submitted. Instead, vegetative vigour and seedling emergence studies have been conducted (see Section B.9.11.2).

B.9.11.2. Testing on non-target plants

No specific non-target plant studies with the formulation BAS 555 01 F I were previously evaluated during EU review of the active substance. A new study investigating the effects of BAS 555 01 F on seedling emergence and two new studies investigating the effects of BAS 555 01 F on vegetative vigour have been submitted. A summary of the three available studies is provided below.

Report:	**CP10.6.2/01. Sack D. (2005)** **BAS 555 01 F: Effects on non-target plants in the greenhouse - A multiple rate test**
Report No.:	2005/1029570
Guidelines:	OECD 208 B (Draft 2000)
GLP:	No
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and Methods:	
Test substance:	BAS 555 01 F (Batch No.: R2066-079, contains 89.2 g/L of the active ingredient metconazole)
Test species :	
Monocotyledons:	onion (*Allium cepa* L.), oats (*Avena sativa* L.)
Dicotyledons:	pea (*Pisum sativum* L.), cabbage (*Brassica napus* L.), sugar beet (*Beta vulgaris*), cucumber (*Cucumis sativus*), tomato (*Lycopersicon esculentum*), carrot (*Daucus carota* L.)
Type of test:	(greenhouse) vegetative vigour test (21 days)
Test design:	4 replicates for the test item treatments and 16 replicates for the control for each plant species. Each replicate consisted of one pot, containing 2 to 5 plants, depending on te species (2 plants per pot for cucumber, 3 plants per pot for sugar beet, cabbage, pea and tomato, 5 plants per pot for carrot, oat and onion).
Applied concentrations:	untreated control; 0.8, 1.6, 2.0 and 2.4 L BAS 555 01 F/ha (corresponding to 72, 144, 180 and 216 g a.s./ha)
Spray volume:	400 L/ha
Test substrate:	Lihof Boden 2003 soil (steam sterilized), sandy loam; 8.1% organic carbon; pH = 6.7
Environmental conditions:	greenhouse conditions temperatures: > 14.0 (night); 20-31°C (day) relative humidity: about 80%

	photoperiod: 16:8 h light:dark light intensity: Plants were illuminated with additional light to ensure 4500 lux on top of the plants watering: according to need. First watering 24h after application of the test item
Statistics:	Descriptive statistics, ANOVA followed by Bonferroni t-test for plant biomass.

Test procedure:
The spray solution was mixed on the day of application, by dilution of the test item in deionized water. BAS 555 01 F was applied post-emergence at growth stage BBCH 13-14 at a water rate of 400 L/ha using a laboratory spray cabin. Deionized water was used as control treatment, applied equivalent to 400 L/ha. Following application, the plants were cultivated for 21 days in the greenhouse. Assessment of phytotoxicity (visible plant damages) was done 20 days after application (DAA). 21 DAA the reduction of plant mass was assessed by measuring the the shoot fresh weight (plant biomass above ground). The plants were cut directly above the ground and weighed immediately after cutting to avoid weight loss by wilting.

Findings:
Phytotoxicity:
The results are summarized in Table B.9.11.2-1. Oat and onion were the only species free of plant injuries after BAS 555 01 F application. The remaining plant species were affected by the treatment. Tomato proved to be most sensitive, showing already damages of 25% (compared to control) at the lowest treatment rate of 0.8 L BAS 555 01 F/ha and 70% damage at the highest treatment rate of 2.4 L BAS 555 01 F/ha. Cucumber reacted very sensitive as well showing damages from 15% at the lowest treatment rate up to 50% at the highest treatment rate of 2.4 L BAS 555 01 F/ha. Cabbage and carrot were also affected by the lowest treatment rate (5% each). The visible damage increased in a dose related manner for tomato, cucumber, cabbage and carrot. Whereas sugar beet was affected by the three highest treatment rates, pea only displayed plant injuries at the highest treatment rate.

Biomass (Fresh weight):
The results are summarized in Table B.9.11.2-1. Dicotyledonous plants showed a statistically significant decrease in plant weight after treatment with BAS 555 01 F when compared to the control (Bonferroni t-test, $\alpha = 0.05$). Plant weight of tomato and cucumber were significantly lower at all treatment rates. Cabbage, carrot, and sugar beet had statistically significantly decreased plant weights at the three highest treatment rates. Pea showed significantly reduced plant weights only at the highest treatment rate of 2.4 L BAS 555 01 F/ha. The weight of the monocotyledonous plants was not significantly decreased after BAS 555 01 F application.

Table B.9.11.2-1: Effects of BAS 555 01 F on plant biomass (20 DAA) and plant conditions (21 DAA)

Test item [L BAS 555 01 F/ha]	Cabbage	Carrot	Cucumber	Oat	Sugar beet	Pea	Tomato	Onion
Mean fresh weight [% of control]								
Control	100	100	100	100	100	100	100	100
0.8	100	94	88*	106	101	108	74*	116
1.6	85*	80*	80*	110	72*	109*	47*	114
2.0	76*	73*	63*	107	57*	99*	36*	109
2.4	48*	53*	51*	107	47*	64*	30*	94
Mean visible damage [% damage compared to the control]								
Control	0	0	0	0	0	0	0	0
0.8	5	5	15	0	0	0	25	0
1.6	20	20	25	0	35	0	55	0
2.0	25	30	40	0	45	0	65	0
2.4	55	50	50	0	55	40	70	0

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

The endpoints derived from the results described above are summarized in Table B.9.11.2-2

Table B.9.11.2-2: NOAER, ER_{25} and ER_{50} of BAS 555 01 F on plant fresh weight and phytotoxicity 21 days (± 1 day) after post-emergence application [L/ha]

Test item [L/ha]	Cabbage	Carrot	Cucumber	Oat	Sugar beet	Pea	Tomato	Onion
	Phytotoxicity							
NOAER	< 0.8	< 0.8	< 0.8	≥ 2.4	1.6	2.0	< 0.8	≥ 2.4
	Plant fresh weight (shoots above ground)							
NOAER	1.6	1.6	< 0.8	≥ 2.4	1.6	2.0	< 0.8	≥ 2.4
ER_{25}	1.85	1.6	1.33	> 2.4	1.75	1.75	0.78	> 2.4
ER_{50}	2.35	> 2.4	> 2.4	> 2.4	> 2.4	> 2.4	1.47	> 2.4

Conclusions:

The NOAER based on plant fresh weight for oat and onion was > 2.4 L BAS 555 01 F/ha, for pea the NOAER was 2.0 L BAS 555 01 F/ha, for cabbage, carrot and sugar beet the NOAER was 1.6 L BAS 555 01 F/ha and for cucumber and tomato the NOAER could not be determined, as the plant weight of those species was even decreased in the lowest concentration tested.

The NOAER based on visible plant damage could not be determined for cabbage, carrot, cucumber and tomato, because the plants showed injuries even in the lowest concentration tested. For sugar beet the NOAER based on plant damage was 1.6 L BAS 555 01 F/ha and 2.0 L BAS 555 01 F/ha for peas. For onions and oat the NOAER was determined to be > 2.4 L BAS 555 01 F/ha.

The ER_{25} for plant weight was 1.85 L BAS 555 01 F/ha for cabbage, 1.33 L BAS 555 01 F/ha for cucumber, 1.6 L BAS 555 01 F/ha for carrot, 1.75 L BAS 555 01 F/ha for sugar beet and pea and 0.78 L BAS 555 01 F/ha for tomato. For oat and onion the ER_{25} was > 2.4 L BAS 555 01 F/ha.

The ER_{50} for plant weight was 2.35 L BAS 555 01 F/ha for cabbage and 1.47 L BAS 555 01 F/ha for tomato. The ER_{50} values for carrot, cucumber, oat, sugar beet, pea and onion were > 2.4 L BAS 555 01 F/ha.

RMS comments:
This study was performed according to OECD Test Guideline 208, which is a precursor of the currently accepted OECD Test Guideline 227. The following validity criteria of OECD Test Guideline 227 are met:

- The seedling emergence was at least 70% in the control and treated groups
- The plants in the controls did not exhibit visible phytotoxic effects
- The mean plant survival in the controls was at least 90% for the duration of the study (measured: 100%)
- Environmental conditions for a particular species in the controls were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source.

The seedling emergence in the control (should be > 70% according to OECD TG 227) is not reported in the study report, and could thus not be assessed. It is also noted that the concentration of test item in the test solution was not confirmed by analytical measurements. Further, the substrate used had a high organic carbon content (8.1%, where OECD TD 227 recommends an organic carbon content of up to 1.5%). Finally, this study was not performed under GLP.

Taking into account the shortcomings listed above, the reliability of the study could be questioned. As another vegetative vigour study is available, which is fully in line with OECD TG 227 and was performed under GLP (Porch *et al.* 2006; CP10.6.2/02), the present study will only be considered as supportive information. Note however that the results from the present study are generally in line with the study by Porch *et al.* (2006).

The lowest endpoints from the present study are listed below, for information:
ER_{50} (*Lycopersicon esculentum*, 21d) = 1.47 L BAS 555 01 F/ha (equivalent to 132.3 g a.s./ha)
NOER (*Lycopersicon esculentum*, 21d) < 0.8 L BAS 555 01 F (equivalent to < 72 g a.s./ha)

Report:	**CP10.6.2/02. Porch J.R, Krueger H.O., Kendall T.Z., Holmes C. (2006a)** **Caramba (BAS 555 01 F): A toxicity test to determine the effects of the test substance on vegetative vigor of ten species of plants**
Report No.:	2006/7007217
Guidelines:	EPA 850.4150, EPA 850.4250
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and Methods:	
Test substance:	BAS 555 01 F (Batch No.: 2030, contains 87.9 g/L of the active ingredient metconazole)
Test species :	
Monocotyledons:	corn (*Zea mays*), onion (*Allium cepa* L.), ryegrass (*Lolium perenne*), wheat (*Triticum aestivum*)
Dicotyledons:	bean (*Phaseolus vulgaris*), cabbage (*Brassica oleracea*), lettuce (*Lactuca sativa*), radish (*Raphanus sativus*), soybean (*Glycine max*), tomato (*Lycopersicon esculentum*)

Type of test:	(greenhouse) vegetative vigour test (21 days)
Test design:	6 replicates for the test item treatments and for the control for each plant species. Each replicate consisted of five plants, with each plant contained in a separate pot. Each replicate set of pots was assigned to a subirrigation tray.
Applied concentrations:	untreated control (water purified by reverse osmosis), 0.08, 0.16, 0.31, 0.62, 1.24 L BAS 555 01 F/ha (equivalent to 7, 14, 28, 56 and 112 g a.s./ha)
Spray volume:	200 L/ha
Test substrate:	sandy loam soil (65% sand, 18% silt, 17% clay); 2.2% organic matter; pH = 7.3
Environmental conditions:	greenhouse conditions
	temperatures: 19.1-34.6°C (mean: 25.1°C) for corn ryegrass, wheat, bean and radish; 17.7-38.7°C (mean: 24.9°C) for onion, cabbage, tomato, lettuce and soybean relative humidity: 21-90% (mean: 60%) for corn, ryegrass, wheat, bean and radish; 24-94% (mean: 66%) for onion, cabbage, tomato, lettuce and soybean photoperiod: 16:8 h light:dark light intensity: Plants were illuminated with additional light to ensure a minimum 16-hour photoperiod watering: subirrigation (to minimize the potential of leaching of the test substance through soil), according to need. First watering on the day of application of the test item
Analytical methods:	HPLC-UV
Statistics:	Descriptive statistics, Dunnett's test.

Test procedure:
For each plant species tested, seeds were planted in round plastic pots measuring approximately 11 cm in diameter and 10 cm deep. After planting, the pots were placed in the greenhouse where the seeds were allowed to emerge and develop into seedlings to be used in the study. Seedlings used in the test were selected based upon visual evaluation of their similarity in size and condition, and were randomly assigned to treatment groups prior to test initiation.
The spray solution was mixed on the day of application, by dilution of the test item in reverse osmosis water. The spray mixtures were sampled after their preparation to provide material for analytical confirmation of the test concentrations. A single application of BAS 555 01 F was made to each treatment group using a calibrated laboratory spray cabin.
Following the application the plants were cultivated for 21 days in the greenhouse. Assessment of phytotoxicity (e.g. leaf curl, necrosis, chlorosis) and plant height were done on Day 0 (prior to application), and 7, 14, and 21 days after application (DAA). 21 DAA the dry weight of the plant biomass above ground was determined.

Findings:
Analytical results: The control samples showed no indication of the presence of the test substance or of the presence of a co-eluting substance at the characteristic retention time of the substance. The measured concentrations for the samples collected from the treatments were within 95.5-98.9% of the nominal concentration.

Biological results: The biological results are summarized in Table B.9.11.2-3 and Table B.9.11.2-4.

No unacceptable adverse effects were observed on any endpoint at test termination for corn, onion, ryegrass, wheat, lettuce or tomato. Apparent treatment-related necrosis was observed on tomato seedlings 7 DAA and 14 DAA, but plants were largely recovered at test termination and only incidental signs of toxicity were observed. Although signs of toxicity such as necrosis, chlorosis and leaf curl were observed on onion, ryegrass, wheat, and lettuce, the severity of the observed signs was slight and not dose-responsive. Therefore the signs of toxicity were not considered treatment-related.

Effects were observed on the four remaining species (bean, cabbage, soybean, and radish) at test termination. Statistically significant differences (Dunnett's test, $\alpha = 0.05$) in mean height compared to the control were observed among treatment groups of bean and cabbage. The significant differences were observed 7 DAA and 14 DAA only. 21 DAA, all treatment groups showed heights and dry weights similar to the control, but effects on plant condition of bean and cabbage were present in the highest treatment group. Effects on soybean were restricted to necrosis and chlorosis at the highest application rate. Slight necrosis, chlorosis, and/or insect damage were observed on plants in the 28 and 56 g a.s./ha treatment groups, but the signs were not dose-responsive and considered incidental to treatment. Mean height and dry weight of radish plants were reduced by up to 14% in the treatment groups compared to the control throughout the study. Although several of the group means were significantly different from the control (Dunnett's test, $\alpha = 0.05$), effects were not dose-related and the magnitude of the reductions was not adequate for determining an ER_{25}. Effects on the condition of radish seedlings such as necrosis, chlorosis, and leaf curl were observed at the three highest application rates 21 DAA.

Since there were no reductions of 25% or greater at test termination in this study, the ER_{25} and ER_{50} estimates were all determined to be greater than 112 g a.s./ha (corresponding to 1.24 L BAS 555 01 F/ha), the highest application rate tested.

Table B.9.11.2-3: Effects of BAS 555 01 F on plant height, dry weight and survival 21 DAA

Test item [g a.s./ha]	Corn	Onion	Ryegrass	Wheat	Lettuce	Tomato	Bean	Cabbage	Soybean	Radish
Plant height [% reduction compared to the control]										
7	4	-7	-1	-3	-2	6	1	3	2	9
14	-2	6	0	-7	-4	-5	0	9	-4	7
28	-2	7	-6	-4	-1	-4	14	6	-4	8
56	0	4	1	0	2	4	18	5	1	8
112	-4	-1	0	-6	-4	6	13	8	3	8
Dry weight [% reduction compared to the control]										
7	8	-2	6	-11	1	-5	-9	-6	6	10
14	1	7	8	-14	-5	-10	-15	4	-2	12
28	3	9	1	-7	-2	3	-11	5	-3	12
56	2	-1	-2	-1	-2	-6	13	3	-4	14*
112	-1	-2	14	-11	0	10	5	13	9	14
Survival [% reduction compared to the control]										
7	0	0	0	0	0	0	0	0	0	-3
14	0	3	0	0	0	3	0	0	0	-3
28	0	0	0	0	3	0	0	0	0	-3
56	0	0	0	0	0	0	0	0	0	-3
112	0	0	0	0	0	0	0	0	0	0

** Statistically significant differences compared to the control (Dunnett's test, $\alpha = 0.05$)*

Table B.9.11.2-4: NOER, ER_{25} and ER_{50} of BAS 555 01 F on plant dry weight, plant height and plant survival/condition 21 days after post-emergence application [g a.s./ha]

Test item [g a.s./ha]	Corn	Onion	Ryegrass	Wheat	Lettuce	Tomato	Bean	Cabbage	Soybean	Radish
	Plant dry weight, plant height, plant condition									
NOER	≥ 112	≥ 112	≥ 112	≥ 112	≥ 112	≥ 112	56 *	56 *	56 *	14 *
	Plant dry weight, plant height									
ER_{25}	> 112	> 112	> 112	> 112	> 112	> 112	> 112	> 112	> 112	> 112
ER_{50}	> 112	> 112	> 112	> 112	> 112	> 112	> 112	> 112	> 112	> 112

** Most sensitive endpoint: plant condition.*

Conclusions:

The application of BAS 555 01 F to seedlings at rates up to 112 g a.s./ha resulted in no unacceptable adverse effects on corn, onion, ryegrass, wheat, lettuce, or tomato at test termination. The ER_{25} for all species was determined to be > 112 g a.s./ha, which was the maximum application rate tested. Effects on plant condition were observed on bean, cabbage, and soybean at the application rate of 112 g a.s./ha, and on the condition of radish at the application rates of 28 g a.s./ha and higher.

RMS comments:

The test was performed according to US EPA Test Guideline 850.4150, which is equivalent to OECD Test Guideline 227; The validity criteria of both test guidelines are met:

- The plants in the controls did not exhibit visible phytotoxic effects
- The mean plant survival in the controls was at least 90% for the duration of the study (measured: 100%)
- Environmental conditions for a particular species in the controls were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source.

Consequently, this study is considered acceptable for use in the risk assessment.

The analytical method used could be sufficiently validated according to the current EU Guidance SANCO/3029/99 rev. 4. The LOQ could be set at 2.3 mg/L (please refer to Vol. 3 (CA), Section B.5.1.2.6 – study no. 21, for further details). The fortification range covers the tested concentrations and the endpoints.

The lowest endpoints derived from this study are presented below:
ER_{50} (several plant species, biomass) > 1.24 L BAS 555 01 F/ha (equivalent to > 112 g a.s./ha)
NOER (*Raphanus sativus*, plant condition) = 0.16 L BAS 555 01 F/ha (equivalent to 14 g a.s./ha)

Report:	**CP10.6.2/03. Porch J.R., Krueger H.O., Krip W., Holmes C. (2006b)** **Caramba (BAS 555 01 F): A toxicity test to determine the effects of the test substance on seedling emergence of ten species of plants**
Report No.:	2006/7007216
Guidelines:	EPA 850.4100, EPA 850.4225
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and methods:	
Test substance:	BAS 555 01 F (Batch No.: 2030, contains 87.9 g/L of the active ingredient metconazole)
Test species :	
Monocotyledons:	corn (*Zea mays*), onion (*Allium cepa* L.), ryegrass (*Lolium perenne*), wheat (*Triticum aestivum*)
Dicotyledons:	bean (*Phaseolus vulgaris*), cabbage (*Brassica oleracea*), lettuce (*Lactuca sativa*), radish (*Raphanus sativus*), soybean (*Glycine max*), tomato (*Lycopersicon esculentum*)
Type of test:	(greenhouse) seedling emergence and seedling growth test (21 days)
Test design:	four replicate for the test item treatment and the control. Each replicated consisted of one pot, with ten seeds planted in each pot
Applied concentrations:	untreated control (water), 0.015, 0.046, 0.14, 0.41 and 1.24 L BAS 555 01 F/ha (equivalent to 1.38, 4.15, 12.4, 37.3 and 112 g a.s./ha) for corn, onion, ryegrass, wheat, bean, radish and soybean. Cabbage, lettuce and tomato were sprayed with these rates plus the additional lower test rate of 0.005 L BAS 555 01 F/ha (equivalent to 0.461 g a.s./ha)
Spray volume:	200 L/ ha
Test substrate:	sandy loam soil (65% sand, 18% silt, 17% clay); 2.2% organic matter; pH = 7.3
Environmental conditions:	greenhouse conditions temperatures: 18.7-34.6°C (mean: 24.9°C) for onion, ryegrass, cabbage, lettuce and tomato; 18.8-38.2°C (mean: 25.1°C) for corn, wheat, bean, radish and soybean relative humidity: 21-90% (mean: 56%) for onion, ryegrass, cabbage, lettuce and tomato; 24-92% (mean: 58%) for corn, wheat, bean, radish and soybean photoperiod: 16:8 h light:dark light intensity: Plants were illuminated with additional light to ensure a minimum 16-hour photoperiod watering: subirrigation (to minimize the potential of leaching of the test substance through soil), according to need. First watering on the day of application of the test item
Analytical methods:	HPLC-UV
Statistics:	Descriptive statistics, Dunnett's test.
Test procedure:	

For each plant species tested, seeds were planted in plastic pots (approximately 16 cm in diameter and 12 cm deep) on the day of test substance application. Ten indiscriminately selected seeds were planted in each pot. Corn, wheat, bean and soybean seeds were planted at a depth of approximately 20 mm, while all other species were planted at a depth of approximately 6 mm.
The spray solution was mixed on the day of application, by dilution of the test item in reverse osmosis water. The spray mixtures were sampled after their preparation to provide material for analytical confirmation of the test concentrations. A single application of BAS 555 01 F was made to each treatment group using a calibrated laboratory spray cabin.
Following the application the plants were cultivated for 21 days in the greenhouse. Assessments for seedling emergence and phytotoxicity were done 7, 14 and 21 days after application (DAA) for all plants. Determination of dry weight and height of the plant biomass above ground was done 21 DAA.

Findings:
Analytical results: The control samples showed no indication of the presence of the test substance or of the presence of a co-eluting substance at the characteristic retention time of the substance. The measured concentrations for the samples collected from the treatments were within 94.7-109% of the nominal concentration.

Biological results: The biological results are summarized in Table B.9.11.2-5 and Table B.9.11.2-6.
The application of BAS 555 01 F caused no unacceptable adverse effect in any species, and there were no reductions of 25% or more when compared to the control for any endpoint. Statistically significant differences have been observed in emergence and survival of bean, height of cabbage and dry weight of radish, but the reductions were not dose-responsive and therefore were determined to be incidental to treatment. Since there were no reductions of 25% or greater at test termination in this study, the ER_{25} and ER_{50} estimates were all determined to be greater than 112 g a.s./ha (corresponding to 1.24 L BAS 555 01 F/ha), the highest application rate tested.

Table B.9.11.2-5: Effects of BAS 555 01 F on seedling emergence, plant height, dry weight and survival 21 DAA

Test item [g a.s./ha]	Corn	Onion	Ryegrass	Wheat	Lettuce	Tomato	Bean	Cabbage	Soybean	Radish
Seedling emergence [% reduction compared to the control]										
0.461	--	--	--	--	3	-8	--	0	--	--
1.38	0	6	3	-22	-3	-27	5	-3	8	3
4.15	0	0	-6	-4	-5	-23	5	3	8	3
12.4	0	0	-8	15	0	-27	3	-3	0	3
37.3	0	-3	6	-22	-5	4	10*	-3	5	3
112	3	14	-3	-19	-3	-8	5	3	5	0
Plant height [% reduction compared to the control]										
0.461	--	--	--	--	-7	5	--	8	--	--
1.38	-15	-5	0	-9	-11	-2	-12	4	1	-4
4.15	-13	5	6	-7	-2	-8	-25	11*	-6	5
12.4	-11	-5	6	-10	-12	9	2	3	0	4
37.3	-6	-7	5	1	-12	-5	3	5	-4	5
112	-12	9	3	5	-2	4	-10	11*	5	12
Dry weight [% reduction compared to the control]										
0.461	--	--	--	--	2	4	--	12	--	--
1.38	-39	-14	13	-2	-1	10	-5	13	-13	-5
4.15	-26	-10	2	-12	1	-8	1	20	1	12
12.4	-24	-8	7	-18	-3	8	0	13	1	-7
37.3	-14	-39	-4	8	-4	-8	-9	9	-12	19*
112	-34	9	-1	-2	1	9	-6	8	-10	9
Survival [% reduction compared to the control]										
0.461	--	--	--	--	0	-8	--	-3	--	--
1.38	0	6	0	-19	-6	-28	5	-3	8	5
4.15	0	0	-6	-8	-8	-28	5	0	8	3
12.4	3	3	-11	15	-3	-32	3	-5	-3	3
37.3	0	0	3	-27	-8	4	10*	3	5	8
112	3	14	-6	-23	-6	-12	5	8	5	0

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

Table B.9.11.2-6: NOER, ER_{25} and ER_{50} of BAS 555 01 F on seedling emergence, plant height, dry weight and survival 21 DAA [g a.s./ha]

Test item [g a.s./ha]	Corn	Onion	Ryegrass	Wheat	Lettuce	Tomato	Bean	Cabbage	Soybean	Radish
NOER	≥ 112	≥ 112	≥ 112	≥ 112	≥ 112	≥ 112	≥ 112	≥ 112	≥ 112	≥ 112
ER_{25}	> 112	> 112	> 112	> 112	> 112	> 112	> 112	> 112	> 112	> 112
ER_{50}	> 112	> 112	> 112	> 112	> 112	> 112	> 112	> 112	> 112	> 112

Conclusions:

A pre-emergence application of BAS 555 01 F at the application rate of 112 g a.s./ha resulted in no unacceptable adverse effects of 25% or more on the ten species tested. Therefore, the NOER for all species was determined to be 112 g a.s./ha, and all ER_{25} estimates were determined to be greater than 112 g a.s./ha, which was the highest application rate tested.

RMS comments:

The test was performed according to US EPA Test Guideline 850.4100, which is equivalent to OECD Test Guideline 208. The validity criteria of US EPA Test Guideline 850.4100 are met:

- The mean survival of emerged control seedlings was at least 90% at test termination (measured: ≥ 96.2%)
- The seedlings in the control did not exhibit visible phytotoxic effects that are the same as due to the test substance
- All test chambers and soil medium used for a particular species are identical

It is however noted that for all plant species, 10 seeds were planted per pot, while the test guideline recommends to limit the number of seeds per pot (15 cm diameter) for species such as corn, soybean and tomato to one to two seeds. Further, the relative humidity range is this study was wider than recommended by the test guideline. As the validity criteria for the control were met, it is considered that these deviations do not impact the acceptability of this study.

It should also be noted that OECD Test Guideline 208 mentions the following additional validity criterion: "The seedling emergence in the controls should be at least 70%". In the present study, this validity criterion was met for all plant species except for wheat (emergence in the control of 67.5%) and for tomato (emergence in the control of 65.0%). For these two species, the validity of the study results could thus be questioned.

Overall, this study is considered acceptable for use in the risk assessment, with the exception of the results for wheat and tomato.

The analytical method used could be sufficiently validated according to the current EU Guidance SANCO/3029/99 rev. 4. The LOQ could be set at 2.3 mg/L (please refer to Vol. 3 (CA), Section B.5.1.2.6 – study no. 21, for further details). The fortification range covers the tested concentrations and the endpoints.

ER_{50} (several plant species) > 1.24 L BAS 555 01 F (equivalent to > 112 g a.s./ha)
NOER (several species) = 1.24 L BAS 555 01 F (equivalent to 112 g a.s./ha)

B.9.11.3. Extended laboratory studies on non-target plants

No extended laboratory studies on non-target plants using the formulated product have been submitted.

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

No semi-field and field tests on non-target plants using the formulated product have been submitted.

B.9.12. RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS

The risk assessment for effects on terrestrial non-target higher plants is conducted according to **the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC** (SANCO/10329/2002 rev. 2).

Toxicity

Non-target higher plant (NTP) toxicity studies have been submitted, which investigate the impact of the active substance metconazole in the representative formulation BAS 555 01 F.

In these toxicity studies, BAS 555 01 F was tested at an application rate of up to 1.24 L product/ha, which is equivalent to 112 g a.s./ha. As this application rate exceeds the proposed application rate in winter and spring cereals and winter oilseed rape, these data can be used for the present risk assessment. The tested plant species cover a range of dicotyledons and monocolyledons from 6 different plant families, which meets the regulatory requirements. No effect > 50% for vegetative vigour and seedling emergence were reported for any of the tested plant species at the highest dose tested. Therefore, the ER_{50} value was estimated to be > 112 g a.s./ha. This value is used in the risk assessment.

Table B.9.12-1: Effects on seedling emergence and vegetative vigour of metconazole in the formulation BAS 555 01 F.

Species	Family	Shoot fresh weight ER_{50}	
		Vegetative vigour study [1] (g a.s./ha)	Seedling emergence study [2] (g a.s./ha)
MONOCOTYLEDONS			
Zea mays (corn)	Poaceae	> 112	> 112
Allium cepa (onion)	Liliaceae	> 112	> 112
Lollium perenne (ryegrass)	Poaceae	> 112	> 112
Triticum aestivum (wheat)	Poaceae	> 112	- [3]
DICOTYLEDONS			
Phaseolus vulgaris (bean)	Fabaceae	> 112	> 112
Brassica oleracea (cabbage)	Brassicaceae	> 112	> 112
Lactuca sativa (lettuce)	Asteraceae	> 112	> 112
Raphanus sativus (radish)	Brassicaceae	> 112	> 112
Glycine max (soybean)	Fabaceae	> 112	> 112
Lycopersicon esculentum (tomato)	Solanaceae	> 112	- [3]

Notes: [1] Porch J.R. et al., 2006a; CP10.6.2/02 ; [2] Porch J.R. et al., 2006b; CP10.6.2/03 ; [3]This species was tested in the available seedling emergence study, but the validity criteria of the test were not met.

Exposure

The critical use patterns relevant for the uses of BAS 555 01 F are given in Table B.9.12-2.

Table B.9.12-2: Use pattern of BAS 555 01 F for use as foliar spray

Crop	Application time (BBCH growth stage)	Max. number of applications	Interval between applications [d]	Application rate per treatment	
				Metconazole [kg a.s./ha]	BAS 555 01 F [L/ha]
Cereals (winter/spring)	30 - 69	1 - 2	21	0.090	1.0
Winter oilseed rape	13 – 20 (autumn) 21 – 71 (spring) [1)]	1 - 2	90	0.072	0.8
	21 – 71 (spring)	1 - 2	14	0.072	0.8

[1)] *According to the GAP a first application in autumn (between BBCH 13 and BBCH 20) is followed by a second application in spring (between BBCH 21 and BBCH 71)*

Spray drift is considered the key exposure route for terrestrial plants located in the vicinity of the treated area. Following the Guidance Document on Terrestrial Ecotoxicology, the Predicted Exposure Rates (PER) are calculated as follows:

PER = application rate (g a.s./ha) x drift factor

The drift factor accounts for the reduced exposure in off-field areas due to drift of plant protection products following spraying. The drift models produced by the BBA for the exposure assessment of aquatic organisms (Ganzelmeier *et al.*, 1995: recalculated by the German BBA and UBA and published by the BBA in 2000) are used as a surrogate to cover the exposure assessment of terrestrial plants. These drift values are obtained from tables in the Guidance Document on Terrestrial Ecotoxicology or appendix IV in the ESCORT II Guidance Document.

For the intended application of BAS 555 01 F in cereals and oilseed rape, the drift factor is derived from the category "field crops" with a drift value of 2.77% at 1 m. The PER values following the highest single proposed application rate of BAS 555 01 F at a drift distance of 1 m for all proposed uses are shown in Table B.9.12-3.

Table B.9.12-3: Predicted exposure rates (PER) for the proposed use of BAS 555 01 F in winter and spring cereals and winter oilseed rape.

Crop use	Application rate (g a.s./ha)	Drift distance (m)	Drift factor	PER (g a.s./ha)
Cereals (winter/spring)	90	1	0.0277	2.493
Winter oilseed rape	72	1	0.0277	1.994

Risk assessment

The risk to NTPs is considered based on TER values (Toxicity Exposure Ratios) which compare the observed toxicity from experimental studies with the predicted off-field exposure rate (based on maximum single exposure rate). Calculation of the TER is based on the following equation:

$$TER = \frac{ER_{50}\ (g\ a.s./ha)}{PER\ (g\ a.s./ha)}$$

TER values below the trigger value of 5 indicate an unacceptable risk to NTPs in the off-field environment. Calculated TER values for the single proposed application of BAS 555 01 F are listed in Table B.9.12-4.

Table B.9.12-4: TER values for non-target plants following the proposed uses of BAS 555 01 F in winter and spring cereals, and winter oilseed rape.

Crop use	Drift distance (m)	PER (g a.s./ha)	ER_{50} (g a.s./ha)	TER
Cereals	1	2.493	> 112	> 44.93
Oilseed rape	1	1.994		> 56.17

Using the standard distance of 1m for all proposed uses, the TER value exceeds the trigger of 5, indicating an acceptable risk.

Conclusion: The risk to non-target terrestrial plants from exposure to metconazole following the intended uses of BAS 555 01 F in winter and spring cereals and oilseed rape is acceptable.

B.9.13. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

Although no longer a data requirement according to Regulation (EU) No. 284/2013, a new acute earthworm toxicity study and a carbon transformation study with the formulation BAS 555 01 F have been submitted. In addition, a litter bag study with the formulation BAS 555 00 F has been submitted. These three studies are summarized below.

Report:	**CP10.7/01. Koelzer U. (2003b)** **Assessment of the side effects of BAS 555 01 F on the activity of the soil microflora, short-term respiration**
Report No.:	2003/1004138
Guidelines:	OECD Guideline No. 217
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and methods:	
Test substance:	BAS 555 01 F (Batch No.: R2066-079, contains 89.2 g/L of the active ingredient metconazole)
Type of test:	soil respiration tests (28 days)
Test soil:	A biologically active agricultural soil: loamy sand soil (57.8% sand, 32.6% silt, 9.6% clay), pH 6.31, 1.10% C_{org}, WHC: 34.1%
Test design:	3 replicates per concentration and control
Applied concentrations:	
Nominal:	Control, 1.116 mg BAS 555 01 F/kg dry soil (corresponding to a single application rate of 0.8 L/ha, equivalent to 72 g a.s./ha), 11.16 mg BAS 555 01 F/kg dry soil (corresponding to an application rate of 8.0 L/ha, equivalent to 720 g a.s./ha). Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm³
Reference material:	Dinoterb (purity: 97.0%), tested at 3.33, 6.67 and 13.33 mg/kg soil dry weight in a separate study
Test conditions:	soil moisture: approximately 45% of maximum water holding capacity (MWHC) at test start. The water content of the soil was adjusted one a week when needed. pH: 6.07-6.73 soil samples were incubated at 20 ± 2°C in the dark

Test procedure:
Test solutions containing the appropriate amount of BAS 555 01 F in water were applied directly to the test soil. The amount of test solution added to the test soil resulted in a final water content of 45% WHC_{max}. The control samples were treated with water only. After addition of the test solutions (BAS 555 01 F) and water (control) to the soil, the soil samples were thoroughly mixed. Each treatment group contained 6000 g moist soil.
After application, three 2000 g soil sub-samples (one for each replicate in each treatment group) were placed in 2000 mL glass bottles. The bottles were closed loosely with screw caps and weighed for the

determination of the starting weight. About every 7 days the amount of moisture loss was determined by reweighing, and water was added to adjust vessels to the starting weight.
At 0, 7, 14 and 28 days after application of the test item, samples of 200 g were taken from the bulk batches for carbon transformation measurements. The carbon transformation was measured after the addition of glucose (500 mg/100g soil wet weight, which was found to be the optimum to obtain maximal short-term rates of respiration). A "OxiTop Control" system was used to measure the oxygen consumption. The incubation period was between 20h and 24h at 20 ± 2 °C. The rate of oxygen consumption during the measurement period was determined within the first 12h, and the O_2 produced per hour per kg dry weight of soil was calculated.

Findings:
No adverse effects of BAS 555 01 F on carbon transformation in soil were observed in both test item concentrations after 28 days. Only slight deviations from the control between -7.72 and 2.43 were measured after 28 days. The results are summarized in Table B.9.13-1.

In a separate study the reference item Dinoterb produced the expected level of effect (-28.8%, -35.6% and -29.5% inhibition).

Table B.9.13-1: Effects of BAS 555 01 F on soil micro-organisms (carbon transformation) on days 7, 14 and 28 of incubation

Soil (days)	Control	1.116 mg BAS 555 01 F per kg dry soil equivalent to 0.8 L/ha		11.16 mg BAS 555 01 F per kg dry soil equivalent to 8.0 L/ha	
	O_2 consumption [mg/h/kg dry soil]	O_2 consumption [mg/h/kg dry soil]	% Deviation from the control [1)]	O_2 consumption [mg/h/kg dry soil]	% Deviation from the control [1)]
Loamy sand soil (7 d)	2.86	2.98	+4.21	3.09	+8.19
Loamy sand soil (14 d)	2.83	2.93	+3.39	2.78	-1.68
Loamy sand soil (28 d)	2.63	2.68	+1.88	2.43*	-7.72*

*[1)] Based on O_2 consumption; - = inhibition, + = stimulation.; * value was calculated on the basis of 2 rather than 3 measured values due to a defect in the measuring device.*

Conclusions:
BAS 555 01 F caused no short-term and long-term effects on carbon transformation (measured as O_2 consumption) in a field soil at concentrations up to 0.72 kg metconazole/ha, equivalent to a field application rate of 8.0 L BAS 555 01 F/ha.

RMS comments:
The coefficients of variation in the control were maximum 11.3%. Thus, the validity criterion of OECD Test Guideline 217 (i.e. the variation between replicate control samples needs to be less than 15%) was met for all sampling dates (0, 7, 14, and 28 days). Consequently, this study is considered acceptable for use in the risk assessment. As a soil respiration study is no longer a data requirement according to Regulation (EU) No. 284/2013, the results of this study will however only be used as supportive information.

BAS 555 01 F had no adverse effect of > 25% on soil carbon turnover at concentrations of up to 11.16 mg BAS 555 01 F/kg soil dry weight (equivalent to 0.95 mg a.s./kg soil dry weight), the highest concentration tested.
NOEC (soil carbon turnover, 28 days) = 11.16 mg form./kg soil dry weight (equivalent to 0.95 mg a.s./kg soil dry weight).

Report:	**CP10.7/02. Vértesi A. (2003)** **Acute toxicity of the BAS 555 01 F to earthworms (*Eisenia fetida*)**
Report No.:	2003/1006392 (03/750-125G / 84 583_2)
Guidelines:	OECD Guideline No. 207; ISO 11268-1
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and methods:	
Test substance:	BAS 555 01 F (Batch No.: R 2066-079, contains 89.2 g/L of the active substance metconazole)
Test species:	earthworm (*Eisenia fetida*)
Number of organisms, weight, age:	4 replicates of 10 earthworms per replicate, adults with clitellum, at least 2 months old, weighing between 300 and 600 mg
Type of test:	acute artificial soil test (14 days)
Applied concentrations:	
Nominal concentrations:	de-ionised water control; 53, 95, 171, 309, 556 and 1000 mg BAS 555 01 F/kg test substrate dw (corresponding to 4.52, 8.10, 14.58, 26.35, 47.41 and 85.28 g a.s./kg test substrate dw)
positive control:	chloroacetamide
Soil type and test conditions:	test substrate: The artificial test soil was prepared with 10 % sphagnum peat (as close to pH 5.5 -6.0 as possible, no visible plant remains, finely ground, dried to measured moisture content), 20 % kaolin clay (kaolinite content above 30 %) and 70 % industrial sand (fine sand was dominant with more than 50 % of the particles between 50 and 200 µm). The air-dry constituents were blended in the correct proportions and mixed thoroughly. pH was adjusted to 6.0 by addition of $CaCO_3$. Test substance solutions were added to give an overall moisture content of 34.17 % of the dry weight and the medium was mixed thoroughly. At the end of the test the water content was found to be 31.84 %.
	pH: 5.9-6.4
	water content: 33.80-34.63% (of dry soil) at test initiation, 30.68-32.59% (of dry soil) at test termination
	temperature: 19.2 – 21.4 °C
	light regime: continuously illuminated (779 lux)

Test procedure:
Immediately before the start of the test, the appropriate amount of the test item in deionised water was mixed thoroughly with the artificial soil so that the moisture of the soil was approximately 35 % of the dry weight. Care was taken to avoid any contamination of the control. The test item concentrations were

applied in increasing order, thus minimizing the risk of contamination. On day 0, after washing and drying gently the individual weight of each worm was determined, then worms were exposed to the treated artificial soil. For each concentration and the control, four replicate test containers each containing 750 g wet weight of artificial soil were used.

Observations:
The mortality was assessed by emptying the test medium onto a suitable tray, sorting worms from the medium and testing their reaction to a mechanical stimulus at their front end. After the 7-day assessment, worms and medium were replaced in the test containers. On day 14 – at the end of the experiment – the same mortality assessment was done and additionally the live weight of the worms was determined after gentle washing and drying. The worm biomass (weight) was determined for each worm individually on day 0 as well as on day 14. Any behavioural or pathological symptoms noted were reported.
At the start of the test, the moisture content of the test medium was adjusted to approximately 35 % of the dry weight, at the start and at the end of the test it was determined and both of them were reported.
The pH of the control and treated soil samples were measured on day 0 and 14 of the study.

Statistical evaluation:
For worm weight, mean values and standard deviations were calculated for each treatment and each replicate at the start and at the end of the test. A precise LC_{50} value of the test item could not be calculated due to the lack of sufficient effect of test item on mortality.
Statistical analysis on mortality and worm biomass was assessed by analysis of variance (ANOVA) Fisher's exact Test and Dunnett's Test ($\alpha = 0.05$) by TOXSTAT software. The premises of ANOVA (at least homogeneity of variance) were tested by adequate test statistics.

Findings:
The results of this test are summarised in Table B.9.13-2. The LC_{50} was determined to be higher than 1000 mg BAS 555 01 F/kg soil dry weight. After 14 days of exposure a statistically not significant mortality of 7.5 % was observed at a test concentration of 1000 mg BAS 555 01 F/kg soil dry weight (Fisher's exact Test, $\alpha = 0.05$) compared to the control. No additional mortality was observed at any other treatment group.

The biomass development was not statistically significantly inhibited at any tested concentration of BAS 555 01 F. (Dunnett's Test, $\alpha = 0.05$). NOAEC based on the statistical analysis of the biomass changes and the mortality data could be determined as 1000 mg BAS 555 01 F/kg soil dry weight.

The 14-day LC_{50} value for chloroacetamide was 33.50 mg/kg soil dry weight (95 % confidence limits: 31.34 – 35.81 mg/kg) and the NOEC was 20 mg/kg (based on mortality and biomass development).

Table B.9.13-2: Effect of BAS 555 01 F on earthworm (*Eisenia fetida*) mortality and biomass 14 days after treatment

Concentration of BAS 555 01 F (mg/kg soil dry weight)	control	53	95	171	309	556	1000
Mortality (%) – 14 day	0.0	0.0	0.0	0.0	0.0	0.0	7.5
Weight change (%)	-9.61	-11.09	-10.70	-12.30	-11.09	14.68	13.94
Endpoints (mg/kg soil dry weight)							
NOAEC (14 day)	1000						
LC_{50}	> 1000						

Mortality: Fisher's exact Test, α = 0.05; statistically not significant compared to the control
Weight change: Dunnett's Test, α = 0.05; statistically not significant compared to the control

Conclusions:
In a 14-day toxicity study with BAS 555 01 F to earthworms (*Eisenia fetida*) the LC_{50} was higher than 1000 mg BAS 555 01 F/kg soil dry weight.
The NOAEC (related to mortality and weight change) was 1000 mg BAS 555 01 F/kg soil dry weight, the highest rate tested.

RMS comments:
The test design was in line with the OECD Test Guideline 207 and the validity criteria are met:
- The mortality in the controls did not exceed 10 % at the end of the test (measured: 0.0 %)

Consequently, this study is considered acceptable for use in the risk assessment. As an earthworm acute toxicity study is no longer a data requirement according to Regulation (EU) No. 284/2013, the results of this study will however only be used as supportive information.

The following endpoints are concluded for metconazole:
LC_{50} (*Eisenia* foetida, 14 days) > 1000 mg BAS 555 01 F/kg soil dw (equivalent > 85.28 mg a.s./kg soil dw)
NOEC (*Eisenia* foetida, 14 days) = 1000 mg BAS 555 01 F/kg soil dw (equivalent to 85.28 mg a.s./kg soil dw)

Report:	**CP10.7/03. Klein S. and Meister A. (2003) Effects of BAS 555 00 F on the decomposition of organic matter enclosed in litter bags in the field**
Report No.:	2003/1012079
Guidelines:	Draft Method for Litter-bag test on decomposition (March 2001) Improvements of the method developed during the EPFES workshop (April 2002)
GLP:	Yes
Previous evaluation:	None (submitted for the purpose of renewal of a.s. approval); relevant for renewal application
Materials and methods:	
Test substance:	BAS 555 00 F (Batch No.: 162940, contains 58.2 g/L of the active substance metconazole)
Test site:	arable field site under conventional use (crop: summer barley (*Hordeum vulgare*)) in Rossdorf, Darmstadt-Dieburg, Germany; total size: about 1050 m^2. The selected area did not receive an application of any crop protection product during the study apart from the test item and the reference item, including the control plots.
Test design:	An arable field site under conventional use was chosen in order to simulate a typical biocoenosis. The experiment was of randomized design with two treatment groups and six replicates (plots) each and 40 litter bags per replicate. The size of each plot was 25 m^2. Within the plots the litter bags were distributed randomly with a distance of at least 10 cm from each other and buried in 5 cm depth. The treatments were assigned randomly to the plots within each replicate.
Endpoints:	total mass loss of straw (in percent of ash-free dry weight)

Treatment groups:	untreated control; test item treatment group: BAS 555 00 F 1st application applied at 1.5 L/ha (corresponding to the long-term PEC; incorporated into soil up to a depth of 10 cm) and 2nd application at 3.0 L/ha (corresponding to the seasonal rate applied at one application; not including crop interception according to FOCUS)
	The 1st application was done onto bare soil and at the same time summer barley was drilled; the 2nd application was done after the litter bags have been buried into the soil (the litter bags were buried at approximately 5 cm deep into the soil 7 days after the 1st application and 3 days before the 2nd application)
Application dates:	1st application: 03.06.2002 (before litter-bags were buried into the soil) 2nd application: 13.06.2002 (after litter-bags were buried into the soil)
Test conditions:	Natural field conditions
	soil type: sandy loamy silt sand, pH 7.2, TOC: 1.16 %, WHC: 47 %. The field was irrigated with approx. 6 mm on 16.06.2002, rainfall within 3 days after the 2nd application was 0.9 mm.
Litter bags:	Litter bags consisted of curtain material with a mesh size of about 7 x 7 mm. The size of a bag was about 10 x 15 cm. Bags were filled with about 3.0 g (dry weight) of untreated dried wheat straw.
Sampling dates:	1st sampling: 09.07.2002 (after 29 days of exposure) 2nd sampling: 16.09.2002 (after 98 days of exposure) 3rd sampling: 15.11.2002 (after 158 days of exposure) 4th sampling: 14.03.2003 (after 277 days of exposure)
Soil sampling:	Treatments were verified by analyzing soil cores that were taken from plots following the 1st application and incorporation of the test item and one day after the 2nd application and submitted to a residue analysis. 5 soil samples of 4 cm diameter were taken from each treated plot and pooled. Soil samples were analyzed in the IBACON laboratory. Quantification of metconazole was done by LC-MS/MS.
Sample processing:	8 bags per plot were taken from the soil, immediately transported to the laboratory and either stored under cooled conditions or immediately submitted to further processing. The bag content was oven-dried at 35 °C for 15 hours; enclosed straw material was separated manually by dry sieving. Then the straw was combusted for 30 min at 600 °C and ash-free weight was determined. Sampling was done at four different time intervals (29, 98, 158 and 277 days after burying of the litter bags).
Statistics:	The mean values per replicate (expressed in % mass loss of ash-free dry weight) were analyzed by Student-t-test ($\alpha = 0.05$).

Findings:

After 29 days of exposure the mass losses in the control (14.6 %) and in the test item group (14.9 %) were comparable. The test item group resulted in slightly higher mass loss values and the decomposition was 102.2 % of the control. According to Student-t Test ($\alpha = 0.05$) this was statistically not significant.

The mean mass loss after 98 days of exposure was 40.7 % in the test item group. In the control group a higher mean mass loss (44.3 %) was observed. The decomposition in the test item group was 92.1 % of the control. The difference was statistically significant compared to the control (Student-t Test, $\alpha = 0.05$).

After 158 days of exposure the mean mass loss of the test item treatment group was 50.5 %, which is comparable to the mass loss value in the control group, where 51.0 % mass loss was found. The comparison of the two treatments resulted in a decomposition of 99.1 % of the control. This was statistically not significant (Student-t Test, $\alpha = 0.05$).

After 277 days of exposure the mean mass loss of the test item treatment group was 59.0 %, which is comparable to the mass loss value in the control group, where 60.3 % mass loss was found. The comparison of the two treatments resulted in a decomposition rate of 97.8 % of the control. This was statistically not significant (Student-t Test, $\alpha = 0.05$).

In order to analyse the decomposition process, the mass loss per day was calculated. The highest rates per day were achieved between the applications and the first sampling date (0.50 % per day in the control and 0.51 % per day in the test item group).

Effects of the treatments on the degradation of buried wheat straw are summarized in Table B.9.13-3.

Table B.9.13-3: Effects of the formulation BAS 555 00 F on the degradation of buried wheat straw (mean mass loss, decomposition expressed as % of control, decomposition per day (%))

Treatment	Sampling date 1	Sampling date 2	Sampling date 3	Sampling date 4
	Mean mass loss (%)			
control	14.6	44.3	51.0	60.3
BAS 555 00 F	14.9	40.7*	50.5	59.0
	Decomposition (% of control)			
BAS 555 00 F	102.2	92.1*	99.1	97.8
	Decomposition per day (%)			
control	0.50	0.43	0.11	0.08
BAS 555 00 F	0.51	0.37*	0.16	0.07

The results represent rounded values calculated on the exact raw data
Decomposition per day: between burying the bags and the 1st sampling and between samplings respectively
Statistics: Student-t-test, one sided, $\alpha = 0.05$ (significantly different compared to the control)*

Conclusions:
BAS 555 00 F caused no adverse long-term effects on functions of soil organisms, i.e. the organic matter decomposition process. The risk to soil inhabiting organisms, such as soil microorganisms, mesofauna and macrofauna, contributing to decomposition of buried organic wheat straw enclosed in litter bags, is low. Validity criteria were reached with 60 % decomposition in the control group at the end of the study.

RMS comments:
The test design was in line with the draft method and the validity criterion was met:

- The mass loss in the control group should be at least 60 % at the end of the experimental phase (measured: 60.3 %)

Consequently, this study is considered acceptable for use in risk assessment.

B.9.14. RISK ASSESSMENT FOR OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

B.9.14.1. Risk assessment for biological methods for sewage treatment

Toxicity

A study on the impact of metconazole on the respiration of sewage sludge (CA8.8/01; Heim D. & Yan Z., 1999) is summarized in Vol. 3 (AS), Section B.9.8, from which an EC_{50} > 1000 mg a.s./L was derived. However, as not all validity criteria of the most recent version of the test guideline were met, this endpoint is not considered acceptable for use in the risk assessment.

Risk assessment

Although the available study on the effect of metconazole on the respiration of sewage sludge is not acceptable, a preliminary risk assessment based on the endpoint derived from this study (EC_{50} > 1000 mg a.s./L) is performed. This EC_{50} value is 38 x 10^3 times greater than the FOCUS Step 1 overall maximum PEC_{SW} (26.367 µg/L) for the proposed uses of BAS 555 01 F. As dilution prior to reaching sewage treatment works would also be expected to reduce the exposure further, the risk to sewage treatment facilities would be considered limited based on the currently available endpoint. However, a fully acceptable study on effects of metconazole on the respiration of sewage sludge is needed to obtain a reliable EC_{50} value, and to confirm that the risk would indeed be acceptable.

B.9.15. BATCHES USED IN THE ECOTOXICOLOGICAL STUDIES

Test species	Test substance	Chemical purity	Batch n°	Reference
Birds - Acute oral toxicity				
Colinus virginianus	Metconazole 83:17 *cis:trans*	95.3% (79.8% *cis*, 15.5% *trans*)	89-01	[redacted] 1992a
Colinus virginianus	Metconazole 85:15 *cis:trans*	97.9% (84.2% *cis*, 13.7% *trans*)	AC 10575-61	[redacted] 1998
Colinus virginianus	Metconazole 95% *cis*	96.7%	ST89/324	[redacted] 1998
Colinus virginianus	Triazolyl acetic acid	98.9%	01893-196	[redacted] 2003
Colinus virginianus	BAS 555 01 F	84.6 g a.s./L	200002	[redacted] 2008
Birds – Subchronic and reproductive toxicity				
Colinus virginianus	Metconazole 83:17 *cis:trans*	95.3% (79.8% *cis*, 15.5% *trans*)	89-01	[redacted] 1991a
Anas platyrhynchos	Metconazole 83:17 *cis:trans*	95.3% (79.8% *cis*, 15.5% *trans*)	89-01	[redacted] 1991b
Colinus virginianus	Mectonazole 95% *cis*	95.2%	12 (F900250)	[redacted] 1992b
Anas platyrhynchos	Mectonazole 95% *cis*	95.2%	12 (F900250)	[redacted] 1992c
Colinus virginianus	Metconazole 85:15 *cis:trans*	97.9% (84.2% *cis*, 13.7% *trans*)	AC 10575-61	[redacted] [redacted]., 1999
Colinus virginianus	Triazolyl alanine	97.5%	TLB 1207	[redacted] [redacted] 1983
Anas platyrhynchos	Triazolyl alanine	97.5%	TLB 1207	[redacted] 1983
Aquatic organisms – Toxicity of the active substance and metabolites				
Oncorhynchus mykiss	Metconazole 83:17 *cis:trans*	95.3% (79.8% *cis*, 15.5% *trans*)	89-01	[redacted] 1990
Pimephales promelas	Metconazole 83:17 *cis:trans*	95.3% (79.8% *cis*, 15.5% *trans*)	89-01	[redacted] 1991a
Cyprinus carpio	Metconazole 85:15 *cis:trans*	97.9% (83.3% *cis*, 14.6% *trans*)	AC 9339-114	[redacted] [redacted] 1996a
Oncorhynchus mykiss	Mectonazole 95% *cis*	95.2%	12 (F900250)	[redacted] 1991b
Cyprinodon variegatus	Metconazole 85:15 *cis:trans*	98.7% (84.3% *cis*, 14.4% *trans*)	AS2122a	[redacted] 2005
Lepomis macrochirus	Metconazole 80:20 *cis:trans*	98.8%	COD-000779	[redacted] 2008a
Danio rerio	Metconazole 80:20 *cis:trans*	98.8%	COD-000779	[redacted] 2008b
Gasterosteus aculeatus	Metconazole 85:15 *cis:trans*	98.1%	AC12140-17	[redacted] 2010
Oncorhynchus mykiss	1,2,4-triazole	91.9%	EN38530	[redacted] 1983
Oncorhynchus mykiss	Metconazole 85:15 *cis:trans*	97.9% (83.3% *cis*, 14.6% *trans*)	AC 9339-114	[redacted] [redacted] 1996b
Oncorhynchus mykiss	Metconazole 85:15 *cis:trans*	97.4% (83.1% *cis*, 14.3% *trans*)	AC 10925-24B	[redacted] 2001
Cyprinodon variegatus	Metconazole 85:15 *cis:trans*	99.4% (84.1% *cis*, 15.3% *trans*)	AS2122a	[redacted] 2009
Pimephales promelas	Mectonazole 95% *cis*	95.2%	12 (F900250)	[redacted] 1992
Oncorhynchus mykiss	1,2,4-triazole	99.9%	NLL 7052-1	[redacted] [redacted] 2002

Test species	Test substance	Chemical purity	Batch n°	Reference
Pimephales promelas	Metconazole 85:15 *cis:trans*	99.1% (85.0% *cis*, 14.1% *trans*)	43707	[redacted] 2008
Pimephales promelas	[triazole-3,5-^{14}C]metconazole	99.44%	10624-1A	[redacted] 1996
Pimephales promelas	[p-Chlorophenyl-U-14C]metconazole	97.2%	10973-2	[redacted] M., 2002
Daphnia magna	Metconazole 83:17 *cis:trans*	95.3% (79.8% *cis*, 15.5% *trans*)	89-01	Toy R., 1990
Daphnia magna	Mectonazole 95% *cis*	95.2%	12 (F900250)	Toy R., 1991b
Daphnia magna	1,2,4-triazole	100.8%	JC16/215854/3	Bell G., 1995
Daphnia magna	Metconazole 85:15 *cis:trans*	97.4% (83.1% *cis*, 14.3% *trans*)	AC 10925-24B	Jatzek H.J., 2002
Daphnia magna	Mectonazole 95% *cis*	95.2%	12 (F900250)	Toy R., 1991c
Chironomus riparius	Metconazole 85:15 *cis:trans*	97.9% (83.3% *cis*, 14.6% *trans*)	AC 9339-114	England D.C. *et al.*, 1997
Pseudokirchneriella subcapitata	Metconazole 83:17 *cis:trans*	95.3% (79.8% *cis*, 15.5% *trans*)	89-01	Toy R., 1990
Pseudokirchneriella subcapitata	Mectonazole 95% *cis*	95.2%	12 (F900250)	Toy R., 1991b
Pseudokirchneriella subcapitata	1,2,4-triazole	99%	R200	Palmer S.J. *et al.*, 2001
Lemna gibba	Metconazole	98.2%	COD-001502	Brzozowska K., 2014
Oncorhynchus mykiss	Metconazole	≥ 97%	Unknown	Konwick B.J. *et al.*, 2006
Aquatic organisms – Toxicity of the preparations				
Oncorhynchus mykiss	BAS 555 01 F	89.2 g a.s./L	R2066-079	Zok S., 2003
Daphnia magna	BAS 555 01 F	92.3 g a.s./L	R1811-181	Oliveiri C.F. *et al.*, 2000a
Pseudokirchneriella subcapitata	BAS 555 01 F	92.3 g a.s./L	R1811-181	Oliveiri C.F. *et al.*, 2000b
Bees – Acute and chronic toxicity of the active substance and metabolites				
Apis mellifera	Metconazole 83:17 *cis:trans*	95.3% (79.8% *cis*, 15.5% *trans*)	89-01	Harrison E.G. & Hillaby J.M., 1991
Bombus terrestris	Metconazole	98.2%	COD-001502	Haupt S., 2015a and b
Apis mellifera	Metconazole	98.7%	COD-001163	Kleebaum K., 2014
Apis mellifera	Metconazole	98.7%	COD-001163	Kleebaum K., 2015a
Apis mellifera	Metconazole	98.7%	COD-001163	Kleebaum K., 2017
Bees – Acute toxicity of the preparations				
Apis mellifera	BAS 555 01 F	89.2 g a.s./L	R2066-079	Schmitzer S. & Weber B., 2002
Apis mellifera	BAS 555 01 F	88.9 g a.s./L	200001	Kling A., 2007
Bees – Semi-field studies				
Apis mellifera	BAS 555 00 F	60.1 g a.s./L	0003255328	Franke M., 2013
Non-target terrestrial arthropods – laboratory tests				
Typhlodromus pyri	BAS 555 01 F	89.2 g a.s./L	R2066-079	Schwiening S. & Buetzler R., 2002
Aphidius rhopalosiphi	BAS 555 01 F	89.2 g a.s./L	R2066-079	Drexler A., 2002a
Chrysoperla carnea	BAS 555 01 F	89.2 g a.s./L	R2066-079	Drexler A., 2002b

Test species	Test substance	Chemical purity	Batch n°	Reference
Aleaochara bilineatia	BAS 555 01 F	89.2 g a.s./L	R2066-079	Buehler A., 2003
Typhlodromus pyri	BAS 555 01 F	89.2 g a.s./L	R2066-079	Eden A., 2003
Typhlodromus pyri	BAS 555 01 F	88.9 g a.s./L	200001	Hanewald N. & Petrik-Steisslinger D., 2007
Aphidius rhopalosiphi	BAS 555 01 F	89.2 g a.s./L	R2066-079	Moll M. & Buetzler R., 2003
Soil organisms - Toxicity of the active substance and metabolites				
Eisenia foetida	Mectonazole 95% *cis*	95.2%	12 (F900250)	Hillaby J.M. & Harrison E.G., 1991
Eisenia foetida	Metconazole 85:15 *cis:trans*	97.9% (84.2% *cis*, 13.7% *trans*)	AC10575-61	Engelhard E.K. *et al.*, 1998a
Eisenia foetida	Metconazole	98.2%	COD-001502	Friedrich S., 2014
Eisenia foetida	1,2,4-triazole	99.9%	NLL7052-1	Moser Th. & Scheffczyk A., 2004
Folsomia candida	Metconazole	98.7%	COD-001163	Ganssmann M., 2013
Folsomia candida	1,2,4-triazole	99.9%	NLL7052-1	Moser Th. & Scheffczyk A., 2002
Hypoaspis aculeifer	Metconazole	98.7%	COD-001163	Schulz L., 2014a
Hypoaspis aculeifer	1,2,4-triazole	99%	R 200	Schulz L., 2014b
Soil organisms – Toxicity of the preparations				
Eisenia foetida	BAS 555 01 F	89.2 g a.s./L	R2066-079	Vértesi A., 2003
Eisenia foetida	BAS 555 01 F	90.2 g a.s./L	FRE-000698	Friedrich S., 2012a
Eisenia foetida	BAS 555 00 F	60.1 g a.s./L	0003255328	Friedrich S., 2012b
Naturally occurring earthworm populations	BAS 555 00 F	58.2 g a.s./L	162940	Luehrs U., 2003
Folsomia candida	BAS 555 01 F	90.2 g a.s./L	FRE-000698	Friedrich S., 2012c
Hypoaspis aculeifer	BAS 555 01 F	90.2 g a.s./L	FRE-000698	Schulz L., 2012
Naturally occurring populations of soil organisms	BAS 555 00 F	58.2 g a.s./L	162940	Klein S. & Meister A., 2003
Soil micro-organisms – Toxicity of the active substance and metabolites				
Micro-organisms	1,2,4-triazole	100%	T4-610-8	Voelkel W., 2000
Soil micro-organisms – Toxicity of the preparations				
Micro-organisms	BAS 555 01 F	89.2 g a.s./L	R2066-079	Koelzer U., 2003a
Micro-organisms	BAS 555 01 F	89.2 g a.s./L	R2066-079	Koelzer U., 2003b
Non-target terrestrial plants – Toxicity of the preparations				
Several plant species	BAS 555 01 F	89.2 g a.s./L	R2066-79	Sack D., 2005
Several plant species	BAS 555 01 F	87.9 g a.s./L	2030	Porch J.R. *et al.*, 2006a
Several plant species	BAS 555 01 F	87.9 g a.s./L	2030	Porch J.R. *et al.*, 2006b
Activated sludge – Toxicity of the active substance				

B.9.16. 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	Previous evaluation
KCP 10.1.1.2/01	[redacted]	2008	Mixture of BAS 507 01 F and BAS 555 01 F - Acute toxicity in the bobwhite quail (Colinus virginianus) after single oral administration (LD50) BASF Report No.: 2008/1032655 [redacted] GLP, not published	Yes	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.1.2.2/01	Anonymous	2015	BAS 555 F - Ecologically relevant chronic toxicity endpoint for the wild mammalian reproductive risk assessment BASF Report No. : 2015/1192591 Not GLP, not published	No	No	Not applicable	BASF	Submitted for the purpose of renewal
KCP 10.2.1/01	[redacted]	2003	BAS 555 01 F - Acute toxicity study on the rainbow trout (Oncorhynchus mykiss) in a static system over 96 hours BASF Report No.: 2003/1014059 [redacted] GLP, not published	Yes	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.2.1/02	Olivieri C.E. et al.	2000a	Acute toxicity of AC 900768 (Metconazole) in a 90 g/L SL formulation (RLF 12307) to Daphnia magna under static test conditions BASF Report No.: MK-560-011 American Cyanamid Co., Princeton NJ, United States of America GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal

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KCP 10.2.1/03	Olivieri C.E. et al.	2000b	Effects of AC 900768 (Metconazole) in a 90 g/L SL formulation (RLF 12307) on growth of the green alga, Selenastrum capricornutum BASF Report No.: MK-560-012 American Cyanamid Co., Princeton NJ, United States of America GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.3.1.1/01	Schmitzer S. Wewer B.	2002	Effects of BAS 555 01 F (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory BASF Report No.: 2002/1012936 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.3.1.1/02	Kling A.	2007	Assessment of side effects of BAS 555 01 F to the honey bee, Apis mellifera L. in the laboratory BASF Report No.: 2007/1050640 Eurofins-GAB GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.3.1.5/01	Franke M.	2013	Effects of BAS 555 00 F on the honeybee Apis mellifera L. under semi-field conditions (tunnel test) with additional assessments on colony and brood development BASF Report No.: 2012/1111494 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal

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	Previous evaluation
KCP 10.3.2.1/01	Schwiening S. Buetzler R.	2002	Effects of BAS 555 01 F on the predatory mite Typhlodromus pyri in the laboratory - Dose response test BASF Report No.: 2002/1012750 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.3.2.1/02	Drexler A.	2002a	Effects of BAS 555 01 F on the parasitoid Aphidius rhopalosiphi (Hymenoptera, Braconidae) in a laboratory trial - Dose-response BASF Report No.: 2002/1012935 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.3.2.1/03	Drexler A.	2002b	Effect of BAS 555 01 F on the green lacewing Chrysoperla carnea (Neuroptera: Chrysopidae) in a laboratory trial - Dose response BASF Report No.: 2002/1008629 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.3.2.1/04	Buehler A.	2003	Effect of BAS 555 01 F on the rove beetle Aleochara bilineata GYLL. (Coleoptera: Staphylinidae) in a laboratory trial BASF Report No.: 2003/1006374 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal

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	Previous evaluation
KCP 10.3.2.2/01	Eden A.	2003	Effects of BAS 555 01 F on the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) in an extended laboratory trial - Dose response BASF Report No.: 2003/1014069 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.3.2.2/02	Hanewald N. Petrik-Steisslinger D.	2007	Evaluation of the duration of effects of BAS 555 01 F on the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) - Aged residue trial BASF Report No.: 2007/1018768 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.3.2.2/03	Moll M. Buetzler R.	2003	Effects of BAS 555 01 F on the parasitoid Aphidius rhopalosiphi, extended laboratory study - Dose response test BASF Report No. : 2003/1006386 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.4.1.1/01	Friedrich S.	2012a	Sublethal toxicity of BAS 555 01 F to the earthworm Eisenia fetida in artificial soil with 5 % peat BASF Report No.: 2012/1182245 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal

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	Previous evaluation
KCP 10.4.1.1/02	Friedrich S.	2012b	Sublethal toxicity of BAS 555 00 F to the earthworm Eisenia fetida in artificial soil with 5 % peat BASF Report No.: 2012/1182243 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.4.1.2/01	Luehrs U.	2003	Field study to evaluate the effects of BAS 555 00 F on earthworms BASF Report No.: 2003/1012039 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.4.2.1/01	Friedrich S.	2012c	Effects of BAS 555 01 F on the reproduction of the collembolan Folsomia candida BASF Report No.: 2012/1182246 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.4.2.1/02	Schulz L.	2012	Effects of BAS 555 01 F on the predatory mite Hypoaspis aculeifer BASF Report No.: 2012/1182247 BioChem agrar Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal

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	Previous evaluation
KCP 10.5/01	Koelzer U.	2003	Assessment of side effects of BAS 555 01 F on the activity of soil microflora, nitrogen turnover BASF Report No.: 2003/1004137 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.6.2/01	Sack D.	2005	BAS 555 01 F: Effects on non-target plants in the greenhouse - A multiple rate test BASF Report No.: 2005/1029570 BASF AG Agrarzentrum Limburgerhof, Limburgerhof, Germany Fed.Rep. GLP, not published	No	No	Not applicable	BASF	Submitted for the purpose of renewal
KCP 10.6.2/02	Porch J.R. et al.	2006a	Caramba (BAS 555 01 F): A toxicity test to determine the effects of the test substance on vegetative vigor of ten species of plants BASF Report No.: 2006/7007217 Wildlife International Ltd., Easton MD, United States of America GLP, not published	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal
KCP 10.6.2/03	Porch J.R. et al.	2006b	Caramba (BAS 555 01 F): A toxicity test to determine the effects of the test substance on seedling emergence of ten species of plants BASF Report No: 2006/7007216 Wildlife International Ltd., Easton MD, United States of America GLP, notpublished	No	Yes	New data for AIR3 renewal; Study required to fulfil new data requirements according to 1107/2009	BASF	Submitted for the purpose of renewal

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	Previous evaluation
KCP 10.7/1	Koelzer U.	2003	Assessment of the side effects of BAS 555 01 F on the activity of the soil microflora, short-term respiration BASF Report No.: 2003/1004138 GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH, Niefern-Oeschelbronn, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal	BASF	Submitted for the purpose of renewal
KCP 10.7/02	Vertesi A.	2003	Acute toxicity of the BAS 555 01 F to earthworms (Eisenia fetida) BASF Report No.: 2003/1006392 TRC - Toxicological Research Centre Ltd., Veszprem, Hungary GLP, not published	No	Yes	New data for AIR3 renewal	BASF	Submitted for the purpose of renewal
KCP 10.7/03	Klein S. Meister A.	2003	Effects of BAS 555 00 F on the decomposition of organic matter enclosed in litter bags in the field BASF Report No. : 2003/1012079 Institut fuer Biologische Analytik und Consulting IBACON GmbH, Rossdorf, Germany Fed.Rep. GLP, not published	No	Yes	New data for AIR3 renewal	BASF	Submitted for the purpose of renewal