Document Title

Summary of the ecotoxicological studies
Aclonifen SC 600 G

Data Requirement(s)

Regulation (EC) No 1107/2009 & Regulation (EU) No 284/2013

Document MCP

Section 10: Ecotoxicological studies

According to the Guidance Document SANCO/10181/2013 for applicants on preparing dossiers for the approval of a chemical active substance

Date

2020-01-16, rev. 2020-07-24

Author(s)

[redacted], [redacted]

Battelle UK Ltd

OWNERSHIP STATEMENT

This document, the data contained in it and copyright therein are owned by Bayer and/or affiliated entities. No part of the document or any information contained therein may be disclosed to any third party without the prior written authorisation of Bayer and/or affiliated entities.

The summaries and evaluations contained in this document are based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority. Other registration authorities should not grant, amend or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either:

- **from Bayer or respective affiliate; or**
- **from other applicants once the period of data protection has expired.**

Version history

Date [yyyy-mm-dd]	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2020-01-16	Initial version	M-677017-01-1
2020-07-24	CP 10.4.2.2 – Inclusion of new statement regarding interpretation of CP 10.4.2.2/01 study results and removal of superceded statements	M-677017-02-1

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4, 'How to revise an Assessment Report'.

Table of Contents

CP 10 ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Aclonifen was included in Annex I to Council Directive 91/414/EEC in 2008 (Directive 2008/116/EC, Entry into Force on 01 August 2009).

The formulation PPP Aclonifen SC 600 G (or Aclonifen 600 g/L), is a suspension concentrate formulation containing 600 g/L of aclonifen. This formulation is registered throughout Europe under trade names such as Bandur (Aclonifen-SC600; AE-F068300-00-SC50-A2; EXP-04209). Aclonifen SC 600 G was already a representative formulation of Bayer for the Annex I inclusion of aclonifen under Council Directive 91/414/EEC.

This present dossier in support of approval renewal includes all the data submitted at the time of the Annex I inclusion, in summaries updated and re-evaluated as necessary to take account of current validity criteria and data requirements.

Use pattern considered in this risk assessment

Table 10-1: Intended application pattern

Crop	Timing of application (BBCH range)	Number of applications	Application interval (days)	Maximum label rate (range) [L/ha]	Maximum application rate (g aclonifen/ha)
Peas	11-30	1	-	1	600
Peas	12-19	1	-	0.5	300

Definition of the residue for risk assessment

Justification for the residue definition for risk assessment is provided in Document M-CA7, Section 7.4.1 and Document M-CA6, Section 6.7.1.

Table 10-2: Definition of the residue for risk assessment

Compartment	Compound / Code
Soil	Aclonifen
Groundwater	Aclonifen
Surface water	Aclonifen
Plant material	Aclonifen

Consideration of metabolites

None of the metabolites of aclonifen is considered as ecotoxicologically relevant. None of the metabolites poses a higher risk to terrestrial and aquatic organisms than the parent compound.

CP 10.1 Effects on birds and other terrestrial vertebrates

CP 10.1.1 Effects on birds

A summary of the avian toxicity endpoints for aclonifen is provided in the following table. Details and a full description of the toxicity studies used in this risk assessment can be found in Document M-CA8, Section CA 8.1.1 of this dossier.

Table 10.1-1: Avian endpoints used in risk assessment

Test item	Risk assessment	Type of exposure	Endpoint	Reference
Aclonifen	Acute risk assessment	Acute oral toxicity on Bobwhite quail	LD_{50} >2000 mg a.s./kg bw	KCA 8.1.1.1/01 M-172009-01-1 [redacted] 1999
	Long-term risk assessment	Subchronic, 6 week dietary (reproduction) on Japanese quail	NOAEC = 1000 ppm NOAEL = 141 mg a.s./kg bw/day	KCA 8.1.1.3/01 M-174897-01-1 [redacted] 1995

Toxicity of the formulation

Aclonifen is of low acute oral toxicity to bobwhite quail with LD_{50} values in excess of 2000 mg a.s./kg bw.

With regard to animal welfare, acute oral studies with formulations are not routinely conducted on birds, but only with the active ingredients. If substances are non-toxic to birds, the LD_{50} data of the active ingredient can be used to reliably predict the toxicity of the formulation.

The LD_{50} of aclonifen confirms aclonifen is non-toxic to birds, therefore, it can be assumed that the product is also non-toxic to birds. Therefore it is justified to waive the acute test with the formulation in birds.

Summary of the risk assessment for birds

The risk assessment for effects of Aclonifen SC 600 G on birds was performed in accordance with the "European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals" (EFSA 2009)[1], (subsequently referred to as the Guidance document (EFSA 2009)). The risk assessment demonstrated acceptable acute and long-term dietary exposure risks following the proposed uses and based on the 'worst-case' screening step.

The risk from consumption of contaminated water was assessed for aclonifen. The acute and long-term risk from drinking water exposure was considered to be acceptable.

Aclonifen has a log P_{ow} of 4.37 which is higher than the trigger value of 3 and hence an assessment of the risk from secondary poisoning was required. The secondary poisoning risk for earthworm-eating and fish-eating birds from the proposed uses of Aclonifen SC 600 G was shown to be acceptable.

Risk assessment for birds

The following avian risk assessment has been conducted in line with EFSA's Bird and Mammal Guidance Document (EFSA Journal 2009; 7(12):1438), referred to in the following as EFSA (2009). No short-term risk assessment is required under EFSA (2009) as this is assumed to be covered by the acute and reproductive (long-term) risk assessment and these are conducted in the sections below.

[1] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal .2009; 7(12):1438. [139 pp.] doi:10.2903/j.efsa.2009.1438. Avalable online: www.efsa.europa.eu

The main potential route of exposure for birds to foliar applied agrochemicals is considered to be through the ingestion of residues on contaminated food, e.g. vegetation, insects and earthworms. The intended GAP for Aclonifen SC 600 G is presented in Table 10-1 above.

Direct exposure of Aclonifen SC 600 G to birds is considered unlikely since at the time of application, and for a period thereafter, most birds will leave the immediate vicinity of spray operations in response to the degree of human disturbance. The greatest levels of exposure will arise in the case of birds foraging in the foliage of the crops some hours after application.

To achieve a concise risk assessment, the risk envelope approach is applied. Here all following assessments have been made for the use of Aclonifen SC 600 G in peas using an application rate of 600 g a.s./ha as this will also cover the risks from use at lower application rates.

Dietary risk assessment

Screening assessment

The first, or screening, step assesses the risk based on a worst-case approach. The risk is considered acceptable, if the ‘Toxicity Exposure Ratio’ (TER) value pass the trigger values of ≥10 for acute exposure and ≥5 for chronic exposure. If the TER values do not pass the trigger values in certain areas, a Tier 1 risk assessment based on more relevant and realistic conditions is performed in those particular areas.

Calculation of Daily dietary Dose (DDD)

The daily dietary dose (DDD) for a single application is given by the following equation:

$$DDD_{single\ application} = Application\ Rate\ [kg/ha]\ x\ Shortcut\ Value\ x\ TWA$$

The Time Weighted Average factor (TWA) is only considered for the long-term exposure. The long-term risk assessment can be based on a TWA = 0.53 (estimates time-weighted exposure over 21 days, assuming a default DT_{50} of 10 days).

Calculation of Toxicity Exposure Ratio (TER)

The assessment of the risks to birds is performed for both acute and long-term exposures using endpoints derived from acute and reproduction studies with birds.

The calculation of acute and long-term toxicity-exposure-ratios (TER) is defined as follows:

Acute risk assessment: $TER_A = LD_{50}/DDD$

Reproductive risk assessment: $TER_{LT} = NOAEL/DDD$

Screening step

According to EFSA (2009), an ‘indicator species’ is used in a screening step to eliminate all those substances that clearly pose a low risk to birds. This ‘indicator species’ is not a real species but, by virtue of its size and feeding habits, is considered to have a higher exposure than (i.e. to be protective of) other species that may occur in a particular crop at a particular time.

For application to the crop relevant for this dossier, peas, the small omnivorous bird should be considered in the screening step using the relevant shortcut values for acute and long-term risk assessments. The shortcut value consists of the food intake rate of the species of concern, its body weight, the concentration of a substance in/on fresh diet and the fraction of diet obtained in the treated area.

Table 10.1-2: Avian indicator species and shortcut values for the screening assessment

Crop	Indicator species	Shortcut value (SV)	
		Acute assessment	Reproductive assessment

Peas	Small omnivorous bird	158.8	64.8

Table 10.1-3: Avian screening acute assessment for the proposed uses of Aclonifen SC 600 G

Crop	Indicator Species	Toxicity (mg a.s./ kg bw)	Appl. rate (kg/ha)	SV	DDD	TER_A	Trigger
Peas	Small omnivorous bird	>2000	0.6	158.8	95.28	20.99	10

SV: Shortcut Value
TER: Toxicity Exposure Ratio
DDD: Daily Dietary Dose

The screening assessment for the acute risks to birds from exposure to Aclonifen SC 600 G after use according to the recommended GAP demonstrate that the risks are acceptable, with the TER_A value calculated to be greater than the trigger of 10, indicating a low potential acute risk to birds from the exposure of Aclonifen SC 600 G. In this occasion, a first-tier assessment was not required.

For the long-term (reproduction) assessment, in accordance with the recommendations of EFSA (2009), the acute oral LD_{50} used in the acute avian assessment was divided by 10 to obtain the $LD_{50}/10$. This was compared to the lowest NOAEL from the avian reproduction studies and the lowest of the $LD_{50}/10$ and NOAEL values was used in the screening assessment.

Acute oral LD_{50} from acute avian assessment >2000 mg a.s./kg bw

$LD_{50}/10$ = >200 mg a.s./kg bw

Lowest NOAEL from avian reproduction studies = 141 mg a.s./kg bw/d

The NOAEL was less than the $LD_{50}/10$, therefore the NOAEL from the avian reproduction studies was used in the long-term assessment.

Table 10.1-4: Avian screening long-term assessment for the proposed uses of Aclonifen SC 600 G

Crop	Indicator Species	Toxicity (mg a.s./kg bw/d)	Appl. rate (kg/ha)	SV	TWA	DDD	TER_A	Trigger
Peas	Small omnivorous bird	141	0.6	64.8	0.53	20.61	6.84	5

SV: Shortcut Value
TWA: Time Weighted Average factor
TER: Toxicity Exposure Ratio
DDD: Daily Dietary Dose

The screening assessment for the long-term risks to birds from exposure to Aclonifen SC 600 G after use according to the recommended GAP demonstrate that the risks are acceptable, with the TER_{LT} value calculated to be greater than the trigger of 5, indicating a low potential long-term risk to birds from the exposure of Aclonifen SC 600 G. Therefore, a first-tier assessment was not required.

Drinking water risk assessment

Exposure of birds or mammals via drinking water is not explicitly included in the DDD calculations of the dietary risk assessment. Therefore, in line with EFSA (2009) an approach is presented that allows estimating the possible risk arising from uptake of contaminated drinking water for two basic scenarios. Due to the incidental nature of occurrence of drinking water reservoirs on agricultural fields (as

compared to the contamination of food items growing or dwelling on those fields), a separate assessment of this exposure route is considered appropriate at least on the first-tier level.

Most birds and mammals can, in principle, satisfy at least parts of their daily water demand via uptake of food. However, this potential depends on the water content of the diet items, which is lowest for seeds. Therefore, the assessment methodology for the risk to birds and mammals of pesticides in drinking water as provided below uses small granivorous animals as indicator species at Tier 1.

EFSA (2009) identifies two scenarios as relevant for assessing the risk of pesticides via drinking water to birds and mammals:

- *Leaf scenario:* Birds taking water that is collected in leaf whorls after application of a pesticide to a crop and subsequent rainfall or irrigation.
- *Puddle scenario:* Birds and 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.

A leaf scenario is clearly the worst-case situation. It is relevant for spray applications only and according to EFSA (2009) should be considered for the following crop types and growth stages:

- Leaf vegetables (forming heads) at principal growth stage 4 until harvest (classification according to BBCH52).
- Other leaf vegetables (e.g. cauliflower) at principal growth stage 4 or later, with a morphology that facilitates collection of rain/irrigation water in reservoirs that are large enough and easily accessible to attract birds and sufficiently stable over some hours.

A leaf scenario is not deemed relevant for small mammals.

As the proposed use for Aclonifen SC 600 G does not include any leaf scenario listed above, the only relevant source of exposure is the puddle scenario.

EFSA (2009) indicates that no specific calculations of exposure and TER are necessary when the ratio of the effective application rate (g/ha) to the relevant endpoint (mg a.s./kg bw/d) does not exceed 50 in the case of less sorptive substances (K_{oc} <500 L/kg) or does not exceed 3000 in the case of more sorptive substances (K_{oc} >500 L/kg).

Rather than the effective application rate, the maximum application rate of 600 g a.s./ha will be used as a worse case consideration. The K_{oc} value for aclonifen is 5727 L/kg and as it is >500 L/kg the trigger of 3000 is acceptable.

Table 10.1-5: Application rate to endpoint ratios for the proposed uses of Aclonifen SC 600 G

Risk assessment	Application rate (g a.s./ha)	Endpoint (mg a.s./kg bw/d)	Ratio	Trigger
Acute	600	>2000	< 0.3	3000
Long-term		141	4.25	

As the ratios of application rate to endpoint are lower than 3000 for both the acute and long-term assessment no specific calculations of exposure to birds via drinking water are necessary. An acceptable risk can be concluded from contaminated drinking water as a result of the proposed use of Aclonifen SC 600 G.

Bioaccumulation and food chain behaviour

Plant protection products with high bioaccumulation potential could theoretically bear a risk of secondary poisoning for birds, if contaminated prey such as fish or earthworms are eaten. According to EFSA (2009), for organic chemicals, a log P_{ow} >3 is used to indicate whether there might be a potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains.

The log P_{ow} of aclonifen was determined to be 4.37 (see Document M-CA2, Section CA 2.7). Therefore, a risk assessment for a generic earthworm-eating birds and a generic fish-eating bird has been performed to evaluate the risk of secondary poisoning from the use of Aclonifen SC 600 G.

a) Food chain from earthworm to earthworm-eating birds:

Dry soil approach

The bioconcentration factor for the earthworm ($BCF_{earthworm}$) was estimated according to the following equation (from the works of Jager, 1998):

$$BCF_{earthworm} = \frac{0.84 + 0.012K_{ow}}{f_{oc}\ x\ K_{oc}}$$

Where:

K_{oc} = Organic carbon adsorption coefficient
f_{oc} = Organic carbon content of soil (default value of 0.02 used)

Table 10.1-6: Calculation of $BCF_{earthworm}$ for Aclonifen SC 600 G

K_{ow}	f_{oc}	K_{oc}	$BCF_{earthworm}$
23442[1]	0.02	5727[2]	2.463

[1]: See Document CA-2, Section CA 2.7
[2]: See Document CA7, Section CA 7.1.3.1

The calculated BCF value along with the PEC_{soil} from the proposed use in peas was used to estimate the residue level in earthworms ($PEC_{earthworm}$) using the following equation:

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

The residue ($PEC_{earthworm}$) was converted into a daily dose by multiplying with the default value for birds 1.05 (calculated on the basis of a 100 g bird eating 104.6 g earthworm fresh per day), according to Smit (2005). The TER_{LT} was then calculated from the daily dose and the long-term NOAEL.

Table 10.1-7: Food chain from earthworm to earthworm-eating birds assessment for the proposed use of Aclonifen SC 600 G

NOAEL (mg/kg bw/d)	PEC_{soil} (mg/kg)	$BCF_{earthworm}$	$PEC_{earthworm}$ (mg/kg)	Daily dose (mg/kg bw/d)	TER_{LT}	Trigger
141	0.5697	2.463	1.403	1.473	95.7	5

The risk from the proposed use of Aclonifen SC 600 G in peas was above the TER_{LT} trigger value of 5, indicating the risk to earthworm-eating birds was acceptable.

b) Food chain from fish to fish-eating birds

The BCF (whole-body) for fish, experimentally determined for the active ingredient aclonifen, is 1349 L kg^{-1} ([redacted] 2019, M-667576-02-1, KCA 8.2.2.3/03).

The residue in fish was estimated according to the following equation with the TWA from the reproductive assessment being used:

$$PEC_{fish} = PEC_{sw}\ x\ TWA\ x\ BCF$$

The residue (PEC_{fish}) was converted into a daily dose by multiplying with the default value for birds 0.159 (calculated on the basis of a 1000 g bird eating 159 g fresh fish per day) according to Smit (2005). The TER_{LT} was then calculated from the daily dose and the long-term NOAEL.

Table 10.1-8: Food chain from fish to fish-eating birds assessment for the proposed uses of Aclonifen SC 600 G

NOAEL (mg/kg bw/d)	PEC_{sw} (mg/L)	TWA	BCF	PEC_{fish} (mg/kg)	Daily dose (mg/kg bw/d)	TER_{LT}	Trigger
141	0.0287[1]	0.53	1349	20.52	3.26	43	5

[1]: Maximum PEC_{sw} from FOCUS Step 1

The TER_{LT} is above the relevant trigger value of 5 demonstrating that there is no unacceptable long-term risk to birds *via* the food chain from fish to fish-eating birds from the proposed uses of Aclonifen SC 600 G.

c) Biomagnification in terrestrial food chains

ADME studies performed on aclonifen (see Document M-CA5, Section CA 5.1.1) showed no evidence of accumulation. As such, in accordance with EFSA (2009), no further assessment of the potential for biomagnification in terrestrial food chains is required.

CP 10.1.1.1 Acute oral toxicity

No studies were performed on the representative formulation as it was considered that the data generated for the active substance, aclonifen, was sufficient to reliably predict the toxicity of the formulation. For details of the studies performed on aclonifen, please refer to Document M-CA8, Section 8.1.1 of this dossier.

CP 10.1.1.2 Higher tier data on birds

No further data are required as no unacceptable risk to birds is anticipated according to the screening risk assessment.

The following generic field monitoring studies were included in the previous submission (Addendum 4 to the DAR, Confirmatory Data, 2011) and accepted as valid for risk assessment purposes. These studies are not required for this submission and hence summaries of these studies are not presented in this dossier.

Data Point:	KCP 10.1.1.2/01
Report Author:	[redacted]
Report Year:	2005
Report Title:	Generic field monitoring of birds and mammals on maize and beet fields in Austria
Report No:	M-252240-01-1
Document No:	M-252240-01-1
Guideline(s) followed in study:	The monitoring was especially designed for the purpose of this study.
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, submitted, not evaluated Study not trackable in DAR 2006, its Addenda 2008 or in Study list relied upon 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

This study has been duplicated in Bayer systems and is no longer valid. For details of the current valid study entry please refer to KCP 10.1.1.2/03.

Data Point:	KCP 10.1.1.2/02
Report Author:	[redacted]
Report Year:	2010
Report Title:	Generic field monitoring of birds on maize and beet fields in Austria - An excerpt from the GLP study WFC/FS017, performed by Christian [redacted] 2005
Report No:	M-365883-01-1
Document No:	M-365883-01-1
Guideline(s) followed in study:	EU 96/46/EC amending 91/414/EEC
Deviations from current test guideline:	Not applicable
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011, report ID 2112695 (RMS: DE)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCP 10.1.1.2/03
Report Author:	[redacted]
Report Year:	2005
Report Title:	Generic field monitoring of birds and mammals on maize and beet fields in Austria
Report No:	WFC/FS 017
Document No:	M-242960-01-1
Guideline(s) followed in study:	The test was specifically designed for this study
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, submitted, not evaluated Study not trackable in DAR 2006, its Addenda 2008 or in Study list relied upon 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

CP 10.1.2 Effects on terrestrial vertebrates other than birds

A summary of the mammalian toxicity endpoints for aclonifen is provided in the following table. Details and a full description of the toxicity studies used in this risk assessment can be found in Document M-CA5 of this dossier.

Table 10.1-9: Mammalian endpoints used in risk assessment

Test item		Type of exposure	Endpoint	Reference
Aclonifen SC 600 G	Acute risk assessment	Acute oral toxicity on rat	LD_{50} = 5596 mg/kg bw 2770 mg a.s./kg bw	KCP 7.1.1/01 & KCP 10.1.2.1/01 M-208817-01-1 [redacted] 1989
Aclonifen	Acute risk assessment	Acute oral toxicity on rat	LD_{50} >5000 mg a.s./kg bw	KCA 5.2.1/01 M-174876-01-1 [redacted], 1981
	Long-term risk assessment	2-generation study on rat	NOAEL = 35 mg a.s./kg bw/day	KCA 5.6.1/01 M-174748-01-1 [redacted], 1985

Summary of the risk assessment for mammals

The risk assessment for effects of Aclonifen SC 600 G on mammals was performed in accordance with the "European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals" (EFSA 2009)[2], (subsequently referred to as the Guidance document (EFSA 2009)). The risk assessment demonstrated acceptable acute dietary exposure risks following the proposed uses and based on the 'worst-case' screening step. Unacceptable long-term risks were shown following the 'worst-case' screening step, however following a first tier assessment, acceptable risk was demonstrated.

The risk from consumption of contaminated water was assessed for aclonifen. The acute and long-term risk from drinking water exposure was considered to be acceptable.

Aclonifen has a log P_{ow} of 4.37 which is higher than the trigger value of 3 and hence an assessment of the risk from secondary poisoning was required. The secondary poisoning risk for earthworm-eating and fish-eating mammals from the proposed uses of Aclonifen SC 600 G was shown to be acceptable.

Risk assessment for mammals

The following mammalian risk assessment has been conducted in line with EFSA's Bird and Mammal Guidance Document (EFSA Journal 2009; 7(12):1438), referred to in the following as EFSA (2009). No short-term risk assessment is required under EFSA (2009) as this is assumed to be covered by the acute and reproductive (long-term) risk assessment and therefore these are conducted in the sections below.

The main potential route of exposure for mammals to foliar applied agrochemicals is considered to be through the ingestion of residues on contaminated food, e.g. vegetation, insects and earthworms. The intended GAP for Aclonifen SC 600 G is presented in Table 10-1 above.

Direct exposure of Aclonifen SC 600 G to mammals is considered unlikely since at the time of application, and for a period thereafter, most mammals will leave the immediate vicinity of spray operations in response to the degree of human disturbance. The greatest levels of exposure will arise in the case of mammals foraging in the foliage of the crops some hours after application.

To achieve a concise risk assessment, the risk envelope approach is applied. Here all following assessments have been made for the use of Aclonifen SC 600 G in peas using an application rate of 600 g a.s./ha as this will also cover the risks from the use at lower application rates.

Toxicity of the formulation

[2] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal .2009; 7(12):1438. [139 pp.] doi:10.2903/j.efsa.2009.1438. Avalable online: www.efsa.europa.eu

EFSA's Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EFSA, 2019)[3] recommends that if the toxicity of the Plant Protection Product (PPP) is at least three times lower than the endpoint derived form a study performed on the active ingredient, the PPP should be considered as more toxic than the active ingredient and the risk assessment should be performed on the formulation endpoint.

The results of the acute oral toxicity on rat studies performed on the active ingredient and the representative formulation showed that the formulation endpoint was a factor of 1.8 lower than that derived using the active ingredient. As this was less than a factor of 3 lower, the risk assessment was performed using the active ingredient endpoints only.

Selection of relevant endpoint for long-term/reproductive risk assessment

The EFSA Scientific Report for Aclonifen (2008)[4] identified a NOAEL of 8 mg a.s./kg bw/d based on the 2-generation reproductive toxicity study on rats ([redacted], 1985, KCA 5.6.1/01) as the relevant endpoint for the long-term/reproductive risk assessment. Since this time more detailed guidance for the risk assessment has been developed (EFSA, 2008[5] and EFSA, 2009[6]) and as such a re-assessment of the relevant endpoint has been undertaken ([redacted], 2019, M-675718-01-1, KCA 8.1.2.2/01).

Based on this re-assessment, the relevant endpoint for the long-term/reproductive risk assessment is concluded to be 35 mg a.s./kg bw/d.

Dietary risk assessment

Screening assessment

The first, or screening, step assesses the risk based on a worst-case approach. The risk is considered acceptable, if the 'Toxicity Exposure Ratio' (TER) value pass the trigger values of ≥ 10 for acute exposure and ≥ 5 for chronic exposure. If the TER values do not pass the trigger values in certain areas, a Tier 1 risk assessment based on more relevant and realistic conditions is performed in those particular areas.

Calculation of Daily dietary Dose (DDD)

The daily dietary dose (DDD) for a single application is given by the following equation:

$$DDD_{single\ application} = Application\ Rate\ [kg/ha]\ x\ Shortcut\ Value\ x\ TWA$$

The Time Weighted Average factor (TWA) is only considered for the long-term exposure. The long-term risk assessment can be based on a TWA = 0.53 (estimates time-weighted exposure over 21 days, assuming a default DT_{50} of 10 days).

Calculation of Toxicity Exposure Ratio (TER)

The assessment of the risks to mammals is performed for both acute and long-term exposures using endpoints derived from acute and reproduction studies with mammals.

[3] EFSA (European Food Safety Authority), 2019. Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117 pp. doi:10.2903/sp.efsa.2019.EN-1673
[4] EFSA Scientific Report (2008) 149, 1-80, Conclusion on the peer review of aclonifen
[5] EFSA Scientific Opinion of the Panel on Plant protection products and their Residues. The EFSA Journal (2008) 734, 1-181
[6] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. doi:10.2903/j.efsa.2009.1438. Available online: www.efsa.europa.eu

The calculation of acute and long-term toxicity-exposure-ratios (TER) is defined as follows:

Acute risk assessment: $TER_A = LD_{50}/DDD$

Reproductive risk assessment: $TER_{LT} = NOAEL/DDD$

Screening step

According to EFSA (2009), an 'indicator species' is used in a screening step to eliminate all those substances that clearly pose a low risk to mammals. This 'indicator species' is not a real species but, by virtue of its size and feeding habits, is considered to have a higher exposure than (i.e. to be protective of) other species that may occur in a particular crop at a particular time.

For application to the crop relevant for this dossier, peas, the small herbivorous mammal should be considered in the screening step using the relevant shortcut values for acute and long-term risk assessments. The shortcut value consists of the food intake rate of the species of concern, its body weight, the concentration of a substance in/on fresh diet and the fraction of diet obtained in the treated area.

Table 10.1-10: Mammalian indicator species and shortcut values for the screening assessment

Crop	Indicator species	Shortcut value (SV)	
		Acute assessment	Reproductive assessment
Peas	Small omnivorous mammal	136.4	72.3

Table 10.1-11: Mammalian screening acute assessment for the proposed uses of Aclonifen SC 600 G

Crop	Indicator Species	Toxicity (mg a.s./ kg bw)	Appl. rate (kg/ha)	SV	DDD	TER_A	Trigger
Peas	Small omnivorous mammal	2770	0.6	136.4	81.84	33.85	10

SV: Shortcut Value
TER: Toxicity Exposure Ratio
DDD: Daily Dietary Dose

The screening assessment for the acute risks to mammals from exposure to Aclonifen SC 600 G after use according to the recommended GAP demonstrate that the risks are acceptable, with the TER_A value calculated to be greater than the trigger of 10, indicating a low potential acute risk to mammals from the exposure of Aclonifen SC 600 G. In this occasion, a first-tier assessment was not required.

Table 10.1-12: Mammalian screening long-term assessment for the proposed uses of Aclonifen SC 600 G

Crop	Indicator Species	Toxicity (mg a.s./kg bw/d)	Appl. rate (kg/ha)	SV	TWA	DDD	TER_{LT}	Trigger
Peas	Small omnivorous mammal	35	0.6	72.3	0.53	22.99	**1.52**	5

SV: Shortcut Value
TWA: Time Weighted Average factor
DDD: Daily Dietary Dose
TER: Toxicity Exposure Ratio
TER values in **bold** are indicating unacceptable risks

The screening assessment for the long-term risks to mammals from exposure to Aclonifen SC 600 G after use according to the recommended GAP demonstrates that the risks from the potential application to bare soil are acceptable, with the TER_{LT} value calculated to be greater than the Annex VI trigger of 5, indicating a low potential long-term risk to mammals from the exposure of Aclonifen SC 600 G.

However, the TER_{LT} for the use in peas showed an unacceptable risk at the screening stage and therefore, a first-tier assessment was required.

1st tier assessment

In the first-tier assessment, more realistic exposure estimates along with a 'generic focal species'is used. In accordance with EFSA (2009), a 'generic focal species' is not a real species, however it is considered to be representative of all those species potentially at risk, i.e. it is based on ecological knowledge of a range of species that could be at risk. It has a high food intake rate and may consume a mixed diet rather than just one as for the indicator species. The diet is not real but is considered to be representative of the species represented and hence EFSA (2009) employs a quartile approach where only the 2, 3 or 4 largest food types have been extrapolated to either 25% or 50% of the total diet. The 'generic focal species' is also considered to be a representative of the types of birds or mammals that occur across Member States.

Table 10.1-13: Mammalian first tier long-term assessment for the proposed uses of Aclonifen SC 600 G

Scenario	Generic focal spp.	SV	TWA	DDD (mg a.s./kg bw)	Endpoint (mg a.s./kg bw)	TER_{LT}	Trigger
BBCH 10-19	Small insectivorous mammal "shrew"	4.2	0.53	1.34	35	26.21	5
BBCH ≥20	Small insectivorous mammal "shrew"	1.9		0.60		57.93	
BBCH 10-49	Large herbivorous mammal “lagomorph”	14.3		4.55		7.70	
BBCH 10-49	Small omnivorous mammal “mouse"	7.8		2.48		14.11	

SV: Shortcut Value
TWA: Time Weighted Average factor
DDD: Daily Dietary Dose
TER: Toxicity Exposure Ratio

Following a Tier 1 assessment, the TER_{LT} values from the use in peas were shown to be greater than the trigger of 5, indicating a low potential long-term risk to mammals from the exposure of Aclonifen SC 600 G.

Drinking water risk assessment

Exposure of birds or mammals via drinking water is not explicitly included in the DDD calculations of the dietary risk assessment. Therefore, in line with EFSA (2009) an approach is presented that allows estimating the possible risk arising from uptake of contaminated drinking water. A leaf scenario is deemed not relevant for small mammals and hence only the puddle scenario has been assessed.

EFSA (2009) indicates that no specific calculations of exposure and TER are necessary when the ratio of the effective application rate (g/ha) to the relevant endpoint (mg a.s./kg bw/d) 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).

Rather than the effective application rate, the maximum application rate of 600 g a.s./ha will be used as a worse case consideration. The K_{oc} value for aclonifen is 5727 L/kg and as it is > 500 L/kg the trigger of 3000 is acceptable.

Table 10.1-14: Application rate to endpoint ratios for the proposed uses of Aclonifen SC 600 G

Risk assessment	Application rate (g a.s./ha)	Endpoint (mg a.s./kg bw/d)	Ratio	Trigger
Acute	600	> 5000	< 0.12	3000
Long-term		35	17	

As the ratios of application rate to endpoint are lower than 3000 for both the acute and long-term assessment no specific calculations of exposure to mammals via drinking water are necessary. An acceptable risk can be concluded from contaminated drinking water as a result of the proposed use of Aclonifen SC 600 G.

Bioaccumulation and food chain behaviour

Plant protection products with high bioaccumulation potential could theoretically bear a risk of secondary poisoning for mammals, if contaminated prey such as fish or earthworms are eaten. According to EFSA (2009), for organic chemicals, a log P_{ow} >3 is used to indicate whether there might be a potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial

food chains. The log P_{ow} of aclonifen was determined to be 4.37 (see Document M-CA2, Section CA 2.7). Therefore a risk assessment for a generic earthworm-eating mammal and a generic fish-eating mammal has been performed to evaluate the risk of secondary poisoning from the use of Aclonifen SC 600 G.

a) Food chain from earthworm to earthworm-eating mammals:

Dry soil approach

The bioconcentration factor for the earthworm ($BCF_{earthworm}$) was estimated according to the following equation (from the works of Jager, 1998):

$$BCF_{earthworm} = \frac{0.84 + 0.012K_{ow}}{f_{oc} \; x \; K_{oc}}$$

Where:
K_{oc} = Organic carbon adsorption coefficient
f_{oc} = Organic carbon content of soil (default value of 0.02 used)

Table 10.1-15: Calculation of $BCF_{earthworm}$ for Aclonifen SC 600 G

K_{ow}	f_{oc}	K_{oc}	$BCF_{earthworm}$
23442[1]	0.02	5727[2]	2.463

[1]: See Document CA-2, Section CA 2.7
[2]: See Document CA7, Section CA 7.1.3.1

The calculated BCF value along with the PEC_{soil} from the proposed use in peas was used to estimate the residue level in earthworms ($PEC_{earthworm}$) using the following equation:

$$PEC_{earthworm} = PEC_{soil} \; x \; BCF_{earthworm}$$

The residue ($PEC_{earthworm}$) was converted into a daily dose by multiplying with the default value for mammals 1.28 (calculated on the basis of a 10 g mammal eating 12.8 g earthworm fresh per day), according to Smit (2005). The TER_{LT} was then calculated from the daily dose and the long-term NOAEL.

Table 10.1-16: Food chain from earthworm to earthworm-eating mammals assessment for the proposed use of Aclonifen SC 600 G

NOAEL (mg/kg bw/d)	PEC_{soil} (mg/kg)	$BCF_{earthworm}$	$PEC_{earthworm}$ (mg/kg)	Daily dose (mg/kg bw/d)	TER_{LT}	Trigger
35	0.5697	2.463	1.403	1.796	19.5	5

The risk from the proposed use of Aclonifen SC 600 G in peas was above the TER_{LT} trigger value of 5, indicating the risk to earthworm-eating mammals was acceptable.

b) Food chain from fish to fish-eating mammals

The BCF (whole-body) for fish, experimentally determined for the active ingredient aclonifen, is 1349 L kg^{-1} ([redacted] 2019, M-667576-02-1, KCA 8.2.2.3/03).

The residue in fish was estimated according to the following equation with the TWA from the reproductive assessment being used:

$$PEC_{fish} = PEC_{sw} \; x \; TWA \; x \; BCF$$

The residue (PEC_{fish}) was converted into a daily dose by multiplying with the default value for mammals 0.142 (calculated on the basis of a 3000 g mammal eating 425 g fresh fish per day) according to Smit (2005). The TER_{LT} was then calculated from the daily dose and the long-term NOAEL.

Table 10.1-17: Food chain from fish to fish-eating mammals assessment for the proposed uses of Aclonifen SC 600 G

NOAEL (mg/kg bw/d)	PEC_{sw} (mg/L)	TWA	BCF	PEC_{fish} (mg/kg)	Daily dose (mg/kg bw/d)	TER_{LT}	Trigger
35	0.0287[1]	0.53	1349	20.52	2.91	12	5

[1]: Maximum PEC_{sw} from FOCUS Step 1

The TER_{LT} is above the relevant trigger value of 5 demonstrating that there is no unacceptable long-term risk to birds *via* the food chain from fish to fish-eating birds from the proposed uses of Aclonifen SC 600 G.

c) Biomagnification in terrestrial food chains

ADME studies performed on aclonifen (see Document M-CA5, Section CA 5.1.1) showed no evidence of accumulation. As such, in accordance with EFSA (2009), no further assessment of the potential for biomagnification in terrestrial food chains is required.

CP 10.1.2.1 Acute oral toxicity to mammals

Data Point:	KCP 10.1.2.1.01
Report Author:	[redacted]
Report Year:	1989
Report Title:	Acute oral toxicity study (and limit test) of EXP4209 in rats
Report No:	C025169
Document No:	M-208817-01-1
Guideline(s) followed in study:	OECD: 401, 1982
Deviations from current test guideline:	Current Guideline: OECD 401, 1987 No deviation
Previous evaluation:	Yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Please refer to the mammalian toxicology section; Document M-CP7, Section CP 7.1.1 for a full summary of this study.

CP 10.1.2.2 Higher tier data on mammals

No further data are required as no unacceptable risk to mammals is anticipated according to the screening and/or first tier risk assessments.

The following generic field monitoring studies were included in the previous submission (Addendum 4 to the DAR, Confirmatory Data, 2011) and accepted as valid for risk assessment purposes. These studies are not required for this submission and hence summaries of these studies are not presented in this dossier.

Data Point:	KCP 10.1.2.2/01
Report Author:	[redacted]
Report Year:	2006
Report Title:	Generic field monitoring of mammals in freshly drilled oilseed rape fields in summer in Germany
Report No:	BAR/FS 036
Document No:	M-281405-01-1
Guideline(s) followed in study:	The test was specifically designed for this study
Deviations from current test guideline:	Not applicable
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCP 10.1.2.2/02
Report Author:	[redacted]; [redacted]
Report Year:	2010
Report Title:	Exposure of mammals in maize fields in France - Attractiveness of maize fields and relevant species
Report No:	R09-012-2
Document No:	M-369149-01-1
Guideline(s) followed in study:	No official test guideline(s) available at present. The study was conducted under consideration of the Scientific Opinion of the Panel on Plant protection products and their residues on risk assessment for birds and mammals Anonymous 2008).
Deviations from current test guideline:	Not applicable
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

A Letter of Access is also provided for this document (KCP 10.1.2.2/03, M-369666-01-1).

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

Please refer to Document M-CA8, Section 8.1.4.

CP 10.2 Effects on aquatic organisms

Studies on the aquatic toxicity have been carried out with aclonifen. Details and a full description of these aquatic toxicity studies can be found in Document M-CA 8 of this dossier.

A summary of the aquatic toxicity endpoints for aclonifen relevant for the risk assessment is provided in Table 10.2-1. The selection of studies and endpoints for the risk assessment is in line with the EFSA Scientific Report for Aclonifen (2008)[7] unless otherwise indicated. Justifications are provided where studies or endpoints differ from the EFSA Scientific Report.

Table 10.2-1: Summary of the effects of aclonifen on aquatic organisms

Test species	Test item	Endpoint	Reference
Acute toxicity to fish			
Rainbow trout (*Oncorhynchus mykiss)*	Aclonifen	**96-Hour LC_{50} = 0.67 mg/L (nom)**	KCA 8.2.1/01 M-174317-01-1 [redacted] 1991
Long-term and chronic toxicity to fish			
Fathead minnow (*Pimephales promelas*)	Aclonifen	**35-Day $NOEC_{survival}$ = 0.0425 mg/L (mm)** 35-Day $NOEC_{growth}$ ≥0.106 mg/L (mm) 35-Day $EC_{10,survival}$ = ND 35-Day $EC_{10,growth}$ = ND	KCA 8.2.2.1/03 M-626723-01-1 [redacted], 2018
Bioconcentration in fish			
Rainbow trout (*Oncorhynchus mykiss)*	Aclonifen	**BCF_{KgL} = 1349 L/kg**	KCA 8.2.2.3/03 M-667576-02-1 [redacted] 2019
Acute toxicity to aquatic invertebrates			
Daphnia magna	Aclonifen	**48-Hour EC_{50} = 1.2 mg/L (nom)**	[redacted] 1991 M-174313-01-1 KCA 8.2.4.1/01
Long-term and chronic toxicity to aquatic invertebrates			
Daphnia magna	Aclonifen	**21-Day $NOEC_{body\ length}$ = 0.0142 mg/L (twa)** 21-Day $EC_{10,body\ length}$ = 0.0193 mg/L (twa)	KCA 8.2.5.1/02 M-573305-02-1 [redacted] 2017
Development and emergence in *Chironomus riparius*			
Chironomus riparius	Aclonifen	**21-Day spiked water $NOEC_{emergence}$ = 0.472 mg/L (im)** 21-Day spiked water $EC_{10,emergence}$ = ND	KCA 8.2.5.3/01 M-174918-01-1 [redacted], 1996

[7] EFSA Scientific Report (2008) 149, 1-80, Conclusion on the peer review of aclonifen

Test species	Test item	Endpoint	Reference
Sediment dwelling organisms			
Chironomus riparius	Aclonifen	**28-Day spiked sediment $NOEC_{emergence}$ = 32 mg/kg (nom)** 28-Day spiked sediment $EC_{10, emergence}$ = 36 mg/kg (nom)	KCA 8.2.5.4/01 M-227300-01-1 [redacted] 2004 & KCA 8.2.5.4/02 M-674905-01-1 [redacted], 2019
Effects on growth of green algae			
Desmodesmus subspicatus	Aclonifen	$NOEC_{growth\ rate}$ (0 – 96h) = 0.0000811 mg/L (mm) E_rC_{10} (0 – 96h) = 0.0104 mg/L (mm) **E_rC_{50} (0 – 96h) = 0.0203 mg/L (mm)** $NOEC_{yield}$ (0 – 96h) = 0.0000811 mg/L (mm) E_yC_{10} (0 – 96h) = 0.0244 mg/L (mm) E_yC_{50} (0 – 96h) = 0.0107 mg/L (mm)	KCA 8.2.6.1/03 M-574872-02-1 [redacted], 2016
Effects on aquatic macrophytes			
Lemna gibba	Aclonifen	$NOEC_{growth\ rate,\ dry\ weight}$ = 0.00200 mg/L (mm) E_rC_{10} (0 – 14d)$_{dry\ weight}$ = 0.000265 mg/L (mm) **E_rC_{50} (0 – 14d)$_{dry\ weight}$ = 0.0136 mg/L (mm)**	KCA 8.2.7/01 M-171423-01-1 [redacted], J.R., 1998 KCA 8.2.7/02 M-255537-01-1 [redacted] 2005
Primary producers (algae & macrophytes)			
Species sensitive distribution utilizing 12 species	Aclonifen	**HC_5 = 0.000595 mg a.s./L**	See justification

Endpoints in **bold** were used in the risk assessment
ND: not determined
nom: nominal test concentrations
mm: mean measured test concentrations
twa: time-weighted average measured test concentrations
im: initial measured test concentrations

Justification of new endpoints

Where endpoints differ from the EFSA Scientific Report for Aclonifen (2008), justifications are provided below:

Long-term and chronic toxicity to fish

The study referenced in the DAR (2006) and EFSA Scientific Report 149 (2008), [redacted] (1997) KCA 8.2.2.1/01, is considered as not valid due to a failure to meet all relevant validity criteria given in

the updated OECD 210 (2013) test guideline. A new study, [redacted] (2018), KCA 8.2.2.1/03, is presented which satisfies all current guideline validity criteria.

The NOEC for the new study is 42.5 µg/L and is considered the relevant endpoint for use in risk assessment.

A detailed justification for this updated endpoint is presented in KCA 8.2.2.1/04.

Bioconcentration in fish

The study referenced in the DAR (2006) and EFSA Scientific Report 149 (2008), [redacted] (1995) KCA 8.2.2.3/01, is considered as not valid due to a failure to meet all relevant validity criteria given in the updated OECD 305-I (2012) test guideline. A new study, [redacted] (2019), KCA 8.2.2.3/03, is presented which satisfies all current guideline validity criteria.

A BCF_{KgL} of 1349 L/kg was determined in the current study and is considered the relevant endpoint for use in risk assessment.

A detailed justification for this updated endpoint is presented in KCA 8.2.2.3/04.

Long-term and chronic toxicity to aquatic invertebrates

The study referenced in the DAR (2006) and EFSA Scientific Report 149 (2008), [redacted] (1991) KCA 8.2.5.1/01, is not valid due to a failure to meet the validity criteria relating to the mean number of living offspring per parent animal of the updated OECD 211 (2012) test guideline. A new study, [redacted] (2017), KCA 8.2.5.1/02, is presented which satisfies all current guideline validity criteria.

The NOEC for the new study is 14.2 µg/L and is considered the relevant endpoint for use in risk assessment.

Effects on growth of green algae

The study referenced in the DAR (2006) and EFSA Scientific Report 149 (2008), [redacted] (1990) KCA 8.2.6.1/01, is not valid due to a failure to meet the validity criteria relating to the coefficient of variation of sectional growth rates in control cultures of the updated OECD 201 (2011) test guideline. A new study, [redacted] (2016), KCA 8.2.6.1/03, is presented which satisfies all current guideline validity criteria.

The E_rC_{50} (0 – 96h) of 0.0203 mg/L determined in this new study is considered the relevant endpoint for use in risk assessment.

Effects on aquatic macrophytes

The E_rC_{50} = 0.012 mg/L and the E_bC_{50} = 0.006 mg a.s./L as listed in the DAR (2006) and EFSA Scientific Report 149 (2008) for *Lemna* were erroneously labelled as growth rate and biomass related endpoints although in fact no calculations for these response variables had been conducted in the frame of the study report.

In order to fulfil the current requirements as set out in Regulation 283/2013 and OECD 221, which ask for the EC_{50} for growth rate of both endpoints; i.e. frond number and dry weight of plants, the endpoints of the original study by [redacted] (KCA 8.2.7/01) were re-calculated by [redacted] (KCA 8.2.7/02). The resulting 14d-E_rC_{50} = 13.6 µg/L for dry weight is considered the relevant endpoint for use in risk assessment.

Primary producers (algae & macrophytes)

Since EFSA Scientific Report 149 (2008) was published, the use of growth endpoints for primary producers in the construction of Species Sensitivity Distributions (SSDs) has gained wide acceptance

and is supported by the EFSA Aquatic Guidance Document[8] and EFSA's Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EFSA, 2015)[9].

A SSD curve has been constructed using the data generated in 12 aquatic primary producer species and resulted in a HC_5 = 5.95 µg a.s./L which is considered the relevant endpoint for use in risk assessment for primary producers.

Effects of Aclonifen SC 600 G on aquatic organisms

Studies on the aquatic toxicity have been carried out with Aclonifen SC 600 G. Details and a full description of these aquatic toxicity studies can be found in Sections CP 10.2.1 and CP 10.2.2 of this dossier. Data for the formulation are summarised in Table 10.2-2.

Table 10.2-2: Summary of the effects of Aclonifen SC 600 G on aquatic organisms

Test species	Test item	Endpoint	Reference
Acute toxicity to fish			
Rainbow trout (*Oncorhynchus mykiss)*	Aclonifen SC 600 G	96-Hour LC_{50} = 1.27 mg product/L [0.61 mg a.s./L] (mm)	KCP 10.2.1/03 M-216973-01-1 [redacted] 1993
Common carp (*Cyprinus carpio*)	Aclonifen SC 600 G	96-Hour LC_{50} = 1.86 mg product/L [0.92 mg a.s./L] (mm)	KCP 10.2.1/04 M-217134-01-1 [redacted] 1993
Long-term and chronic toxicity to fish			
Rainbow trout (*Oncorhynchus mykiss)*	Aclonifen SC 600 G	21-Day NOEC = 0.140 mg product/L [0.068 mg a.s./L] (mm)[1] 21-Day LC_{10} = 0.315 mg product/L [0.166 mg a.s./L] (mm)[1] 21-Day LC_{50} = 0.406 mg product/L [0.196 mg a.s./L] (mm)[1]	KCP 10.2.2/01 M-216971-01-1 [redacted] 1993
Acute toxicity to aquatic invertebrates			
Daphnia magna	Aclonifen SC 600 G	48-Hour EC_{50} = 2.4 mg product/L [1.2 mg a.s./L] (mm)	KCP 10.2.1/05 M-216843-01-1 [redacted] 1993
Long-term and chronic toxicity to aquatic invertebrates			
Daphnia magna	Aclonifen SC 600 G	21-Day $NOEC_{reproduction}$ = 0.104 mg product/L [0.052 mg a.s./L] (mm)[2] 21-Day $EC_{10,reproduction}$ = ND	KCP 10.2.2/02 M-216975-01-1 [redacted] 1993
Daphnia magna	Aclonifen SC 600 G	21-Day $NOEC_{reproduction\ and\ dry\ weight}$ = 0.060 mg product/L [0.0261 mg a.s./L] (gmm) 21-Day $EC_{10\ dry\ weight}$ = 0.072 mg product/L [0.0312 mg a.s./L](gmm)	KCP 10.2.2/03 M-597212-02-1 [redacted], 2017

[8] 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. doi:10.2903/j.efsa.2013.3290
[9] 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.

Test species	Test item	Endpoint	Reference
Effects on growth of green algae			
Selenastrum capricornutum (currently known as *Raphidocelis subcapitata*)	Aclonifen SC 600 G	$NOEC_{growth\ rate}$ (0 – 96h) = 0.00733 mg product/L [0.00362 mg a.s./L] (gmm) E_rC_{10} (0 – 96h) = 0.00903 mg product/L [0.00447 mg a.s./L] (gmm) E_rC_{50} (0 – 96h) = 0.043 mg product/L [0.021 mg a.s./L] (gmm) $NOEC_{yield}$ (0 – 96h) = 0.00733 mg product/L [0.00362 mg a.s./L] (gmm) E_yC_{10} (0 – 96h) = 0.00611 mg product/L [0.00301 mg a.s./L] (gmm) E_yC_{50} (0 – 96h) = 0.012 mg product/L [0.0061 mg a.s./L] (gmm)	KCP 10.2.1/02 M-217128-01-1 [redacted] 1993 & KCP 10.2.1/06 M-674903-01-1 [redacted], 2019
Effects on aquatic macrophytes			
Lemna gibba	Aclonifen SC 600 G	NOEC (0 – 7d)$_{frond\ number}$ = 0.004 mg product/L [0.002 mg a.s./L] (mm) E_rC_{10} (0 – 7d)$_{frond\ number}$ = 0.003 mg product/L [0.0015 mg a.s./L] (mm) E_rC_{50} (0 – 7d)$_{frond\ number}$ = 0.043 mg product/L [0.021 mg a.s./L] (mm)	KCP 10.2.1/01 M-222986-01-1 [redacted], 2003

[1]: Study design and endpoint no longer required for the registration of plant protection products in the EU
[2]: Study does not meet the validity criteria of OECD 211 (2012)

ND: not determined
nom: nominal test concentrations
mm: mean measured test concentrations
im: initial measured test concentrations
gmm: geometric mean measured test concentrations

Relative toxicity of the formulation

In accordance with EFSA's Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EFSA, 2019)[10] an assessment of the toxicity of the formulation relative to that of the active substance was undertaken.. Where the endpoint of the formulation (expressed in terms of the active substance) is at least three times lower than the equivalent endpoint for the active substance, the formulation should be considered as more toxic.

Table 10.2-3: Relative toxicity of Aclonifen SC 600 G and the active substance, aclonifen for aquatic organisms

Test species	Endpoint (mg a.s./L)	Test item		Relative toxicity*
		Aclonifen	Aclonifen SC 600 G	
Rainbow trout	96-Hour LC_{50}	0.67	0.61	1.09
Common carp	96-Hour LC_{50}	1.7	0.92	1.85
Rainbow trout	21-Day NOEC	0.0924	0.068	1.36

[10] EFSA (European Food Safety Authority), 2019. Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117 pp. doi:10.2903/sp.efsa.2019.EN-1673

Daphnia magna	48-Hour EC_{50}	1.2	1.2	1.00
Daphnia magna	21-Day NOEC	0.0142	0.0261	0.54
Raphidocelis subcapitata	E_rC_{50} (0 – 96h)	0.0203	0.021	0.97
Lemna gibba	E_rC_{50} (0 – 7d)	0.0136	0.021	0.65

*: Relative toxicity = active substance endpoint/formulation endpoint

Based on the calculated concentration for the active substance, effect values for the formulation were no greater than 1.93 times those for the active substance in acute and 21-Day chronic studies performed with fish and daphnia, an algal growth inhibition test and a 7-day growth test with an aquatic macrophyte. The formulation Aclonifen SC 600 G does not therefore exhibit higher toxicity to aquatic organisms than expected from its active substance content.

Summary of the Risk Assessment for Aclonifen on aquatic organisms

The risk assessment for effects of Aclonifen SC 600 G on aquatic organisms was performed in accordance with the "Guidance of tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters" (EFSA Journal 2013; 11(7):3290)[11].

Based on the maximum FOCUS Step 3 PECs, PEC:RAC ratios were shown to be less than 1 for fish (acute), invertebrates (acute) and sediment dwelling organisms, indicating acceptable risk. However, for invertebrates (chronic), algae and aquatic macrophytes, some PEC:RAC ratios were in excess of 1 and hence for these organisms the risk assessment required refinement.

In view of the substantial amount of data available for primary producers (algae and macrophytes) and the comparability of growth rate endpoints between algae and aquatic macrophytes, it was possible to calculate an HC_5 based on the available E_rC_{50} data for primary producers.

Following the refinement of the endpoint for primary producers, an acceptable risk was still not shown and hence mitigation methods were suggested. As the RAC for invertebrates (chronic) was lower than the refined RAC for primary producers, mitigation required for invertebrates (chronic) covered the risk for the less sensitive species also.

Risk was shown to be acceptable for aquatic organisms for the proposed uses of Aclonifen SC 600 G when 75% drift reduction was applied. Alternatively, a 5 m buffer zone with no drift reduction would be sufficient to mitigate the risk.

Risk assessment for aquatic organisms

The following risk assessment has been conducted in line with the "Guidance of tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters" (EFSA Journal 2013; 11(7):3290), hereafter referred to as EFSA Aquatic Guidance Document, 2013.

Exposure

Aquatic organisms may be exposed to aclonifen through spray drift, run-off and drainage from the application site into adjacent water bodies. Exposure of aquatic organisms from these routes was estimated by calculating Predicted Environmental Concentrations in surface water (PEC_{sw}) and sediment (PEC_{sed}) for aclonifen. The predicted concentrations of aclonifen were calculated at FOCUS Steps 1, 2 and 3 using FOCUS version 3.2 software.

To achieve a concise risk assessment, the risk envelope approach is applied. Here all following assessments have been made for the use of Aclonifen SC 600 G in peas using an application rate of 600 g a.s./ha as this will also cover the risks from the use at lower application rates.

[11] 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. doi:10.2903/j.efsa.2013.3290

Table 10.2-4: FOCUS Step 1, 2 and 3 PEC_{sw} values for the application of Aclonifen SC 600 G in peas

PEC_{sw} (µg/L)													
FOCUS 1	FOCUS 2		FOCUS 3										
	NE	SE	D3/ ditch	D4/ pond	D4/ stream	D5/ pond	D5/ stream	D6/ ditch	R1/ pond	R1/ stream	R2/ stream	R3/ stream	R4/ stream
28.7	5.52	7.48	3.12	0.125	2.54	0.126	2.60	3.12	0.133	2.16	2.87	3.05	2.15

NE: Northern Europe
SE: Southern Europe

Regulatory Acceptable Concentration (RAC_{sw}) values based on the toxicity endpoints from the most sensitive species were compared to the maximal PEC_{sw} and sediment PEC_{sed} values derived from the FOCUS Step 1, 2 and 3 values for aclonifen. Full details of the calculation of the PEC values are provided in Document M-CP9, Section CP 9.2.5.

As the formulation was shown to be no more toxic than the active substance, the risk assessment was performed using the data generated on the active substance alone as this will be protective of the use of the formulation. PEC:RAC ratios of greater than 1 indicate an unacceptable risk.

Table 10.2-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen for each organism group for the application of Aclonifen SC 600 G in peas

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Sed. Dwell. Prolonged	Green Algae	Aquatic macrophyte		Sed. Dwell. Prolonged
Test species		*Oncorhynchus mykiss*	*Pimephales promelas*	*Daphnia magna*	*Daphnia magna*	*Chironomus riparius*	*Desmodesmus subspicatus*	*Lemna gibba*		*Chironomus riparius*
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	NOEC	E_rC_{50}	E_rC_{50}		NOEC
(µg/L)		670	42.5	1200	14.2	472	24.6	13.6		32000
AF		100	10	100	10	10	10	10		10
RAC (µg/L)		6.7	4.25	12	1.42	47.2	2.46	1.36		3200
FOCUS Scenario	**PEC $_{sw-max}$ (µg/L)**								**PEC $_{sed-max}$ (µg/kg)**	
Step 1										
	28.7	**4.284**	**6.753**	**2.392**	**20.211**	0.608	**14.138**	**21.103**	1326	**2.556**
Step 2										
N-Europe	5.52	0.824	**1.299**	0.460	**3.887**	0.117	**2.719**	**4.059**	220	0.424
S-Europe	7.48	**1.116**	**1.760**	0.623	**5.268**	0.158	**3.685**	**5.500**	413	0.796
Step 3										
D3/ditch	3.12	0.466	0.734	0.260	**2.197**	0.066	**1.537**	**2.294**	-	-
D4/pond	0.125	0.019	0.029	0.010	0.088	0.003	0.062	0.092	-	-
D4/stream	2.54	0.379	0.598	0.212	**1.789**	0.054	**1.251**	**1.868**	-	-
D5/pond	0.126	0.019	0.030	0.011	0.089	0.003	0.062	0.093	-	-
D5/stream	2.60	0.388	0.612	0.217	**1.831**	0.055	**1.281**	**1.912**	-	-
D6/ditch	3.12	0.466	0.734	0.260	**2.197**	0.066	**1.537**	**2.294**	-	-
R1/pond	0.133	0.020	0.031	0.011	0.094	0.003	0.066	0.098	-	-
R1/stream	2.16	0.322	0.508	0.180	**1.521**	0.046	**1.064**	**1.588**	-	-
R2/stream	2.87	0.428	0.675	0.239	**2.021**	0.061	**1.414**	**2.110**	-	-
R3/stream	3.05	0.455	0.718	0.254	**2.148**	0.065	**1.502**	**2.243**	-	-
R4/stream	2.15	0.321	0.506	0.179	**1.514**	0.046	**1.059**	**1.581**	-	-

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

Based on the maximum FOCUS Step 3 PECs, the above calculations show PEC:RAC ratios in excess of 1 for invertebrates (chronic), algae and aquatic macrophytes. For these organisms a refined risk assessment is presented below.

Refined risk assessment

Calculation of the species-sensitivity distribution (SSD) with growth rate endpoints for all primary producer species

In view of the substantial amount of data available for primary producers (algae and macrophytes) and the comparability of growth rate endpoints between algae and aquatic macrophytes, it is considered acceptable to calculate an HC_5 based on the available E_rC_{50} data for primary producers.

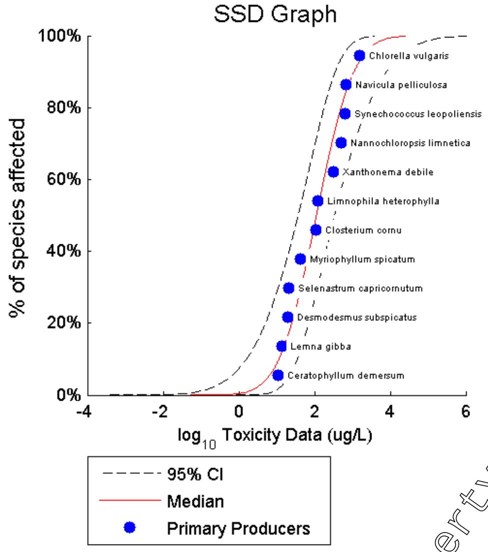
The SSD was calculated following the recommendations of the EFSA Aquatic Guidance Document, 2013 using the DEFRA webfram tool (https://webfram.com/home.aspx). In particular, unbound values should not be included in an SSD, however in cases where the unbound value relates to a species for which no other data is available, the unbound value can be used (without the < or > sign) if it is outside the range of all other available toxicity values.

In the following table all primary producer E_rC_{50} endpoints are listed, along with the applicability of these for use in the calculation of the SSD.

Table 10.2-6: Primary producer endpoints and applicability to SSD calculation

Reference	Species	Endpoint, E_rC_{50} (µg a.s./L)
KCA 8.2.7/03	*Ceratophyllum demersum*	10.8
KCA 8.2.7/01 KCA 8.2.7/02	*Lemna gibba*	13.6
KCA 8.2.6.1/03	*Desmodesmus subspicatus*	20.3
KCA 10.2.1/04	*Selenastrum capricornutum*	21.482
KCA 8.2.7/09 KCA 8.2.7/10	*Myriophyllum spicatum*	42.01
KCA 8.2.7/05	*Cabomba caroliniana*	> 79.5[1]
KCA 8.2.7/07	*Heteranthera zosterifolia*	> 98.5[1]
KCA 8.2.6.2/02	*Closterium cornu*	112
KCA 8.2.7/06	*Limnophila heterophylla*	122
KCA 8.2.7/08	*Egeria densa*	> 221[1]
KCA 8.2.7/04	*Elodea canadensis*	> 306[1]
KCA 8.2.6.2/02	*Xanthonema debile*	319
KCA 8.2.6.2/02	*Nannochloropsis limnetica*	513
KCA 8.2.6.2/03	*Synechococcus leopoliensis*	644
KCA 8.2.6.2/04	*Navicula pelliculosa*	672
KCA 8.2.6.2/07	*Chlorella vulgaris*	> 1583[2]
HC_5		**5.95**

[1]: unbound endpoint within the range of available toxicity values, not used in SSD calculation
[2]: unbound value outside of the range of available toxicity value, used in SSD calculation



GoF Results

Kolmogorov Smirnov

P-Values	Critical Values For Test Statistic	Calculated Test Statistic	Accepted or Rejected
0.1	0.819	0.6030	Accepted
0.05	0.895	0.6030	Accepted
0.025	0.995	0.6030	Accepted
0.01	1.035	0.6030	Accepted

Cramer Von Mises

P-Values	Critical Values For Test Statistic	Calculated Test Statistic	Accepted or Rejected
0.1	0.104	0.0556	Accepted
0.05	0.126	0.0556	Accepted
0.025	0.148	0.0556	Accepted
0.01	0.179	0.0556	Accepted

Anderson Darling

P-Values	Critical Values For Test Statistic	Accepted or Rejected
0.1	0.631	Accepted
0.05	0.752	Accepted
0.025	0.873	Accepted
0.01	1.035	Accepted

AD Stat: 0.4217
AD P-Val: 0.6777

Figure 10.2-1: Updated SSD curve based on growth rate endpoints for all species (HC_5 = 5.95 µg/L)

In accordance with the recommendations of EFSA, 2103, the SSD-RAC for primary producers was calculated using the median HC_5 of 5.95 µg a.s./L and applying an Assessment Factor (AF) of 3. The resultant SSD-RAC was calculated to be 1.98 µg a.s./L.

Table 10.2-7: Primary producers: acceptability of risk (PEC/RAC < 1) for aclonifen based on refined toxicity data for primary producers (HC5 = 5.95 µg a.s./L) for the application of Aclonifen SC 600 G in peas

Group		**Primary Producers**
Test species		**SSD**
Endpoint		HC_5
(µg/L)		5.95
AF		3
RAC (µg/L)		1.98
FOCUS Scenario	**PEC_{gl-max} (µg/L)**	
Step 1		
	28.7	**14.446**
Step 2		
N-Europe	5.52	**2.779**
S-Europe	7.48	**3.765**
Step 3		
D3/ditch	3.12	**1.570**
D4/pond	0.125	0.063
D4/stream	2.54	**1.279**
D5/pond	0.126	0.063
D5/stream	2.60	**1.309**
D6/ditch	3.12	**1.570**
R1/pond	0.133	0.067
R1/stream	2.16	**1.087**
R2/stream	2.87	**1.445**
R3/stream	3.05	**1.535**
R4/stream	2.15	**1.082**

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

PEC/RAC ratios above the relevant trigger of 1 are shown in **bold** indicating unacceptable risk

Following the refinement of the endpoint for primary producers, an acceptable risk was still not shown and hence mitigation methods are suggested. The RAC for invertebrates (chronic) of 1.42 µg/L, see Table 10.2-5, was lower than the refined RAC for primary producers (1.98 µg/L) and hence the mitigation required for invertebrates (chronic) will cover the risk for the less sensitive species also.

Table 10.2-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen based on toxicity data for invertebrate chronic (NOEC = 14.2 µg a.s./L) for the application of Aclonifen SC 600 G in peas considering mitigation methods

Intended use		**Peas**						**RAC (µg/L)**					
Active substance		**Aclonifen**						**1.42**					
Application rate (g/ha)		**1 x 600 g/ha**						**PEC/RAC ratio**					
Nozzle reduction	**No-spray buffer (m)**	**0**	**5**	**10**	**20**	**10**	**20**	**0**	**5**	**10**	**20**	**10**	**20**
	Vegetated filter strip (m)	**-**	**-**	**-**	**-**	**10**	**20**	**-**	**-**	**-**	**-**	**10**	**20**
None	D3/ditch	3.1200	1.0200	0.5410	0.2810	0.5410	0.2810	**2.20**	0.72	0.38	0.20	0.38	0.20
50%		1.5600	0.5100	0.2700	0.1400	0.2700	0.1400	**1.10**	0.36	0.19	0.10	0.19	0.10
75%		0.7780	0.2550	0.1350	0.0700	0.1350	0.0700	0.55	0.18	0.10	0.05	0.10	0.05
90%		0.3110	0.1020	0.0540	0.0280	0.0540	0.0280	0.22	0.07	0.04	0.02	0.04	0.02
None	D4/pond	0.1250	0.1120	0.0810	0.0540	0.0810	0.0540	0.09	0.08	0.06	0.04	0.06	0.04
50%		0.0630	0.0560	0.0400	0.0270	0.0400	0.0270	0.04	0.04	0.03	0.02	0.03	0.02
75%		0.0310	0.0280	0.0200	0.0130	0.0200	0.0130	0.02	0.02	0.01	0.01	0.01	0.01
90%		0.0130	0.0110	0.0080	0.0070	0.0080	0.0070	0.01	0.01	0.01	0.00	0.01	0.00
None	D4/stream	2.5400	1.0700	0.5650	0.2940	0.5650	0.2940	**1.79**	0.75	0.40	0.21	0.40	0.21
50%		1.2700	0.5330	0.2820	0.1470	0.2820	0.1470	0.89	0.38	0.20	0.10	0.20	0.10
75%		0.6330	0.2660	0.1410	0.0730	0.1410	0.0730	0.45	0.19	0.10	0.05	0.10	0.05
90%		0.2530	0.1060	0.0560	0.0510	0.0560	0.0510	0.18	0.07	0.04	0.04	0.04	0.04
None	D5/pond	0.1260	0.1120	0.0810	0.0540	0.0810	0.0540	0.09	0.08	0.06	0.04	0.06	0.04
50%		0.0630	0.0560	0.0400	0.0270	0.0400	0.0270	0.04	0.04	0.03	0.02	0.03	0.02
75%		0.0310	0.0280	0.0200	0.0140	0.0200	0.0140	0.02	0.02	0.01	0.01	0.01	0.01
90%		0.0130	0.0110	0.0080	0.0050	0.0080	0.0050	0.01	0.01	0.01	0.00	0.01	0.00
None	D5/stream	2.6000	1.0900	0.5780	0.3000	0.5780	0.3000	**1.83**	0.77	0.41	0.21	0.41	0.21
50%		1.3000	0.5450	0.2890	0.1500	0.2890	0.1500	0.92	0.38	0.20	0.11	0.20	0.11
75%		0.6480	0.2720	0.1440	0.0750	0.1440	0.0750	0.46	0.19	0.10	0.05	0.10	0.05
90%		0.2590	0.1090	0.0580	0.0300	0.0580	0.0300	0.18	0.08	0.04	0.02	0.04	0.02
None	D6/ditch	3.1200	1.0200	0.5410	0.2810	0.5410	0.2810	**2.20**	0.72	0.38	0.20	0.38	0.20
50%		1.5600	0.5100	0.2700	0.1400	0.2700	0.1400	**1.10**	0.36	0.19	0.10	0.19	0.10
75%		0.7790	0.2550	0.1350	0.0700	0.1350	0.0700	0.55	0.18	0.10	0.05	0.10	0.05
90%		0.3110	0.1020	0.0540	0.0430	0.0540	0.0430	0.22	0.07	0.04	0.03	0.04	0.03
None	R1/pond	0.1330	0.1190	0.0880	0.0790	0.0830	0.0550	0.09	0.08	0.06	0.06	0.06	0.04
50%		0.0790	0.0790	0.0790	0.0790	0.0430	0.0280	0.06	0.06	0.06	0.06	0.03	0.02
75%		0.0790	0.0790	0.0790	0.0790	0.0320	0.0160	0.06	0.06	0.06	0.06	0.02	0.01
90%		0.0790	0.0790	0.0790	0.0790	0.0320	0.0160	0.06	0.06	0.06	0.06	0.02	0.01
None	R1/stream	2.1600	0.9070	0.7070	0.7070	0.4810	0.2500	**1.52**	0.64	0.50	0.50	0.34	0.18
50%		1.0800	0.7070	0.7070	0.7070	0.3180	0.1660	0.76	0.50	0.50	0.50	0.22	0.12
75%		0.7070	0.7070	0.7070	0.7070	0.3180	0.1660	0.50	0.50	0.50	0.50	0.22	0.12
90%		0.7070	0.7070	0.7070	0.7070	0.3180	0.1660	0.50	0.50	0.50	0.50	0.22	0.12

None	R2/stream	2.8700	1.2100	0.6390	0.3320	0.6390	0.3320	**2.02**	0.85	0.45	0.23	0.45	0.23
50%		1.4300	0.6020	0.3190	0.1860	0.3190	0.1660	**1.01**	0.42	0.22	0.13	0.22	0.12
75%		0.7150	0.3010	0.1860	0.1860	0.1590	0.0830	0.50	0.21	0.13	0.13	0.11	0.06
90%		0.2860	0.1860	0.1860	0.1860	0.0850	0.0440	0.20	0.13	0.13	0.13	0.06	0.03
None	R3/stream	3.0500	1.2800	0.6800	0.5020	0.6800	0.3530	**2.15**	0.90	0.48	0.35	0.48	0.25
50%		1.5200	0.6410	0.5020	0.5020	0.3400	0.1760	**1.07**	0.45	0.35	0.35	0.24	0.12
75%		0.7610	0.5020	0.5020	0.5020	0.2290	0.1200	0.54	0.35	0.35	0.35	0.16	0.08
90%		0.5020	0.5020	0.5020	0.5020	0.2290	0.1200	0.35	0.35	0.35	0.35	0.16	0.08
None	R4/stream	2.1500	1.1600	1.1600	1.1600	0.5160	0.2680	**1.51**	0.82	0.82	0.82	0.36	0.19
50%		1.1600	1.1600	1.1600	1.1600	0.5160	0.2680	0.82	0.82	0.82	0.82	0.36	0.19
75%		1.1600	1.1600	1.1600	1.1600	0.5160	0.2680	0.82	0.82	0.82	0.82	0.36	0.19
90%		1.1600	1.1600	1.1600	1.1600	0.5160	0.2680	0.82	0.82	0.82	0.82	0.36	0.19

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

Based on the NOEC of 14.2 µg/L for invertebrates (chronic), 75% drift reduction would be sufficient to mitigate the risk for the intended use in peas. Alternatively, a 5 m buffer zone with no drift reduction would be sufficient to mitigate the risk.

CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Studies on the toxicity of the formulation Aclonifen SC 600 G to fish, aquatic invertebrates and algae have been conducted and presented below.

Data Point:	KCP 10.2.1/03
Report Author:	[redacted]
Report Year:	1993
Report Title:	Acute toxicity to rainbow trout Bandur
Report No:	C024470
Document No:	M-216973-01-1
Guideline(s) followed in study:	OECD: 203 (1984)
Deviations from current test guideline:	Current guideline: OECD 203, 2019 No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The acute toxicity of Bandur (aclonifen 600 g/L) to rainbow trout, *Oncorhynchus mykiss*, was determined in a 96-hour, flow-through exposure. Test solutions were prepared using stock solutions prepared in tap water treated to remove chlorine and reduce hardness to within the range 200 – 250 mg/L as $CaCO_3$. Ten rainbow trout per test group were exposed to an untreated control and nominal Bandur concentrations of 0.58, 1.16, 2.31, 4.63, 9.25, 18.5, 37 and 74 mg/L. The total test period was 96 hours. Samples for analytical confirmation of actual exposure concentrations were taken at the start and after 48 and 96 hours of exposure.

Dissolved oxygen, pH, and temperature were measured in the controls and each test concentration at the beginning of the test and end of the test. Daily observations were made of mortality and treatment related effects.

Measured concentrations of test exposure solutions at the start of the test range from 74 to 85% of nominal, with the exception of 9.25 mg/L which was 61%. At 48 and 96 hours concentrations ranged from 73 to 107% of nominal. Overall mean measured concentrations at the levels of biological interest (0.58 to 2.31 mg/L) range between 82 to 91% of nominal, confirming the stability of the test item over the period of the test.

The 96-hour LC_{50} of Bandur to rainbow trout, *Oncorhynchus mykiss*, based on the mean measured test concentrations was estimated to be 1.27 mg/L (confidence limits 1.05 – 1.90 mg/L). The NOEC, based on mortality, was 0.519 mg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item:	Bandur (aclonifen 600g/L) 2-chloro-6-nitro-3-phenoxyaniline
Batch no.:	OP 920521
Active Ingredient / Purity:	582 g/L
Density:	1.205 g/cm³
Appearance:	Yellow opaque viscous liquid
Date received:	16 October 1992
Storage:	Room temperature, in the dark
Expiry date:	8 July 1994
2. Test Organism:	Rainbow trout, *Oncorhynchus mykiss*
Mean length:	4.9 cm (± 0.6 cm)
Mean weight:	1.75g (± 0.57g)
Source:	[redacted] Fish were acclimated for 14 days in aerated dilution water, under flow-through conditions Mortalities in 14 days prior to test start were <2.5%
Feeding:	Commercial trout pellets daily. Discontinued 24 hours prior to study start
3. Test water:	Treated tap water. Treatment involved blending tap water previously filtered through activated carbon to remove chlorine, with tap water that had been softened and treated by reverse osmosis to reduce hardness
Total hardness:	234 - 250 mg/L as $CaCO_3$

B. STUDY DESIGN AND METHODS

1. In-life phase:	16 to 20 November 1992
2. Exposure conditions	
Test vessels:	15 litre capacity glass aquaria, containing 10 L test solution.
Experimental design:	Eight test concentrations (0.58, 1.16, 2.31, 4.63, 9.25, 18.5, 37 and 74 mg/L) plus one control

Loading:	Not specified
Temperature:	13.9 ± 0.6ºC
pH:	7.9 – 8.2
Dissolved oxygen:	>9.6 mg O_2/L
Aeration:	Continuous flow
Photoperiod:	16 h light: 8 h dark

3. Administration of the test item

Stock solutions were prepared daily by addition of test material directly into dilution water. Test solutions were prepared by further diluting aqueous stock solutions with water using electronically controlled dosing apparatus.

Concentrated stock solutions were contained in glass aspirators connected to the dosing apparatus. Water (nominally 40 mL) was drawn into a syringe followed by a pre-programmed volume of stock solution (2 to 22 mL), followed by dilution water until syringe is completely filled (200 mL). Contents of the syringe were then discharged via silicon and glass tubing to appropriate test vessel. When all vessels had received appropriate dose (200 mL) the apparatus paused until selected cycle time elapsed and the apparatus restarted.

Dosing was begun 24 hours prior to test start (addition of fish). Ten fish were allocated to each test vessel.

4. Measurements and observations

Observations for mortality were made after 2 hours and daily thereafter (24, 48, 72 and 96 hours). Mortality was defined as absence of respiratory movement and absence of response to physical stimulation.

Temperature, pH and dissolved oxygen were measured at the start of the test and daily thereafter. Total hardness was determined in the control and selected concentrations at the start and end of the test.

Samples were taken from the aqueous stock solutions and from each test vessel prior to the addition of fish and again after 48 and 96 hours. All samples were diluted to within the aqueous solubility of the active ingredient before analysis. Analysis was performed by HPLC with a spectrophotometric detector.

5. Statistics/Data evaluation

The LC_{50} and associated 95% confidence limits were calculated following the method described by Stephan (1977, 1982). The No Observed Effect Concentration (NOEC) was determined by visual inspection of the data.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Analysis of the aqueous stock solutions were between 90 to 102% of nominal. Measured concentrations of test exposure solutions at the start of the test range from 74 to 85% of nominal, apart from 9.25 mg/L which was 61%. At 48 and 96 hours concentrations ranged from 73 to 107% of nominal.

Overall mean measured concentrations at the levels of biological interest (0.58 to 2.31 mg/L) range between 82 to 91% of nominal, confirming the stability of the test item over the period of the test. Mean measured concentrations were 0.519, 1.05, 1.90, 3.59, 6.89, 16.5, 28.2 and 69.9 mg/L.

The results of analysis of aqueous stock solutions used to prepare exposure solutions is summarised below:

Table: Measured concentrations of Bandur (aclonifen) aqueous stock solutions

Nominal concn (mg/L)	0 hour		48 hours		96 hours*	
	Measured concn (mg/L)	% of nominal	Measured concn (mg/L)	% of nominal	Measured concn (mg/L)	% of nominal
58	53.8, 56.7	95	57.1, 56.3	98	55.9, 55.0	96
700	722, 712	102	635, 622	90		

* 24 hours after preparation

The results of analysis of test solutions is summarised below

Table: Measured concentrations of Bandur (aclonifen)

Nominal concn (mg/L)	0 hour		48 hours		96 hours		Overall	
	Measured concn (mg/L)	% of nominal	Measured concn (mg/L)	% of nominal	Measured concn (mg/L)	% of nominal	Mean	% of nominal
Control	n.d.	-	n.d.	-	n.d.	-	-	-
0.58	0.428, 0.440	75	0.568, 0.610	102	0.533, 0.537	92	0.519	89
1.16	0.909, 0.925	79	1.10, 1.13	96	1.10, 1.11	95	1.05	91
2.31	1.72, 1.68	74	2.09, 2.15	92	1.90, 1.87	82	1.90	82
4.63	3.93, 3.94	85	3.40, 3.53	77	3.42, 3.30	73	3.59	78
9.25	5.74, 5.54	61	8.38, 7.90	88	-	-	6.89	74
18.5	13.8, 13.9	75	19.3, 19.1	104	-	-	16.5	89
37	28.5, 29.0	78	27.5, 27.7	75	-	-	28.2	76
74	62.0, 58.6	81	78.4, 80.5	107		-	69.9	94

n.d. = none detected,

\- all fish died, no further analysis conducted

The validated method is summarised in Document M-CP5 (CP 5.1.2/01).

B. BIOLOGICAL DATA

The highest mean measured concentration showing no mortality and the lowest at which there was 100% mortality was 0.519 and 1.90 mg/L respectively. The cumulative mortality of rainbow trout after 2, 24, 48, 72 and 96 hours are presented in the following table:

Table: Cumulative mortality for rainbow trout from the exposure to Bandur

Measured concentration (mg/L)	Cumulative mortality				
	2h	24h	48h	72h	96h
Control	0	0	0	0	0
0.519	0	0	0	0	0
1.05	0	0	0	0	0
1.90	0	2	7	8	10
3.59	0	3	8	9	10

6.89	0	6	9	10	10
16.5	0	4	10	10	10
28.2	0	5	9	10	10
69.9	0	5	10	10	10

Treatment related effects, other than death, were swimming at the dark pigmentation, oedema, lethargy, loss of coordination, overturned and immobility on the bottom or at the surface. Treatment related effects were progressive and were seen at all exposure concentrations. At and above 1.90 mg/L the majority of fish were severely affected (dark pigmentation, lethargy, oedema and/or loss of coordination) or were dead within 48 hours. At 1.05 mg/L seven fish were affected within 24 hours (dark pigmentation, lethargy, and/or loss of coordination), these effects were sustained and progressive and all fish were affected by the end of the test. Two fish were immobile by the end of the test. At 0.519 mg/L, two fish were dark and showed loss of coordination, with periods of erratic swimming. These symptoms were progressive, and all fish were affected by the end of the test. Consequently, a NOEC based on treatment related effects could not be established and was less than the lowest measured concentration (0.519 mg/L).

All chemical and physical parameters in the definitive test were within expected ranges.

LC_{50} values were estimated by non-linear interpolation between two concentrations bracketing 50% effect. These concentrations were taken to be 95% confidence limits. It was not possible to calculate results using moving average angle or probit methods.

Based on the observed mortality, the LC_{50} values at each observation point were determined to be:

Table: LC_{50} values from the exposure of rainbow trout *Oncorhynchus mykiss* to Bandur

Time (Hours)	Nominal		Measured	
	LC_{50} (mg/L)	95% confidence limits (mg/L)	LC_{50} (mg/L)	95% confidence limits (mg/L)
24	>4.63	-	>3.59	-
48	1.97	1.16 – 2.31	1.66	1.05 – 1.90
72	1.86	1.16 – 2.31	1.58	1.05 – 1.90
96	1.44	1.16 – 2.31	1.27	1.05 – 1.90
NOEC (mortality)	0.58	-	0.519	-

C. VALIDITY CRITERIA

Validity criteria	Required (OECD 203, 2019)	Achieved
Mortality in controls	<10%	0%
Dissolved oxygen concentration at the end of the test	>60% ASV	>9.6 mg/L >93% ASV
Analytical measurement of test concentrations	Compulsory	Performed

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Measured concentration (mg/L)

LC_{50} (96 hours)	1.27
95% confidence limits	1.05 – 1.90
NOEC (mortality)	0.519

III. CONCLUSION

The 96-hour LC_{50} of Bandur to rainbow trout, *Oncorhynchus mykiss*, based on the mean measured test concentrations was estimated to be 1.27 mg/L (confidence limits 1.05 – 1.90 mg/L). The NOEC, based on mortality, was 0.519 mg/L.

[redacted] (1993)

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

The 96-hour LC_{50} of Bandur to rainbow trout, *Oncorhynchus mykiss*, based on the mean measured test concentrations was estimated to be 1.27 mg/L (confidence limits 1.05 – 1.90 mg/L). The NOEC, based on mortality, was 0.519 mg/L.

In terms of the active ingredient content, based on the reported product density of 1.205 and an active ingredient content of 582 g/L, the 96-hour LC_{50} was estimated to be 0.61 mg a.s./L (confidence limits 0.0.51 – 0.92 mg a.s./L). The NOEC, based on mortality, was 0.251 mg a.s./L.

Assessment and conclusion by RMS:

Data Point:	KCP 10.2.1/04
Report Author:	[redacted]
Report Year:	1993
Report Title:	Bandur: Acute toxicity to carp
Report No:	C024554
Document No:	M-217134-01-1
Guideline(s) followed in study:	OECD 203 (1984)
Deviations from current test guideline:	Current guideline: OECD 203, 2019 No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The acute toxicity of aclonifen to common carp, *Cyprinus carpio*, was determined in a 96-hour, semi-static exposure. Test solutions were prepared using stock solutions prepared in tap water treated to remove chlorine and reduce hardness to within the range 200 – 250 mg/L as $CaCO_3$. Ten carp per

test group were exposed to an untreated control and nominal Bandur concentrations of 0.31, 0.63, 1.25, 2.5, 5 and 10 mg/L. The total test period was 96 hours. Samples for analytical confirmation of actual exposure concentrations were taken at the start and after 44 and 96 hours of exposure.

Dissolved oxygen, pH, and temperature were measured in the controls and each test concentration at the beginning of the test and end of the test. Daily observations were made of mortality and treatment related effects.

Results for the two sets of freshly-prepared dilutions indicated that at the levels of biological significance (ie. Concentrations between the NOEC, 0.63 mg/L, and the lowest level causing 100% mortality, 2.5 mg/L) intended exposure concentrations of Bandur were achieved (between 80 and 142% of their nominal values). Analysis of test media 48 hours after preparation confirmed that these levels had been adequately maintained (between 88 and 121% of nominal). Overall, mean measured concentrations at biologically significant levels ranged between 85 and 113% of their nominal values.

At 0.31 mg/L, measured levels were variable (from 68 to 155% of nominal) although the levels measured generally did not exceed those obtained at the next higher exposure level. At 5 mg/L, the intended concentration was both achieved and maintained (between 72 and 80% of nominal). At 10 mg/L measured levels were lower than intended and decreased over the 48 hour exposure period (to 69% of the initial level).

These results suggest that at concentrations above 5 mg/L, the aqueous solubility of Bandur was exceeded; this was supported by the appearance of the test media at 10 mg/L.

The 96-hour LC_{50} of Bandur to common carp, *Cyprinus carpio*, based on the mean measured test concentrations was determined to be 1.86 mg/L (confidence limits 1.23 – 2.82 mg/L). The NOEC, based on mortality, was 1.23 mg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	Bandur (aclonifen 600g/L) 2-chloro-6-nitro-3-phenoxyaniline
	Batch no.:	OP 889348
	Active Ingredient / Purity:	600 g/L (49.4% a.s.)
	Appearance:	Yellow to brown suspension
	Date received:	7 May 1991
	Storage:	Room temperature, in the dark
	Expiry date:	January 1993
2.	**Test Organism:**	Common carp, *Cyprinus carpio*
	Mean length:	2.3 cm
	Mean weight:	0.4g
	Source:	[redacted] Fish were acclimated for 14 days in aerated dilution water, under flow-through conditions Mortalities in 14 days prior to test start were <5%

Feeding:	Commercial trout pellets daily. Discontinued 26 hours prior to study start
3. Test water:	Treated tap water. Treatment involved blending tap water previously filtered through activated carbon to remove chlorine, with tap water that had been softened and treated by reverse osmosis to reduce hardness
Total hardness:	208 - 214 mg/L as $CaCO_3$

B. STUDY DESIGN AND METHODS

1. In-life phase:	15 to 19 July 1991
2. Exposure conditions	
Test vessels:	15 litre capacity glass aquaria containing 10 L test solution
Experimental design:	Six test concentrations (0.31, 0.63, 1.25, 2.5, 5 and 10 mg/L) plus one control
Loading:	0.88 g bodyweight/L (static volume) 0.10 g bodyweight/L (volume in 24 hours)
Temperature:	21.2 ± 0.7°C
pH:	7.6 – 8.3
Dissolved oxygen:	> 7.3 mg O_2/L
Aeration:	Static, gentle aeration
Photoperiod:	16 h light: 8 h dark

3. Administration of the test item

Test solutions were prepared adding appropriate weights of test substance to 10 litres of dilution water in the test vessels. Test and control media were renewed after 48 hours.

4. Measurements and observations

Observations for mortality were made after 2 and 4 hours and daily thereafter (24, 48, 72 and 96 hours). Mortality was defined as absence of respiratory movement and absence of response to physical stimulation.

Temperature, pH and dissolved oxygen were measured at the start of the test and daily thereafter. Total hardness was determined in the control and selected concentrations at the start and end of the test.

Samples were taken from each test vessel prior to the addition of fish, after 48 and 96 hours. Analysis was performed by HPLC with a spectrophotometric detector.

5. Statistics/Data evaluation

The LC_{50} and associated 95% confidence limits were calculated following the method described by Stephan (1977, 1982). The No Observed Effect Concentration (NOEC) was determined by visual inspection of the data.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Results for the two sets of freshly prepared dilutions indicated that at the levels of biological significance (i.e. concentrations between the NOEC, 0.63 mg/L, and the lowest level causing 100% mortality, 2.5 mg/L) intended exposure concentrations of Bandur were achieved (between 80 and 142% of their nominal values). Analysis of test media 48 hours after preparation confirmed that these levels had been adequately maintained (between 88 and 121% of nominal). At 10 mg/L measured levels were lower than intended and decreased over the 48 hour exposure period (to 69% of the initial level). These results suggest that at concentrations above 5 mg/L, the aqueous solubility of Bandur was exceeded; this was supported by the hazy appearance of the test media at 10 mg/L.

Overall, mean measured concentrations at biologically significant levels ranged between 85 and 113% of their nominal values.

The results of analysis of aqueous stock solutions used to prepare exposure solutions is summarised below:

Table: Measured concentrations of Bandur aqueous stock solutions

Nominal concn (mg/L)	0 hour		48 hours		96 hours*	
	Measured concn (mg/L)	% of nominal	Measured concn (mg/L)	% of nominal	Measured concn (mg/L)	% of nominal
58	53.8, 56.7	95	57.1, 56.3	98	55.9, 55.0	96
700	722, 712	102	635, 622	90	-	-

* 24 hours after preparation

The results of analysis of test solutions is summarised below

Table: Measured concentrations of Bandur

Nominal concn (mg/L)	0 hour		48 hours (old solutions)		48 hours (fresh solutions)		96 hours (old solutions)		Mean measured concn (mg/L)
	Measured concn (mg/L)	% of nom	Measured concn (mg/L)	% of nom	Measured concn (mg/L)	% of nom	Measured concn (mg/L)	% of nom	
Control	n.d.	-	n.d.	-	n.d.	-	-	-	-
0.31	0.212 0.212	68	0.229 0.228	74	0.497 0.463	155	0.476 0.475	153	0.349
0.63	0.499 0.496	80	0.548 0.554	88	0.535 0.538	85	0.556 0.568	90	0.537
1.25	1.40 1.40	112	1.16 1.18	94	1.11 1.12	89	1.23 1.23	98	1.23
2.5	3.57 3.55	143	3.01 3.02	121	2.38 2.31	94	2.37 2.32#	94	2.82
5.0	4.01 4.03	80	3.58 3.61	72	-	-	-	-	3.81
10	7.66 6.92	73	5.08 5.05	51	-	-	-	-	6.18

n.d. = none detected,

= samples taken at 72 hours following death of all fish

- No analysis, all fish dead

The validated method is summarised in Document M-CP5 (CP 5.1.2/03).

B. BIOLOGICAL DATA

The highest nominal concentration showing no mortality and the lowest at which there was 100% mortality was 1.25 and 2.5 mg/L, respectively. The cumulative mortality of common carp after 2, 4, 24, 48, 72 and 96 hours are presented in the following table:

Table: Cumulative mortality for common carp from the exposure to Bandur

Measured concentration (mg/L)	Cumulative mortality					
	2h	4h	24h	48h	72h	96h
Control	0	0	0	0	0	0
0.31	0	0	0	0	0	0
0.63	0	0	0	0	0	1
1.25	0	0	0	0	0	0
2.5*	0	0	7	10	11	11
5.0	0	0	6	10	10	10
10	0	0	9	10	10	10

- 11 fish exposed at 2.5 mg/L. in error

Treatment related effects, other than death, were swimming at the dark pigmentation, oedema, lethargy, loss of coordination, overturned and immobility on the bottom or at the surface. Treatment related effects were progressive and were seen at exposure concentrations 1.25 mg/L and above.

At 10 mg/L two fish were lethargic and showed dark pigmentation or loss of coordination with 2 hours of exposure. Nine fish were dead within 24 hours and all fish were dead after 48 hours.

At 2.5 and 5.0 mg/L, all fish were normal at 4 hours but were lethargic or dead after 24 hours.

At 1.25 mg/L one fish showed dark pigmentation, by the end of the study two fish showed dark pigmentation.

Consequently, a NOEC based on treatment related effects was estimated to be 0.63 mg/L, based on nominal concentrations.

All chemical and physical parameters in the definitive test were within expected ranges.

LC_{50} values were estimated by non-linear interpolation between two concentrations bracketing 50% effect. These concentrations were taken to be 95% confidence limits. It was not possible to calculate results using moving average angle or probit methods.

Based on the observed mortality, the LC_{50} values at each observation point were determined to be:

Table: LC_{50} values from the exposure of common carp *Cyprinus carpio* to Bandur (aclonifen 600g/L)

Time (Hours)	Nominal		Measured	
	LC_{50} (mg/L)	95% confidence limits (mg/L)	LC_{50} (mg/L)	95% confidence limits (mg/L)
24	2.23	1.25 – 2.5	2.45	1.23 – 2.82
48	1.89	1.25 – 2.5	2.02	1.23 – 2.82

72	1.76	1.25 – 2.5	1.86	1.23 – 2.82
96	1.76	1.25 – 2.5	1.86	1.23 – 2.82
NOEC (mortality)	1.25	-	1.23	-

C. VALIDITY CRITERIA

Validity criteria	Required (OECD 203, 2019)	Achieved
Mortality in controls	<10%	0%
Dissolved oxygen concentration at the end of the test	>60% ASV	>7.3 mg/L >82% ASV
Analytical measurement of test concentrations	Compulsory	Performed

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Mean measured concentration (mg/L)
LC_{50} (96 hours)	1.86
95% confidence limits	1.23 – 2.82
NOEC (mortality)	1.23

III. CONCLUSION

The 96-Hour LC_{50} of Bandur to common carp, *Cyprinus carpio*, based on the mean measured test concentrations was estimated to be 1.86 mg/L (confidence limits 1.23 – 2.82 mg/L). The NOEC, based on mortality, was 1.23 mg/L.

[redacted] *(1993)*

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

The 96-hour LC_{50} of Bandur to common carp, *Cyprinus carpio*, based on the mean measured test concentrations was estimated to be 1.86 mg/L (confidence limits 1.23 – 2.82 mg/L). The NOEC, based on mortality, was 1.23 mg/L.

In terms of the active ingredient content, based on a reported active ingredient content of 49.4%, the 96-hour LC_{50} was estimated to be 0.92 mg a.s./L (confidence limits 0.61 – 1.39 mg a.s./L). The NOEC, based on mortality, was 0.61 mg a.s./L.

The results were based on the arithmetic mean measured test concentrations. EFSA's Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EFSA, 2015)[12] recommends that mean measured concentrations are calculated using the geometric mean rather than the arithmetic mean. The geometric mean measured concentrations calculated from the reported analysis results were 0.324, 0.536, 1.22, 2.77, 3.80 and 6.07 mg/L. Given that the difference between the geometric mean measured and arithmetic mean measured concentrations was less than 5%, it was

[12] 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.

considered that recalculation of the study endpoints based on the geometric mean measured concentrations was not necessary.

Assessment and conclusion by RMS:

Data Point:	KCP 10.2.1/05
Report Author:	[redacted]
Report Year:	1993
Report Title:	Bandur: Acute toxicity to Daphnia magna
Report No:	C024396
Document No:	M-216843-01-1
Guideline(s) followed in study:	OECD: 202 (1984)
Deviations from current test guideline:	Current guideline: OECD 202, 2004 No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The acute toxicity of Bandur (aclonifen 600g/L) to *Daphnia magna*, was determined in a 48-hour, static exposure. Test solutions were prepared by direct addition of test substance to dilution water. Twenty *Daphnia* per test group were exposed to an untreated control and nominal Bandur concentrations of 0.31, 0.63, 1.25, 2.5, 5 and 10 mg/L. The total test period was 48 hours. Samples for analytical confirmation of actual exposure concentrations were taken at the start and after 48 hours of exposure.

Dissolved oxygen, pH, and temperature were measured in the controls and each test concentration at the beginning of the test and daily thereafter. Daily observations were made of immobilisation.

Analytical results indicate that at the levels of biological significance (i.e. NOEC and EC_{50}) intended exposure concentrations were achieved (between 75 -95% of nominal) and were adequately maintained during the test (96 - 104% of starting concentrations). At 10 mg/L, mean measured values at the start and end of the test were 58 and 41% of nominal, respectively; suggesting that the aqueous solubility of the test material had been exceeded.

The 48-hour EC_{50} of Bandur (aclonifen 600g/L) to *Daphnia magna* was determined to be 2.40 mg/L (confidence limits 2.19 – 3.69 mg/L). The NOEC was 0.30 mg/L. Results were based on the mean measured concentrations.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	Bandur (aclonifen 600 g/L) 2-chloro-6-nitro-3-phenoxyaniline
	Batch no.:	OP 880348
	Active Ingredient / Purity:	600 g/L (49.4% a.s.)
	Appearance:	Yellow to brown suspension
	Date received:	7 May 1991
	Storage:	Room temperature, in the dark
	Expiry date:	January 1993
2.	**Test Organism:**	*Daphnia magna*
	Age:	Juvenile *Daphnia* between 6 and 24 hours old at start of the test
	Source:	[redacted]
	Feeding:	Adult cultures fed at least 5 times/week with green alga, *Chlorella vulgaris*, and yeast. No feeding during test.
3.	**Test water:**	Treated tap water. Treatment involved blending tap water previously filtered through activated carbon to remove chlorine, with tap water that had been softened and treated by reverse osmosis to reduce hardness to 200 – 250 mg/L as $CaCO_3$
	Total hardness:	222 – 240 mg/L as $CaCO_3$

B. STUDY DESIGN AND METHODS

1. In-life phase: 10 to 12 July 1991

2. Exposure conditions

Test vessels:	Glass crystallising dishes (150 mL) covered with a watch-glass
Experimental design:	Six test concentrations (0.31, 0.63, 1.25, 2.5, 5 and 10 mg/L) plus one control; 4 replicates each containing 5 *Daphnia*
Loading:	*ca.* 30 mL of media per *Daphnia*
Temperature:	20.8 – 21.4°C
pH:	7.3 – 8.6
Dissolved oxygen:	8.6 – 8.9 mg O_2/L
Aeration:	None
Photoperiod:	16 h light: 8 h dark

3. Administration of the test item

The highest nominal test exposure concentration (10 mg/L) was prepared by addition of test material to dilution water. This was further diluted to prepare lower test concentrations.

Each exposure concentration and the control comprised 4 replicates each containing 5 *Daphnia*.

4. Measurements and observations

The number of immobilised daphnids was assessed after 24 and 48 hours from the beginning of the test. The criterion for the effect (immobility) was the inability to swim within 15 seconds after gentle prodding with a glass rod.

Temperature, dissolved oxygen concentrations and pH values were measured in all the test groups and the control vessels at the beginning and end of the test. The total hardness and alkalinity were also measured at the start and end of the test. Measurements were from the excess solutions remaining after filling test vessels at the start of the test and from pooled replicates at the end of the test.

Samples were taken from each test concentration and control for analysis. The samples were collected at 0 hours from fresh test solutions and at the end of the test from pooled replicates of the aged test solutions.

5. Statistics/Data evaluation

The EC_{50} and its associated 95% confidence limits were calculated using a computer program following [redacted] (1982). The No Observed Effect Concentration (NOEC) was determined by visual inspection of the data.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Analytical results indicate that at the levels of biological significance (i.e. NOEC (0.31 mg/L) and lowest concentration causing 100% immobility (5 mg/L)) intended exposure concentrations were substantially achieved (between 75 -95% of nominal) and were adequately maintained during the test (96 - 104% of starting concentrations). At 10 mg/L mean measured values at the start and end of the test were 58 and 41% of nominal respectively; suggesting that the aqueous solubility of the test material had been exceeded.

The results of analysis of test solutions is summarised below:

Table: Measured concentrations of Bandur

Nominal concn (mg/L)	0 hour		48 hours		Mean measured concn (mg/L)
	Measured concn (mg/L)	% of nominal	Measured concn (mg/L)	% of nominal	
Control	n.d.	-	n.d.	-	-
0.31	0.297, 0.299	95	0.310, 0.306	98	0.30
0.63	0.547, 0.548	88	0.574, 0.568	91	0.56
1.25	1.07, 1.08	86	1.11, 1.11	89	1.09
2.5	2.14, 2.16	86	2.23, 2.24	89	2.19
5.0	3.76, 3.75	75	3.61, 3.63	72	3.69
10	5.73, 5.87	58	4.14, 4.06	41	4.95

n.d. = none detected

Although results varied by more than 20% at the biologically relevant concentrations closest to the EC_{50} and NOEC concentrations remained within ± 20%, results were based on mean measured test concentrations.

The validated method is summarised in Document M-CP5 (CP 5.1.2/04).

B. BIOLOGICAL DATA

The number of immobilized daphnids and the percentage of immobilization at 24 and 48 hours of exposure are presented in the following table:

Table: Numbers of mobile, immobile and floating *Daphnia magna* after exposure to Bandur

Nominal concn (mg/L)	No. of Daphnia							
	24 hours				48 hours			
	Mobile		Immobile		Mobile		Immobile	
	Submerged	Floating	Submerged	Floating	Submerged	Floating	Submerged	Floating
Control	20	0	0	0	20	0	0	0
0.31	20	0	0	0	20	0	0	0
0.63	19	0	1	0	19	0	1	0
1.25	17	0	3	0	16	0	4	0
2.5	17	1	2	0	12	1	7	0
5.0	11	0	8	1	0	0	20	0
10	1	0	13	0	0	0	29	0

All chemical and physical parameters in the definitive test were within expected ranges.

EC_{50} values were estimated by non-linear interpolation between two concentrations bracketing 50% effect. These concentrations were taken to be 95% confidence limits. It was not possible to calculate results using moving average angle or probit methods.

Based on the observed immobilisation, the EC_{50} values at each observation point were determined to be:

Table: EC_{50} values from the exposure of *Daphnia magna* to Bandur (aclonifen 600g/L)

Time (Hours)	Nominal		Measured	
	EC_{50} (mg/L)	95% confidence limits (mg/L)	EC_{50} (mg/L)	95% confidence limits (mg/L)
24	5.94	5 – 10	3.97	3.69 – 4.95
48	2.83	2.5 - 5	2.40	2.19 – 3.69
NOEC (48 hours)	0.31	-	0.30	-

C. VALIDITY CRITERIA

Validity criterion	Required (OECD 202, 2004)	Achieved
Mortality in controls	<10%	0%
Dissolved oxygen concentration at the end of the test	>3 mg/L	> 96% ASV equivalent to 8.5 mg O_2/L

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Mean measured concentration (mg/L)
EC_{50} (48 hours)	2.40
95% confidence limits	2.19 – 3.69
NOEC	0.30

III. CONCLUSION

The 48-hour EC_{50} of Bandur (aclonifen 600g/L) to *Daphnia magna* was determined to be 2.40 mg/L (confidence limits 2.19 – 3.69 mg/L). The NOEC was 0.30 mg/L. Results were based on measured concentrations.

[redacted] (1993)

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

The 48-hour EC_{50} to *Daphnia magna* based on mean measured concentrations was determined to be 2.40 mg/L (confidence limits 2.19 – 3.69 mg//L). The NOEC was 0.30 mg/L.

In terms of the active ingredient content, based on a reported active ingredient content of 49.4%, the 48-hour EC_{50} was estimated to be 1.19 mg a.s./L (confidence limits 1.08 – 1.82 mg a.s./L). The NOEC, based on mortality, was 0.15 mg a.s./L.

The results were based on the arithmetic mean measured test concentrations. EFSA's Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EFSA, 2015) recommends that mean measured concentrations are calculated using the geometric mean rather than the arithmetic mean. The geometric mean measured concentrations were determined to be 0.30, 0.56, 1.1, 2.2, 3.7 and 4.9 mg/L. Given that the difference between the geometric mean measured and arithmetic mean measured concentrations was less than 1%, it was considered that recalculation of the study endpoints based on the geometric mean measured concentrations was not necessary..

Assessment and conclusion by RMS:

Data Point:	KCP 10.2.1/01
Report Author:	[redacted]
Report Year:	1993
Report Title:	Bandur: Determination of its EC50 to Selenastrum capricornutum
Report No:	C024552
Document No:	M-217128-01-1
Guideline(s) followed in study:	OECD: 201 (1984)
Deviations from current test guideline:	Current Guideline: OECD 201, 2011 No deviation Current method guideline: SANCO/3029/99 rev.4 Yes, no recovery experiments were performed during method validation
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The effects of Bandur (600 g/L aclonifen) on the unicellular green alga, *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*), was determined in a 96-hour exposure. Algae were exposed to an untreated control, and nominal Bandur concentrations of 3.2, 10, 32, 100, 320 and 1000 µg/L. The total test period was 96 hours. Samples for analytical confirmation of actual exposure concentrations were taken at the start and after 96 hours of exposure.

Temperature and pH were measured in the control and each test concentration at the beginning and end of the test. Daily observations were of algal cell density. Samples were chemical analysis were taken at the start and end of the test.

The analytical limit of detection was determined to be 5 µg/L, therefore, it was not possible to determine the concentration of aclonifen in the nominal 3.2 µg/L test exposure solutions. Similarly, results were variable at the nominal 10 µg/L exposure concentration as this was close to the analytical limit of detection. At and above 32 µg/L, measured concentrations were lower than expected, but were maintained between 78 and 115% of starting concentrations, indicating aclonifen stability over the course of the study.

The 96-hour E_rC_{50} of Bandur (600 g/L aclonifen) to the green alga *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*), was determined to be 58 µg/L using initial mean measured concentrations. Using nominal concentrations, the 96-hour E_rC_{50} of Bandur (600 g/L aclonifen) was determined to be 69 µg/L (confidence limits 55 - 88 µg/L). The NOEC was 10 µg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Bandur
Active ingredient: 2-chloro-6-nitro-3-phenoxyaniline
Batch no.: OP 880348
Active Ingredient / Purity: 49.4% a.s.
Appearance: Yellow to brown suspension
Date of production: 19 January 1989
Storage: Room temperature, in the dark
Expiry date: January 1993

2. Test Organism: *Seleniastrum capricornutum* (currently known as *Raphidocelis subcapitata*)
Strain: CCAP no. 278/4
Source: [redacted]
Culture was stored in an illuminated incubator at 25°C until required
Initial density:
Culture and test conditions: Gallenkamp temperature controlled orbital incubator at 20-25°C, with shaking at 175 rpm. Light intensity was approximately 10,850 lux

3. Treatment: Sterile OECD medium

4. Test vessels: 250 mL conical flasks, containing 50 mL test solution. Flasks had been sterilised by autoclaving. After addition of test solution vessels were closed with a non-absorbent cotton wool plug

Test water: Sterile OECD medium

Shaking: 175 rpm

5. Environmental conditions:

Temperature: 21.8 – 21.9°C (start of test)
22.8 – 23.9°C (96 hours)

pH 8.0 – 8.1 (start of test
7.2 – 8.0 (96 hours)

Photoperiod Continuous illumination, approx. 10,850 lux

B. STUDY DESIGN AND METHODS

1. In-life phase: 17 to 7 August 1990

2. Exposure conditions

The test started (0 hours) by addition of 1.0 x 10^4 cells/mL to each test flask. Algae were from laboratory cultures. The test was performed with six control replicates and three replicates of the test concentrations.

3. Administration of the test item

Test solutions were by serial dilution of nominal 100 mg/L stock solution prepared in sterile OECD medium. Aliquots of 1 mL test solution was added to each test vessel to give nominal exposure levels of 3.2, 10, 32, 100, 320 and 1000 µg/L. Test vessels were randomly allocated to a numbered position in the test incubator

4. Measurements and observations

The cell density in each replicate was determined daily (days 1,2 and 3) during the test period by haemocytometer.

The temperature and pH was measured daily in an additional vessel from each test and control group. This flask was also used to take samples for chemical analysis. The pH and temperature was measured in all test and control vessels at the end of the test.

At the end of the test approximately 2 mL samples were taken from each test flask at the three highest exposure concentrations where algal growth had been inhibited and from the control. Samples from each group of vessels were pooled and the combined suspensions were used to inoculated fresh OECD medium (1mL/100mL). Flasks were plugged and incubated and cell density assessed after 3,7 and 10 days to determine any algistatic/algicidal effect.

At the start of the test, duplicate samples were taken from the additional flask at each exposure concentration and the control. After 96 hours, the contents of the vessels at each exposure level were pooled and duplicate samples taken for analysis. OECD medium from the control was used as a blank.

Samples were analysed by HPLC with a spectrophotometric detector.

5. Statistics/Data evaluation

The EC_{50} and effects of Bandur were determined by examining growth rate, following the equations outlined in the OECD guidance. Statistical comparisons of average specific growth rates in the control and test concentrations were carried out by Dunnett's multi comparison test (1955, 1964), using a multiple t-test comparing each treatment with the control using a common estimate of experimental error. Dunnett's t-statistic tables were used to assess significance at 95% level of probability.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

The analytical limit of detection was determined to be 5 µg/L, therefore, it was not possible to determine the concentration of aclonifen in the nominal 3.2 µg/L test exposure solutions. Similarly, results were variable at the nominal 10 µg/L exposure concentration as this was close to the analytical limit of detection. At and above 32 µg/L, measured concentrations were lower than expected, but were maintained between 78 and 115% of starting concentrations, indicating aclonifen stability over the course of the study.

Test results were calculated using initial measured concentrations.

The validated method is summarised in Document M-CP5 (CP 5.1.2/05).

Table: Measured concentrations of BANDUR

Nominal concentration (µg/L)	0 hours		96 hours		% of 0 hours
	Measured concn (µg/L)	% of nominal	Measured concn (µg/L)	% of nominal	
Control	-	-	-	-	-
3.2	-	-	-	-	-
10	6.41, 15.1	108	-	-	-
32	23.8, 14.7	60	19.9, 18.3	60	100
100	63.8, 65.6	65	78.2, 70.7	74	115
320	314, 253	89	224, 216	69	78
1000	797, 797	80	722, 704	71	89

- = none detected (<5 µg/L)

B. BIOLOGICAL DATA

A significant reduction in growth rate and biomass compared to the control was observed at levels above 10 µg/L ($p < 0.05$). A statistically significant impact was shown at the nominal 3.2 µg/L test exposure concentration, but this was not considered biologically meaningful as there was no effect observed at the next highest test concentration (10 µg/L). The NOEC for both growth rate and biomass was 10 µg/L, nominal test concentration.

The 96-hour EC_{50} for average specific growth rate (E_rC_{50}) and mean biomass (E_bC_{50}), based on nominal concentrations, was 69 µg/L (95% confidence limits 55 and 88 µg/L) and 21 µg/L (95% confidence limits 16 and 26 µg/L), respectively, determined by moving average angle.

The E_rC_{50} and E_bC_{50}, based in initial mean measured levels were 58 (95% confidence limits 50 and 68 µg/L) and 16 µg/L (approximate value based on non-linear interpolation between 10.8 and 19.3 µg/L).

Mean area under the curve and growth rates are presented in the following tables.

Table: Mean average specific growth rate and biomass after 96 hours of exposure

Nominal concentration (µg/L)	0-96h	
	Average specific growth rate	Biomass
Control	5.611	5937
3.2	5.040	4026
10	5.565	5539
32	4.010	1722
100	2.114	260
320	0.318	41
1000	0.272	34

Sub-cultures in freshly prepared sterile OECD medium from nominal test exposure concentrations 100, 320 and 1000 µg/L showed normal growth after 10 days incubation, indicating that at these levels the test material was algistatic.

All chemical and physical parameters in the definitive test were within expected ranges.

Table: EC_{50} values from the exposure of green alga *Selenastrum capricornutum* (currently known as *Raphidocelis subcapitata*) to Aclonifen

Endpoint (96 hours)	Based on nominal test concn (µg/L)	95% confidence limits (µg/L)	Based on initial measured concn (µg/L)
E_rC_{50}	69	55 – 88	58
E_bC_{50}	21	16 - 26	16
No Observed Effect Concentration (96 hours) = 10 µg/L			

C. VALIDITY CRITERIA

Validity criterion	Required (OECD 202, 2004)	Achieved
Biomass in control should increase exponentially by factor of ≥16 within 72-h test period.	≥16	106
Mean coefficient of variation for section by section specific growth rates (days 0-1, 1-2 & 2-3) for 72-h test) in controls must be ≤35%	≤35%	21%
Coefficient of variation of average specific growth rates during whole test period in replicate controls must be ≤7%	≤7%	3.8%

Study was valid according to the validity criteria set out in the OECD 201 guideline (2011) and is therefore considered an acceptable study.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Growth rate (96 hours)			
	Nominal concentration (µg/L)	95% confidence limit	Initial measured concentration (µg/L)	95% confidence limit
E_rC_{50}	69	55 - 88	58	n.d.
NOEC	10		10.8	
LOEC	32		19.2	

n.d. Not determined

III. CONCLUSION

The 96-hour EC_{50} for average specific growth rate (E_rC_{50}), based on nominal concentrations, was 69 µg/L (95% confidence limits 55 and 88 µg/L). The 96-hour NOEC for growth rate was 10 µg/L and the corresponding LOEC was 32 µg/L, based on nominal test concentrations.

The 96-hour E_rC50 was calculated to be 58 µg/L, based on initial measured concentrations.

[redacted] *(1993)*

Data Point:	KCP 10.2.1/06
Report Author:	[redacted]
Report Year:	2019
Report Title:	Bandur: Determination of its EC50 to Selenastrum capricornutum statistical re-analysis of Jenkins, 1993 (M-217128-01-1) study
Report No:	VCA19/027/001
Document No:	M-674903-01-1
Guideline(s) followed in study:	Not applicable. Report is a re-evaluation of previously generated study data
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The report for the algal growth inhibition test of BANDUR to *Selenastrum capricornutum* (M-217128-01-1, [redacted] 1993) only provided EC_{50} values expressed in terms of the test item concentration (formulation). Data from the study has been re-analysed in order to provide EC_{10}, EC_{20} and EC_{50} values along with the LOEC and NOEC based on geometric mean measured concentrations of the formulation and the active substance, aclonifen.

Analysis of the test solutions (fresh and spent) for the determination of the content of BANDUR was performed in samples taken at 0 and 96 hours after application.

The analytical limit of detection was determined to be 5 µg/L, therefore, it was not possible to determine the concentration in the nominal 3.2 µg/L test exposure solutions. Similarly, results were variable at the

nominal 10 µg/L exposure concentration as this was close to the analytical limit of detection. At and above 32 µg/L, measured concentrations were lower than expected, but were maintained between 78 and 115% of starting concentrations, indicating stability over the course of the test.

The geometric mean measured concentrations of the formulation were calculated using the measured concentrations at 0 and 96 hours. Where concentrations were below the LOD of the analytical method, the LOD value (5 µg/L) was substituted to enable calculation of the geometric mean measured concentration.

As a result of the LOD being higher than the lowest nominal test concentration, the calculated geometric mean measured concentration for the 3.2 µg/L test exposure solutions was considered to be unreliable and was not used in the determination of the EC_x values.

Geometric mean measured concentrations of the active substance were calculated from the geometric mean measured concentrations of the formulation using an active substance content of 49.4% aclonifen.

The geometric mean measured concentrations for the 10, 32, 100, 320 and 1000 µg formulation/L test exposure solutions were determined to be 7.33, 19.2, 69.4, 250 and 754 µg formulation/L. In terms of the active substance, the geometric mean measured test concentrations were determined to be 3.62, 9.5, 34.3, 123 and 372 µg aclonifen/L.

Statistical analysis of the data was carried out using ToxRat Professional version 3.3.0 (ToxRat Solutions GmbH, 2018). ECx values were determined by Probit analysis using linear max. likelihood regression. NOEC/LOEC values were determined by multiple sequentially-rejective Welsh-t-test after Bonferroni-Holm (α = 0.050, one-sided smaller), or Williams multiple sequential t-test procedure (α = 0.050, one-sided smaller).

Statistical analyses of the available data resulted in the calculation of the following EC_x, LOEC and NOEC values:

Formulation

Parameter	Yield (96 hours)			Growth Rate (0 – 96 hours)			Biomass integral (0 – 96 hours)		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_rC_{10}	E_rC_{20}	E_rC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}
Value (µg/L)	6.105	7.771	12.239	9.031	15.489	43.475	7.110	9.029	14.259
Lower 95%-cl	3.183	4.802	9.654	5.436	10.681	35.256	4.489	6.413	12.053
Upper 95%-cl	8.151	9.877	15.584	12.684	20.158	53.929	9.020	10.905	16.508
LOEC	19.2			19.2			19.2		
NOEC	7.33			7.33			7.33		

Aclonifen

Parameter	Yield (96 hours)			Growth Rate (0 – 96 hours)			Biomass integral (0 – 96 hours)		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_rC_{10}	E_rC_{20}	E_rC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}
Value (µg/L)	3.014	3.838	6.095	4.470	7.662	21.482	3.511	4.460	7.051
Lower 95%-cl	1.569	2.370	4.770	2.691	5.284	17.423	2.215	3.166	5.959
Upper 95%-cl	4.026	4.881	7.708	6.278	9.971	26.641	4.456	5.389	8.166
LOEC	9.5			9.5			9.5		
NOEC	3.62			3.62			3.62		

[redacted] *(2019)*

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

The analytical limit of detection was determined to be 5 µg/L, therefore, it was not possible to determine the concentration of aclonifen in the nominal 3.2 µg/L test exposure solutions. Similarly, results were variable at the nominal 10 µg/L exposure concentration as this was close to the analytical limit of detection. At and above 32 µg/L, measured concentrations were lower than expected, but were maintained between 78 and 115% of starting concentrations, indicating aclonifen stability over the course of the study.

EC_{10} or EC_{20} values were not presented in the original study report. These values along with the EC_{50}, LOEC and NOEC based on geometric mean measured concentrations of the formulation and active ingredient were determined by re-evaluation of the original study data (M-674903-01-1) and gave the following results:

Formulation

Parameter	Yield (96 hours)			Growth Rate (0 – 96 hours)			Biomass integral (0 – 96 hours)		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_rC_{10}	E_rC_{20}	E_rC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}
Value (µg/L)	6.105	7.771	12.239	9.031	15.489	43.475	7.110	9.029	14.259
Lower 95%-cl	3.183	4.802	9.654	5.436	10.681	35.256	4.489	6.413	12.053
Upper 95%-cl	8.151	9.877	15.584	12.684	20.158	53.929	9.020	10.905	16.508
LOEC	19.2			19.2			19.2		
NOEC	7.33			7.33			7.33		

Aclonifen

Parameter	Yield (96 hours)			Growth Rate (0 – 96 hours)			Biomass integral (0 – 96 hours)		
	E_yC_{10}	E_yC_{20}	E_yC_{50}	E_rC_{10}	E_rC_{20}	E_rC_{50}	E_bC_{10}	E_bC_{20}	E_bC_{50}
Value (µg/L)	3.014	3.838	6.095	4.470	7.662	21.482	3.511	4.460	7.051
Lower 95%-cl	1.569	2.370	4.770	2.691	5.284	17.423	2.215	3.166	5.959
Upper 95%-cl	4.026	4.881	7.708	6.278	9.971	26.641	4.456	5.389	8.166
LOEC	9.5			9.5			9.5		
NOEC	3.62			3.62			3.62		

For risk assessment purposes, the E_rC_{50} (0 - 96 hours) value of 43.475 mg formulation/L (21.482 mg a.s./L) is considered appropriate.

Assessment and conclusion by RMS:

Data Point:	KCP 10.2.1/02
Report Author:	[redacted]
Report Year:	2003
Report Title:	Toxicity of Bandur (formulation of Aclonifen, code: AE F068300 00 SC50 A204) to the aqualtic plant Lemna gibba in a growth inhibition test.
Report No:	C037483
Document No:	M-222986-01-1
Guideline(s) followed in study:	OECD: 221; USEPA (=EPA): OPPTS850.4400,1996
Deviations from current test guideline:	Current Guideline: OECD 221, 2006 No deviation Current method guideline: SANCO/3029/99 rev.4 No deviations
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary:

The effects of Bandur (formulation of aclonifen, code: AE F068300 00 SC50 A204), on the growth and reproduction of the aquatic monocotyledonous plant *Lemna gibba,* were investigated in an exposure to nominal concentrations of 0.005, 0.01, 0.02, 0.04, 0.08 and 0.16 mg test item/L.

Fronds of *Lemna gibba* were exposed to Bandur for seven days in a semi-static system with test medium renewal on days 3 and 5. The effect was expressed in terms of percent inhibition in frond number and biomass relative to the blank control on day seven of the study.

In-the freshly prepared stock solution samples between 80 and 90% of nominal were determined. In the test media low recovery rates, probably caused by lack of homogeneity of test item in test water due to precipitation or settling of the test item, were observed. This is substantiated by the observation of precipitation of test item in the stock solutions after stirring. Since the determined test concentrations were below the nominal concentrations, all reported results are related to mean measured concentrations, calculated as the average over all measurements per test concentration. ·Therefore, the mean measured test concentration was used to calculate the study endpoints.

The test item Bandur (formulation of aclonifen, code: AE F068300 00 SC50 A204) had a statistically significant inhibitory effect on the growth of *Lemna gibba* after 7 days exposure period at the mean measured concentration of 0.009 mg test item/L (Dunnett tests, one-sided, a = 0.05). Thus, this test concentration was determined as the 7-day LOEC (lowest concentration tested with toxic effects). The 7-day NOEC (highest concentration tested without toxic effects after a test period of 72 hours) was determined to be the mean measured concentration of 0.004 mg test item/L, since in this test concentration the growth rate and biomass gain of *Lemna gibba* was statistically not significantly lower than in the control. The EC_{50} values were calculated for the parameters; area under the growth curve (biomass integral), growth rate and biomass gain (based on dry weight). After 7 days these were determined to be 0.020, 0.043 and 0.010 mg/L, respectively. The corresponding EC_{10} values were

0.004, 0.003 and 0.004 mg/L (area under the growth curve (biomass integral), growth rate and biomass gain, respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test material:**	Bandur (formulation of aclonifen, code: AE F068300 00 SC50 A204)
	Batch no.:	OP 220331
	Purity:	591 g/L
	Density:	1.196 g/cm^3
2.	**Test organism:**	*Lemna gibba*
	Strain:	G3
	Source:	Ökotox Moser & Pickl GbR, Stuttgart, Germany
3.	**Treatment:**	Nominal test concentrations of 0.005, 0.01, 0.02, 0.04, 0.08 and 0.16 mg test item/L
4.	**Test vessels:**	Glass flasks, 250 mL volume with 150 mL test solution, covered with glass dishes
	Test water:	20X AAP growth medium
5.	**Environmental conditions:**	
	Temperature:	23 – 14°C
	pH:	7.6 (start of test) 7.5 – 8.8 during test in control medium)
	Photoperiod:	Continuous illumination by fluorescent tubes located above test vessels, 8740 lux (mean), range 8570 – 89810 lux

B. STUDY DESIGN AND METHODS

1. In-life phase: 13 to 20 June 2003

2. Test organism assignment and treatment

Colonies consisting of 4 fronds were transferred from the inoculum culture. Each test vessel contained a total of 12 fronds, with 3 replicates per treatment. The test vessels were placed in a random order and were repositioned each day of measurement to minimize differences in light intensity. A semi-static test procedure was used and the test media were renewed on days 3 and 5. Test vessels were re-positioned each working day.

3. Dose preparation

At the start of the test and at each test medium renewal, stock solutions of nominal 50 mg/L were prepared by dissolving 51 mg test item into 1000 mL test water with intense stirring for *ca*. 10 minutes. Volumes of stock solution were diluted with test water to prepare the test media. The stock solutions and the test media were renewed on days 3 and 5 to keep the concentration of Bandur (formulation of aclonifen, code: AE F068300 00 SC50 A204) as high as possible in the test medium. The test media were freshly prepared just before introduction of the aquatic plants. The control comprised test dilution water only.

4. Measurements and observations

Frond counts were made on Days 0, 3, 5, and 7. Biomass was determined at the completion of the study using all replicates from the test treatments and blank control. *Lemna gibba* G3 growth measurement was determined by visually counting the number of fronds per flask.

The pH-values of the test media were measured in all freshly prepared and old test concentrations and the control at the start and at each observation day. The temperature was measured daily in a test vessel filled with test medium and incubated under the same conditions as the test flasks. The light intensity was measured once during the study.

One sample was taken from the freshly prepared stock solution and duplicate samples were taken from the freshly prepared test media of all test concentrations and the control at the start of the test and on days 3 and 5. For the determination of the stability of the test item under the test conditions duplicate samples of all aged test media and controls were sampled at each test medium renewal. Any samples not analysed immediately were stored refrigerated until analysis. Analysis of achieved concentration for media was undertaken at the start and end of the exposure. Samples were analysed by HPLC using a UV-vis detector.

5. Statistics

The EC_{50} (the concentrations of the test item corresponding to 50% inhibition of growth rate, area under the growth curve and dry weight compared to the control), and their 95% confidence limits were calculated by Probit analysis using ToxRat Professional Version 2.07. The NOEC and LOEC were determined by the multiple Dunnett-test using ToxRat Pro Version 2.07 after analysis of variance (ANOVA). All results were calculated were based on mean measured concentration.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

In-the freshly prepared stock solution samples between 80 and 90% of nominal were determined. In the test media low recovery rates, probably caused by lack of homogeneity of test item in test water due to precipitation or settling of the test item, were observed. This is substantiated by the observation of precipitation of test item in the stock solutions after stirring. Since the determined test concentrations were below the nominal concentrations, all reported results are related to mean measured concentrations, calculated as the average over all measurements per test concentration. ·Therefore, the mean measured test concentration was used to calculate the study endpoints.

The validated method is summarised in Document M-CP5 (CP 5.1.2/08).

Table: Mean measured concentrations (mg/L) of Bandur (aclonifen) in the exposure solutions

Nominal concn (mg/L)	Mean measured concn (mg/L)
0.005	0.004
0.01	0.009
0.02	0.014
0.04	0.024
0.08	0.051
0.16	0.088

B. BIOLOGICAL DATA

Growth inhibition

Mean frond numbers are presented in the following table:

Table: Mean number of healthy fronds during 7-day exposure

Nominal conc (mg/L)	Day 3		Day 5		Day 7	
	Mean	SD	Mean	SD	Mean	SD
Control	42.3	3.79	87.0	8.54	182	28.2
0.005	42.3	2.89	96.7	10.5	197	16.4
0.01	43.0	2.00	83.7	11.9	127	20.6
0.02	37.0	3.61	58.0	7.00	65.7	8.74
0.04	28.0	3.61	39.3	1.53	44.0	3.46
0.08	22.7	0.58	34.3	1.16	40.7	1.53
0.16	21.0	0.00	34.3	2.08	43.3	2.08

Day 0 = 12 fronds per test flask, 3 replicates

Table: Growth rate based on frond number and % inhibition

Mean measured concn (mg/L)	0 - 3 days		0 - 5 days		0 – 7 days	
	Growth rate (r)	% inhibition	Growth rate (r)	% inhibition	Growth rate (r)	% inhibition
Control	0.419	-	0.396	-	0.387	-
0.004	0.420	-0.1	0.416	-5.3	0.399	-3.1
0.009	0.425	-1.4	0.387	2.1	0.336*	13.2
0.014	0.374	10.7	0.314*	20.6	0.242*	37.5
0.024	0.281*	33.1	0.237*	40.0	0.185*	52.1
0.051	0.212*	49.5	0.210*	46.9	0.174*	55.0
0.088	0.187*	55.5	0.210*	46.9	0.183*	52.7

Negative inhibition = increase in growth relative to control
* Significant difference from control

Table: Biomass (AUC) based on frond number and % inhibition

Mean measured concn (mg/L)	0 - 3 days		0 - 5 days		0 – 7 days	
	Biomass	% inhibition	Biomass	% inhibition	Biomass	% inhibition
Control	45.5	-	150.8	-	395.8	-
0.004	45.5	0.0	160.5	-6.4	429.8	-8.6
0.009	46.5	-2.2	149.2	1.1	336.2	15.1
0.014	37.5	17.6	108.5*	28.1	208.2*	47.4
0.024	24.0*	47.3	67.3*	55.4	126.7*	68.0
0.051	16.0*	64.8	49.0*	67.5	100.0*	74.7
0.088	13.5*	70.3	44.8*	70.3	98.5*	75.1

Negative inhibition = increase in growth relative to control
* Significant difference from control

Shape of fronds

The shape of fronds and colonies after 7 days was not different to those in the control at the mean measured concentration of 0.004 mg test item/L. At concentrations of 0.009 to 0.088 mg/L the roots of the plants were shorter than in the control. At concentrations of 0.014 to 0.088 mg/L the fronds were smaller and showed necrosis and some fronds were single. At the three highest test concentrations (0.024, 0.051 and 0.088 mg/L) some fronds were coloured red or without any colour.

Table: Biomass gain (as dry weight) and % inhibition

Mean measured concn (mg/L)	0 - 7 days	
	Biomass gain	% inhibition
Control	27.17	-
0.004	26.83	1.2
0.009	13.17*	51.5
0.014	8.50*	68.7
0.024	4.50*	83.4
0.051	1.83*	93.3
0.088	1.50*	94.5

* Significant difference from control

C. VALIDITY CRITERIA

Validity criterion	Required (OECD 221, 2006)	Achieved
Doubling time of frond number in the control <2.5 days (60 h), corresponding to approximately 7-fold increase in 7 days	2.5d	1.8d

The above validity criterion was met and the study is considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Parameter	Mean measured concn (mg/L)					
	Growth rate (frond number)	95% confidence limit	Biomass integral (frond number)	95% confidence limit	Biomass gain (dry weight)	95% confidence limit
EC_{50}	0.043	0.017 – 5.995	0.020	0.009 – 0.046	0.010	0.007 – 0.014
EC_{10}	0.003	n.d. – 0.011	0.004	0.000 – 0.009	0.004	0.001 – 0.006
NOEC	0.004		0.009		0.004	-
LOEC	0.009	-	0.014	-	0.009	-

III. CONCLUSION

The test item Banduc (formulation of aclonifen, code: AE F068300 00 SC50 A204) had a statistically significant inhibitory effect on the growth of *Lemna gibba* after 7 days exposure period at the mean measured concentration of 0.009 mg test item/L (Dunnett tests, one-sided, a = 0.05). Thus, this test concentration was determined as the 7-day LOEC (lowest concentration tested with toxic effects). The 7-day NOEC (highest concentration tested without toxic effects after a test period of 72 hours) was determined to be the mean measured concentration of 0.004 mg test item/L, since in this test concentration the growth rate and biomass gain of *Lemna gibba* was statistically not significantly lower than in the control. The EC_{50} values were calculated for the parameters; area under the growth curve (biomass integral), growth rate and biomass gain (based on dry weight). After 7 days these were determined to be 0.020, 0.043 and 0.010 mg/L, respectively. The corresponding EC_{10} values were 0.004, 0.0003 and 0.004 mg/L (area under the growth curve (biomass integral), growth rate and biomass gain, respectively.

[redacted] *(2003)*

Assessment and conclusion by applicant:

Validity criterion was met and study is acceptable

The 7-day NOEC for Bandur (aclonifen 600 g/L) was determined to be 0.004 mg test item/L, based on mean measured concentrations. The E_rC_{50} value for growth rate after 7 days was determined to be 0.043 mg/L. The corresponding E_rC_{10} value was 0.003 mg/L, based on mean measured concentrations.

EFSA's Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EFSA, 2015)[13] recommends that measured concentrations are calculated using the geometric mean. A summary of the arithmetic and geometric mean measured concentrations is provided in the following table:

Table: Measured concentrations from the exposure of *Lemna gibba* to Bandur

Nominal concentration (mg/L)	Measured concentration (mg/L)			
	Arithmetic mean	% Nominal	Geometric mean	% Nominal
0.0050	0.0040	80	0.0038	77
0.010	0.0085	85	0.0083	83
0.020	0.0142	71	0.0140	70
0.040	0.0239	60	0.0229	57
0.080	0.0513	64	0.0502	63
0.16	0.0882	55	0.0819	51

Given that the geometric mean measured test concentrations were within 10 µg/L of the arithmetic mean measured test concentrations it was considered that recalculation of the study endpoints based on the geometric mean measured concentrations was not necessary. Consequently, the E_rC_{50} of 0.043 mg/L is used for risk assessment.

In terms of the active ingredient content, based on the reported product density of 1.196 g/cm^3 and an active ingredient content of 591 g/L, the E_rC_{50} and E_rC_{10} values were 0.021 and 0.0015 mg a.s./L respectively. The NOEC was 0.0020 mg a.s./L.

Assessment and conclusion by RMS:

CP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

[13] 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.

Data Point:	KCP 10.2.2/01
Report Author:	[redacted]
Report Year:	1993
Report Title:	Bandur: 21-Day rainbow trout toxicity study under flow-through conditions
Report No:	C024469
Document No:	M-216971-01-1
Guideline(s) followed in study:	OECD: 204 (1984)
Deviations from current test guideline:	Not applicable as OECD 204 guideline has been deleted and there is no equivalent current test guideline Current method guideline: SANCO/3029/99 rev.4 Yes, no recovery experiments were performed during method validation
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

In the previous submission (DAR, 2006), this study was evaluated and accepted as valid for risk assessment purposes. This study design and endpoint is no longer required for the registration of plant protection products in the EU and hence a summary of this study is not presented in this dossier.

Assessment and conclusion by RMS:

Data Point:	KCP 10.2.2/02
Report Author:	[redacted]
Report Year:	1993
Report Title:	Bandur: Daphnia magna - 21-Day reproduction test (semistatic conditions)
Report No:	C024471
Document No:	M-216975-01-1
Guideline(s) followed in study:	OECD: 202
Deviations from current test guideline:	Current Guideline: OECD 211, 2012 Adult Daphnia were not held in individual test vessels; pH increased by >0.3 pH unit between each renewal; Growth measurements (e.g. body length) were not included in the test design (recommendation but not a requirement); Other parameters such as time to first brood, number and size of broods were recorded but not analysed (recommendation but not a requirement) Current method guideline: SANCO/3029/99 rev.4 Yes, no recovery experiments were performed during method validation
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

The objectives of this study were to determine the effects of Bandur (aclonifen 600 g/L) on the survival and reproduction of the water flea *Daphnia magna.* Treatment groups of 40 *Daphnia* in 4 replicates were exposed to the test item at 10, 32, 100, 320 and 1000 µg/L (nominal) plus a dilution water control. The test was performed in a semi-static system with test substance renewal on days 3, 6, 8, 10, 13, 15, 17 and 20.

Exposure levels were monitored by an HPLC method of analysis: the limit of the assay in terms of Bandur was estimated to be 8 ug/L. Results for duplicate samples from three sets of freshly-prepared and three-day-old test dilutions indicated that intended exposure concentrations of Bandur were achieved and adequately maintained. Mean measured concentrations were 10.6, 30.1, 104, 350 and 1190 µg/L (94% to 119% of their nominal values).

The numbers of mobile, immobile and dead parental *Daphnia* were recorded each day, and the numbers of juveniles produced were counted three times each week. Mortality of parental *Daphnia* after 21 days in groups exposed to Bandur ranged from 20% at the lowest concentration (10.6 ug/L) to 100% at the highest level (1190 µg/L). Mortality of the parental control *Daphnia* after 21 days was 15%.

The 21-day median lethal concentration (LC_{50}) of Bandur to parental *Daphnia*, calculated using mean measured concentrations, was 446 µg/L. Statistical comparisons (Dunnett's test) of the numbers of mobile parental *Daphnia* present on Day 21 showed that at mean measured concentrations of 350 and 1190 µg/L, the survival of the parental generation was significantly lower ($p < 0.05$) compared to the control group. The no-observed-effect-concentration (NOEC) for parental mortality, based mean measured levels, was therefore 104 ug/L.

Statistical comparisons (Dunnett's test) of the total numbers of juveniles produced in each vessel, and the mean cumulative number produced by each surviving adult by Day 21, showed that at measured Bandur concentrations of 10.6, 30.1 and 104 µg/L, there was no significant difference in production ($p > 0.05$) compared to the control group. At 350 µg/L, juvenile production was significantly lower ($p < 0.05$) than in the control group.

The 21-day EC_{50} values for inhibition of reproduction were 153 µg/L based on the total number of juveniles produced at each concentration, and 147 µg/L based on the mean number of juveniles produced per adult. Overall, the no-observed-effect concentration for reproduction was 104 µg/L.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test material:**	Bandur (aclonifen 600g/L) 2-chloro-6-nitro-3-phenoxyaniline
	Batch no.:	OP 880348
	Active ingredient / Purity:	600 g/L (49.4% a.s.)
	Appearance	Yellow to brown suspension
	Date received:	7 May 1991
	Storage:	Room temperature, in the dark
	Expiry date:	January 1993
2.	**Test organism**	*Daphnia magna* Straus, Clone V
	Age:	>6 - <24 hours old (First-instar)
	Source:	[redacted]
	Feeding:	Cultures fed at least 3 times per week with unicellular green algae (*C. vulgaris*) and yeast. Each culture received 2 – 8 x 10^5 cells/mL algae and 0.04 – 0.05 mg/L of 100 mg/L yeast suspension During the test *Daphnia* were fed daily according to the following schedule Test day 1: 50% culturing volume algae and yeast Test day 2 – 7: 100% culturing volume algae and yeast Test day 8 – 20: 150% culturing volume algae and yeast Feeding reduced to 50% when >50% parental *Daphnia* had died
3.	**Treatment:**	Control, 10, 32, 100, 320 and 1000 µg/L (nominal) 4 replicates per treatment, 10 *Daphnia*/replicate
	Test vessels:	Glass vessels (500 mL) covered with clear Perspex sheet.
	Loading:	ca. 50mL of media per *Daphnia*
	Test water:	Elendt M4 medium (pH adjusted and ranged at 7.78 and 7.99)
4.	**Environmental conditions**	
	Temperature:	19.2 – 20.6°C
	pH:	7.51 – 7.85 (new solutions) 7.58 – 8.66 (aged solutions)
	Dissolved oxygen:	85 – 101% ASV
	Total hardness:	Fresh dilution water: 210 – 242 mg/L as $CaCO_3$ Aged control solution: 204 – 222 mg/L as $CaCO_3$
	Photoperiod:	16 h light : 8 h darkness (1100 - 1250 Lux)

B. STUDY DESIGN AND METHODS

1. In-life phase: 8 – 29 September 1992

2. Test organism assignment and treatment

Freshly hatched daphnids less than 24 hours old were used for the test. At test start, *Daphnia* were inserted into test vessels in a randomized order. Based on the results of a non GLP range-finding test, the following nominal concentrations were tested in the main test: 10, 32, 100, 320 and 1000 µg/L (nominal) and a control. The test was performed with 4 vessels for each test item concentration and the

control, containing 10 *Daphnia* each. Each treatment group and the control group consisted of 40 daphnids in total. The reproduction test was performed semi-statically with renewal of the test solutions three times per week.

The duration of the test was 21 days. Assessments on mortality and other effects were performed each day. Offspring was counted and removed daily after appearance of first brood. Measurement of body length was performed for all adult daphnia alive at the end of the test (21 days).

3. Dose preparation

A nominal 1 mg/L (1000 µg/L) stock solution was prepared by addition of 5 mg test material to 5 litres of dilution water. This was further diluted to provide lower test concentrations. The control comprised dilution water only.

Two sets of vessels were employed alternately, with one set filled with test media and Daphnia at the start of the test. Thereafter, the test dilutions were renewed three times per week (days 3, 6, 8, 10, 13, 15, 17 and 20). On each occasion and dead animals were discarded and surviving parental Daphnia transferred to fresh test dilutions in the second set of test vessels. Juveniles present in the old test solutions were counted and discarded.

4. Measurements and observations

Within the 21-day reproduction test, effects on mortality and reproductive outputs were evaluated. The mortality, the time of the first production of offspring and the number of offspring were compared with the corresponding parameters in the control. Body length of surviving adult *Daphnia* was also measured at test end.

The numbers of mobile, immobile and dead parental *Daphnia* in each vessel was recorded daily and the presence of juvenile Daphnia was recorded on days between renewal.

Analytical samples taken at day 0 (fresh), day 3 (aged), Day 10 (fresh), Day 13 (aged), Day 17 (fresh), Day 20 (aged)) were analysed from controls and all test item concentrations. Samples (2 x 20 ml) of test medium at each exposure level were taken immediately after preparation and again before renewal on three occasions. Samples of freshly prepared media were removed from the preparation flasks after the test vessels had been filled and samples of aged media were taken from the pooled contents of the replicate vessels at each level.

Temperature, pH and dissolved oxygen concentration of the test solutions (all concentrations and controls), were measured at the start and at every test solution renewal day.

5. Statistics

The median lethal concentrations (LCBOs) for the parental generation at intervals during the test were calculated by the computer program of [redacted] (1977, 1982) using the number of parental *Daphnia* and the number of dead animals at each nominal and mean measured concentration. The median effect concentrations (EC50s) for reproduction were similarly calculated using the total number of juveniles produced at each concentration and the cumulative number of juveniles produced per adult. In both cases, the numbers of juveniles were expressed as percentages of the mean number in the control group, which were then subtracted from 100 to give the percentage reduction. The numbers of dead parental *Daphnia,* the total number of juveniles and the mean number of juveniles produced per adult in the

control group were compared with those in test groups, by Dunnett's multi-comparison test (1955, 1964) which uses a multiple t-test with a common estimate of experimental error. Dunnett's t-Statistic tables were used to assess significance at the 95% level of probability.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Mean measured concentrations determined in freshly prepared test media ranged between 90 and 126% of nominal values. Measured levels in aged media ranged between 76 and 127% of nominal. Results were most variable at the lowest exposure level (10 ug/L), due to its proximity to the limit of quantification. Overall, mean measured values were 10.6, 30.1, 104, 350 and 1190 g/1 (between 94 and 119% of their nominal values).

The validated method is summarised in Document M-CP5 (CP 5.1.2/07).

Table: Measured concentrations of Bandur

Sampling day	Nominal concentration (ug/L)				
	10	32	100	320	1000
0 (new)	12.5, 12.6	32.2, 35.7	118, 119	380, 387	1210, 1220
3 (old)	8.87, 9.30	27.2, 29.7	104, 104	358, 363	1160, 1170
10 (new)	12.3, 12.9	32.7, 28.8	106, 106	330, 334	-
13 (old)	11.8, 13.6	30.0, 33.2	103, 109	368, 365	-
17 (new)	8.48, 9.54	30.1, 30.8	98.4, 101	332, 341	-
20 (old)	7.69, 7.47	25.2, 25.1	90.8, 88.2	318, 320	-
Overall mean	10.6	30.1	104	350	1190
% of nominal	106	94	104	109	119

- = No analysis, all parental *Daphnia* dead
No test material detected in any control samples on any sampling occasion

B. BIOLOGICAL DATA

Adult mortality

After 21 days, 15% of the parental control *Daphnia* had died. In groups exposed to Bandur, mortality ranged from 20% at the lowest exposure level (10.6 ug/1) to 100% at the highest test level (1190 µg/L).

The results are summarized in the following tables.

Table: Cumulative mortality of parental daphnids during the 21-day exposure period

Measured concn (µg/L)	Cumulative number of dead parental daphnids on day:													%[a]
	0-3	4	5-7	8	9-11	12	13-14	15	16	17	18	19	20	21	
Control	0	0	0	0	0	1	2	5	5	6	6	6	6	6	15
10.6	0	0	0	0	0	0	0	4	5	6	6	6	8	8	20
30.1	0	0	0	0	0	0	2	4	5	5	6	6	10	10	25
104	0	0	0	0	0	0	0	5	6	10	10	11	12	12	30
350	0	0	0	1	2	2	3	4	4	6	6	6	9	13	32.5*
1190	20	37	40	40	40	40	40	40	40	40	40	40	40	40	100*

a % dead on day 21

40 *Daphnia* per test treatment and control at the start of the test

* Significant difference from control (Dunnett's test, P >0.05)

Juvenile production

Gravid *Daphnia* were first observed on Day 5 in one test vessel each at 10.6 and 30.1 µg/L. On Day 6, gravid *Daphnia* were observed in each of the control vessels and in all test vessels at 10.6, 30.1 and 104 µg/L. At 350 µg/L, gravid animals were not observed until Day 15. At 1190 µg/L, all the parental *Daphnia* had died by Day 5.

On Day 8, juveniles were present in each of the test vessels at 30.1 and 104 µg/L; at 10.6 µg/L there were juvenile *Daphnia* in two of the vessels. Between Days 9 and 10, the parental *Daphnia* in each of the control vessels and test vessels up to and including 104 µg/L produced juvenile animals. At 350 µg/L, juveniles were first noted in three of the vessels on Day 19; the adults in the fourth vessel did not produce young during the test period.

The data were derived by dividing the number of juveniles present at each renewal by the number of mobile adults present on the day of the previous renewal of test dilutions, and this was then added to the previous number(s) to give the cumulative number produced per parental *Daphnia.*

The 21-day EC50 value of Bandur, calculated by the moving average method using the total number of juveniles produced at each mean measured concentration, was 153 µg/L (95% confidence limits of 132 and 178 µg/L).

Based on the cumulative number of juveniles produced per adult, the 21-day EC50 was 147 µg/L (95% confidence limits, 127 and 171 µg/L; moving average method).

There was no significant difference ($p > 0.05$) in the numbers produced in the control group and the three lowest concentrations but at 350 µg/L significantly fewer juveniles were produced ($p < 0.05$). Overall, the no-observed-effect concentration for reproduction was 104 µg/L.

Table: Cumulative and mean numbers of juvenile Daphnia per adult during the 21-day exposure period

Measured concn (µg/L)	Cumulative number of total juveniles and juveniles per adult on day:													
	8		10		13		15		17		20		21	
	T	M	T	M	T	M	T	M	T	M	T	M	T	M
Control	0	0	51	1.3	809	20.2	1127	28.7	1622	42.5	2120	57.1	2123	57.2
10.6	4	0.1	197	4.9	927	23.2	1297	32.4	1722	44.3	2206	58.8	2224	59.3
30.1	49	1.2	499	12.5	1338	33.5	1690	43.1	1912	49.2	2199	57.5	2201	57.6
104	30	0.8	505	12.6	1359	34.0	1497	37.4	1643	41.7	1758	45.4	1759	45.4
350	0	0	0	0	0	0	0	0	0	0	30	0.9	46	1.4*
1190	0	0	0	0	0	0	0	0	0	0	0	0	0	0

T = total no. of juveniles
M = Mean no. juveniles per adult on day x
* Significant difference from control (Dunnett`s test, P >0.05)

The overall NOEC was 100 µg/L (nominal) or 104 µg/L (measured), including mortality of adults and reproduction and body length.

C. TOXICITY ENDPOINT

The median lethal concentrations (LC_{50}s) of Bandur to parental *Daphnia* were calculated at intervals. Approximate values, obtained by nonlinear interpolation between the two concentrations which bracket the 50% effect level, have been quoted because valid statistical results could not be calculated using either the moving average or probit methods.

Table: Toxicity endpoints of the test item Bandur

	Mortality of Adults		Total number offspring	Alive offspring per adult
	nominal	measured	measured	measured
NOEC	100	104	104	-
LC_{50} /EC_{50}	446 (350 – 1190)	401 (320 – 1000)	153 (132-147)	147 (127 – 171)

D. VALIDITY CRITERIA

Validity criterion	**Required (OECD 211, 2012)**	**Achieved**
Control mortality of parent animals (female *Daphnia*)	<20%	15%
Control reproduction: The mean number of live offspring produced per parent animal alive at the end of the test	≥60	57.2

Study met validity criteria for guideline followed (OECD 202, 1984) but fails to meet reproductive criterion according to current guideline (OECD 211, 2012).

III. CONCLUSION

The numbers of mobile, immobile and dead parental *Daphnia* were recorded each day, and the numbers of juveniles produced were counted three times each week. Mortality of parental *Daphnia* after 21 days in groups exposed to Bandur ranged from 20% at the lowest concentration (10.6 ug/l) to 100% at the highest level (1190 µg/L). Mortality of the parental control *Daphnia* after 21 days was 15%.

The 21-day median lethal concentration (LC_{50}) of Bandur to parental *Daphnia,* calculated using mean measured concentrations, was 446 µg/L. Statistical comparisons (Dunnett's test) of the numbers of mobile parental *Daphnia* present on Day 21 showed that at mean measured concentrations of 350 and 1190 µg/L, the survival of the parental generation was significantly lower ($p < 0.05$) compared to the control group. The no-observed-effect-concentration (NOEC) for parental mortality, based mean measured levels, was therefore 104 ug/1.

Statistical comparisons (Dunnett's test) of the total numbers of juveniles produced in each vessel, and the mean cumulative number produced by each surviving adult by Day 21, showed that at measured Bandur concentrations of 10.6, 30.1 and 104 µg/L, there was no significant difference in production ($p > 0.05$) compared to the control group. At 350 µg/L, juvenile production was significantly lower ($p < 0.05$) than in the control group.

The 21-day EC_{50} values for inhibition of reproduction were 153 µg/L based on the total number of juveniles produced at each concentration, and 147 µg/L based on the mean number of juveniles produced per adult. Overall, the no-observed-effect concentration for reproduction was 104 µg/L.

[redacted] *(1993)*

Assessment and conclusion by applicant:

Study met validity criteria for guideline followed (OECD 202, 1984) but fails to meet reproductive criterion according to current guideline (OECD 211, 2012).

The LC_{50} value for mortality was 401 µg/L, based on measured concentrations. The EC_{50} values for reproduction were 153 µg/L based on total number of juveniles produced and 147 µg/L based on mean number of juveniles produced per adult (measured). The overall NOEC for reproduction was 104 µg/L (measured).

In terms of the active ingredient content, based on a reported active ingredient content of 49.4%, LC_{50} for mortality was estimated to be 198 µg a.s./L. The EC_{50} values for reproduction were 74 µg a.s./L based on total number of juveniles produced and 71 µg a.s./L based on mean number of juveniles produced per adult (measured). The overall NOEC for reproduction was 50 µg a.s./L (measured).

EC_{10} and EC_{20} values were not calculated in the study, however as the study is presented for information only it is considered that these are not required.

Assessment and conclusion by RMS:

Executive Summary

In a 21-day chronic test first instars of *Daphnia magna* (<24 hours old) were exposed to Aclonifen SC 600 with nominal concentrations of control, 15.0, 30.0, 60.0, 120 and 240 µg a.s./L for 21 days under static-renewal conditions. Stock and test solutions were prepared on days 0, 2, 5, 7, 9, 12, 14, 16 and 19.

Each test treatment comprised 10 replicates with 1 adult Daphnia per replicate. Observations for sublethal effects and survival were made daily. Observations of reproductive output (neonates counts) occurred three times per week including day 21. Growth determinations were made at the end of the exposure. Sublethal effects; adult survival (immobilization), time (days) to first brood release, reproduction (neonates per adult at start of the study, neonates per adult reproduction day) and growth (length and dry weight at study termination) were recorded.

Geometric mean measured recoveries were within the range of 86 to 91% of nominal concentrations. Results are based on nominal concentrations in µg aclonifen SC 600/L and on geometric mean measured test concentrations in µg aclonifen/L.

The NOEC and LOEC were calculated based on nominal concentrations in µg aclonifen SC 600/L and on geometric mean measured test concentrations in µg aclonifen/L. The 21-day exposure to aclonifen SC 600 resulted in a NOEC of 60.0 µg aclonifen SC 600/L (26.1 µg a.s.) and a LOEC of 120 µg aclonifen SC 600/L (50.8 µg a.s.) based on dry weight and offspring per parent. The lowest EC10 and associated 95% confidence limits was calculated to be 72.5 (49.1 to 85.7) µg aclonifen SC 600/L and 31.2 (21.7 to 36.3) µg a.s./L, corresponding to dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test material:**	Aclonifen SC 600
	Batch no.:	EV56006446
	Active ingredient / Purity:	595.1 g/L (49.4% a.s.)
	Appearance	Yellow dispersion
	Certificate of analysis date:	7 October 2016
	Storage:	Room temperature
	Expiry date:	7 October 2018
2.	**Test organism**	*Daphnia magna* (Culture lot no. ECT042215)
	Age:	<24 hours old (First-instar)
	Source:	[redacted]
	Feeding:	Cultures were fed a combination of green algae (*Pseudokirchneriella subcapitata*) and blended Tetrafin® flaked fish food. Algae fed daily and fish food fed 3 days per week. During the study, green algae (*Pseudokirchneriella subcapitata*) at a density of ≥2.0 x 108 algal cells/L at increasing rates throughout the study
3.	**Treatment:**	Control, 15.0, 30.0, 60.0, 120 and 240 µg a.s./L (nominal) 10 replicates per treatment, 1 *Daphnia*/replicate
	Test vessels:	Borosilicate glass beakers, approx. 200 mL test solution
	Loading:	ca. 200mL of media per *Daphnia*
	Test water:	Hard water (blended spring and reverse osmosis)
4.	**Environmental conditions**	
	Temperature:	19.1 – 20.7ºC
	pH:	7.8 – 9.0
	Dissolved oxygen:	8.6 – 10.8 mg/L (95-118%)
	Total hardness:	164 – 180 mg/L as $CaCO_3$
	Photoperiod:	16 h light : 8 h darkness (Mean 1010 lux, range 947 – 1068 lux)

B. STUDY DESIGN AND METHODS

1. In-life phase: 19 April to 10 May 2017

2. Test organism assignment and treatment

Freshly hatched daphnids less than 24 hours old were used for the test. At test start, *Daphnia* were inserted into test vessels in a randomized order. Based on the results of a non GLP range-finding test, the following nominal concentrations were tested in the main test: 15.0, 30.0, 60.0, 120 and 240 µg a.s./L and a control. The test was performed with 10 vessels for each test item concentration and the control, containing 1 *Daphnia* each. Each treatment group and the control group consisted of 10

daphnids in total. The reproduction test was performed semi-statically with renewal of the test solutions three times per week.

The duration of the test was 21 days. Assessments on mortality and other effects were performed each day. Offspring was counted and removed daily after appearance of first brood. Measurement of body length was performed for all adult daphnia alive at the end of the test (21 days).

3. Dose preparation

Stock solutions were prepared on days 0, 2, 5, 7, 9, 12, 14, 16, and 19 in an aspirator bottle and were stirred until homogenous. Dilution water used was hard process water. Test solutions were prepared by addition of appropriate volume of stock solution to three litres of dilution water. Test solutions were inverted several times to mix. Stock solutions were prepared on days 0, 2, 5, 7, 9, 12, 14, 16, and 19 in glass volumetric flasks.

4. Measurements and observations

Within the 21-day reproduction test, effects on mortality and reproductive outputs were evaluated. The mortality, the time of the first production of offspring and the number of offspring were compared with the corresponding parameters in the control. Body length of surviving adult *Daphnia* was also measured at test end.

The numbers of mobile, immobile and dead parental *Daphnia* in each vessel was recorded daily and the presence of juvenile *Daphnia* was recorded on days between renewal.

Water samples for analysis were taken from batch solutions (new test solutions) at each concentration on days 0, 9, and 19 and from composites of replicates (old test solutions) at each concentration on days 2, 12, and 21.

Dissolved oxygen and pH measurements were taken from batch solutions (new test solutions) at each concentration on days 0, 5, 12, and 19 and from composites of replicates (old test solutions) at each concentration on days 5, 12, 19, and 21. Temperature was measured continuously throughout the exposure period. Hardness measurements were taken at the start of the study from new test solutions and at the end of the study from composites of replicates (old test solutions) at each concentration. Hardness measurements were also taken from the hard water batch on days 0, 2, 5, 7, 9, 12, 14, 16, and 19.

5. Statistics

Data analysis was conducted based on nominal concentrations in µg of aclonifen SC 600/L and on geometric mean measured test concentrations in µg aclonifen/L. The geometric mean concentrations were calculated following OECD guideline 23. The replicate test vessels were considered to be the smallest experimental unit based on the design of the test system and were used for statistical analysis of each endpoint. Appropriate tests were used to determine if the data had equal variances and normal distribution. Endpoints showing monotonic trends were analysed with William's Test. Endpoints not showing monotonic trends were analysed with Dunnett's Multiple Comparison Test. For the data that did not pass the test for normality or homogeneity of variance, the Jonkheere-Terpstra Step-Down Test was used. The results were used to determine the No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC). ECx values were calculated where applicable using

linear interpolation/nonlinear regression. All data analysis was conducted using CETIS statistical software.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Mean measured concentrations determined in freshly prepared test media ranged between 86 and 91% of nominal values. Overall, geometric mean measured values were 6.71, 13.3, 26.1, 50.8 and 103 µg a.s./L (between 94 and 119% of their nominal values).

Table: Measured concentrations of aclonifen

Nominal conc (ug aclonifen SC 600/L)	Nominal conc (ug a.s./L)	Measured concentrations (ug a.s./L)						Geometric mean measured conc (ug a.s./L)	% of nominal
		Day 0 (new)	Day 2 (ages	Day 9 (new)	Day 12 (aged)	Day 19 (new)	Day 21 (aged)		
Control	-	<0.7	<0.7	<0.7	0.7	<0.7	<0.7	-	-
15	7.41	7.73	7.04	7.03	5.34	8.93	5.46	6.71	91
30	14.8	14.6	14.1	14.2	10.7	17.0	11.2	13.3	90
60	29.6	27.3	26.8	31.5	20.8	28.4	23.5	26.1	88
120	59.3	58.3	54.4	50.7	46.2	59.0	41.0	50.8	86
240	119	117	110	116	87.9	117	91.3	103	87

Limit of Quantification (LoQ) = 0.7 ug a.s./L)
New = newly prepared solutions
Aged = solutions replaced during each renewal

The validated method is summarised in Document M-CP5-(CP 5.12/16).

B. BIOLOGICAL RESULTS

Adult mortality

Percent adult survival of *Daphnia* was analysed statistically to determine if there were any treatment related adverse effects. Survival of adult daphnids ranged from 60 to 100%. Statistical analysis indicated significant effects compared to the controls in the highest treatment level. The results are summarized in the following table.

Table: Adult survival at day 21

Nominal conc (ug aclonifen SC 600/L)	Nominal conc (ug a.s./L)	Geometric mean measured concn (ug a.s./L)	% survival
Control	-	-	80
15	7.41	6.71	100
30	14.8	13.3	100
60	29.6	26.1	60
120	59.3	50.8	80
240	119	103	60*

* Significant difference from control (non-parametric, Jonkheere-Terpstra step-down test)

10 adult Daphnia per treatment at study start

Time to first brood

The time to first brood was analysed statistically to determine if there were any related adverse effects. The mean time to first brood ranged from 8.0 to 8.9 days across all treatment levels. Statistical analysis indicated significant effects compared to the controls in the highest treatment level. The results are summarized in the following table.

Table: Time to first brood

Nominal conc (ug aclonifen SC 600/L)	Nominal conc (ug a.s./L)	Geometric mean measured concn (ug a.s./L)	Mean time to 1st brood (days)
Control	-	-	8.1
15	7.41	6.71	8.7
30	14.8	13.3	8.0
60	29.6	26.1	8.5
120	59.3	50.8	8.6
240	119	103	8.9*

* Significant difference from control (parametric, Dunnett's multiple comparison test)

Total live offspring per adult and Neonates per adult reproduction day

Neonate production throughout the exposure per adult present at the start of the test was analysed. Neonates per adult was determined by dividing the total number of living neonates produced by the number of adults present in the replicate vessel at the start of the test. Neonates produced per adult ranged from 35 to 135. Statistical analysis showed significant differences from the controls in the highest treatment group.

On day 21, the number of neonates produced per adult reproduction day was analysed. Neonates per adult reproduction day is determined by dividing the total number of neonates by the number of days adults are capable of reproducing (begins with release of first brood). Neonates produced per adult reproduction days (average daily offspring) ranged from 6.1 to 10.2. Statistical analysis indicated significant effects compared to controls in the highest treatment level.

Table: Total living neonates/adult and neonates/adult reproduction day

Nominal conc (ug aclonifen SC 600/L)	Nominal conc (ug a.s./L)	Geometric mean measured concn (ug a.s./L)	Mean living neonates/adult at study start	Neonates / adult reproduction day
Control	-	-	106	8.3
15	7.41	6.71	135	10.2
30	14.8	13.3	132	9.5
60	29.6	26.1	128	9.5
120	59.3	50.8	83	7.3
240	119	103	35*	6.1**

* Significant difference from control (parametric, William's test)

** Significant difference from control (non-parametric, Jonkheere-Terpstra step-down test)

10 adult Daphnia per treatment at study start

Growth

On day 21, the lengths were measured using a dissecting light microscope and slide micrometer. Mean replicate lengths ranged from 3.76 to 4.53 mm. Statistical analysis indicated significant effects from controls in the highest treatment level.

After the termination of the study dry weights were measured and ranged from 0.400 to 0.929 mg. Statistical analysis indicated significant effects from controls in the highest treatment level.

Table: Adult growth (length and dry weight) at day 21

Nominal conc (ug aclonifen SC 600/L)	Nominal conc (ug a.s./L)	Geometric mean measured concn (ug a.s./L)	Length (mm)	Adult dry weight (mg)
Control	-	-	4.30	0.692
15	7.41	6.71	4.53	0.929
30	14.8	13.3	4.50	0.850
60	29.6	26.1	4.51	0.786
120	59.3	50.8	4.14	0.572
240	119	103	3.76*	0.400**

* Significant difference from control (parametric, Dunnett's multiple comparison test)

** Significant difference from control (parametric, William's test)

10 adult *Daphnia* per treatment at study start

C. TOXICITY ENDPOINT

The median lethal concentrations (LC_{50}s) of Bandur to parental *Daphnia* were calculated at intervals. Approximate values, obtained by nonlinear interpolation between the two concentrations which bracket the 50% effect level, have been quoted because valid statistical results could not be calculated using either the moving average or probit methods.

Table: Toxicity endpoints of the test item Aclonifen SC 600

Endpoint	Measured concentration as µg aclonifen SC 600/L (Measured concentration as µg a.s./L)

	Time to 1st brood	Neonates / adult repro day (average daily offspring)	Total living offspring / adult	Adult survival	Adult body length	Adult dry weight
NOEC	120 (50.8)	120 (50.8)	60 (26.1)	120 (50.8)	120 (50.8)	60 (26.1)
LOEC	240 (103)	240 (103)	120 (50.8)	240 (103)	240 (103)	120 (50.8)
EC_{10} (95% CI)	Measured concentration as µg aclonifen SC 600/L					
	>240 (n/a)	88.1 (57.0 – 122)	77.8 (66.5 – 95.0)	110 (n/a – 208)	159 (110 – 194)	72.5 (49.1 – 85.7)
	Measured concentration as µg a.s./L					
	>103 (n/a)	37.7 (23.4 – 51.3)	33.4 (28.5 – 39.4)	47.4 (n/a – 90.0)	67.8 (47.8 – 83.7)	31.2 (21.7 – 36.3)

n/a = not applicable

D. VALIDITY CRITERIA

Validity criterion	Required (OECD 211, 2012)	Achieved
Control mortality of parent animals (female *Daphnia*)	<20%	20%
Control time to 1st brood (ASTM criterion)	7 – 10 days	8.1 days
Control reproduction: The mean number of live offspring produced per parent animal alive at the end of the test	≥60	106

Study met validity criteria for guideline OECD 211 (2012) and can be considered a valid study.

III. CONCLUSION

The NOEC and LOEC were calculated based on nominal concentrations in µg aclonifen SC 600/L and on geometric mean measured test concentrations in µg aclonifen/L.

The 21-day exposure to aclonifen SC 600 resulted in a NOEC of 60.0 µg aclonifen SC 600/L (26.1 µg a.s.) and a LOEC of 120 µg aclonifen SC 600/L (50.8 µg a.s.) based on dry weight and offspring per parent.

The lowest EC_{10} and associated 95% confidence limits was calculated to be 72.5 (49.1 to 85.7) µg aclonifen SC 600/L and 31.2 (21.7 to 36.3) µg a.s./L, corresponding to dry weight.

[redacted] *(2017)*

Assessment and conclusion by applicant:

Study met validity criteria for guideline OECD 211 (2012) and can be considered a valid study.

The 21-day exposure to aclonifen SC 600 resulted in a NOEC of 60.0 µg aclonifen SC 600/L (26.1 µg a.s.) and a LOEC of 120 µg aclonifen SC 600/L (50.8 µg a.s.) based on dry weight and offspring per parent.

The lowest EC_{10} and associated 95% confidence limits was calculated to be 72.5 (49.1 to 85.7) µg aclonifen SC 600/L and 31.2 (21.7 to 36.3) µg a.s./L, corresponding to dry weight.

EFSA's Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EFSA, 2019)[14] recommends that the lowest of the EC_{10} and NOEC values be used for risk assessment purposes. In this study, as the NOEC was lower than the EC_{10}, the NOEC is the most appropriate endpoint for risk assessment.

Assessment and conclusion by RMS:

CP 10.2.3 Further testing on aquatic organisms

No studies were necessary based on the current data requirements. Please refer to Document M-CA8, Section 8.2.

CP 10.3 Effects on arthropods

CP 10.3.1 Effects on bees

The available bee toxicity data for the active substance and Aclonifen SC 600 G are summarised in the following table.

Table 10.3-1: Summary of toxicity data to bees

Test Species	Test Item	Time-scale / Test type / substrate	Endpoint	Reference
Honey bee *Apis mellifera* L.	Aclonifen	48 h Acute oral	**LD_{50} > 106.8 µg a.s./bee**	KCA 8.3.1.1.1/01 KCA 8.3.1.1.2/01 M-174936-01-1 [redacted], 1999
		48 h Acute contact	**LD_{50} > 100 µg a.s./bee**	
Honey bee *Apis mellifera L.*	Aclonifen SC 600 G	48 h Acute oral	LD_{50} > 115.36 µg a.s./bee	KCP 10.3.1.1.1/01 KCP 10.3.1.1.2/01 M-227865-01-1 [redacted], 2003
		48 h Acute contact	LD_{50} > 100 µg a.s./bee	
Honey bee *Apis mellifera L.*	Aclonifen SC 600 G	24 h Acute oral	LD_{50} > 141 µg product/bee	KCP 10.3.1.1.1/02 KCP 10.3.1.1.2/02 KCP 10.3.1.5/01 M-174869-01-2 [redacted], 1991
Bumble bee *Bombus terrestris* L.	Aclonifen	48 h Acute oral	LD_{50} > 130.36 µg a.s./bee	KCA 8.3.1.1.1/02 KCA 8.3.1.1.2/02 M-567133-01-1 [redacted], 2016
		48 h Acute contact	LD_{50} > 150 µg a.s./bee	
Honey bee *Apis mellifera L.*	Aclonifen SC 600 G	10 d Chronic oral	**NOEDD = 36.55 µg a.s./bee /day**	KCP 10.3.1.2/01 M-601664-01-1 [redacted], 2017

[14] EFSA (European Food Safety Authority), 2019. Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117 pp. doi:10.2903/sp.efsa.2019.EN-1673

Test Species	Test Item	Time-scale Test type / substrate	Endpoint	Reference
				& KCP 10.3.1.2/01 M-567602-01-1 [redacted], 2017
Honey bee *Apis mellifera* L	Aclonifen	8 d repeated exposure Larval toxicity	NOED = 25.0 µg a.s./larval stage	KCA 8.3.1.3/01 M-600773-01-1 [redacted], 2017
Honey bee *Apis mellifera* L	Aclonifen	22 d repeated exposure Larval toxicity	**NOED = 40.0 µg a.s./larva**	KCA 8.3.1.3/02 M-578600-01-1 [redacted], 2017
Honey bee *Apis mellifera L.*	Aclonifen SC 600 G	27 d semi-field conditions Honey bee brood	No effect on honey bees or honey bee brood at 2.4 kg a.s./ha	KCP 10.3.1.5/02 M-621305-01-1 [redacted], 2016

Endpoints in **bold** were used in the risk assessment

Summary of the risk assessment for Aclonifen SC 600 G and bees

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

The risk assessment showed no unacceptable acute or chronic risks arising from the use of Aclonifen SC 600 G according to the proposed GAP.

Risk assessment for bees

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

Bayer recognizes the need to review the bee pollinator risk assessment based on scientific progress. However, the EFSA Bee Guidance Document issued in 2013 has not been noted and therefore is not a realistically feasible way forward for assessing the chronic risk to honeybees. Therefore, the risk assessment below has been conducted following the EPPO 2010 scheme which provides a comparable level of protection to the EFSA approach and is based on the current scientific state of the art for bee pollinator risk assessment.

Application scenario

According to the GAP, Table 10-1, Aclonifen SC 600 G is proposed to be applied to peas at 0.30 and 0.60 kg a.s/ha (1 application), during BBCH 11-30 and BBCH 12-19, respectively. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of Aclonifen SC 600 G in peas using an application rate of 0.60 kg a.s/ha also covers the risk for bees from the intended use at the lower application rate..

Hazard quotients for bees

Acute contact and oral hazard quotients (Q_H) for the maximum proposed application rate of aclonifen are presented in the following table:

Table 10.3-2: Acute risk assessment for bees arising from the use of Aclonifen SC 600 G in peas

Intended use	Peas, 1.0 L prod./ha, BBCH 11 - 30		
Active substance	Aclonifen		
Application rate (g a.s./ha)	1 x 600		
Test design	**LD_{50} (µg/bee)**	**Single application rate (g a.s./ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>106.8	600	<5.6
Contact toxicity	>100		<6.0

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact toxicity. Q_H values in **bold** breach the relevant trigger

Hazard quotients for both oral and contact toxicity were below the trigger value of 50 thereby indicating no unacceptable risks to honey bees from the use of Aclonifen SC 600 G according to the GAP.

Chronic risk assessment

The chronic oral and development risks to honeybee adults and larvae have been evaluated in accordance with the EPPO guidance (EPPO 2010). These long-term assessments are considered to address potential exposure via nectar and pollen from the treated crop and flowering weeds, and encompass potential exposure from systemic activity.

Chronic risk to honeybee adults

In accordance with the revised EPPO scheme (OEPP/EPPO 2010) the chronic risk to adult bees and larvae can be evaluated by comparing the NOED to an estimate of daily residue consumption to give a toxicity exposure ratio (TER). The EPPO assessment trigger value is 1, whereby a TER >1 indicates a low risk based on the use evaluated. Daily residue consumption data are available from the draft EFSA guidance (2013)[15]. The worst-case screening SV for adult bees and larvae, taken from the EFSA guidance (2013), is applied in the assessment. The equation applied in the risk assessment is as follows and the TER calculations are presented in Table 10.3-3:

$$TER = \frac{NOED}{\text{Application rate (kg a.s./ha) x consumption (SV)}}$$

Table 10.3-3: Chronic risk assessment for bees arising from the use of Aclonifen SC 600 G in peas

Intended use	Peas, 1.0 L prod./ha, BBCH 11 - 30				
Active substance	Aclonifen				
Application rate (kg a.s./ha)	1 x 0.6				
Assessment	**Toxicity[1]**	**Single application rate (kg a.s./ha)**	**Daily consumption (SV)[2]**	**Daily exposure[3]**	**TER criterion: >1**
Adult - chronic	36.55	0.6	7.6	4.56	8.0
Larvae - chronic	40		4.4	2.64	15.2

TER values in **bold** breach the relevant trigger
[1]: NOEDD (µg a.s./bee/day) for adults; NOED (µg a.s./larva) for larvae
[2]: Worst-case short-cut value (90th percentile) for daily exposure (downwards spraying) from the EFSA Guidance (EFSA, 2013)

[15] "Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)" EFSA Journal 2013; 11(7):3295

[3]: Daily exposure expressed as µg a.s./bee/day for adults and µg a.s./larva for larvae

TER values were above the EPPO trigger value of 1 on the basis of worst-case daily exposure estimates, therefore it is considered that aclonifen will pose acceptable chronic risk to adult honey bees and honey bee larvae following application of Aclonifen SC 600 G in accordance with the proposed use.

Risk to non-*Apis* bees

There are no testing requirements for any bee other than the honey bee within the current implemented Regulation (EC) No. 1107/2009.

An acute oral and contact toxicity test has however been performed by the applicant on bumble bees (M-567133-01-1). The acute endpoints for bumble bees $LD_{50\ oral}$ >130.36 µg a.s./bee, $LD_{50\ contact}$ >150 µg a.s./bee) are in line with those obtained for honey bees and therefore the two species would not appear to show any difference in sensitivity to aclonifen.

Higher-tier risk assessment for bees (tunnel test)

A honey bee semi-field study (OECD 75) assessing the potential effect of Aclonifen SC 600 G applied at a rate of 2.4 kg a.s./ha on honey bee colonies indicated no adverse effects on foraging activity, brood, or colony development and strength (M-621305-01-1).

These results confirmed that aclonifen will pose no unacceptable risks to honey bees following application of Aclonifen SC 600 G in accordance with the proposed GAP.

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

Executive Summary

An acute test was conducted to determine the acute oral and contact effect of aclonifen (AE F068300 00 SC50 A203) on mortality and behaviour of the honey bee, *Apis mellifera*. The test was a limit test conducted at the highest recommended dose (100 µg a.s./bee, corresponding to an actual intake of 115.36 µg a.s./bee) over 48 hours and included a control plus four concentrations of the toxic standard, (dimethoate, 0.2 µg a.s./bee). Bees were assessed for any behavioural effects.

The contact test was 48 hours duration. There was 20% mortality in the single aclonifen test treatment and the 48-hour LD_{50} was >100 µg a.s./bee. Some traumatised bees were observed after 4 hours exposure, it was assumed these bees subsequently died. There was 0% mortality in the control.

The oral test was 48 hours duration. There was 30% mortality in the single aclonifen test treatment and the 48-hour LD_{50} was >115.36 µg a.s./bee. Some traumatised bees were observed after 4 hours exposure, it was assumed these bees subsequently died. There was 0% mortality in the control.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Item:** BANDUR / AE F068300 00 SC50 A203
   Batch no.: I.OT V00403017
   Active Ingredient / Purity: 48.81% w/w (600 g/L)
   Appearance: Yellow liquid

	Storage:	Room temperature in the dark
	Expiry date:	23 April 2003
2.	**Reference item:**	Perfekthion / BAS 152 11I
	Batch no.:	2002-1
	Active Ingredient / Purity:	400 g/L dimethoate (401.2 g/L analysed)
3.	**Test Organism:**	Young adult worker honey bee *Apis mellifera* L.
	Age:	Not specified
	Source:	[redacted]
	Feeding:	Not specified Commercial ready-to-use syrup for honey bees (50% saccharose, 31% glucose, 39% fructose). Supplied by Apiinvert, Co. Südzucker AG, D-97199 Ochsenfurt

A. STUDY DESIGN AND METHODS

1.	**In-life phase:**	19 to 21 November 2002
2.	**Exposure conditions**	
	Test vessels:	Stainless steel cages 10 cm x 8.5 cm x 5.5 cm (length x width x height) with transparent pane for observation and perforated board on bottom of cage to allow sufficient air supply
	Experimental design:	*Contact:* Control (tap water), test item 100 µg a.s./bee; Dimethoate (toxic standard) 0.10, 0.15, 0.23, 0.34 µg a.s./bee *Oral:* Control (50% w/v aqueous sucrose solution); test item 100 µg a.s./bee; Dimethoate (toxic standard) 0.06, 0.09, 0.14, 0.23 µg a.s./bee
	Replicates:	5 replicates per test item dose level, controls and toxic standard, consisting of 10 bees
	Temperature:	24.2 – 26.1°C
	Relative humidity:	44.5 – 58.0%
	Photoperiod:	Darkness (except during observation)

3. Administration of the test item

Contact toxicity test

Test substance was dissolved in tap water. Bees were anaesthetised with CO_2 until completely immobilised immediately before application of test treatments. A single 2 µL droplet was placed on the thorax of each bee using a micro applicator. After application bees were returned to test cages and fed *ad libitum* with untreated 50% aqueous sucrose solution.

Oral toxicity test

Test substance was dissolved in tap water and final dose was prepared by mixing aliquot of stock solution in tap water with 50% aqueous sucrose solution such that the intended dose per bee was found in 20 µL test solution. Concentration in feeding solution was 25% higher than necessary to achieve intended dose to compensate for potential decrease in food uptake by bees. Before feeding started bees were starved for 2 hours. Test solution (250 µL) was offered for 6 hours in each cage of 10 bees to ensure sufficient uptake. Amount consumed (mean for 10 bees) was determined by weighing feeders before and after feeding. After feeding of test solutions, bees were fed *ad libitum* with untreated 50% aqueous sucrose solution.

4. Measurements and observations

Observation of the bees was undertaken at the following times:

- 4 hours (first day)
- 24 hours, 48 hours following days

Any cases of mortality and/or poisoning or behavioural abnormalities of the bees (e.g. food refusal, apathy, moving coordination problems) were recorded.

5. Statistics/Data evaluation

Average mortality of all replicates was calculated after correction for control mortality according to [redacted] (1947). LD50 and 95% confidence intervals were determined by probit analysis, using statistical program SAS, release V8.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

No analytical verification of the dosing solutions was performed.

B. BIOLOGICAL DATA

Contact toxicity test

Some traumatised bees were observed after 4 hours exposure, it was assumed these bees subsequently died.

Table: Mean mortality and behavioural abnormalities of the bees in the contact toxicity test

Dose (µg a.s./bee)	Mortality (%) 24h	Mortality (%) 48h
Control	0	0
Test substance		
100	26	30
Toxic standard		
0.10	10	12
0.15	42	48
0.23	70	74
0.34	88	94

Oral toxicity test

Some traumatised bees were observed after 4 hours exposure, it was assumed these bees subsequently died.

Table: Mean mortality and behavioural abnormalities of the bees in the oral toxicity test

Dose (µg a.s./bee)	Intake (µg a.s./bee)	Mortality (%)	
		24h	48h
Control	-	0	0
Test substance			
100	115.36	18	20
Toxic standard			
0.06	0.06	4	10
0.09	0.09	26	30
0.14	0.12	58	76
0.23	0.20	90	94

C. VALIDITY CRITERIA

Validity criteria	Required (OECD 213/214, 1998)	Achieved
Mortality in controls	≤10%	0% (contact test) 0% (oral test)
Oral LD_{50} of the toxic standard (dimethoate)	0.10 – 0.35 µg a.i./bee	0.10 µg a.i./bee after 48h
Contact LD_{50} of the toxic standard (dimethoate)	0.10 – 0.30 µg a.i./bee	0.16 µg a.i./bee after 48h

The OECD 213/214 validity criteria regarding control mortality were met. Therefore, it is considered that this study is valid for risk assessment purposes.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoints (µg a.s./bee)		24h		48h	
		LD_{50}	95% confidence interval	LD_{50}	95% confidence interval
Contact	Test substance (AE F068300 00 SC50 A203)	>100	-	>100	-
	Toxic standard	0.18	0.16 – 0.20	0.16	0.15 – 0.18
Oral	Test substance (AE F068300 00 SC50 A203)	>115.36	-	>115.36	-
	Toxic standard	0.12	0.11 – 0.13	0.10	0.09 – 0.11

III. CONCLUSION

The toxicity of aclonifen (AE F068300 00 SC50 A203) was tested in both an acute contact and an oral toxicity test on honey bees. The LD_{50} (48 h) was >100 µg a.s./bee in the contact toxicity test. The LD_{50} (48 h) was >115.36 µg a.s./bee in the oral toxicity test.

[redacted] *(2003)*

Assessment and conclusion by applicant:

The OECD 213/214 validity criteria regarding control mortality were met. Therefore, it is considered that this study is valid for risk assessment purposes.

The toxicity of aclonifen (AE F068300 00 SC50 A203) was tested in both an acute contact and an oral toxicity test on honey bees. The LD_{50} (48 h) was >100 µg a.s./bee in the contact toxicity test. The LD_{50} (48 h) was >115.36 µg a.s./bee in the oral toxicity test.

Assessment and conclusion by RMS:

In the previous submission (DAR, 2006), this study was evaluated and not accepted as valid for risk assessment purposes. Therefore a summary of this study is not presented in this dossier.

CP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to Section 10.3.1.1.1/01 for a full summary of this study.

In the previous submission (DAR, 2006), this study was evaluated and not accepted as valid for risk assessment purposes. Therefore a summary of this study is not presented in this dossier.

CP 10.3.1.2 Chronic toxicity to bees

Data Point:	KCP 10.3.1.2/01
Report Author:	[redacted]
Report Year:	2017
Report Title:	Aclonifen SC 600 - Assessment of effects on the adult honey bee, Apis mellifera L., in a 10 day chronic feeding test under laboratory conditions
Report No:	S15-00363
Document No:	M-601664-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 (2009) Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.SUPP
Deviations from current test guideline:	Current guideline: OECD 245, 2017 No Deviation
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCP 10.3.1.2/02
Report Author:	[redacted]; [redacted]
Report Year:	2016
Report Title:	Final report - Aclonifen SC 600 - Assessment of effects on the adult honey bee, apis mellifera L., in a 10 day chronic feeding test under laboratory conditions
Report No:	P62164704
Document No:	M-567602-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 of the European Parliament and the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC European Commission Guidance Document for Generating and Reporting Methods of Analysis in Support of Pre-Registration data Requirements for Annex II (part A, Section 4) and Annex III (part A, section 5) of directive 91/414, SANCO/3029/99 rev. 4, 11/07/00 Guidance document on residue analytical methods, SANCO/825/00/rev. 8.1, European Commission, Directorate General Health and Consumer Protection 16/11/2010 US EPA Residue Chemistry Test Guideline OCSPP 860.1340: Residue Analytical Method
Deviations from current test guideline:	Current guideline: OECD 245, 2017 No Deviation
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The study was conducted to determine possible effects of aclonifen SC 600 on the honey bee, *Apis mellifera* L. after 10-day chronic feeding test under laboratory conditions. The test included a control, a

solvent control, the test item (target 1050, 1366, 1775, 2308 and 3000 mg a.s./kg) and reference item groups (dimethoate, 0.90 mg a.s./kg). Additionally, bees were assessed for any behavioural effects.

Analysis of the feeding solutions determined mean measured concentrations ranged from 93 to 98% of nominal.

The mean consumption of feeding solution per day (corrected for evaporation), the mean uptake of test item per day over the 10-day test period and the accumulated mean uptake of test item are presented in the following table. After 10 days of continuous exposure the accumulated mean uptake of aclonifen SC 600 at the treatment levels of 1050, 1366, 1775, 2308 and 3000 mg a.s./kg feeding solution was 268.54, 365.52, 421.24, 562.54 and 712.08 µg a.s./bee, respectively. The corresponding average daily dose was therefore 26.85, 36.55, 42.12, 56.25 and 71.21 µg a.s./bee/day.

The overall mean daily consumption of feeding solution (i.e. the average consumption/bee over 10 days) in the test item treatment groups was reduced compared to the overall mean daily consumption of feeding solution in the solvent control group (25.6, 26.8, 24.0, 24.4 and 23.7 mg/bee/day at 1050, 1366, 1775, 2308 and 3000 mg a.s./kg feeding solution, respectively, compared to 33.8 mg/bee/day in the solvent control group).

The NOEC for mortality after 10 days of continuous exposure was determined to be 1366 mg a.s./kg feeding solution. The corresponding NOEDD, based on the actual consumption of the respective feeding solutions, was determined to be 36.55 µg a.s./bee/day.

The LC_{50} after 10 days of continuous exposure was determined to be >3000 mg a.s./kg feeding solution. The corresponding LDD_{50}, based on the actual consumption of the respective feeding solutions, was determined to be >71.21 µg a.s./bee/day.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Item:** Aclonifen SC 600
   Batch no.: EV56005993
   Active Ingredient / Purity: 596.7 g/L (49.5% w/w) analysed
   Appearance: Yellow liquid
   Storage: Room temperature in the dark
   Expiry date: 9 February 2017

2. **Reference item:** Perfekthion / BAS 152 11 I
   Batch no.: FRE-000926
   Active Ingredient / Purity: Dimethoate (400 g/L)

3. **Test Organism:** Young adult worker honey bees (*Apis mellifera* L.)
   Age: 1 - 2 days old (newly hatched)
   Source: [redacted]
   Feeding: *ad libitum* with 50% (w/v) aqueous sucrose solution

B. STUDY DESIGN AND METHODS

1. In-life phase: 9 – 23 June 2015

2. Exposure conditions

Test vessels: Easy to clean and well-ventilated stainless steel cages were used. The size of the test cages (approximately 8 x 4 x 6 cm) provided adequate space for the bees. Each test unit was labelled with the study number and all necessary additional information to ensure unique identification. The units were equipped with a transparent pane to enable observation. The bottom of the cage was perforated steel to enable sufficient air supply. Cages were lined with filter paper

Experimental design: Control, solvent control (0.1% xanthan); test item 1050, 1366, 1775, 2308 and 3000 mg a.s./kg feeding solution;

Dimethoate 0.9 mg a.s./kg feeding solution

Replicates: Four replicates of 10 bees were used. Therefore, a total number of 40 bees for each control, test item concentration treatment and for the reference treatment were set up

Additionally, 4 test units without bees but with full food syringes for daily dose verification

Temperature: Target: 33 ± 2 ºC; Achieved: 31.7 – 34.6ºC

Relative humidity: Target: 60 ± 10%; Achieved: 36.7 – 64.2%

Photoperiod: Darkness (except during application and observation)

3. Administration of the test item

Dose preparation

Stock solutions of the test item in 50% (w/v) sucrose solution containing 0.1% xanthan were prepared with deionised water and stored refrigerated (6 ± 2ºC) for a maximum of 4 days. Reference treatment stock solution was prepared using deionised water at the start of the test and stored refrigerated (6 ± 2ºC). Further dilution of the stock solution to achieve desired concentrations was carried out on the day of use. Definitive solutions were freshly prepared daily from stock solution with 50% (w/v) aqueous sucrose solution for the reference treatments and with 50% (w/v) aqueous sucrose solution plus 0.1% xanthan for the test item treatments.

Oral treatment

Approximately 3-4 mL feeding solution was offered to the test organisms of each test unit in plastic syringes (approx. 5mL). The tip of each feeder was removed to all bees had access to the feeding solution. Syringes were replaced every day throughout the 10-day test period with freshly prepared feeding solutions. Therefore, the bees were continuously exposed to the feeding solution over a ten day period (D0 - D10). The amount of food consumed was determined by weighing the syringes before being introduced into the test units and after they were replaced by new ones in order to enable the calculation of the amount of effectively ingested dose. The dose consumed per bee was calculated by

dividing the consumed amount of aqueous sucrose solution by the number of surviving bees. Food consumption was corrected accounting the loss by evaporation.

4. Measurements and observations

Mortality and behavioural abnormalities were assessed daily from D1 to D10. Any immobile bees not reacting at contact with a fine brush were considered dead. At the feeding time, dead bees were systematically removed from the cages.

Behavioural abnormalities in the test item treatment (e.g. moribund, affected, cramps, apathy or regurgitating) were assessed during the course of the study. Bees in the reference group were not assessed for behavioural abnormalities as it was assumed that moribund bees would die by the end of the study.

Analytical samples and retain samples of the feeding solutions in the control and test item treatments were taken daily after preparation of feeding solutions. The weight of each samples was determined and recorded. No samples of reference feeding solutions were taken. Samples were stored frozen (-18oC) with 45 minutes of sampling until required for analysis.

Analytical determination was conducted by [redacted]

5. Statistics/Data evaluation

The percent cumulative mortality was calculated for each treatment group and was corrected for control mortality according to the formula of [redacted] (1947).

Fisher's Exact Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there are significant differences between the mortality data of the solvent control and the test item treatment group and to determine the NOEC and NOEDD based on mortality. Probit analysis using linear maximum likelihood regression was used to calculate the LC10, LC20, LDD10 and LDD20.

Statistical calculations were made by using the statistical program TOXRAT Professional 3.2.1.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

The mean measured concentrations determined in the feeding solutions ranged from 93 to 98% of nominal.

Table: Analytical verification of feeding solutions

Nominal concentration (mg a.s./kg)	Measured concentration (g/L)	% of nominal concentration
Control	<LoQ	-
1050	1028	98
1366	1337	98
1775	1651	93
2308	2216	96
3000	2849	95

LoQ (limit of quantification) = 0.01 mg/kg
LoD (limit of detection) = 0.005 mg/kg

The validated method is summarised in Document M-CP5 (CP 5.1.2/02).

B. BIOLOGICAL DATA

In the test item group, a cumulative mortality of 5, 12.5, 15, 32.5 and 45% was observed at the concentrations of 1050, 1366, 1755, 2308 and 3000 mg a.i./kg feeding solution, respectively. Mortality was statistically significantly different when compared to the control at 1775, 2308 and 3000 mg a.s./kg. The mortality in the dimethoate reference treatment (nominally 0.90 mg a.s./kg) was 100% by the end of the 10-day exposure period.

In the test item treatment groups, some affected bees were observed from assessment day 4 to 10 at all tested concentrations. A few apathetic and moribund bees were observed in the three highest concentrations of 1775, 2308 and 3000 mg a.s./kg feeding solution.

Table: Food uptake and mortality at the end of the test

Nominal test concentration (mg/kg)	Cumulative mortality (%)									
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
Control	0	0	0	0	2.5	2.5	2.5	2.5	5	5
Solvent control	0	0	0	0	0	0	0	0	0	0
Reference item (Dimethoate)[1]	0	0	0	0	15.4	43.6	74.4	92.3	97.4	100
1050	0	0	0	0	0	0	0	5	5	5
1366	0	0	0	0	2.5	2.5	2.5	10	12.5	12.5
1775	0	0	0	0	2.5	5	5	7.5	15	15*
2308	0	0	0	7.5	10	10	12.5	17.5	22.5	32.5*
3000	0	0	0	0	0	5	10	27.5	40	45*

1 – mortality corrected for corresponding control mortality ([redacted] 1947)

* Statistically significant difference compared to the control; Fisher's Exact Test ([redacted] corrected, one-sided, α = 0.05

Food consumption and uptake of test item

The mean consumption of feeding solution per day (corrected for evaporation), the mean uptake of test item per day over the 10-day test period and the accumulated mean uptake of test item are presented in the following table. After 10 days of continuous exposure the accumulated mean uptake of aclonifen SC 600 at the treatment levels of 1050, 1366, 1775, 2308 and 3000 mg a.i./kg feeding solution was 268.54, 365.52, 421.24, 562.54 and 712.08 µg a.i./bee, respectively. The corresponding average daily dose was therefore 26.85, 36.55, 42.12, 56.25 and 71.21 µg a.i./bee/day.

The overall mean daily consumption of feeding solution (i.e. the average consumption/bee over 10 days) in the test item treatment groups was reduced compared to the overall mean daily consumption of feeding solution in the solvent control group (25.6, 26.8, 24.0, 24.4 and 23.7 mg/bee/day at 1050, 1366, 1775, 2308 and 3000 mg a.i./kg feeding solution, respectively, compared to 33.8 mg/bee/day in the solvent control group).

Table: Mean food consumption and test item uptake over the 10-day test exposure

Nominal test concentration (mg/kg)	Mean consumption of feeding solution (mg/bee)	Mean uptake of active ingredient (µg a.s./bee/day)	Accumulated mean uptake of active ingredient (µg a.s./bee/day)
Control	30.3	-	
Solvent control	33.8	-	-
Reference item	16.9	0.02	0.17
1050	25.6	26.85	268.54
1366	26.8	36.55	365.52
1775	24.0	42.12	421.24
2308	24.4	56.25	562.54
3000	23.7	71.21	712.08

Reference item = dimethoate (0.090 mg/kg)

The NOEC for mortality after 10 days of continuous exposure was determined to be 1366 mg a.s./kg feeding solution. The corresponding NOEDI, based on the actual consumption of the respective feeding solutions, was determined to be 36.55 µg a.s./bee/day.

The LC_{50} after 10 days of continuous exposure was determined to be >3000 mg a.s./kg feeding solution. The corresponding LDD_{50}, based on the actual consumption of the respective feeding solutions, was determined to be >71.21 µg a.s./bee/day.

C. VALIDITY CRITERIA

Validity criterion	Required (OECD 245, 2017)	Achieved
Average mortality in control treatment	≤15%	10%
Average mortality in reference item treatment	≥50%	100%

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint		LC_x [95% Confidence Limits]
Day 10	NOEC[1]	1366 mg a.s/kg feeding solution
	NOEDD[1,2]	36.55 µg a.i./bee/day
	LC_{10}	1342.01 mg a.i./kg feeding solution [953.94 – 1594.29]
	LC_{20}	1824.40 mg a.i./kg feeding solution [1516.96-2109.65]
	LC_{50}	> 3000 mg a.i./kg feeding solution (n.d.)
	LDD_{10}	34.29 µg a.i./bee/day [25.30 – 40.04]
	LDD_{20}	45.21 µg a.i./bee/day [38.30 – 51.52]
	LDD_{50}	> 71.21 µg a.i./bee/day (n.d.)

1 = based on mortality (not significantly different compared to control)

2 = based on actual doses

(n.d) = not determined due to mathematical or inappropriate data

III. CONCLUSION

Continuous *ad libitum* feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item aclonifen SC 600 at the treatment levels of 1050, 1366, 1775, 2308 and 3000 mg a.s./kg feeding solution resulted in dose-dependent effects regarding mortality.

The NOEC for mortality after 10 days of continuous exposure was determined to be 1366 mg a.s./kg feeding solution. The corresponding NOEDD, based on the actual consumption of the respective feeding solutions, was determined to be 36.55 µg a.s./bee/day.

The LC_{50} after 10 days of continuous exposure was determined to be >3000 mg a.s./kg feeding solution. The corresponding LDD_{50}, based on the actual consumption of the respective feeding solutions, was determined to be >71.21 µg a.s./bee/day.

[redacted] *(2017)*

<u>Assessment and conclusion by applicant:</u>

All validity criteria were satisfied and therefore this study can be considered to be valid.

The NOEC for mortality after 10 days of continuous exposure was determined to be 1366 mg a.s./kg feeding solution. The corresponding NOEDD, based on the actual consumption of the respective feeding solutions, was determined to be 36.55 µg a.s./bee/day.

The LC_{50} after 10 days of continuous exposure was determined to be >3000 mg a.s./kg feeding solution. The corresponding LDD_{50}, based on the actual consumption of the respective feeding solutions, was determined to be >71.21 µg a.s./bee/day.

<u>Assessment and conclusion by RMS:</u>

CP 10.3.1.3 Effects on honey bee development and other honey bee life stages

No data available on the formulated product.

CP 10.3.1.4 Sub-lethal effects

No data available on the formulated product.

CP 10.3.1.5 Cage and tunnel tests

Data Point:	KCP 10.3.1.1.1/02
Report Author:	[redacted]
Report Year:	1991
Report Title:	Laboratory study to determine the side effects of SAG 12701D in the honey bee, Apis mellifera
Report No:	C025164
Document No:	M-174869-01-2
Guideline(s) followed in study:	BBA: IV, 23-1, part IV, 23-1 (1991)
Deviations from current test guideline:	Current Guideline: OECD 213 and 214 (1998) The contact toxicity testing method employed was not in accordance with current guideline methodology.
Previous evaluation:	yes, evaluated, not accepted Source: DAR, Vol. 3 B9 (9.4.1.2), August 2006 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

In the previous submission (DAR, 2006), this study was evaluated and not accepted as valid for risk assessment purposes. Therefore, a summary of this study is not presented in this dossier.

Data Point:	KCP 10.3.1.5/02
Report Author:	[redacted]
Report Year:	2016
Report Title:	Aclonifen SC 600A G (600 g/L): Effects on honey bee brood (Apis mellifera L.) under semi-field conditions - Tunnel test
Report No:	98811033
Document No:	M-621305-01-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP Not Applicable OECD No. 75 (2007) and OEPP/EPPO No. 170 (4)(2010)
Deviations from current test guideline:	Current guideline: OECD GD 75 (2007) After application of test item bees remained in the test tunnel for 3 days, rather than recommended 7 days. This was due to lack of available food caused by action of test item on bee food crop. To prevent starvation bees were fed a defined amount of commercially available ready-to-use syrup (Apiinvert). These deviations are not considered to have affected the integrity or outcome of the study
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

To assess the potential effects of aclonifen SC 600A G (600 g/L) on honey bee colonies including brood development, 4.85 kg product in 400 L tap water/ha (corresponding to 2.4 kg a.s. aclonifen/ha), tap water for the control and a reference item were applied to a full-flowering and highly bee-attractive crop (*Phacelia tanacetifolia*) under semi-field (tunnel) conditions during bee-flight.

No biological relevant adverse effects on mortality of worker bees or pupae were observed. Foraging activity, behaviour, nectar- and pollen storage as well as queen survival were not affected. No effects on colony development, colony strength or bee brood were observed.

Based on the results of this study, it can be concluded that aclonifen SC 600A G (600 g/L) does not adversely affect honey bees and honey bee brood when applied at a rate 4.85 kg product in 400 L tap water/ha (corresponding to 2.4 kg a.s. aclonifen/ha), during honey bees actively foraging on a bee-attractive, flowering crop.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	Aclonifen SC 600A G (600g/L)
	Batch no.:	EV56005993
	Active Ingredient / Purity:	49.5% w/w (596.7 g/L), according to certificate of analysis
	Appearance:	Yellow liquid
	Storage:	Room temperature in the dark
	Expiry date:	9 February 2017

2.	**Reference item:**	Insegar (Fenoxycarb)
	Batch no.:	L166662
	Active Ingredient / Purity:	250 g/kg (nominal), 257 g/kg (according to certificate of analysis
3.	**Test Organism:**	Young adult worker honey bee, *Apis mellifera* L.
	Age:	Not specified
	Source:	[redacted]
	Feeding:	Day 15 following application 1.5L commercial ready-to-use syrup (Apiinvert) was supplied to each colony. During 4[th] colony assessment it was noted that insufficient nectar/honey stores were available. Consequently, a small amount of supplemental food was supplied to all colonies to prevent starvation and a decline in colonies. It was considered that very limited natural resources available to colonies at the open field location led to shortage of food. Water was offered in each tunnel via a drinking trough, except during test item application

A. STUDY DESIGN AND METHODS

1.	**In-life phase:**	29 June to 29 July 2015

2. Exposure conditions

Test tunnels	Semi-circular tunnels (20m x 5.5m x 2.5m, length x width x height) constructed of tubular steel frame with synthetic gauze mesh. Tunnels were placed over flowering plants before experimental start date with a distance of ≥2m between tunnels
Plants	*Phacelia tanacetifolia,* 'Balo'. Flowering *Phacelia* is highly attractive to honey bees. Seeds were sown *ca.* 8 weeks before study start at a rate of 9 kg/ha. Plots of *ca.* 75m² prepared per replicate prior to setting of tunnels Plants were *ca.* 80-90 cm, 90% flowering, 100% vegetation cover at test start
Set up of plots	Each plot was subdivided in the middle of the tunnel (allowing access for application and maintenance of test system
Location of test field	Darmstadt-Dieburg, Germany
Temperature:	Natural conditions, recorded whole experimental time
Relative humidity:	Natural conditions, recorded whole experimental time

Precipitation	Natural conditions, recorded whole experimental time
Wind	Natural conditions, recorded during test item application
Clouding	Recorded daily during exposure phase

3. Administration of the test item

Target concentration was 2.4 kg a.s aclonifen/ha via 400 L water/ha. 4.85 kg test substance (aclonifen SC 600A was mixed with tap water and applied to the flowering Phacelia crop. The control group was treated with tap water at a rate equivalent to 400 L/ha. The reference substance target concentration was 300 g a.s. fenoxycarb/ha via 400 L water/ha.

4. Measurements and observations

Mortality

Mortality was assessed by collecting dead bees from gauze strips placed on bare ground in the middle and at both ends of the tunnel and from dead-bee traps in front of each hive. Dead bees were recorded as adult worker bees, larvae, pupae or drones (males).

Mortality evaluation intervals were as follows:

Day	Exposure period	Frequency
-3 to -1	Pre-exposure	1 x per day
0	Day of application	1 x before application 1 x approx. 2 hours after application 1 x after application (evening)
1 to 3	In tunnels	1 per day
Up to +28 days, after brood area fixing day	Outside tunnels	1 x per day (bee traps only)

Foraging activity

Foraging activity on the plants was recorded within each tunnel at three different 1m^2 areas. Foraging honey bees in these areas were counted to assess the number of bees foraging on flowering plants per unit area. Counting was for approximately 10-15 seconds.

Foraging activity evaluation intervals were as follows:

Day	Exposure period	Frequency
-3 to -1	Pre-exposure	1 x per day
0	Day of application	1 x before application 4 x within 1 hour after application 1 x approx. 2 hours after application 1 x approx. 4 hours after application 1 x approx. 6 hours after application
1	Day after application	3 x per day (morning, midday, afternoon)
2 to 3	In tunnels	1 x per day

Behavioural abnormalities

Sub-lethal effects such as symptoms of poisoning, abnormal behaviour at hive entrance or on plants were assessed during mortality and foraging activity evaluations.

Symptoms were assessed in comparison to the control and include:

Symptom	Observation
Moribund	Cannot walk, show only feeble movements of legs and antennae, weak response to stimulation (e.g. light or blowing). Moribund bees may recover but usually die
Affected	Upright and attempting to walk but showing signs of reduced coordination
Cramps	Contracting abdomen or entire body
Apathy	Low or delayed reactions to stimulation (e.g. light or blowing), sitting motionless in unit, able to walk but not correctly
Intensive cleaning	
Aggressiveness	

Condition and strength of the colonies

Status of the brood (eggs, young and old larvae, closed brood) and status of pollen/nectar stores was estimated quantitively. Brood area and pollen/nectar storage area estimated as percentage of total area. Presence of a healthy queen was determined by the presence of eggs and/or queen cells.

Strength of colonies was estimated quantitatively as percentage of bees occupying each side of the frame. Percentages were converted to real numbers by multiplying by 9 (i.e. 100% = 900 bees/frame side, according to [redacted] (1987), [redacted] (1999).

Colony condition and strength evaluation were conducted during brood development assessments (of individual marked cells as follows:

Exposure period	Frequency
Before application	1 day before application (Brood Area Fixing Day, BFD0)
After application	Days 4, 8, 15, 21 and 27 (Brood Fixing Day 5, 9, 22 and 28)

Development of bee brood

One complete honey bee development period was assessed at different expected stages in individual marked cells. One day before application, 250 eggs were taken out of each colony and a digital photograph of the comb(s) was taken, automatically numbered and marked using an image analysis program ([redacted] For each subsequent assessment the comb was removed from the hive and photographed. The different brood stages were transcribed into indices (0 = empty, 1 = egg, 2 = young larva, 3 = old larva, 4 = pupa, N = nectar, P = pollen). If not enough eggs were found on one side of comb then the second side or an additional comb was inspected and recorded accordingly.

Bee brood assessment evaluation was conducted as follows:

Assessment date	Expected brood stage
1 day pre application (BFD0)	Eggs (1)
5 days after BFD0 (= BFD +5)	Young (2) to old (3) larvae, capped cells (4)
9 days BFD0 (= BFD +9)	Capped cells (4)
16 days after BFD0 (= BFD + 16)	Closed brood (4) shortly before hatch
22 days after BFD0 (+ BFD +22)	Empty cells or cells with eggs or young larvae or food

BFD = Brood Area Fixing Day

Brood termination rate

Calculation of brood termination rate was split into two categories:

- Bee brood in observed cell reached expected brood stage at the different assessment days or was found empty or containing an egg or a small larvae after hatch of the adult on BFD +22 (egg stage) = successful development
- Bee brood in observed cell did not reach expected brood stage at one of the assessment days, was empty or food was stored in cell during BFD +5 - +22 = termination of bee brood development

The percentage of brood not developing to an adult bee successfully was determined.

Compensation index

Brood compensation index is an indicator of recovery (compensation) of the colony after potential brood loss, based on identified growth stage at assessment day. Brood index was calculated for each assessment day and classified from 0 to 5 (where 0 = empty cells, 1 = egg stage, 2 = young larvae, 3 = old larvae, 4 = capped cell, 5 = empty after hatching or again filled with brood (eggs or small larvae)).

For the final calculation, the values of all individual cells are summed and divided by the number of observed cells to obtain the average brood compensation index.

Brood index

Brood index is an indicatory of bee brood development. Brood index was calculated for each assessment day and colony. Brood index was calculated for each assessment day and classified from 0 to 5 (where 0 = empty cells, 1 = egg stage, 2 = young larvae, 3 = old larvae, 4 = capped cell, 5 = empty after hatching or again filled with brood (eggs or small larvae)).

Assessment date	Expected brood index
BFD 0	1
BFD +5	2,3 or 4
BFD +9	4
BFD +16	4
BFD +22	5

BFD = Brood fixing day

For the final calculation, the values of all individual cells are summed and divided by the number of observed cells to obtain the average brood index.

5. Statistics/Data evaluation

Data were tested for normal distribution using Shapiro-Wilk's test and variance of homogeneity using Levene's test.

Mortality and foraging activity

Before application: A pairwise and two-sided comparison (α = 0.05) was used for adult mortality (daily and overall), pupae, colony strength and foraging activity (overall) before application using Student or Welch t-test for homogeneous variances.

After application: A pairwise comparison (α = 0.05) was for comparison of mortality (one-sided greater, daily (adults) and overall (adults and pupae), foraging activity and colony strength data after application

(one-sided smaller, overall) using Student t-test for homogeneous variances or Welch t-test for non-homogeneous variances.

Brood development

A pairwise comparison (α = 0.05) was carried out for comparison of brood termination rate (one-sided, greater) and the brood compensation index/brood index (one-sided smaller) using Student t-test for homogeeous variances.

The computer program used was TOX Rat Professional, Version 2.10.05, ([redacted] 2010).

II. RESULTS AND DISCUSSION

A: ANALYTICAL VERIFICATION

No analytical verification of the dosing solutions was performed.

B: BIOLOGICAL DATA

Mortality

Worker bee mortality before application in the test and reference treatment groups was not statistically different to the control (Student t-test, pairwise comparison to the control, two-sided, α = 0.05).

On the day of application and days 1 and 2 mean mortality rates in the test item groups was higher than in the control but were not found to be statistically significant (Student t-test, pairwise comparison, α = 0.05, one-sided greater). On day 3 a statistically significant difference in mean mortality from the control was observed (Student t-test, pairwise comparison, α = 0.05, one-sided greater). The overall daily mortality (days 0 to 3) was not found to be statistically different to the control.

Overall evaluation of post-application day 4 to 27 did not show a statistically significant difference between the control and the test item treatment (Student t-test, pairwise comparison, α = 0.05, one-sided greater).

Taking into account dead bees for the overall evaluation of post-application day 0 to 27 there was no statistically significant difference (Student t-test, pairwise comparison, α = 0.05, one-sided greater).

Table: Summary of mortality date for worker bees

Time[a]	Water treated control Dead bees				Aclonifen SC 600A G Dead bees					Reference item Dead bees				
	Total[b]	Mean[c]		SD	Total	Mean		SD	Statistics	Total[b]	Mean[c]		SD	Statistics
Day -3	106	26.5	±	18.8	99	24.8	±	20.6	n.s.	126	31.5	±	49.3	n.s.
Day -2	497	124.3	±	83.9	444	111.0	±	113.8	n.s.	623	155.8	±	224.2	n.s.
Day -1	393	98.3	±	43.6	346	86.5	±	58.8	n.s.	391	97.8	±	110.2	n.s.
Day 0 b.a.[d]	**239**	**59.8**	±	**34.4**	**279**	**69.8**	±	**67.4**	**n.s.**	**404**	**101.0**	±	**118.5**	**n.s.**
Daily mean Day -3 to 0 b.a.[d]	**309**	**77.2**	±	**43.9**	**285**	**71.1**	±	**39.7**	**n.s.**	**386**	**96.5**	±	**50.9**	**n.s.**
Day 0 a.a.[e]	**462**	**115.5**	±	**47.0**	**718**	**179.5**	±	**55.2**	**n.s.**	**615**	**153.8**	±	**123.0**	**n.s.**
Day 1	126	31.5	±	13.3	155	38.8	±	19.5	n.s.	247	61.8	±	90.9	n.s.
Day 2	438	109.5	±	66.9	553	138.3	±	79.5	n.s.	536	134.0	±	129.8	n.s.
Day 3	506	126.5	±	36.1	1088	272.0	±	128.6	*	965	241.3	±	197.1	n.s.
Daily mean Day 0 to 3 a.a.[e]	**383**	**95.8**	±	**43.4**	**629**	**157.1**	±	**96.7**	**n.s.**	**591**	**147.7**	±	**73.9**	**n.s.**
Day 4	2	0.5	±	0.6	4	1.0	±	1.4	n.s.	12	3.0	±	0.8	*
Day 5	19	4.8	±	3.9	34	8.5	±	7.3	n.s.	91	22.8	±	14.8	*
Day 6	8	2.0	±	2.0	10	2.5	±	2.4	n.s.	35	8.8	±	12.2	n.s.
Day 7	1	0.3	±	0.5	2	0.5	±	0.6	n.s.	6	1.5	±	1.3	n.s.
Day 8	5	1.3	±	1.5	2	0.5	±	0.6	n.s.	3	0.8	±	1.5	n.s.
Day 9	10	2.5	±	2.1	12	3.0	±	3.5	n.s.	27	6.8	±	4.3	n.s.
Day 10	17	4.3	±	2.5	11	2.8	±	1.3	n.s.	35	8.8	±	6.1	n.s.
Day 11	6	1.5	±	0.6	6	1.5	±	1.3	n.s.	4	1.0	±	0.8	n.s.

Day 12	2	0.5	±	0.6	1	0.3	±	0.5	n.s.	4	1.0	±	0.8	n.s.
Day 13	6	1.5	±	1.7	10	2.5	±	1.9	n.s.	13	3.3	±	1.5	n.s.
Day 14	17	4.3	±	5.9	20	5.0	±	3.2	n.s	39	9.8	±	8.6	n.s.
Day 15	3	0.8	±	1.0	15	3.8	±	2.2	*	25	6.3	±	3.8	*
Day 16	127	31.8	±	22.2	359	89.8	±	70.4	n.s.	126	31.5	±	35.0	n.s.
Day 17	20	5.0	±	4.8	38	9.5	±	11.0	n.s.	5	1.3	±	1.0	n.s.
Day 18	3	0.8	±	1.0	0	0.0	±	0.0	n.d.	2	0.5	±	1.0	n.s.
Day 19	1	0.3	±	0.5	4	1.0	±	2.0	n.s.	3	0.8	±	1.0	n.s.
Day 20	2	0.5	±	0.6	7	1.8	±	1.7	n.s.	11	2.8	±	1.7	*
Day 21	4	1.0	±	0.8	6	1.5	±	1.3	n.s.	4	1.0	±	1.2	n.s.
Day 22	7	1.8	±	3.5	14	3.5	±	2.9	n.s.	12	3.0	±	2.7	n.s.
Day 23	2	0.5	±	1.0	1	0.3	±	0.5	n.s.	2	0.5	±	0.6	n.s.
Day 24	0	0.0	±	0.0	1	0.3	±	0.5	n.s.	3	0.8	±	1.0	n.s.
Day 25	0	0.0	±	0.0	0	0.0	±	0.0	n.s.	3	0.8	±	1.0	*
Day 26	1	0.3	±	0.5	2	0.5	±	0.6	n.s.	1	0.3	±	0.5	n.s.
Day 27	1	0.3	±	0.5	1	0.3	±	0.5	n.s.	0	0.0	±	0.0	n.d.
Daily mean Day 4 to 27 a.a.	**11.0**	**2.8**	**±**	**6.4**	**23.3**	**5.8**	**±**	**18.0**	**n.s.**	**19.4**	**4.9**	**±**	**7.6**	**n.s.**
Daily mean Day 0 to 27 a.a.	**64.1**	**16.0**	**±**	**36.6**	**109.8**	**27.4**	**±**	**65.0**	**n.s.**	**101.0**	**25.3**	**±**	**57.0**	**n.s.**

[a] days -3 to -1 = days before application; day 0 = application day; day 1 to 27 = days after application (= day 28 after BFD0)
[b] total = sum of four tunnels per treatment group; c mean = mean values (rounded) of four tunnels per treatment group
[d] b.a.= before application; e a.a.= after application; "n.d." = not determined due to "0" response
n.s. = not statistically significant compared to the control; * = statistically significant compared to the control
Statistics: Student t-test, pairwise comparison, two-sided (before application), one-sided greater (after application), α = 0.05

Foraging activity

No statistically significant differences in foraging activity were observed before application (Student t-test, α = 0.05, two-sided). Foraging intensity indicated colonies were vital and active.

For the first two days following application foraging activity was not reduced compared to the control. From day 2 onwards foraging activity was reduced due to the fading attractiveness of the crop as a result of herbicide action on the plants. By day 3 foraging activity was decreased compared to the control group and the colonies were removed from the tunnels on the evening of day 3. Mean foraging activity over first three days post application of the test substance was not statistically significant when compared with the control (Student t-test, pairwise comparison, α = 0.05, one-sided smaller).

Table: Summary of foraging activity

Time[a]	Water treated control Mean number of bees per m² [b]			Aclonifen SC 600A G Mean number of bees per m² [b]			Statistics	Reference item Mean number of bees per m² [b]			Statistics
Day -3	19.3	±	2.1	21.9	±	7.1	-	17.8	±	3.6	-
Day -2	18.8	±	2.6	21.7	±	6.3	-	17.7	±	1.1	-
Day -1	31.1	±	8.7	27.3	±	6.8	-	24.7	±	9.1	-
Mean Day 0 b.a.[c]	**24.0**	**±**	**6.3**	**24.5**	**±**	**6.0**	**-**	**26.8**	**±**	**5.6**	**-**
Daily Mean Day -3 to 0 b.a.	**23.3**	**±**	**5.7**	**23.8**	**±**	**2.6**	**n.s.**	**21.7**	**±**	**4.7**	**n.s.**
Mean Day 0 a.a.[d]	**23.0**	**±**	**2.9**	**23.4**	**±**	**7.0**	**-**	**21.8**	**±**	**3.4**	**-**
Day 1	22.9	±	3.0	24.9	±	3.5	-	22.9	±	1.6	-
Day 2	31.3	±	3.6	26.7	±	6.7	-	28.7	±	5.2	-
Day 3	21.5	±	2.6	14.0	±	4.9	-	15.3	±	6.3	-
Daily Mean Day 0 a.a. to Day 3	**24.7**	**±**	**4.5**	**22.2**	**±**	**5.7**	**n.s.**	**22.2**	**±**	**5.5**	**n.s.**

[a] days -3 to -1 = days before application; day 0 = application day; day 1 to 3 = days after application
[b] mean values (rounded) of four tunnels per treatment group
[c] b.a.= before application; [d] a.a.= after application
n.s. = not statistically significant compared to the control; * = statistically significant compared to the control
"-" = no statistics were performed
Statistics: Student t-test, pairwise, two-sided (before application); one-sided smaller (after application), α = 0.05

Behavioural abnormalities

No test item related behavioural abnormalities occurred at any time during the assessment period up to day 27.

Colony conditions

In order to assess the condition of the colonies during one whole brood cycle of the bees, 6 brood assessments were carried out. At the beginning of the trial, all queens (or eggs) and brood stages (eggs, larvae and closed brood) was found in all colonies as an indication of healthy colonies. Moreover the amount of food reserves (nectar and pollen) was sufficient to ensure colony viability and brood status but also allowed that enough space was available for exposure of the brood to new food sources.

On day 15 following the application, 1.5 L commercial ready-to-use syrup (Apiinvert) was supplied to each of the colonies. During the 4th colony assessment, it was observed that some of the colonies started to have an insufficient amount of nectar/honey stores. Therefore, in order to prevent artefacts from insufficient food supply/starvation, it was necessary to provide an exactly dosed, small amount of supplemental food to all colonies in order to avoid a decline of the colonies.

All queens and/or a sufficient presence of eggs were found in the test item treated colonies during all following brood checks indicating that the queens were alive and healthy.

At the end of the 3rd day after application, the hives were relocated from their tunnels. In general, the test item treated colonies developed in the same manner as the control colonies. Compared to the control, a similar amount of brood could be found during the assessments with no indication of a test item related effect. All test item treated colonies remained vital with increasing bee numbers and healthy brood. There was no indication of any effect of the test item on the condition of the bee colonies.

In contrast to this, the development of the larvae and pupae in the reference item colonies was distinctly decreased at least for the first brood cycle after the application.

Strength of the colonies

The mean number of bees per colony in all groups (control, test item and reference item) one day before application was between 6278 and 6739 bees per colony and did not differ statistically. By setting the initial mean number of bees per treatment group before the application to 100%, the relative increase or decrease of bees were determined.

The mean colony strength on day +27 compared to day -1 was higher in all colonies of the control and test item treated group, showing that all colonies were able to grow during the duration of the study. The increase of the control and test item treated colonies followed the same pattern. At the end of the test, the relative numbers of bees per colony in the control and test item group were 127% and 129%, respectively. 99% was found in the reference item treatment, compared to the initial value (=100%). Since the increase in the test item treated group was very similar to the control group, there was no statistically significant difference in the test item treated group compared to the control group (Student t-test, pairwise comparison, one-sided smaller). Accordingly, the test item had no influence on the colony strength.

Overall, no adverse effects of the test item on colony strength and population development have been observed throughout the study. Compared to the development of the colony strength observed in the control group, the development in the reference item group was decreased.

Table: Summary of colony strength

Time (days)[a]	Control	Test item (aclonifen)	Reference
	Number of bees	Number of bees	Number of bees

	Mean	SD	%	Mean	SD	%	Stats	Mean	SD	%	Stats
-1	6278	376	100	6345	362	100	n.s.	6739	789	100	n.s.
+4	7178	868	114	7144	894	113	n.s.	7436	2045	110	n.s.
+8	81089	1463	129	8190	1039	129	n.s.	8741	543	130	n.s.
+15	9394	2012	150	8393	558	132	n.s.	7976	850	118	n.s.
+21	8370	810	133	7830	899	123	n.s.	8145	419	121	n.s.
+27	7999	1410	127	8213	843	129	n.s.	6683	633	99	n.s.

a Time in relation to application (-1 is pre-application, =4 is post-application
b Mean value of 4 colonies
c SD = standard deviation
d In relation to 1st assessment on day -1
e Statistics: Student t-test, pair-wise, two-sided (before application), one-sided small (after application), α = 0.05
n.s. Not statistically significant compared to the control

Development of brood

Brood termination rate

Following the assessment of single cells from the egg stage to the successfully hatched worker bee, the mean termination rate at Brood Fixing Day 22 in the test item group of 10.5% clearly lower compared to the control group (29.7%). However, this lower Brood Termination Rate in the test item group was not statistically significantly different compared to the control group. Treatment with the reference item Insegar (a.s.: fenoxycarb) caused a clear decrease of brood development of the marked eggs, resulting in a termination rate of 88.3%. This decrease was statistically significantly different compared to the control group (Student t-test, pair-wise comparison to the control, one-sided greater, α = 0.05).

Table: Bee brood termination rates

Treatment group	Replicate	Eggs BFD0	22 days after BFD0 brood terminated			
			Number terminated cells	%	Mean %	Stats
Control	1	250	54	21.6	29.7	-
	2	250	74	29.6		
	3	250	60	24.0		
	4	250	109	43.6		
Test item	1	250	14	5.6	10.5	n.s.
	2	250	3	1.2		
	3	250	30	12.0		
	4	250	58	23.2		
Reference	1	250	174	69.6	88.3	*
	2	250	250	100		
	3	250	250	100		
	4	250	209	83.6		

BFD0 = Brood Fixing Day 0
n.s. Not statistically significant compared to the control
* Statistically significant compared to the control
Statistics: Student t-test, pair-wise comparison, one-sided greater, α = 0.05

Brood compensation rate

Brood Compensation Indices of the test item group were all higher compared to the corresponding indices of the control group and most of the few terminated brood cells were refilled with new eggs which developed successfully. The mean brood compensation indices in the test item group were 2.8,

3.7, 3.6 and 4.6 at BFD +5, BFD +9, BFD +16 and BFD +22, respectively compared with 2.5, 3.0, 3.0 and 3.9 in the control. There was no statistically significant difference compared to the control (Student t-test, pairwise comparison to the control, one-sided smaller, α = 0.05). No adverse effects of the test item on brood development have been observed throughout the study, following the labelling of the egg stage up to day 21 after application (BFD+22).

The high termination rate of the marked cells after treatment with the reference item Insegar (a.s.: fenoxycarb) is also reflected by the statistically significantly lower Brood Compensation Indices in the reference item group when compared to the control. (Student t-test, pair-wise comparison to the control, one-sided smaller, α = 0.05).

Table: Brood compensation index

Treatment		Mean Brood Compensation Indices (Eggs)				
		BFD 0	**BFD 5**	**BFD 9**	**BFD 16**	**BFD 22**
Control	Mean	1.0	2.5	2.4	2.4	3.1
	SD	-	0.2	0.4	0.4	0.5
	Stats	-	-	-	-	-
Test item	Mean	1.0	2.8	3.7	3.6	4.6
	SD	-	0.3	0.3	0.3	0.3
	Stats	-	n.s.	n.s.	n.s.	n.s.
Reference	Mean	1.0	0.7	0.8	1.0	2.3
	SD	-	0.6	0.6	0.3	0.7
	Stats	-	*	*	*	*
Nominal max.		1.0	2.0 – 3.0	3.0 – 4.0	4.0	5.0

BFD = Brood Area Fixing Date
Statistics: Student t-test, pair-wise comparison, one-sided smaller, α = 0.05
n.s. Not statistically significant compared to the control
* Statistically significant compared to the control

Brood index

The Brood Index as an indicator for the bee brood development facilitates a comparison between the different treatments. Mean Brood Indices of the test item group indicated a continuous brood development with values higher when compared to the control group between BFD +5 to BFD +22.

The mean brood indices in the test item group were 2.8, 3.6, 3.6 and 4.5 at BFD +5, BFD +9, BFD +16 and BFD +22, respectively compared with 2.4, 2.8, 2.8 and 3.5 in the control group. This was not statistically significantly different compared to the control (Student t-test, pair-wise comparison to the control, one-sided smaller, α = 0.05).

No adverse effects of the test item on brood development were observed throughout the study.

After treatment with the reference item Insegar (a.s.: fenoxycarb), the mean Brood Indices were statistically significant lower compared to the control indices (Student t-test, pair-wise comparison to the control, one-sided smaller, α = 0.05).

Table: Brood index

Treatment		Mean Brood Indices (Eggs)				
		BFD 0	**BFD 5**	**BFD 9**	**BFD 16**	**BFD 22**
Control	Mean	1.0	2.4	2.3	2.3	2.8
	SD	-	0.3	0.4	0.4	0.5
	Stats	-	-	-	-	-

Test item	Mean	1.0	2.8	3.6	3.6	4.5
	SD	-	0.3	0.4	0.4	0.5
	Stats	-	n.s.	n.s.	n.s.	n.s.
Reference	Mean	1.0	0.5	0.6	0.5	0.6
	SD	-	0.5	0.7	0.6	0.7
	Stats	-	*	*	*	*
Nominal max.		1.0	2.0 – 3.0	3.0 – 4.0	4.0	5.0

BFD = Brood Area Fixing Date
Statistics: Student t-test, pair-wise comparison, one-sided smaller, $\alpha = 0.05$
n.s. Not statistically significant compared to the control
* Statistically significant compared to the control

C: VALIDITY CRITERIA

The study was based on OECD guidance document 75 (2007), and the recommendations of AG Bienenschutz (2011). Validity set out in the study were qualitative, stating that control mortality should not be considerable and that there should be a high number of impacted bees in the reference test treatments.

Over the course of the study (day 0 – 27) there was a daily mean mortality of 64.1, from this it can be inferred that the validity criterion for control mortality was met.

The reference item treatment showed significant impact compared to the control in relation to brood termination rate, brood compensation rate and brood index, and from this it can be inferred that the validity criterion for considerable impact in the reference test treatment was met.

Therefore, it is considered that this study is valid for risk assessment purposes.

III. CONCLUSION

To assess the potential effects of aclonifen SC 600A G (600 g/L) on honey bee colonies including brood development, 4.85 kg product in 400 L tap water/ha (corresponding to 2.4 kg a.s. aclonifen/ha), tap water for the control and a reference item were applied to a full-flowering and highly bee-attractive crop (*Phacelia tanacetifolia*) under semi-field (tunnel) conditions during bee-flight.

No biological relevant adverse effects on mortality of worker bees or pupae were observed. Foraging activity, behaviour, nectar- and pollen storage as well as queen survival were not affected. No effects on colony development, colony strength or bee brood were observed.

Based on the results of this study, it can be concluded that aclonifen SC 600A G (600 g/L) does not adversely affect honey bees and honey bee brood when applied at a rate 4.85 kg product in 400 L tap water/ha (corresponding to 2.4 kg a.s. aclonifen /ha), during honey bees actively foraging on a bee-attractive, flowering crop.

[redacted] *(2016)*

Assessment and conclusion by applicant:

The study was based on OECD guidance document 75 (2007), and the recommendations of AG Bienenschutz (2011). Validity set out in the study were qualitative, stating that control mortality should not be considerable and that there should be a high number of impacted bees in the reference test treatments.

Over the course of the study (day 0 – 27) there was a daily mean mortality of 64.1, from this it can be inferred that the validity criterion for control mortality was met.

The reference item treatment showed significant impact compared to the control in relation to brood termination rate, brood compensation rate and brood index, and from this it can be inferred that the validity criterion for considerable impact in the reference test treatment was met.

Therefore, it is considered that this study is valid for risk assessment purposes.

No biological relevant adverse effects on mortality of worker bees or pupae were observed. Foraging activity, behaviour, nectar- and pollen storage as well as queen survival were not affected. No effects on colony development, colony strength or bee brood were observed.

Based on the results of this study, it can be concluded that aclonifen SC 600A G (600 g/L) does not adversely affect honey bees and honey bee brood when applied at a rate 4.85 kg product in 400 L tap water/ha (corresponding to 2.4 kg a.s. aclonifen /ha), during honey bees actively foraging on a bee-attractive, flowering crop.

Assessment and conclusion by RMS:

CP 10.3.1.6 Field tests with honeybees

No data available on the formulated product.

CP 10.3.2 Effects on non-target arthropods other than bees

A summary of the non-target arthropod toxicity endpoints for Aclonifen SC 600 G is provided in the following table.

Table 10.3-4: Non-target arthropod endpoints used in risk assessment

Test item	Test species	Time-scale Test type / substrate	Endpoint	Reference
Aclonifen SC 600G	*Aphidius rhopalosiphi*	48 h Mortality Glass plate (2D	**LR_{50} > 2930 g a.s./ha**	KCA 8.3.2.1/01 KCP 10.3.2.1/01 M-172247-01-1 [redacted], 1999
		48 h Reproduction Glass plate (2D	**ER_{50} > 2930 g a.s./ha**	
Aclonifen SC 600 G	*Typhlodromus pyri*	7-d Mortality Glass plate (2D	LR_{50} < 2930 g a.s./ha	KCA 8.3.2.2/01 KCP 10.3.2.1/02 M-172210-01-1 [redacted] 1999
		7-d Reproduction Glass plate (2D	ER_{50} < 2930 g a.s./ha	
Aclonifen SC 600 G	*Typhlodromus pyri*	7-d Mortality Glass plate (2D	**LR_{50} = 102 g a.s./ha**	KCA 8.3.2.2/02 KCP 10.3.2.1/03 M-232137-01-1 [redacted], 2003
		7-d Reproduction Glass plate (2D	ER_{50} > 150 g a.s./ha	
		5-d	LR_{50} > 3300 g a.s./ha[1]	

Aclonifen SC 600 G	*Aleochara bilineata*	Mortality Quartz sand (2D)		KCA 8.3.2/01 KCP 10.3.2.1/04 M-174575-01-1 [redacted] 1992
		5-d Reproduction Quartz sand (2D)	ER_{50} > 3300 g a.s./ha[1]	
Aclonifen SC 600 G	*Poecilus cupreus*	14-d Mortality Quartz sand (2D)	**LR_{50} > 3300 g a.s./ha**	KCA 8.3.2/02 KCP 10.3.2.1/05 M-174573-01-1 [redacted] 1992
Aclonifen SC 600 G	*Pardosa sp.*	28-d Mortality Quartz sand (2D)	41.7% mortality after 2 weeks and 58.3% mortality after 4 weeks at 3300 g a.s./ha	KCA 8.3.2/05 KCP 10.3.2.1/06 M-174577-01-1 [redacted] 1992
Aclonifen SC 600 G	*Chrysoperla carnea*	13-d Mortality Glass plate (2D)	**LR_{50} > 720 g a.s./ha**	KCA 8.3.2/03 KCP 10.3.2.1/07 M-177360-01-1 [redacted], 1999
		13-d Reproduction Glass plate (2D)	**ER_{50} > 720 g a.s./ha**	
Aclonifen SC 600 G	*Typhlodromus pyri*	14-d Mortality Leaf disc (2D)	LR_{50} > 120 g a.s./ha	KCA 8.3.2.2/03 KCP 10.3.2.2/01 M-238634-01-1 [redacted], 2000
		14-d Reproduction Leaf disc (2D)	ER_{50} > 120 g a.s./ha	
Aclonifen SC 600 G	*Pardosa sp.*	14-d Mortality Natural soil (2D)	**LR_{50} > 2970 g a.s./ha**	KCA 8.3.2/04 KCP 10.3.2.2/02 M-238654-01-1 [redacted], 2000
Aclonifen SC 600 G	*Chrysoperla carnea*	28-d Mortality Leaf disc (2D)	**LR_{50} > 2364 g a.s./ha**	KCP 10.3.2.2/03 M-221161-01-1 [redacted] 2003
		28-d Reproduction Leaf disc (2D)	**ER_{50} > 2364 g a.s./ha**	
Aclonifen SC 600 G	*Aleochara bilineata*	82-d Reproduction Natural soil (2D)	**ER_{50} > 2400 g a.s./ha**	KCP 10.3.2.2/04 M-561614-01-1 [redacted], 2016
Aclonifen SC 600 G	*Typhlodromus pyri*	14-d Mortality Leaf disc (2D)	**LR_{50} = 2336 g a.s./ha**	KCP 10.3.2.2/05 M-588206-01-1 [redacted], R. U., 2017
		14-d Reproduction Leaf disc (2D)	**ER_{50} = 601 g a.s./ha**	
Aclonifen SC 600 G	*Typhlodromus pyri*	14-d Mortality Potato plant (3D) aged residues	600 g a.s./ha Mortality of 9.0% at 0 DAT and 4.25% at 14 DAT	KCP 10.3.2.2/06 M-574023-01-1 [redacted], 2016
		14-d Reproduction Potato plant (3D) aged residues	600 g a.s./ha Reduction of 19.5% reproduction at 0 DAT and -12.8% at 14 DAT	
Aclonifen SC 600 G	*Typhlodromus pyri*	14-d Mortality Potato plant (3D) aged residues	1.8 kg a.s./ha Mortality of 47.4% at 0 DAT and 18.2% at 14 DAT	KCP 10.3.2.2/07 M-639666-01-1 [redacted] 2018

		14-d Reproduction Potato plant (3D) aged residues	1.8 kg a.s./ha Reduction of 25.5% reproduction at 0 DAT and 13.8% at 14 DAT	

Values in **bold** used in risk assessment
[1]: Study not used in risk assessment as performed according to outdated test guideline
DAT: Day after treatment

Summary of the risk assessment for Aclonifen SC 600 G on non-target terrestrial arthropods

Predicted environmental rates (PER), in-field and off-field, were determined (according to ESCORT 2, [redacted] 2000[16]) for the proposed uses of Aclonifen SC 600 G.

Based on the hazard quotients calculated based on Tier I (glass plate) LR_{50} values for *Typhlodromus pyri*, Aclonifen SC 600 G poses an unacceptable risk to the indicator species following the proposed uses as the calculated in-field HQ values were greater than the trigger value of 2.

Tier II extended laboratory studies performed on *Typhlodromus pyri*, *Aleochara bilineata*, *Poecilus cupreus*, *Pardosa* and *Chrysoperla carnea* were performed and indicated acceptable risk based on LR_{50} values. Potential concerns regarding the effects on reproduction for *Typhlodromus pyri* were addressed by the results of aged residue studies.

It can therefore be concluded that no long-lasting effects on foliage dwelling non-target arthropods with a sensitivity similar to *Typhlodromus pyri* are to be expected from the exposure to Aclonifen SC 600 G according to the proposed use pattern.

Concerning the effects on soil dwelling non-target arthropods the studies on *Aleochara bilineata, Poecilus cupreus* and *Pardosa* indicated no adverse effects even above the maximum intended application rate of 600 g a.s./ha.

Therefore, it can be concluded that the application of Aclonifen SC 600 G with application rates up to 600 g a.s./ha will not result in unacceptable adverse effects on non-target arthropods.

Risk assessment for other non-target arthropods

The risk assessment for non-target arthropods has been conducted in line with ESCORT 2 ([redacted] 2000).

In-field

Non-target arthropods can be exposed to residues from Aclonifen SC 600 G by direct contact either as a result of over-spray or through contact with residues on soil or in food items. Aclonifen SC 600 G is applied at a proposed maximum application of 0.06 kg a.s./ha (600 g a.s./ha) in peas.

The in-field exposure (predicted environmental residue, PER) is calculated according to ESCORT 2 using the following equation:

$$In-field\ PER = Application\ rate\ x\ MAF$$

The MAF is a generic multiple application factor, which is used to take into account the potential build-up of applied active substances between applications and is based on the application interval, the DT_{50} value on foliage and the number of applications. As Aclonifen SC 600 G is only applied once, the MAF value is 1.0 and hence the in-field PER is 600 g a.s./ha.

16 [redacted] (2000) Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods. From the ESCORT 2 workshop (European Standard Characteristics Of non-target arthropod Regulatory Testing)

Off-field

Risk assessment of areas immediately surrounding the crop is considered important since these areas represent potential natural reservoirs for immigration, emigration and reproduction of arthropod species and provide increased species diversity in the natural community. Exposure of non-target arthropods living in off-field areas to Aclonifen SC 600 G will mainly be due to spray drift from field applications. Off-field areas are assumed to be densely vegetated, and thus, spray drift is unlikely to reach bare ground.

The off-field exposure (predicted environmental residue, PER) is calculated according to ESCORT 2 using the following equation:

$$Off-field\ PER = Application\ rate\ x\ MAF\ x\ \frac{drift\ factor}{vegetation\ distribution\ factor}\ x\ correction\ factor$$

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, a vegetation distribution or dilution factor is incorporated into the equation when calculating PERs to be used in conjunction with toxicity endpoints derived from two-dimensional (glass plate or leaf disc) studies. A dilution factor of 10 is recommended by ESCORT 2 and will be associated to endpoints from studies in 2D systems, while in case of 3D systems no vegetation distribution factor is used.

Drift factor: The drift factor value (%) at different distances varies depending on the crop and total number of applications; since a single application is intended, the drift value at 90th percentile of 2.77% in field crops at 1 m distance is used (Appendix VI, ESCORT 2, Candolfi et al. 2000).

Correction factor: As recommended by ESCORT 2, correction factors of 10 and 5 are used respectively for Tier I and Tier II assessments.

Table 10.3-5: Calculation of Tier I off-field PER value for Aclonifen SC 600 G

Crop	Max single application rate (g a.s./ha)	Drift factor %	Vegetation distribution factor	Correction factor	MAF	Off-field PER (g a.s./ha)
Tier I assessment (based on 2D studies)						
Peas	600	2.77	10	10	1.0	16.62

Calculation of the Tier I in-field and off-field Hazard Quotients (HQ)

The risk to non-target arthropods is assessed using the approach recommended in the published ESCORT 2 document ([redacted] 2001 and SANCO/10329/2002).

The potential risk of Aclonifen SC 600 G to non-target arthropods was assessed by calculation of the hazard quotient (HQ) using the equation below. The input values were based on the predicted environmental residue (PER) and the lowest lethal rate (LR_{50}) values for both sensitive species exposed to Aclonifen SC 600 G.

$$HQ = \frac{PER}{LR_{50}}$$

The HQ values based on Tier I laboratory studies are evaluated against a trigger value of 2. If values are above the trigger a risk to non-target arthropods is indicated and further higher-tier assessment to address the potential risk is required. The resulting $HQ_{in-field}$ and $HQ_{off-field}$ values for non-target arthropods are presented in the following table.

Table 10.3-6: Tier I In-field and Off-field HQs for non-target arthropods exposed to Aclonifen SC 600 G

Species	LR_{50} (g a.s./ha)	In-field PER (g a.s./ha)	$HQ_{in-field}$	Off-field PER (g a.s./ha)	$HQ_{off-field}$	Trigger
Aphidius rhopalosiphi	>2930	600	<0.20	16.62	<0.006	2
Typhlodromus pyri	102		**5.88**		0.163	

Values in **bold** indicate unacceptable risks

The in-field and off-field HQ values for *Aphidius rhopalosiphi*, and the off-field HQ values for *Typhlodromus pyri* were below the Tier I trigger value of 2. However the in-field HQ values for *Typhlodromus pyri* were below the Tier I trigger value and hence a Tier II assessment is necessary and is presented below.

Tier II in-field assessment (extended laboratory study)

As the HQ value for *Typhlodromus* exceeds the trigger value for the in-field habitats, higher-tier testing is required. According to ESCORT 2 one additional species should be tested if the HQs are only exceeded for the in-field risk assessment. In the case of Aclonifen SC600 G (early spray application on bare soil) *Aleochara bilineata* should preferably be used. The following four additional species have been tested with the 600 g/L SC formulation of aclonifen: *Aleochara bilineata*, *Poecilus cupreus*, *Pardosa spec*. and *Chrysoperla carnea*.

Tier II extended laboratory studies were performed with *Typhlodromus pyri* with dosing of the product onto cowpea leaves. In the initial study performed using application rates of 120 and 2970 g a.s./ha significant mortality (60%) was observed at 2970 g a.s./ha however the mortality at 120 g a.s./ha was shown not to be significant. In order to obtain a LR_{50} value, a second study was performed using five applications rates. This study showed an LR_{50} of 2336 g a.s./ha which was used in the Tier II risk assessment.

Table 10.3-7: Tier II assessment of the in-field risk for non-target organisms exposed to Aclonifen SC 600 G

Species	Rate with ≤ 50% effect (g a.s./ha)	In-field PER (g a.s./ha)	In-field PER below rate with ≤ 50% effect?
Typhlodromus pyri	601	600	Yes
Aleochara bilineata	> 2400		Yes
Poecilus cupreus	> 3300		Yes
Pardosa spec.	> 2970		Yes
Chrysoperla carnea	> 2364		Yes

For *Aleochara bilineata*, *Poecilus cupreus*, *Pardosa spec*. and *Chrysoperla carnea* no harmful or detrimental effects were observed on any of the parameters measured which included mortality, feeding and fecundity. However, in studies performed with *T. pyri* severe effects in reproduction were observed which cannot be overlooked.

Additional higher-tier risk assessment

Refinement for predatory mites

The second extended laboratory study (M-588206-01-1) resulted in an LR_{50} of 2336 g a.s./ha and an ER_{50} of 601 g a.s./ha indicating that the intended application rate of 600 g a.s./ha will have no

unacceptable effect on mortality but may have effects close to 50% on reproduction. Therefore, as a precautionary step, the potential for recovery has been evaluated.

To demonstrate that effects on predatory mites due to an exposure on treated plants will not be long-lasting, aged residue studies (M-574023-01-1 and M-639666-01-1) have been conducted with potato plants at application rates of 600 and 1800 g a.s./ha respectively. Due to the fact that whole plants (3D application) were treated in the aged residue studies, the initial effects were lower as compared to the extended lab study (M-588206-01-1) with the 2D application onto single leaves. The second bioassay of the aged residue study performed at an application rate of 600 g a.s./ha (M-574023-01-1) that was started 14 days after the application indicated no relevant adverse effects (< 5%) anymore.

It can therefore be concluded that no long-lasting effects on foliage dwelling non-target arthropods with a sensitivity similar to *Typhlodromus pyri* are to be expected from the exposure to Aclonifen SC 600 G according to the proposed use pattern.

Concerning the effects on soil dwelling non-target arthropods the studies on *Aleochara bilineata*, *Poecilus cupreus* and *Pardosa* indicated no adverse effects even above the maximum intended application rate of 600 g a.s./ha.

Therefore, it can be concluded that the application of Aclonifen SC 600 G with application rates up to 600 g a.s./ha will not result in unacceptable adverse effects on non-target arthropods.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

Data Point:	KCP 10.3.2.1/01
Report Author:	[redacted]
Report Year:	1999
Report Title:	Effects of EXP 04209E on the aphid parasitoid Aphidius rhopalosiphi (Hymenoptera, Aphidiidae) in the laboratory
Report No:	R006177
Document No:	M-172247-01-1
Guideline(s) followed in study:	IOBC/WPRS 1988
Deviations from current test guideline:	Current Guideline: IOBC/WPRS 1988 None
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of EXP 04209E (aclonifen, 586 g/L) on mortality of the parasitoid, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae), after 48 hours of exposure according to IOBC/WPRS 1988. Additionally, an assessment for significant sublethal effects (parasitisation activity) was made.

The parasitoids were exposed to a dose rate of 5 L EXP 04209E/ha (equivalent to 2930 g a.s./ha). Adult parasitoids were exposed to dried spray residues on glass plates for 48 hours. A toxic reference (Perfekthion (dimethoate a.s.)) was included with the test. Mortality was assessed after 1, 2, 24 and 48 hours of exposure. Four replicates each containing 10 parasitoids were used per treatment group. For the reproduction assessment surviving females were removed from the exposure units and their reproductive capacity was assessed by confining them individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The females were removed after 24 hours and the aphid-infested plants left for further 8 - 11 days before the numbers of aphid mummies that had developed were assessed.

One of the 40 parasitoids died after 48 hours exposure to EXP 04209E. No parasitoid died in the control group. All parasitoids were dead after 48 hours exposure to the toxic standard. There were no statistically significant differences in mortality between the control and the test item treatments.

Surviving parasitoids produced a mean of 13.3 mummies per female in the group treated with EXP 04209E and a mean of 9.5 mummies per female in the control group. There were no statistically significant differences in fecundity between the control and the test item treatments.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	EXP 04209E
	Lot no.:	OP980353
	Active Ingredient / Purity:	Aclonifen, 586 g/L
	Appearance:	Yellow liquid
	Storage:	In original container, at room temperature, in the dark
	Expiry date:	07 May 2000
2.	**Reference item:**	Perfekthion EC
	Batch no.:	98-1
	Active Ingredient / Purity:	Dimethoate, 396 g/L
3.	**Test Organism:**	*Aphidius rhopalosiphi* (DeStefani-Perez)
	Age:	Adults, less than 48 hours old
	Source:	[redacted]
	Acclimatisation:	Approximately 2 days under test conditions
	Feeding:	Honey in water (1:3)

B. STUDY DESIGN AND METHODS

1. In-life phase: 22 February – 08 March 1999

2. Exposure conditions

Test vessels:	*Exposure units:* 2 treated glass plates (13 cm x 13 cm) which were held apart by an untreated aluminium frame (13 cm x 1.5 cm x 1 cm per side) held together by two clamps. *Post-exposure units:* potted barley plants, infested with the host aphids of all developmental stages (*Rhopalosiphum padi*) was enclosed by a polyacrylic cylinder (30 cm high and 10 cm in diameter) with a fine gauze on the top.
Experimental design:	3 experimental groups: control (tap water), test item (5 L EXP 04209E/ha, equivalent to 2930 g aclonifen/ha) and toxic standard (dimethoate).
Replicates:	exposure period: 4 units per treatment group post-exposure period: 20 units
Loading:	exposure period: 10 per replicate (5 females and 5 males per replicate) post-exposure period: 1 female per replicate
Temperature:	acclimatisation period: 18.5 - 20.5 °C exposure period: 18.5 - 20.5 °C post-exposure period: 19.5 - 24 °C
Relative humidity:	acclimatisation period: 70 - 80% exposure period: 68 - 80% post-exposure period: 78.5 - 88.7%, within the post-exposure units
Photoperiod:	16 h light: 8 h dark
Light intensity:	acclimatisation period: 800 lux exposure period: 780 - 910 lux post-exposure period: 2520 - 3000 lux
Ventilation:	Exposure units were ventilated with a small pump

3. Administration of the test item

Dose preparation

The test item was applied to an inert substrate (glass plate). Treatments were applied in a spray volume equivalent to 200 L water/ha. The spraying dilution was applied to the glass plates using laboratory-spraying equipment.

Test organism assignment and exposure

The study encompassed 3 treatment groups (test item, control, reference item) with 4 replicates each containing 10 adult parasitoids. The parasitoids were exposed to dried residues on treated glass plates. Survival of the parasitoids was assessed after 1, 2, 24 and 48 hours. At 48 hours, for treatment groups where ≥ 50% of parasitoids survived they were removed and their reproductive capacity was assessed by confining females individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The females were removed after 24 hours and the aphid-infested plants left for further 8 - 11 days before the numbers of aphid mummies that had developed were assessed.

4. Measurements and observations

Observations of mortality were recorded approximately 1, 2, 24 and 48 hours after test initiation. The number of parasitoids alive and dead were recorded. Number of aphid mummies was counted 8 and 11 days after the 24 hour parasitisation period. Reproduction was performed in the groups where the corrected mortality (Mcorr) was ≤50%. Due to the high mortality no reproduction testing was performed with the reference item.

5. Statistics/Data evaluation

Mortality of the treated and untreated series was compared with Bonferroni-U-Test (α = 0.05), reproduction was compared with Student-T-Test (α = 0.05).

The computer program used to perform the statistical analyses was EASY ASSAY Multiple Testing (Ratte, 1995).

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Analytical verification was not required.

B. BIOLOGICAL DATA

One of the 40 parasitoids died after 48 hours exposure to EXP 04209E. No parasitoid died in the control group. All parasitoids were dead after 48 hours exposure to the toxic standard. There were no statistically significant differences in mortality between the control and the test item treatments.

Surviving parasitoids produced a mean of 13.3 mummies per female in the group treated with EXP 04209E and a mean of 9.5 mummies per female in the control group. There were no statistically significant differences in fecundity between the control and the test item treatments.

Table: Effects of EXP 04209E on mortality and parasitisation efficiency of the parasitoid, *Aphidius rhopalosiphi*, exposed to fresh dried residue in the laboratory

Nominal Application Rate (g a.s./ha)	Mortality (%)	Mortality corr.[1] (%)	Parasitisation rate (mummies/female)
Control	0.0	-	9.5
2930	2.5	2.5	13.3
Reference item	100.0*	100.0	-

[1]: Corrected mortality according to Abbott and improvements by [redacted]
*: Significantly different from the control group (Bonferroni-U-Test, α = 0.05)

C. VALIDITY CRITERIA

Validity criterion	Required	Achieved
Control mortality	≤12.5%	0.0%
Reference item mortality	≥50%	100%

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Nominal Application Rate (g a.s./ha)

$LR_{50\ mortality}$	> 2930
$ER_{50\ reproduction}$	> 2930
NOEC	2930

III. CONCLUSION

Under worst-case conditions of exposure in the laboratory, spray treatments of EXP04209E at a rate of 5 L/ha (equivalent to 2930 g aclonifen/ha) had no significant effects on mortality or reproduction rate of adults of the parasitic wasp *Aphidius rhopalosiphi*.

[redacted] *(1999)*

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

Spray treatments of EXP04209E at a rate of 5 L/ha (equivalent to 2930 g aclonifen/ha) had no significant effects on mortality or reproduction rate of adults of the parasitic wasp *Aphidius rhopalosiphi*. Correspondingly, the $LR_{50\ mortality}$ and $ER_{50\ reproduction}$ of EXP04209E to *Aphidius rhopalosiphi* was estimated to be greater than 2930 g a.s./ha.

Assessment and conclusion by RMS:

Data Point:	KCP 10.3.2.1/02
Report Author:	[redacted]
Report Year:	1999
Report Title:	Effects of EXP04209E on the predatory mite Typhlodromus pyri Scheuten (Acari, Phytoseiidae) in the Laboratory
Report No:	R006155
Document No:	M-172210-01-1
Guideline(s) followed in study:	IOBC/WPRS 1988; Louis/Ufer improvements 1995
Deviations from current test guideline:	Current Guideline: IOBC/WPRS 1988 None
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of EXP 04209E (aclonifen, 586 g/L) on mortality of the predatory mite, *Typhlodromus pyri*, after 7 days of exposure according to [redacted] 1988 and [redacted] improvements 1995. Additionally, an assessment for significant sublethal effects (reproduction assessment) was made.

The predatory mites were exposed to a dose rate of 5 L EXP 04209E/ha (equivalent to 2930 g a.s./ha). Mites were exposed to dried spray residues on glass plates for 14 days. A toxic reference (Perfekthion (dimethoate a.s.)) was included with the test. Mortality was assessed after , 3, 7, 9, 11 and 14 days of exposure. Five replicates each containing 20 mites were used per treatment group.

After 7 days of exposure, mortalities in the control, the test item and the toxic standard groups were 15%, 98% and 80% respectively. Differences in mortality between the control and the test item group were statistically significant.
Reproduction was not evaluated due to the mortality of 98% in the test item group.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Item:** EXP 04209E
   Batch no.: OP980353
   Active Ingredient / Purity: Aclonifen, 586 g/L
   Appearance: Yellow liquid
   Storage: In original container, at room temperature, in the dark
   Expiry date: 07 May 2000

2. **Reference item:** Perfekthion EC
   Batch no.: 98-1
   Active Ingredient / Purity: Dimethoate, 396 g/L

3. **Test Organism:** Predatory mites (*Typhlodromus pyri* Scheuten)
   Age: Protonymphs, about 2 days old
   Source: [redacted]
   Acclimatisation: Under test conditions
   Feeding: A mixture of pine (*Pinus nigra*) and birch (*Betula* sp.) pollen (3:1) *ad libitum*

B. STUDY DESIGN AND METHODS

1. In-life phase: 12 April – 26 April 1999

2. Exposure conditions

Test vessels: *Test units:* formed by two cover slides (glass, 24 x 60 mm) fixed by gluing small cover slides (glass, 18x18 mm) to both side-ends with a barrier of sticky material (Tanglefoot) to keep the mites on this test arena.
Test container: plastic trays (1 1 x 1 1 x 6 cm) with a foam rubber and a glassplate on top covered by tissue paper, half filled with water, tissue paper in contact with the water.

Experimental design:	3 experimental groups: control (tap water), test item (5 L EXP 04209E/ha, equivalent to 2930 g aclonifen/ha) and toxic standard (dimethoate).
Replicates:	5 per treatment group
Loading:	20 individuals per unit
Temperature:	24 – 25.5 °C
Relative humidity:	76 – 78%
Photoperiod:	16 h light: 8 h dark
Light intensity:	640 – 2050 lux

3. Administration of the test item

Dose preparation

The test item was applied to an inert substrate (glass plate). Treatments were applied in a spray volume equivalent to 200 L water/ha. The spraying dilution was applied to the glass plates using laboratory-spraying equipment.

Test organism assignment and exposure

The encompassed 3 treatment groups (test item, control, reference item) with 5 replicates each containing 20 mites. The mites were exposed to dried residues on treated glass plates. Impartially selected mites were introduced with a fine brush following the spraying application. The duration of exposure was 2 weeks.

4. Measurements and observations

Survival of the mites was assessed after 1, 3, 7, 9, 11 and 14 days. Reproduction was not evaluated due to the mortality of 98% in the group treated with EXP 04209E.

5. Statistics/Data evaluation

Mortality on the treated and untreated series was compared with Dunnett-Test.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Analytical verification was not required.

B. BIOLOGICAL DATA

After 7 days of exposure, mortalities in the control, the test item and the toxic standard groups were 15%, 98% and 80%, respectively. Differences in mortality between the control and the test item group were statistically significant.

Reproduction was not evaluated due to the mortality of 98% in the test item group.

Table: Effects of EXP 04209E on mortality and reproduction of adult *Typhlodromus pyri* exposed to fresh dried residue in the laboratory

Nominal Application Rate (g a.s./ha)	Mortality[1] (%)	Corrected Mortality[2] (%)	Reproduction (eggs per female)
Control	15.0	-	N/A
2930	98.0*	97.65	N/A
Reference item	80.0*	-	-

[1]: 1 week after application
[2]: Corrected mortality according to Abbott and improvements by [redacted]
N/A: not evaluated due to the mortality of 98% in the group treated with EXP 04209E
*: Significantly different from the control group (Dunnett-Test, $\alpha = 0.05$)

C. VALIDITY CRITERIA

Validity criterion	Required	Achieved
Control mortality	≤20%	15.0%
Reference item mortality	≥50%	80.0%

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Nominal Application Rate (g a.s./ha)
$LR_{50\ mortality}$	<2930
95% confidence limits	-
NOEC	<2930

III. CONCLUSION

Under the worst-case conditions of exposure used in this study, spray applications of EXP 04209E at a rate of 5 L/ha (corresponding to 2930 g aclonifen/ha) had severe lethal effects on the predatory mite *Typhlodromus pyri*.

[redacted] *(1999)*

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

Spray applications of EXP 04209E at a rate of 5 L/ha (corresponding to 2930 g aclonifen/ha) had severe lethal effects on the predatory mite *Typhlodromus pyri*. Consequently, The $LR_{50\ mortality}$ of EXP 04209E to *T. pyri* was determined to be less than 2930 g a.s./ha. Due to the severe lethal effects observed in the study the $LR_{50\ reproduction}$ could not be determined.

Assessment and conclusion by RMS:

Data Point:	KCP 10.3.2.1/03
Report Author:	[redacted]
Report Year:	2003
Report Title:	Toxicity to the predatory mite Typhlodromus pyri Scheuten (Acari, Phytoseidae) in the laboratory Aclonifen water miscible suspension concentrate 600 g/L code: AE F068300 00 SC50 A203
Report No:	C032823
Document No:	M-232137-01-1
Guideline(s) followed in study:	ESCORT: [redacted] 2001; IOBC: [redacted] 2000
Deviations from current test guideline:	Current Guideline: IOBC (Blümel et al. 2000) None
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A rate-response study was conducted to determine the effect of AE F068300 00 SC50 A203 (aclonifen, 48.8%) on mortality of the predatory mite, *Typhlodromus pyri*, after 7 days of exposure according to IOBC ([redacted] 2000) and the recommendations of ESCORT 2 ([redacted] 2001). Additionally, an assessment for significant sublethal effects (reproduction assessment) was made.

The test item was applied at rates of 0.125; 0.25; 0.5; 1.0 and 4.0 L product/ha (equivalent to 75, 150, 300, 600 and 2400 g a.s./ha) and the effects were compared to a toxic reference (a.i.: dimethoate) applied at 3.0 g a.i./ha, and a water treated control.

Mortality of the nymphs was assessed 1, 3 and 7 days after exposure. The reproduction rate of the surviving mites was then evaluated over the period 7-14 days after treatment by counting the total number of offspring (eggs and larvae) produced.

The mortality / escaping rate in the control chambers up to day 7 after treatment was 5.0%. The mortality of the nymphs ranged from 30.3 to 90.8%.

Based on the results of this study the LD_{50} for AE F068300 00 SC50 A203 was 0.174 L product/ha (equivalent to 102 g a.s./ha). The mean offspring production was reduced by less than 50% compared to the control at the rates tested.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Item:** AE F068300 00 SC50 A203
   Batch no.: LOT V00403017
   Active Ingredient / Purity: Aclonifen, 48.8% w/w

	Expiry date:	23 April 2003
	Density:	1.207 g/mL
2.	**Reference item:**	AE F020846 00 EC37 A203 = BAS 152 11 I
	Batch no.:	99-1
	Active Ingredient / Purity:	Dimethoate, 417.5 g/L = 38.77% w/w
3.	**Test Organism:**	Predatory mites (*Typhlodromus pyri* Scheuten)
	Age:	Protonymphs, less than 5 days old
	Source:	[redacted]
	Acclimatisation:	Under test conditions
	Feeding:	A mixture of pine and birch pollen

B. STUDY DESIGN AND METHODS

1. In-life phase: 14 October – 11 November 2002

2. Exposure conditions

Test vessels:	One unit consisted of round cover glasses (diameter: 45 mm, thickness approx. 0.1mm) floating in petridish bottoms of glass with an outer diameter of 54 mm and an orifice in the middle, diameter 6 mm. Six of these petridishes are arranged in a stainless steel tray. The inner tray has 6 x 10 orifices, distributed uniformly on the basal area, with legs 13 mm in height. Distance bars (6 per petridish) of welding wire of stainless steel, diameter 1.5-2 mm, 140 mm in length are arranged, 2 between the bottoms of 2 petridishes and the inner tray. Silicone plugs are arranged in such a way (12 per inner tray) that 3 plugs enclose a petridish. The plugs are conical, diameter 15/20 mm and 25 mm in height. 3 or 4 inner trays are placed into a rectangular plastic outer tray (waterproof), e.g. internal dimensions 450 x 300 mm x 50 mm, with water outlet device (or draining by gravity over the edge of the dish) at the test location
Experimental design:	7 experimental groups: control (tap water), test item (0.125; 0.25; 0.5; 1.0 and 4.0 L product/ha) and toxic standard (dimethoate).
Replicates:	4 per treatment group
Loading:	20 individuals per unit
Temperature:	24 – 25.5 ºC
Relative humidity:	60 – 72%
Photoperiod:	16 h light: 8 h dark
Light intensity:	1750 - 2090 lux

3. Administration of the test item

Dose preparation
The test concentrations were prepared stepwise. Deionized water was used as diluent for the test and reference item. In accordance with the guideline the items were then applied in the equivalent of 200 L water/ha. The sprayer was calibrated beforehand to deliver 200 L/ha by spraying petridishes and weighing them immediately after in order to determine the actual volume of water applied. The measured application rate was 200 L/ha calculated on the basis of the mean value of 10 weighed petridishes.

The suspensions for the test and reference items were prepared on the day of application. They were applied to the test cover glasses using a sprayer. Prior to application, each glass was placed on a 60 mm plastic Petri dish lid and bottom with a small edge on the flat outside, labelled with a waterproof crayon and laid with the flat side upwards.

After the spray coating had dried, the cover glasses were moved from the treatment dishes into the corresponding petri dishes with the aid of a microscope needle. A small volume of deionized water was then poured slowly into the surrounding plastic vessel so that the glass arenas were lifted from the bottom and floated at approximately half the height of the dish edges.

Test organism assignment and exposure
After the test units were set up the protonymphs were placed onto the glass surface. The mites were transferred with a fine brush under a stereomicroscope and immediately afterwards examined to ensure they were undamaged and in good health. Then pollen (birch - pine mixture) was supplied as food and the units were kept under test conditions.

4. Measurements and observations
Day 1 and 3: The number of dead and living mites was counted. Dead mites were removed. The number of escaped mites was calculated.

Day 7.10 and 12: The number of dead and living mites was counted. Dead mites were removed. The number of escaped mites was calculated. The number of females, males, eggs and juveniles was counted. Males were added from another replicate from the same treatment if the sex ratio was more than 5 females : 1 male. Eggs and juveniles were removed.

Day 14: The number of dead and living stages was counted and dead animals removed.

5. Statistics/Data evaluation
Statistical methods were used to compare mortality between the control and treatment groups.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION
Analytical verification was not required.

B. BIOLOGICAL DATA

After 7 days of exposure, mortalities in the control, the test item and the toxic standard groups were 5.0%, 30.3 – 90.8% and 69.7% respectively. Based on these results the LD_{50} for AE F068300 00 SC50 A203 to predatory mites was 0.174 L product/ha (equivalent to 102 g a.s./ha).

The mean offspring production was reduced by less than 50% compared to the control at the rates tested.

Table: Effects of AE F068300 00 SC50 A203 residues on the survival and reproduction of *Typhlodromus pyri*

Treatment (L/ha)		Mortality (%)		Reproduction (%)	
		Uncorrected	Abbott	Rate	Rel. to control
Control	0.000	5.0	0	7.7	0
Test item	0.125	33.8	30.3	5.4	30.5
	0.250	63.8	61.8	5.1	33.8
	0.500	85.0	84.2	n.d.	n.d.
	1.000	86.3	85.5	n.d.	n.d.
	4.000	91.3	90.8	n.d.	n.d.
Reference item	0.006	71.3	69.7	n.d.	n.d.

n.d. = not determined

C. VALIDITY CRITERIA

Validity criterion	Required	Achieved
Control mortality	<20%	5.0%
Average number of eggs per female in the control group	>4	7.7

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Nominal Application Rate (g a.s./ha)
$LR_{50\ mortality}$	102
$ER_{50\ reproduction}$	>150
NOEC	74

III. CONCLUSION

Based on the results the LD_{50} for AE F068300 00 SC50 A203 to predatory mites was 0.174 L product/ha (equivalent to 102 g a.s./ha). The mean offspring production was reduced by less than 50% compared to the control at the rates tested.

[redacted] *(2003)*

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

The $LR_{50\ mortality}$ of AE F068300 00 SC50 A203 to *T. pyri* was determined to be 102 g a.s./ha.

The mean offspring production was reduced by less than 50% compared to the control at the rates tested and hence the $ER_{50\ reproduction}$ was considered to be greater than 150 g a.s./ha.

Assessment and conclusion by RMS:

Data Point:	KCP 10.3.2.1/04
Report Author:	[redacted]
Report Year:	1992
Report Title:	A study of the acute toxicity for aleochara bilineata (staphylinidae) of SAG 127 01 H
Report No:	R007268
Document No:	M-174575-01-1
Guideline(s) followed in study:	IOBC/WPRS (Samsoe-Petersen)
Deviations from current test guideline:	Current Guideline: Grimm et al., 2000 The test was performed over a 5-Day exposure period rather than the current requirement of 28 days
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

In the previous submission (DAR, 2006), this study was evaluated and accepted as valid for risk assessment purposes. The study was performed to an outdated test design with a 5-Day exposure period rather than a 28-Day exposure period as per the current test guideline ([redacted] 2000).

Therefore, as this study does not meet the requirements of the current guideline, it should be considered as supportive only and hence no summary for this study is provided.

Assessment and conclusion by RMS:

Data Point:	KCP 10.3.2.1/05
Report Author:	[redacted]; [redacted]
Report Year:	1992
Report Title:	A study of the acute toxicity for Poecilus cupreus (Carabidae) of SAG 127 01
Report No:	R007267
Document No:	M-174573-01-1
Guideline(s) followed in study:	BBA: VI 23-2.1.8
Deviations from current test guideline:	Current Guideline: BBA VI 23-2.1.8 None
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of SAG 127 01 H (aclonifen, 600 g/L) on mortality of the beetle, *Poecilus cupreus* (Carabidae), after 14 days of exposure according to BBA VI 23-2.1.8.

The test item, SAG 127 01 H, was sprayed in a green house on test vessels containing quartz sand. Adult beetles of the species Poecilus cupreus (L. 1758) were exposed to it in the laboratory for 14 days at a concentration corresponding to the highest recommended rate for a single field application of 5.5 L/ha. (equivalent to 3300 g a.s./ha).

Mortality was recorded 3 times on the first day and 1, 2, 4, 7, 11, and 14 days after application. Also recorded was the number of fly pupae consumed by each beetle in the test vessels in comparison to the control beetles.

At the end of the test no test animals were recorded as dead. No animals died in the controls. Behavioural changes of the beetles (e.g. coordination problems when walking) were not recorded.

On the average each beetle in the test vessels ate 3.69 fly pupae compared to 3.79 pupae in the control vessels. In total, the beetles in the test vessels ate 111 pupae in comparison to 114 pupae fed by the beetles in the control vessels.

In conclusion, under field conditions a spray treatment of 5.5 L/ha SAG 12701 H (equivalent to 3300 g a.s./ha) will not pose a risk on ground beetles as represented by *Poecilus cupreus*.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	SAG 12701 H
	Batch no.:	OP 880348

Active Ingredient / Purity:	Aclonifen, 600 g/L
Storage:	Dry and cool
Expiry date:	Not provided

2.	**Reference item:**	Afugan
	Batch no.:	07181669
	Active Ingredient / Purity:	Pyrazophos, 294 g/L

3.	**Test Organism:**	*Poecilus cupreus* (L. 1758), Kupferiger Schulterlaufer
	Age:	Adults, 4 weeks old
	Source:	[redacted]
	Acclimatisation:	2 days under test conditions
	Feeding:	*Calliphora* or *Musca* fly pupae

B. STUDY DESIGN AND METHODS

1. In-life phase: Not reported

2. Exposure conditions

Test vessels:	Plastic vessels of the following dimensions: 18.3 cm x 13,6 cm x 6 cm high. Each test vessel was filled with 250 g quartz sand (grain size 0.1 - 0.4 mm; 99.7% Silicium dioxide). In the center (width of edge: 1 cm) of the transparent covers of the vessels a hole was cut, covered by a coarse net (width of mesh: 2 mm).
Experimental design:	3 experimental groups: control (tap water), test item (5.5 L SAG 12701 H /ha, equivalent to 3300 g aclonifen/ha) and toxic standard
Replicates:	5 units per treatment group
Loading:	3 male and 3 female per replicate
Temperature:	21 ± 2 °C
Relative humidity:	35 - 45%
Photoperiod:	16 h light; 8 h dark
Light intensity:	500 - 1500 lux

3. Administration of the test item

Dose preparation

The test item was applied using the highest recommended rate for a single field application. The applied amount of water was 400 L/ha like required by the BBA. The amount of water applied to 1 cm^2 ground was 4 uL.

The test item was applied in a greenhouse at the [redacted] During the application of the test item the temperature was approximately 21 °C. Light intensity: 50 - 75 lux

Test organism assignment and exposure

Six beetles (3 males and 3 females) were placed in each test vessel. They were acclimatized to the test conditions for a period of two days during which time they were not fed. Shortly before the beginning of the test, the vessels were checked for injured animals by bringing up all beetles to the surface of the sand. All beetles showing any abnormalities were replaced by normal carabids of the same sex.

Thereafter, a soil moisture of approximately 70% of the maximum water holding capacity (= 4 6 mL water per 250 g quartz sand) was established in each test vessel. Then the carabids were fed with one fly pupa (generally slightly punctured, frozen *Musca*) per animal. Immediately afterwards the application process began.

4. Measurements and observations

Every two to three days (e.g. Monday, Wednesday, Friday) the beetles were fed with one fly pupa (slightly punctured) per surviving animal. On the same days the sand was watered to replace lost moisture.

Dead carabids were first removed after 6 h and afterwards at each assessment. If a beetle showed abnormal behaviour, e.g. lying on its back for prolonged periods or uncoordinated movements, it was laid in a corner of the test vessel. If it did not recover, it was removed at the next assessment (not before 24 h) and recorded as dead. Also, remnants of the fly pupae were removed. The number of pupae eaten by the carabids was recorded at every assessment. All these observations were made without disturbing up the sand, considering, that in almost all cases the beetles die on the surface of the test substrate. On the other hand, it is normal for *P. cupreus* to sit for days (sometimes even for one to two weeks) in self-dug holes. Only on the last (fifteenth) day of the test the sand was checked for hidden animals.

5. Statistics/Data evaluation

No statistical analysis of the generated data was performed.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Analytical verification was not required.

B. BIOLOGICAL DATA

At the end of the test no test animals were recorded as dead. No animals died in the controls. Behavioural changes of the beetles (e.g. coordination problems when walking) were not recorded.

On the average each beetle in the test vessels ate 3.69 fly pupae compared to 3.79 pupae in the control vessels. In total, the beetles in the test vessels ate 111 pupae in comparison to 114 pupae fed by the beetles in the control vessels.

In conclusion, under field conditions a spray treatment of 5.5 L/ha SAG 12701 H (equivalent to 3300 g a.s./ha) will not pose a risk on ground beetles as represented by *Poecilus cupreus*.

Table: Effects of SAG 127 01 H on *Poecilus cupreus* exposed to fresh dried residue in the laboratory

Nominal Application Rate (g a.s./ha)	Mortality after 14 days (%)	Feeding rate (Average no. of pupae/beetle)
Control	0	3.79
3300	0	3.69
Reference item	96.7	1.10

C. VALIDITY CRITERIA

Validity criterion	Required	Achieved
Control mortality	≤10%	0%
Reference item mortality	≥60%	96.7%
Number of fly pupae eaten per beetle per week (control)	>1.5	3.79

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Nominal Application Rate (g a.s./ha)
LR_{50} (mortality)	> 3300
NOEC	3300

III. CONCLUSION

Under field conditions a spray treatment of 5.5 L/ha SAG 12701 H (equivalent to 3300 g a.s./ha) will not pose a risk on ground beetles as represented by *Poecilus cupreus*.

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

SAG 127 01 H at 5.5 L/ha (equivalent to 3300 g a.s./ha) caused no lethal or sublethal effects to *Poecilus cupreus*. Correspondingly, the $LR_{50\ mortality}$ of SAG 127 01 H to *Poecilus cupreus* was estimated to be greater than 3300 g a.s./ha.

Assessment and conclusion by RMS:

Data Point:	KCP 10.3.2.1/06
Report Author:	[redacted]; [redacted]; [redacted]; [redacted]
Report Year:	1992
Report Title:	A study of the acute toxicity for pardosa sp. (spiders) of SAG 127 01
Report No:	R007269
Document No:	M-174577-01-1
Guideline(s) followed in study:	BBA (July 28, 1987)
Deviations from current test guideline:	Current Guideline: BBA VI, 23-2.1.8, 1991 None
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of SAG 127 01 (aclonifen, 600 g/L) on mortality of the spider, *Pardosa sp.* (wolf spiders), after 28 days of exposure according to BBA VI 23-2.1.8.

The test item, SAG 127 01 was sprayed in a green house on test vessels containing quartz sand and spiders. Adult spiders of the genus *Pardosa sp.* were exposed to it in the laboratory for 28 days at a concentration corresponding to the highest recommended rate for a single field application of 5.5 L/ha. (equivalent to 3300 g a.s./ha).

After the end of the normal test duration of 14 days 10 out of 24 animals sprayed with SAG 127 01 were counted as dead in the test containers (41.7%). Since 4 of these 10 spiders died within the second week the test was extended for another 14 days. After the fourth week in total 14 spiders died (58.3%).

In the control using water 1 animal died within the first 15 days (4.2%; additionally one spider was eaten by other spiders and therefore was not considered in the evaluation of the mortality). This amount did not exceed the value recommended by A. Wehling and U. Heimbach ([redacted] 1992, [redacted]) for a 14 day-test period (15%). After 28 days 4 spiders were recorded as dead (16.7%). Since no experience is available for an elongated test period it cannot be rated. It has to be mentioned that the water control was run two times (the second time two days after the first run) since probably due to an application error nearly all animals died within the first days in the first control. Only the data of the second run are reported.

Behavioural changes of the spiders (e.g. coordination problems) were recorded only on the first day after application of SAG 127 01. Some spiders had coordination problems when walking for some hours.

The evaluation of the feeding rate shows that on the average each spider sprayed with SAG 127 01 ate 2.96 Drosophila fruitflies compared to 3.66 fruitflies ate by spiders of the control.

In conclusion, mortality of *Pardosa sp*. was effected by 41.7% and 58.3% when exposed to SAG 127 01 at a rate of 5.5 L/ha (equivalent to 3300 g a.s./ha) for 2 and 4 weeks respectively.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	SAG 12701
	Batch no.:	OP 880348
	Active Ingredient / Purity:	Aclonifen, 600 g/L
	Storage:	Dry and cool
	Expiry date:	Not provided
2.	**Reference item:**	Karate
	Batch no.:	01202J
	Active Ingredient / Purity:	lambda-Cyhalothrin (50 g/L)
3.	**Test Organism:**	*Pardosa sp.*, wolf spiders
	Age:	Adults, 4 weeks old
	Source:	[redacted]
	Acclimatisation:	At least 10 days under test conditions
	Feeding:	Wingless *Drosophila melanogaster* (var. *vestigial*) fruitflies or flies of the species *Delia antiqua*

B. STUDY DESIGN AND METHODS

1. In-life phase: 29 June – 29 July 1992

2. Exposure conditions

Test vessels:	Plastic containers filled with moist sand (about 70% of the maximum water holding capacity). The walls of the test containers were smeared with Fluon on inner side before to avoid escaping of the spiders and their food (fruitflies)
Experimental design:	3 experimental groups: control (tap water), test item (5.5 L SAG 12701 /ha, equivalent to 3300 g aclonifen/ha) and toxic standard.
Replicates:	6 units per treatment group
Loading:	4 females per replicate
Temperature:	19 – 22.7 °C
Photoperiod:	16 h light: 8 h dark

3. Administration of the test item

Dose preparation

The test item was applied on the spiders using the highest recommended rate for a single field application of 5.5 L/ha of SAG 127 01. The applied amount of water was 400 L/ha. To avoid differences between test and field concentration of the test item, the concentration of the water - test item mixture in the plot sprayer was calculated according to the absolute amount of test item per hectare.

The test item was applied in a greenhouse at the Battelle-Institute, Frankfurt. During the application of the test item the temperature was approximately 26 °C.

Test organism assignment and exposure
Three days before beginning of the test the spiders were acclimatized in plastic containers filled with moist sand (about 70% of the maximum water holding capacity). The walls of the test containers were smeared with Fluon on inner side before to avoid escaping of the spiders and their food (fruitflies).

Due to the natural phenology of the spiders only few males occur in field. The same effect could be observed in the culture of Battelle. In agreement with the BBA only female spiders were used in this test.

Into each test container four females were placed. They were acclimatized to the test conditions for a period of three days during which time they were fed every day. Shortly before the beginning of the test, the containers were checked for injured animals or animals which cast their skin. All spiders showing any abnormalities were replaced by normal animals of the same sex.

4. Measurements and observations
Nearly every day spiders were fed with 5 fruitflies per surviving spider to avoid cannibalism. At the same time the mortality and the behavioural changes were recorded. The sand was watered to replace lost moisture if necessary.

Dead spiders were first removed after 24 h and afterwards at each assessment. If a spider showed abnormal behaviour, e.g. laying without movements, it was laid in a corner of the test container. If it did not recover, it was removed at the next assessment and recorded as dead. Also, not eaten (living and dead) fruitflies were removed. The number of fruitflies eaten by the spiders was calculated by counting the not eaten flies and was recorded at the assessments.

According to the BBA Guideline Part VI, 23-2.1.8 for testing carabids ([redacted] 1991) and the BBA Draft Guideline for testing spiders ([redacted] 1992) which was used as a base for this test the normal test duration is 14 days. Since more than two spiders died within the second week the test duration was extended for another 14 days.

5. Statistics/Data evaluation
No statistical analysis of the generated data was performed.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION
Analytical verification was not required.

B. BIOLOGICAL DATA

After the end of the normal test duration of 14 days 10 out of 24 animals sprayed with SAG 127 01 were counted as dead in the test containers (41.7%). Since 4 of these 10 spiders died within the second week the test was extended for another 14 days. After the fourth week in total 14 spiders died (58.3%).

In the control using water 1 animal died within the first 15 days (4.2%; additionally one spider was eaten by other spiders and therefore was not considered in the evaluation of the mortality). This amount did not exceed the value recommended by [redacted] ([redacted] 1992, [redacted]) for a 14 day-test period (15%). After 28 days 4 spiders were recorded as dead (16.7%). Since no experience is available for an elongated test period it cannot be rated. It has to be mentioned that the water control was run two times (the second time two days after the first run) since probably due to an application error nearly all animals died within the first days in the first control. Only the data of the second run are reported.

Behavioural changes of the spiders (e.g. coordination problems) were recorded only on the first day after application of SAG 127 01. Some spiders had coordination problems when walking for some hours.

The evaluation of the feeding rate shows that on the average each spider sprayed with SAG 127 01 ate 2.96 Drosophila fruitflies compared to 3.66 fruitflies ate by spiders of the control.

Behavioural changes of the spiders were observed only on the first day after application. Some spiders had co-ordination problems.

In conclusion, mortality of *Pardosa sp.* was effected by 41.7% and 58.3% when exposed to SAG 127 01 at a rate of 5.5 L/ha (equivalent to 3300 g a.s./ha) for 2 and 4 weeks respectively.

Table: Effects of SAG 127 01 H on *Pardosa sp*. exposed to fresh dried residue in the laboratory

Nominal Application Rate (g a.s./ha)	Mortality after 2 weeks (%)	Mortality after 4 weeks (%)	Feeding rate (Average no. of flies/spider)
Control	4.2	16.7	3.66
3300	41.7	58.3	2.96
Reference item	100	100	0

C. VALIDITY CRITERIA

Validity criterion	Achieved
Control mortality – Day 15	4.2%
Control mortality – end of test	16.7%
Number of fruitflies eaten per animal (control)	3.66

At the time of study performance, it was not possible to present scientifically based values for the above parameters. However, in the experience of the laboratory performing the study, these numbers appeared to be in the normal range that could be expected. Therefore, this study can be considered to be valid.

III. CONCLUSION

Mortality of *Pardosa sp*. was effected by 41.7% and 58.3% when exposed to SAG 127 01 at a rate of 5.5 L/ha (equivalent to 3300 g a.s./ha) for 2 and 4 weeks respectively.

Assessment and conclusion by applicant:

Validity criteria were considered to have been satisfied and therefore this study can be considered to be valid.

Mortality of *Pardosa sp*. was effected by 41.7% and 58.3% when exposed to SAG 127 01 at a rate of 5.5 L/ha (equivalent to 3300 g a.s./ha) for 2 and 4 weeks respectively.

Assessment and conclusion by RMS:

Data Point:	KCP 10.3.2.1/07
Report Author:	[redacted]
Report Year:	1999
Report Title:	Final Report - Effects of EXP04209E on the Lacewing Chrysoperla carhea Steph. (Neuroptera, Chrysopidae) in the Laboratory
Report No:	R008586
Document No:	M-177360-01-1
Guideline(s) followed in study:	IOBC/WPRS 1988; ring-test group (Vogt 1995, Vogt et al. in prep.)
Deviations from current test guideline:	Current Guideline: IOBC/WPRS 1988 None
Previous evaluation:	Yes, evaluated and accepted Source: DAR Vol. 3 B9 (9.5 table 9.5-7), August 2006 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of EXP04209E (aclonifen, 586 g/L) on mortality and reproductive efficiency of the lacewing, *Chrysoperla carnea*, after 13 days of exposure according to IOBC/WPRS 1988. The test item, EXP 04209E was applied at a rate of 1.23 L/ha (equivalent to 720 g a.s./ha).

Two larvae and two cocoons of the 30 larvae (13.3%, corrected mortality 3.7%) died during exposure in the test substance treated group. In the control group three cocoons of the 30 larvae (10.0%) died during exposure and 29 larvae of the 30 larvae (96.7%) in the toxic standard group. Surviving *C. carnea* produced 21.1 fertile eggs per female per day in the test substance treated group. In the control group surviving *C. carnea* produced 14.3 fertile eggs per female per day. No reproduction testing was performed in the toxic standard group due to the high mortality.

In conclusion, pre-imaginal mortality and reproduction of the lacewing *C. carnea* were not negatively affected by the maximum field rate of EXP 04209E of 1.23 L/ha (equivalent to 720 g a.s./ha).

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	EXP 04209E
	Batch no.:	OP980353
	Active Ingredient / Purity:	Aclonifen, 586 g/L
	Appearance:	Yellow liquid
	Storage:	In original container, at room temperature (+2 °C - + 30 °C), in the dark
	Expiry date:	07 May 2000
2.	**Reference item:**	Perfekthion EC (BAS 152 11 I)
	Batch no.:	98-1
	Active Ingredient / Purity:	Dimethoate, 396 g/L
3.	**Test Organism:**	*Chrysoperla carnea*, lacewings (Neuroptera: Chrysopidae)
	Age:	Approximately 2 days old larvae
	Source:	[redacted]
	Acclimatisation:	2-3 days under test conditions
	Feeding:	larvae: fresh *Sitotroga* eggs, *ad libitum* adults: artificial diet: 1 egg, 1 egg yolk, 15 mL condensed milk, 20 g fructose, 30 g honey, 30 g brewer's yeast, 50 g wheatgerm and aqua dest (approximately 45 mL), *ad libitum*

B. STUDY DESIGN AND METHODS

1. In-life phase: 02 April – 29 October 1999

2. Exposure conditions

Test vessels: *Exposure cages:* 1 treated glass plate (approximately 51 cm x 44 cm) covered with 1 acrylic glass plate (approximately 51 cm x 44 cm) with 30 holes and 30 acrylic glass cylinders treated with Fluon (1.5 cm high and 7.3 cm in diameter), cylinders

	tightly fit in the holes and guarantee a fixed position on glass plate *Post-exposure cages:* *hatching period:* plastic boxes (18.3 cm x 13.6 cm x 6 cm;length, width, height) *pre-oviposition and oviposition period:* an acrylic cylinder (15 cm high /10 cm in diameter) with a cotton net on the top for egg-laying and a hole (diameter approximately 1 - 2 cm) on the bottom to provide water through a cotton plug
Experimental design:	3 experimental groups: control, test item (1.23 L EXP 04209E /ha, equivalent to 720 g aclonifen/ha) and toxic standard.
Replicates:	exposure period: 30 units per treatment group oviposition period: 1 unit per treatment group
Loading:	exposure period: 1 per unit oviposition period: 26 per unit (control, test substance)
Temperature:	exposure: 20 - 30 °C post-exposure: 21 - 32 °C
Relative humidity:	exposure: 51 - 90% post-exposure: 38-93%
Photoperiod:	16 h light: 8 h dark
Light intensity:	exposure: 2360 - 5100 lux post-exposure: 3010-5120 lux

3. Administration of the test item

Dose preparation

The test item was applied to an inert substrate (glass plate). Treatments were applied in a spray volume equivalent to 200 L water/ha. The spraying dilution was applied to the glass plates using laboratory-spraying equipment.

Test organism assignment and exposure

The encompassed 3 treatment groups (test item, control, reference item) with 30 replicates each containing individual. The test organisms were exposed to dried residues on treated glass plates. Impartially selected larvae were introduced with a fine brush following the spraying application. The duration of exposure was 13 days until cocoons were transferred to petri dishes.

4. Measurements and observations

The number of living and dead larvae and number of cocoons formed were determined at least working daily after test start and number of adults hatched were checked regularly.

During the reproduction phase, the number of eggs counted after 24 hours egg-laying periods (checks), 8 checks in regular intervals within 4 week oviposition period; number of larvae was determined after hatching of all larvae and the hatching rate was calculated.

5. Statistics/Data evaluation

Mortality and reproduction were tested for normality and homogeneity using R/s-Test and Cochran-Test. Because mortality data were not normally distributed and homogenous, Bonferroni- U-Test (multiple comparison), α = 0.05, was used. Because reproduction data were normally distributed and homogenous, Student- T-Test (pairwise comparison), α = 0.05, was used.

The computer program used to perform the statistical analyses was EASY ASSAY Multiple Testing (Ratte, 1995).

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Analytical verification was not required.

B. BIOLOGICAL DATA

Two larvae and two cocoons of the 30 larvae (3.7% corrected mortality) died during exposure in the test substance treated group and surviving *C. carnea* produced 21.1 fertile eggs per female per day.

Larval and pupal viability were not affected by the rate of 1.23L /ha of EXP04209E (equivalent to 720 g a.s./ha).

Table: Effects of EXP 04209E on mortality and reproduction of adult *Chrysoperla carnea* exposed to fresh dried residue in the laboratory

Nominal Application Rate (g a.s./ha)	Mortality[1] (%)	Corrected Mortality[2] (%)	Reproduction (eggs / female / day)
Control	10.0	-	14.3
720	13.3	3.7	21.1
Reference item	96.7	96.3	-

[1]: 13 days after application
[2]: Corrected mortality according to Abbott and improvements by [redacted]

C. VALIDITY CRITERIA

Validity criterion	Required	Achieved
Control mortality	≤20%	10.0%
Reference item mortality	≥50%	96.7%

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Nominal Application Rate (g a.s./ha)
$LR_{50\ mortality}$	> 720
$ER_{50\ reproduction}$	> 720
NOEC	720

III. CONCLUSION

Larval and pupal viability were not affected by the rate of 1.23 L/ha of EXP04209E (equivalent to 720 g a.s./ha).

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

Larval and pupal viability were not affected by the rate of 1.23 L/ha of EXP04209E (equivalent to 720 g a.s./ha). Correspondingly, the $LR_{50\ mortality}$ and $ER_{50\ reproduction}$ of EXP 04209E to *Chrysoperla carnea* was estimated to be greater than 720 g a.s./ha.

Assessment and conclusion by RMS:

CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

Data Point:	KCP 10.3.2.2/01
Report Author:	[redacted]
Report Year:	2000
Report Title:	EXP04209E: An Extended Laboratory Study to Evaluate the Effects on the predaceous Mite Typhlodromus pyri Scheuten (Acari: Phytoseiidae)
Report No:	B002976
Document No:	M-238634-01-1
Guideline(s) followed in study:	-
Deviations from current test guideline:	Current Guideline: Bakker et al. 1992 Age of protonymphs is not exactly known, but is expected to be less than 24 hours
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of EXP04209E (aclonifen, 594 g/L) on mortality of the predatory mite, *Typhlodromus pyri*, after 7 days of exposure. Additionally, an assessment for significant sublethal effects (reproduction assessment) was made. No specific guideline existed for this test, but the choice of test organism and design of the test were in accordance with internationally acknowledged

SETAC Guidelines ([redacted] 1994). Assay procedures were based on methods described in [redacted] (1992) and [redacted] (1998), however cowpea leaves were used as a natural substrate.

The predatory mites were exposed to EXP04209E at nominal concentrations of 5 L product/ha, the recommended field rate, and 200 mL product/ha, representing a 4% drift rate, with a spray application volume of respectively 193 and 201 L/ha to excised cowpea (*Vigna sinensis*, L.) leaves. The control was treated with demineralised water. Dimethoate was used as toxic standard.

Typhlodromus pyri Scheuten was confined to the test item residues, kept in ventilated Munger cages, in 10 groups of 10 individuals in all treatments. Mortality was assessed after a 7-day exposure period. At this point the mortality of the toxic reference and the highest test product concentration were above 50%, so these tests were ended, therefore effects of fecundity were not determined. All surviving test animals of the water control and the 200 mL EXP04209E/ha were transferred to untreated glass plates. Reproduction success was determined during 7 days in total.

EXP 04209E applied at 5 L/ha (equivalent to 2970 g a.s./ha) resulted in a statistically significant mortality of 60% after Abbott's correction. When applied at a rate of 200 mL/ha (equivalent to 120 g a.s./ha), EXP 04209E resulted in a corrected mortality of 14% and a reduction in fecundity of 18%, both of which were not significant different from the control.

It is concluded that at a drift rate of 200 mL/ha (equivalent to 120 g a.s./ha), EXP 04209E is harmless to *Typhlodromus pyri*.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Item:** EXP04209E
   Batch no.: OP990851
   Active Ingredient / Purity: Aclonifen, 394 g/L
   Expiry date: 15 September 2001

2. **Reference item:** Brabant dimethoaat
   Batch no.: 00000
   Active Ingredient / Purity: Dimethoate 400 g/L (nominal)

3. **Test Organism:** Predatory mites (*Typhlodromus pyri*)
   Age: Protonymphs
   Acclimatisation: Under test conditions
   Feeding: Pollen of broad bean was collected in April 1999 from dismembered young flowers. The flowers were dried at 30 °C and the pollen was cleaned with the use of a sieve. The pollen can be stored in a refrigerator at 4 °C more than a year without loosing quality as food. Test organisms received food every 2 to 4 days during the entire experiment

B. STUDY DESIGN AND METHODS

1. In-life phase: 04 January – 18 January 2000

2. Exposure conditions

Test vessels: *Exposure phase:* Test units consisted of: (1) a top and bottom glass plate (length:10 cm, width: 5 cm and height: 0.2-0.3 cm), and (2) a Plexiglas plate (length: 10 cm, width: 5 cm and height: 1 cm) with a cylindrical hole (diameter approximately 3.5 cm). A treated, detached cowpea leaf, under-side facing upwards, was pressed between the bottom glass plate and the Plexiglas, such that the leaf covered the hole in the Plexiglas. A water moistened filter paper, about 2-4 cm longer than the glass plates, was kept under the leaf. The top glass plate had 2 holes (diameter about 0.6 cm). Two of them were used to connect the cells to a ventilation system and to administer food. The glass plate was positioned such that the two holes connecting the cell to the ventilation system were positioned above the cylindrical hole of the Plexiglas. The cell was assembled using clamps. All test units of one treatment group (replicates) were kept together in a plastic tray lined with wet cotton wool or water to keep the filter papers moistened.

Reproduction phase: Test units for determining effects on oviposition rates consisted of upturned glass Petri-dishes with a diameter of 9 cm, covered by a wet filter paper of the same size (barrier for the test animals) with a 3 cm (diameter) hole in the middle, which was the mite arena ([redacted] 1988) An additional barrier of glue was put around the glass arena (the opening in the paper) on the wet filter paper. The Petri-dishes rested on a holder (height about 2-3 cm) in a water tray. Strips of filter paper (about 1 cm wide) hung from the wet filter paper in the water tray to ensure that the filter paper remained moistened during the test.

Experimental design: 4 treatments: a water control, the test item at 2 rates and a toxic reference

Replicates: 10 per treatment group

Loading: 10 individuals per unit

Temperature: 24 - 25°C

Relative humidity: 55 - 70%

Light intensity: 200 - 1880 lux

3. Administration of the test item

Dose preparation

The test solutions were obtained by dispersing 6.25 mL product up to a total volume of 250 mL solvent and 250 mL up to a total volume of 250 mL. With a formulated concentration of 594 g a.s./L and actual application volumes of 193 L/ha and 201 L/ha, respectively, the actual test rates were 2866.1 g a.s./ha and 119.4 g a.s./ha, respectively.

The test solutions were applied to the underside of the cowpea leaves using calibrated laboratory spraying equipment, i.e. a Schachtner laboratory track sprayer, which produced a very fine homogeneous spray deposit that was documented by a water sensitive paper with demineralised water during application.

Test organism assignment and exposure

After drying of the residues and assembling of the units 10 protonymphs were introduced into each of the 10 cells for all treatments. The sequence of entering test animals into test cages was such that treatments alternated (e.g. water, test product at different concentrations, toxic standard, water etc.). Three days after exposure food was added to all test units and water was added to the set-up.

4. Measurements and observations

Mortality was assessed seven days after exposure. The sex of the surviving individuals was determined and eggs were counted. The toxic standard treatment and the EXP04209E 5 L/ha were stopped at this point, because more than 50% mortality was observed. Test animals of treatments causing less than 50% mortality (i.e. water and EXP04209E at 200 mL/ha) were transferred to oviposition units.).

The fecundity phase started on day 7 after exposure. The animals from 2 exposure unit were combined into 1 oviposition unit. In two units of the water treatment, males from an other water treatment unit were added, to accomplish a 1:5 male:female sex-ratio. Food was added to all units. The number of eggs and young juveniles retained in the exposure units was counted. After 3, 6 and 7 days the number of surviving females, males, eggs and (young) juveniles were counted again. On these occasions except at the last inspection, eggs and young juveniles were removed and animals were fed pollen of *Vicia faba* L.

5. Statistics/Data evaluation

Juvenile mortality was compared pairwise to controls using Fisher's exact test ([redacted] 1992). The replication chosen should ensure a minimum power of 80% ([redacted] 1997) to detect differences of 30%.

Effects on fecundity (total number of eggs per female) were analyzed in a one-way ANOVA, with treatment as a grouping factor. ANOVA assumptions, homogeneity of group variances and normality of residuals, were tested with Bartlett's test and Lilliefors' test, respectively. Outliers, defined as observations with a chance of occurring lower than 1% (P < 0.01), were identified by referencing studentized residuals against a t-distribution. No outliers were found.

Statistically significant differences were considered at the 5% level (alpha=0.05). Systat 5.2 for the Macintosh was used for all statistical analyses.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Analytical verification was not required.

B. BIOLOGICAL DATA

Concentrations of EXP04209E caused corrected mortality of 14% at the 200 mL product/ha treatment and 60% at the 5 L product/ha treatment. Test animals exposed to both concentrations showed a difference in development: the percentage of juvenile survivors on day 7 were 12% and 59%,

respectively, whereas in the water control 9% juveniles were observed at this point. Mortality and development in EXP04209E treatment 200 mL product/ha were not significantly different (P=0.144 and P=0.592, respectively, Fisher's exact test) from the water control, whereas the mortality and development in the EXP04209E treatment 5 L product/ha were significantly different from the water control treatment (P<0.001 and P=0.011, respectively, Fisher's exact test).

Mortality in the toxic standard (corrected for control mortality) was 90%, showing that test animals were sufficiently sensitive and that potential adverse effects of exposure to test substance residues could be detected with the set-up used in this experiment.

Reproductive performance in the control group was 8.7 eggs/female and in accordance with the validity criteria (mean total fecundity above 4 eggs per female). For the EXP04209E 200 mL/ha treatment this was 7.2 eggs per female. The difference of 18% was not significantly different from the water control (p=0.537, ANOVA). Anova assumptions were met and no outliers were identified.

Table: Effects of EXP 04209E on mortality and reproduction of adult *Typhlodromus pyri* exposed to fresh dried residue in an extended laboratory study

Nominal application rate (mL product/ha)	Nominal application rate (g a.s/ha)	Mortality at 7 DAT[1] (%)	Corrected mortality at 7 DAT[2] (%)	Reproduction 7-14 DAT (eggs per female)	Effect on reproduction (%)
Control	-	20	-	8.70	-
200	120	31	14	7.17	18
5000	2970	68	60	-	-
Reference item	2.2	92	90	-	-

[1]: DAT (Days After Treatment)
[2]: Corrected mortality according to Abbott

C. VALIDITY CRITERIA

Validity criterion	Required	Achieved
Control mortality	≤20%	20%
Reference item mortality	≥50%	92%
Control reproduction	>4 eggs per female	8.70

All validity criteria were satisfied and therefore this study can be considered to be valid.

III. CONCLUSION

EXP 04209E applied at 5 L/ha (equivalent to 2970 g a.s./ha) resulted in a statistically significant mortality of 60% after Abbott's correction. When applied at a rate of 200 mL/ha (equivalent to 120 g a.s./ha), EXP 04209E resulted in a corrected mortality of 14% and a reduction in fecundity of 18%, both of which were not significant different from the control.

It is concluded that at a drift rate of 200 mL/ha (equivalent to 120 g a.s./ha), EXP 04209E is harmless to *Typhlodromus pyri*.

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

Under laboratory conditions, EXP 04209E applied at 5 L/ha (equivalent to 2970 g a.s./ha) resulted in a statistically significant mortality of 60%.

When applied at a rate of 200 mL/ha (equivalent to 120 g a.s./ha), EXP 04209E resulted in a corrected mortality of 14% and a reduction in fecundity of 18%, both of which were not significant different from the control.

It is concluded that at a drift rate of 200 mL/ha (equivalent to 120 g a.s./ha), EXP 04209E is harmless to *Typhlodromus pyri*.

Assessment and conclusion by RMS:

Data Point:	KCP 10.3.2.2/02
Report Author:	[redacted]
Report Year:	2000
Report Title:	Effects of EXP04209E on the wolf spider Pardosa sp (Aranеae, Lycosidae) in the Laboratory - Extended Laboratory Study
Report No:	B002997
Document No:	M-238654-01-1
Guideline(s) followed in study:	BBA: VI, 23-2.1.9 (1994) Draft
Deviations from current test guideline:	Current Guideline: BBA VI, 23-2.1.9 (1994) Draft A natural soil (LUFA 2.1) was used instead of quartz sand as the substrate. The time interval of checks for mortality, sublethal effects and food consumption were slightly changed. Deionized water rather than tap water was used as the test vehicle. Acceptable control mortality was reduced from 10% to 8.8%. The acclimatisation period was 3 days before the start of the experiment rather than 7 days. The above deviations were considered not to have had any adverse scientific effect on the outcome of the study.
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of EXP04209E (aclonifen, 594 g/L) on mortality of the spider, *Pardosa sp.* (wolf spiders), after 14 days of exposure according to BBA VI 23-2.1.9.

Under laboratory conditions *Pardosa spec*. (34 spiders per treatment group) were sprayed with the maximum field rate (5 L/ha) and 4% of the maximum field rate (drift rate) (0.2 L/ha) in 400 L water/ha (equivalent to 2970 and 120 g a.s./ha respectively). Natural soil (LUFA 2.1) was used as a substrate.

Endpoints were mortality and food consumption of the survivors. The control animals were sprayed with deionized water and Perfekthion (900 g Dimethoate in 400 L water/ha) was used as a toxic standard.

None of the 34 spiders died after a 14 days exposure to the maximum field rate (5 L/ha) and 4% maximum field rate (0.2 L/ha) of EXP04209E on natural soil (LUFA 2.1). In the control group none of the 34 spiders died by the end of the experiment. All spiders died after application of 900 g Dimethoate/ha which served as a toxic standard. No adverse effect of EXP04209E on food consumption on spiders *Pardosa spec*, occurred either in the maximum or the 4% maximum field rate.

In conclusion, EXP04209E in the maximum and 4% maximum field rate (equivalent to 2970 and 120 g a.s./ha respectively) is harmless to wolf spiders *Pardosa spec.* if exposed to natural soil (LUFA 2.1).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: EXP04209E
Batch no.: OP990851
Active Ingredient / Purity: Aclonifen, 594 g/L
Appearance: Luminous yellow opaque liquid
Storage: In original container, at room temperature (+2 to 30 °C), in the dark
Expiry date: 15 September 2001

2. Reference item: Perfekthion EC (BAS 152 11 I)
Batch no.: 98-1
Active Ingredient / Purity: Dimethoate, 395.7 g/L

3. Test Organisms: *Pardosa sp.*, wolf spiders
the following species composition was found after the determination at the end of the experiment: *Pardosa prativaga* (67.4%), *Pardosa palustris* (2.2%), *Pardosa amentata* (24.4%), *Pardosa pullata* (5.9%)
Age: Adults
Source: [redacted]

Acclimatisation: 3 days before test start under room temperature
Feeding: With alive flies *Drosophila spec, ad libitum* during the pre-experimental time of keeping and with deep frozen *Drosophila spec*, at day 0, 1, 2, 3, 7 and 10 after application at a rate of 5 flies per spider; no feeding during acclimatisation

B. STUDY DESIGN AND METHODS

1. In-life phase: 10 April – 29 May 2000

2. Exposure conditions

Test vessels:	Plastic boxes (11.5cm x 11.5cm x 6cm; length, width, height); containing a layer of about 1 cm dry natural soil (LUFA 2.1) (125 ± 1 g dry soil) were moistened at the beginning to about 55% ± 5 of its maximum water holding capacity (= ca. 30%); (corresponding to 20 g deionized water per test unit), the upper part of the boxes was painted with Fluon to impede the spiders and the flies from escaping, a plastic lid was cut out up to approx. 1 cm to the edges and covered again by a net (mesh size about 2 mm), during application the walls of the boxes were protected with an inlet.
Experimental design:	4 experimental groups: control (deionized water), test item (5 L/ha and 0.2 L/ha) and toxic standard.
Replicates:	34 units per treatment group
Loading:	1 per replicate, 17 females and 17 males per treatment group
Temperature:	19 – 22 °C
Relative humidity:	40 – 70%
Photoperiod:	16 h light: 8 h dark
Light intensity:	820 – 1270 lux

3. Administration of the test item

Dose preparation

The test item was applied on the spiders using the highest recommended rate for a single field application of 5 L/ha of EXP04209E and 4% maximum field rate (0.2 L/ha). The applied amount of water was 400 L/ha.

The test item was applied in a singular application onto the soil of the trays and the spiders according to agricultural practice.

Test organism assignment and exposure

The test organisms were introduced 3 days before application in the readily prepared test units, using a glass tube. Selection of the spiders was impartially performed.

4. Measurements and observations

The number of living and dead spiders were counted at day 0 (ca. 2 hours after application), 1, 2, 3, 4, 7, 10 and 14 after application. Damaged spiders were placed at one corner of the trays and were counted as dead, if they were still there 24 hours later.

The number of damaged spiders (e.g. uncoordinated movements, crookedness, drawing up the legs) counted at day 0 (ca. 2 hours after application), 1, 2, 3, 4, 7, 10 and 14 after application.

The number of flies consumed or untouched (sucked out and kneaded to a lump), missing flies at day 1, 2, 3, 4, 8 and 11 after application are denoted consumed; untouched flies were removed and replaced for fresh ones.

5. Statistics/Data evaluation

Mean food consumption per living spider was listed per sex and box for each treatment group and assessment date; mortality was calculated as the sum of the recorded separate mortalities. Correction of mortality in the test item and toxic standard groups according to [redacted]1947, was not necessary due to absence of control mortality.

No statistical analysis of the generated data was performed.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Analytical verification was not required.

B. BIOLOGICAL DATA

None of the spiders in the control or test item treated groups died after 14 days exposure. All spiders in the toxic standard group had died by the end of the test.

There were no test item related behavioural abnormalities.
Slightly enhanced food consumption was found in both test item groups as compared to the control.

Table: Effects of EXP 042096E on *Pardosa sp.* exposed to fresh dried residue in an extended laboratory test

Nominal Application Rate (g a.s./ha)	Mortality after 2 weeks (%)	Feeding rate (Average no. of flies/spider)
Control	0.0	2.5
2970	0.0	2.7
120	0.0	2.6
Reference item	100	0.4

C. VALIDITY CRITERIA

Validity criterion	Required	Achieved
Control mortality	≤8.8%	0.0%
Toxic standard mortality	65% ± 35%	100%

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Nominal Application Rate (g a.s./ha)

$LR_{50\ mortality}$	> 2970
NOEC	2970

III. CONCLUSION

No influence of the test item was observed for both test item groups, the max. field rate and the 4% drift rate group (equivalent to 2970 and 120 g a.s./ha respectively). No adverse effects of the test item could be found on food consumption.

EXP04209E is not toxic to spiders of the genus *Pardosa spec*, if natural soil is used as a substrate.

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

The test item had no adverse influence on mortality or food consumption of spiders of the genus *Pardosa spec* when applied at rate of 5 L/ha and 0.2 L/ha ((equivalent to 2970 and 120 g a.s./ha respectively). Correspondingly, the $LR_{50\ mortality}$ of EXP04209E to *Pardosa spec* was estimated to be greater than 2970 g a.s./ha.

Assessment and conclusion by RMS:

Data Point:	KCP 10.3.2.2/03
Report Author:	[redacted]
Report Year:	2003
Report Title:	Acute dose-response (LR5) of AE F068300 00 SC50 A204 to the green lacewing Chrysoperla carnea (Steph.) under extended laboratory conditions
Report No:	C036655
Document No:	M-221161-01-1
Guideline(s) followed in study:	IOBC: [redacted] 2000
Deviations from current test guideline:	Current Guideline: IOBC guideline (2000) Adaptation to the extended laboratory test. Short-term deviations of temperature and relative humidity. These deviations were considered not to have had any adverse scientific effect on the outcome of the study.
Previous evaluation:	yes, evaluated and accepted Source: DAR, Vol. 3 B9 (9.5 table 9.5-8), August 2006 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of AE F068300 00 SC40 A204 (aclonifen, 591 g/L) on mortality and reproductive efficiency of the lacewing, *Chrysoperla carnea*, after 28 days of exposure. The test item was applied at rates of 250, 500, 1000, 2000 and 4000 mL product/ha in 200 L deionised water/ha on excised bean leaves. The control was treated with deionised water (200 L/ha). Dimethoate EC 400 (30 mL product/ha in 200 L water/ha) was used as toxic reference treatment.

Larvae of *Chrysoperla carnea* were exposed in 40 replicates of 1 larva (per treatment group) to the residues of the test item, reference item and control. During the assessments the larvae were fed with UV-sterilised eggs of *Sitotroga cerealella*. The number of surviving larvae and hatched adults, and the number of eggs laid and larvae hatched (F1) were recorded over a period of 41 days. From these data the endpoints mortality and fecundity as well as the dose-response relationship in regard to mortality (LR_{50}) were calculated where applicable.

In all test item groups there were no statistically significant differences compared to the control in mortality and reproduction. The toxic reference treatment resulted in 82.9% corrected mortality.

There was no or low mortality in all test treatment groups. A calculation of the LR_{50} was therefore not possible. The LR_{50} is estimated to be above the highest tested concentration of the test item, 4000 mL product/ha.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Item:** AE F068300 00 SC50 A204
   Batch no.: OP220331
   Active Ingredient / Purity: Aclonifen, 591 g/L
   Appearance: Luminous yellow opaque liquid
   Storage: Room temperature (between +2 and +30 °C), dark and dry
   Expiry date: 17 January 2005

2. **Reference item:** Dimethoate EC 400
   Batch no.: 2002-1
   Active Ingredient / Purity: Dimethoate, 400 g/L

3. **Test Organism:** *Chrysoperla carnea*, lacewings (Neuroptera: Chrysopidae)
   Age: 2 - 3 days old larvae
   Source: [redacted]
   Acclimatisation: 2-3 days under test conditions
   Feeding: larvae: fresh *Sitotroga* eggs

adults: artificial diet: 1 egg, 1 egg yolk, 15 mL condensed milk, 20 g fructose, 30 g honey, 30 g brewer's yeast, 50 g wheatgerm and 45 mL deionized water

B. STUDY DESIGN AND METHODS

1. In-life phase: 06 May – 16 June 2003

2. Exposure conditions

Test vessels: *Exposure cages:* glass cylinder (4 cm diameter, 4 cm high) with gauze cover, with a treated bean leaf on moistened filter paper as bottom, fixed to a glass plate and an acrylic plate (both 25 cm x 25 cm and untreated)
Post-exposure cages:
Oviposition cage 3-l-glass beaker (28 cm high, 14 cm diameter) covered with cotton gauze during egg laying
Hatching cage: plastic cage (Bellaplast) with a clear cover

Experimental design: 3 experimental groups: control, test item (250, 500, 1000, 2000 and 4000 mL product/ha) and toxic standard.

Replicates: exposure period: 40 units per treatment group
oviposition period: 1 unit per treatment group

Loading: exposure period: 1 per unit
oviposition period: 29 - 31 per unit (control, test item)

Temperature: 21 - 38 °C

Relative humidity: 68-93%

Photoperiod: 16 h light: 8 h dark

Light intensity: 2200 lux

3. Administration of the test item

Dose preparation

The required amounts of test item were mixed with deionized water without the addition of solubility mediators, immediately before application. The spray liquids were applied once at a rate of 200 L/ha on excised leaves using an automatic application cabin to ensure a standard high level of uniform deposit (200 L/ha = 2 mg/cm^2, ± 10%). The spray cabin was calibrated before use after adjusting the application speed or spray pressure. The amount of test solution per area was checked by weighing four glass plates (4.9 cm x 4.9 cm) placed at representative spots of the application cabin (levelled with the test leaves) before and immediately after application. These glass plates were used to determine the accuracy of the application only.

Test organism assignment and exposure

In the exposure phase of the test, undamaged primary leaves from the kidney bean (*Phaseolus vulgaris*), with a diameter of about 4 cm (cultivated under laboratory conditions), were used as substrate. Only the most vital leaves were used and cut in a short time before application.

After air drying of the spray deposits (at room temperature for about 1 hour) the leaves were laid, with the treated side upward, on moistened filter paper on glass plates. Acrylic plates with drill holes (4.2 cm diameter) were placed on top of the glass plates with the leaves of the different treatment groups. Glass cylinders (4 cm diameter, 4 cm high) were then fitted into the holes over the treated leaves as confinements for the green lacewing larvae during the test. One impartially selected larva (2-3 days old) and a small quantity UVsterilized eggs of *Sitotroga cerealella* were transferred to each confinement. The inner walls of the glass cylinders were coated with Fluon® to prevent green lacewing larvae from climbing, thus warranting full exposure to the dried spray deposits over the entire test period. The test units were placed in a well-ventilated climatic room.

4. Measurements and observations
Mortality was recorded for larvae and pupae and was summed up for an overall mortality until hatch of the adults.

The reproductive performance of the lacewings was assessed for the control and the test item treatment groups, in which > 50% of the larvae exposed to the test item survived and successfully completed their metamorphosis. The reproduction phase was started with adults from a treatment group hatched within a period of up to seven days and without deformations. These adults were sexed and put together in one oviposition cage The oviposition started about one week after the first egg laying has been observed because the last hatched adults need some days to mature (pre-oviposition period).

The egg samples were taken twice over a one-week period. Each sample covered an egg laying period of 24 hours, i.e. the oviposition cages were covered with new cotton gauze for 24 hours. Eggs which were laid on the walls of oviposition cage, were counted as well. The number of eggs was counted after renewal of the gauze. After 2-3 days of incubation of the eggs on the gauze in a hatching box, food (*Sitotroga cerealella* eggs) was added. The hatched larvae were counted after 3-4 days.

5. Statistics/Data evaluation
Mortality (total number of dead larvae and pupae) was calculated in% for each treatment group. The corrected mortality (M-value) in the treatment groups was calculated according to [redacted] (1925).

The average number of eggs laid per female per day was determined by dividing the total number of eggs laid by the average number of viable females in that group (corrected for mortality during egg laying).

For statistical calculation of the results the computer programs EASY ASSAY, Multiple Testing and Critical Values ([redacted] 1998 as well as ToxRatPro) were used. For statistical calculation of mortality the Fisher's Exact Binominal Test was used. The significance level was $p < 0.05$.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION
Analytical verification was not required.

B. BIOLOGICAL DATA

The results of the control group indicated that the test organisms were in a good condition (mortality: 12.5%, 21.1 eggs per female/day and 81% mean hatching rate). The results of the toxic standard group indicated that the test system was sensitive to harmful substances (corrected mortality: 82.9%).

Regarding mortality and reproduction (mean number of eggs/female) there were no statistically significant differences in all test item groups compared to the control group.

Because of no or low mortality in all test item treatment groups, a calculation of the LR_{50} was not possible. The LR_{50} has to be regarded above the highest tested concentration of the test item, 4000 mL product/ha.

No abnormalities regarding larvae or hatched adults were observed in any treatment group during the test.

Table: Effects of AE F068300 00 SC50 A204 on mortality and reproduction of adult *Chrysoperla carnea* exposed to fresh dried residue in an extended laboratory test

Nominal Application Rate (mL./ha)	Mortality[1] (%)	Corrected Mortality[2] (%)	Reproduction (eggs / female / day)	Hatching rate (%)
Control	12.5	-	21.1	81
250	12.5	0	20.9	79
500	12.5	0	22.0	81
1000	15.0	2.9	21.0	81
2000	15.0	2.9	22.8	79
4000	20.0	8.6	21.6	78
Reference item	85	82.9	-	-

[1]: 28 days after application
[2]: Corrected mortality according to Abbott

C. VALIDITY CRITERIA

Validity criterion	Required	Achieved
Control mortality	≤20%	12.5%
Number of eggs / female / day	≥15	21.1
Mean hatching rate	≥70%	81%
Reference item mortality	>50%	85%

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Nominal Application Rate (mL/ha)
$LR_{50\ mortality}$	>4000
$ER_{50\ reproduction}$	>4000

NOEC	4000

III. CONCLUSION

There was no or low mortality in all test treatment groups. A calculation of the LR_{50} was not possible. The LR_{50} is estimated to be above the highest tested concentration of the test item, 4000 mL product/ha.

[redacted] (2003)

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

Larval and pupal viability were not affected by the rate of 4000 mL/ha of AE F068300 00 SC50 A204 (equivalent to 2364 g a.s./ha). Correspondingly, the $LR_{50\ mortality}$ and $ER_{50\ reproduction}$ of AE F068300 00 SC50 A204 to *Chrysoperla carnea* in an extended laboratory test was estimated to be greater than 2364 g a.s./ha.

Assessment and conclusion by RMS:

Data Point:	KCP 10.3.2.2/04
Report Author:	[redacted]
Report Year:	2016
Report Title:	Effects on the reproduction of rove beetles Aleochara bilineata in an extended laboratory study - Aclonifen SC 600 g/L - Final report
Report No:	98811071
Document No:	M-561614-01-1
Guideline(s) followed in study:	GRIMM ET AL. 2000; US EPA OCSPP not applicable
Deviations from current test guideline:	Current guideline: Grimm et al. (2000) No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of aclonifen, 600 g/L) on the reproduction of staphylinid rove beetles (*Aleochara bilineata*) exposured via treated natural soil LUFA 2.1.

The test item, the control and the reference item were sprayed via laboratory spray applicator on the soil surface at a water amount of 400 L water/ha. The test item rates were 240, 427, 759, 1350 and 2400 g a.s./ha in 400 L water/ha. The beetles were introduced into the exposure units immediately after treatment. Each replicate contained 10 female and 10 male beetles and 4 replicates per treatment. The

beetles were exposed to control, test and reference items for 28 days. On day 7, 14, and 21 approx. 500 pupae of *Delia antiqua* were buried into the soil of each replicate to be parasitized by the larvae of the beetles. On day 28 the adults were separated from the soil and the soil with the pupae was allowed to dry for seven days. On day 35 the pupae were washed out of the natural soil and transferred into an emergence container. The emergence of the F1-generation of beetles was observed from day 37 - 82 and the effect on reproduction of *Aleochara bilineata* was assessed.

The ER_{50} was estimated to be above the highest test concentration, ≥ 2400 g a.s./ha. The NOER (no observed effect rate) for reproduction was ≥ 2400 g a.s./ha.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	Aclonifen SC 600 g/L
	Batch no.:	EV56005993
	Active Ingredient / Purity:	Aclonifen, 596.7 g/L (49.5% w/w)
	Appearance:	Yellow liquid
	Storage:	Room temperature (between +2 and +30 °C), dark
	Expiry date:	9 February 2017
2.	**Reference item:**	Dimethoate EC 400 g/L
	Batch no.:	FRE-001226
	Active Ingredient / Purity:	Dimethoate, 420.3 g/L (analytical)
3.	**Test Organism:**	*Aleochara bilineata* Gyll. Rove beetles (Coleoptera: Staphylinidae) Host organism: *Delia antiqua* Meig. pupae (Diptera, Anthomyiidae
	Age:	3 – 5 days old adult
	Source:	[redacted]
	Acclimatisation:	3 – 5 days under test conditions After arrival at the test facility the parasitized fly pupae containing the test beetles were separated with emerged beetles in a glass beaker and the pupae on the separating funnel. Emerged beetles were counted and separated daily so that cohorts of discrete aged test beetles were available. After counting they were held in plastic boxes on moistened tissue paper under test conditions; food was added *ad libitum* One day before test start the sex of the beetles was determined by observing the copulating pairs. Until introduction they were held in glass beakers on moistened tissue paper and were fed *ad libitum*; each glass beaker contained 10 male and 10 female beetles
	Feeding:	adults: *Delia antiqua* larvae

A. STUDY DESIGN AND METHODS

1. In-life phase: 17 February to 9 May 2016

2. Exposure conditions

Test vessels:	Plastic boxes (18.3 cm x 13.6 cm x 6 cm; length, width, height), covered with perforated plastic lids, filled with soil (600 mL LUFA 2.1 soil), moistened to 35 ± 5% of its maximum water holding capacity with deionised water. Moistened soil was approximately 4 cm deep and soil surface area was 190 cm². During application of the test and reference item the walls of the exposure units were protected with a plastic inlet to avoid an increase of the concentration of the spray liquid on the soil by run-off from the walls. The inlet was removed after application. The natural soil substrate, represents a worst-case scenario with a high proportion of sand and a low content of organic matter
Experimental design:	3 experimental groups: control, test item (250, 500, 1000, 2000 and 4000 mL product/ha) and toxic standard.
Replicates:	4 replicates 20 per replicate (10 male + 10 female)
Temperature:	Acclimatisation: 19-21°C Exposure: 19-22°C Post-exposure: 19-22°C
Relative humidity:	Acclimatisation: 67 – 74% Exposure: 66 – 75% Post-exposure: 67 – 76%
Photoperiod:	16 h light: 8 h dark
Light intensity:	Acclimatisation: 460 – 560 lux Exposure: 670 – 950 lux Post-exposure: 540 – 950 lux%

3. Administration of the test item

Dose preparation

The required amounts of test item were mixed with deionized water without the addition of solubility mediators, immediately before application. The test item was applied as a single application into test units filled with the soil. The spray liquids were applied once at a rate of 400 L/ha. The spray equipment was calibrated before use. deviation in the spray deposit did not exceed ± 10% of the target rate (400 L/ha) in a run of 5 repetitions without changing the adjustment.

Test organism assignment and exposure

10 pairs of beetles (max. 4 days old) were introduced into each test unit within the first hour following application of the test item.

Once a week *ca.* 500 *Delia antiqua* pupae per container were added (days 7, 14 and 21 after application). The number of pupae was estimated by weight: at the beginning, 500 pupae were counted and weighed; the established weight of the 500 pupae was used to introduce the pupae into the test units. The pupae were carefully mixed into the soil (depth *ca.* 2-3 cm) and homogeneously distributed within the test unit so that they were completely covered with the substrate.

The adult test organisms were exposed to the test item for 28 days. After 28 days all surviving adult beetles were removed from the substrate. The substrate and the parasitized onion fly pupae were returned to the controlled environment room in the original test units with a perforated lid for one further week and the substrate allowed drying.

After 35 days the pupae were washed out of the soil and the pupae of each replicate were transferred into a separate emergence container. Emerging beetles were counted and removed from the emergence containers at least 3 times per week; emergence of the F1-generation was monitored until the control treatment fell below a rate of two beetles per replicate per day.

4. Measurements and observations

The reproduction efficiency was assessed by counting the total number of beetles emerged from the offered fly pupae until the emerging of the F1-generation was finished.

5. Statistics/Data evaluation

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk`s test ($\alpha = 0.05$) and Levene`s test ($\alpha = 0.05$). Reproduction data were normally distributed and homogenous, therefore, Dunnett`s multiple t-test, one-sided smaller, $\alpha = 0.05$, was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH .

II. RESULTS AND DISCUSSION

A: ANALYTICAL VERIFICATION

Analytical verification was not required.

B: BIOLOGICAL DATA

The data for reproduction from each test treatment and replicate are presented below:

Table: Number of emerged rove beetles, *Aleochara bilineata* (F1-generation)

Nominal application rate (g a.s./ha)	Replicate				Mean	SD	R^2*
	1	2	3	4			
Control	632	692	669	613	652	32	-
240	508	608	475	534	581	43	18.5*
427	645	563	552	564	581	43	10.8
759	583	634	633	685	634	42	2.7
1350	559	656	597	455	567	85	13.0

Nominal application rate (g a.s./ha)	Replicate				Mean	SD	R^2*
	1	2	3	4			
2400	619	572	567	631	597	32	8.3
Reference item	7	10	1	3	5	4	99.2

*: Statistically significant (Dunnett's multiple T-test, α = 0.05, one-sided smaller)

The statistically significant difference in the 240 g a.s./ha treatment group was considered to be incidental and not a treatment effect as the test item data did not indicate any dose response at all higher test rates were compared to the control group.

C. VALIDITY CRITERIA

Validity criterion	Required	Achieved
Mean no. emerged beetles in control	≥400 per replicate	652
Effect on reproduction in reference item compared to control	≥50%	99.2%

Validity criteria according to [redacted] (2000) were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Nominal Application Rate (g a.s./ha)
$ER_{50, reproduction}$	>2400
$NOER_{reproduction}$	≥2400

III. CONCLUSION

The ER_{50} was estimated to be above the highest test concentration, >2400 g a.s./ha. The NOER (no observed effect rate) for reproduction was ≥ 2400 g a.s./ha.

The study met the validity criteria according to [redacted] (2000).

[redacted] *(2016)*

Assessment and conclusion by applicant:

Validity criteria according to [redacted] (2000) were satisfied and therefore this study can be considered to be valid .

The ER_{50} was estimated to be above the highest test concentration, >2400 g a.s./ha. The NOER (no observed effect rate) for reproduction was ≥ 2400 g a.s./ha.

Due to the lack of significant effects it was not possible to determine ER_{10} or ER_{20} values.

Assessment and conclusion by RMS:

Data Point:	KCP 10.3.2.2/05
Report Author:	[redacted]
Report Year:	2017
Report Title:	Toxicity to the predatory mite Typhlodromus pyri (Acari:phytoseiidae) using an extended laboratory test on bean - aclonifen SC 600 g/L
Report No:	CW16/005
Document No:	M-588206-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable [redacted] (2000) modified. Use of natural substrate (detached bean leaves) instead of glass plate: [redacted] (2001)
Deviations from current test guideline:	Current guideline: [redacted] 2000 No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to investigate the lethal and sub lethal toxicity of aclonifen SC 600 g/L to the predatory mite *Typhlodromus pyri* when exposed to treated leaf surfaces. This species was chosen as it is currently one of the two standard species required for EU registration. The use of leaf surfaces rather than glass provides a more relevant test substrate for the dispersion of the test item and thus a more realistic exposure of non-target arthropods to the product.

The test item was applied onto detached bean leaves (Phaseolus vulgaris) at rates of 240, 427, 759, 1350 and 2400 g a.s./ha and the effects on the predatory mite Typhlodromus pyri were compared to those of a deionised water treated control. A toxic reference (active substance: dimethoate) applied at 20 g a.s./ha was included to indicate the relative susceptibility of the test organisms and the test system.

Mortality of 100 predatory mites, protonymphs at study start (5 replicates with 20 individuals per test group), was assessed 4, 7, 8, 11 and 14 days after exposure by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed.

The reproduction rate of surviving mites was then evaluated from Day 7 until Day 14 after treatment by counting the total number of offspring (eggs and larvae) produced.

The mortality / escaping rate in the control exposure units up to day 7 after treatment was 15.0%.

At the test item rates of 240, 427, 759 and 1350 g a.s./ha, a corrected mortality of 8.2%, -2.4%, 15.3% and 13.2% has been observed, respectively. At the highest rate of 2400 g a.s./ha, a corrected mortality of 51.8% occurred. Only the highest test item rate showed a statistically significantly different mortality compared to the control.

Reproduction was assessed for all rates of aclonifen SC 600 g/L. At 240 and 427 g a.s./ha the reproduction was reduced by 23.0% and 23.1%, respectively. At 759 and 1350 g a.s./ha the reduction was 50.8% and 56.3%, respectively. At the highest rate of 2400 g a.s./ha, a reduction of 89.9% has been observed. All test item rates showed a statistically significant reduction of reproduction.

The LR_{50} was calculated to be 2336 g a.s./ha. The NOER for mortality was 1350 g a.s./ha.

The ER_{50} was calculated to be 601 g a.s./ha. The NOER for reproduction was < 240 g a.s./ha.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	Aclonifen SC 600 g/L
	Batch no.:	EV56005993
	Active Ingredient / Purity:	Aclonifen, 49.5% w/w (596.7 g/L)
	Expiry date:	09 February 2017
2.	**Reference item:**	Dimethoate EC 400 g/L
	Batch no.:	BAS 152 11 I
	Active Ingredient / Purity:	Dimethoate: 420.3 g/L
3.	**Test Organism:**	Predatory mites (*Typhlodromus pyri*)
	Age:	Protonymphs
	Acclimatisation:	Under test conditions
	Feeding:	Pollen mixture (one part birch:one part pine)

A. STUDY DESIGN AND METHODS

1. In-life phase:	29 January – 10 March 2016

2. Exposure conditions

Test vessels:	A treated Phaseolus vulgaris leaf disc was laid on a layer of wet filter paper on top of a water soaked floral foam. A circle of insect glue (ø approx. 40 mm) was formed on the leaves. Sets of such units were placed on a plastic tray such that the filter paper was constantly provided with deionised water.
Experimental design:	4 treatments: a water control, the test item at 2 rates and a toxic reference
Replicates:	10 per treatment group
Loading:	10 individuals per unit
Temperature:	24 - 25°C
Relative humidity:	55 - 70%

Light intensity: 200 - 1880 lux

3. Administration of the test item

Dose preparation

The test solutions were obtained by separately dispersing 0.242, 0.431, 0.767, 1.364 and 2.424 g of test item in 100 g deionised water to give the required spray solutions. Application of the equivalent of 200 L/ha of each of these spay solutions gave the required application rates of 240, 427, 759, 1350 and 2400 g a.s./ha.

The test solutions were applied to the underside of the cowpea leaves using calibrated laboratory spraying equipment.

Test organism assignment and exposure

After the test units were set up the protonymphs were placed onto the exposure units by test group within one and a half hour after application. The mites were transferred with a fine brush under a stereomicroscope and immediately afterwards examined to ensure they were undamaged and in good condition. Then pollen (birch - pine mixture) was supplied as food and the units were maintained under the climatic conditions of the test. The water supply for the mites was ensured by sticking a pin into each of the leaves.

4. Measurements and observations

Day 4: The number of dead and living mites was counted. Dead mites were removed with a fine brush. The number of escaped mites was calculated. Food was replenished.

Day 7, 8, 11 and 14: The number of dead and living mites was counted, the dead mites were removed and the number of escaped mites was calculated. The number of females, males, eggs and juveniles was counted. Eggs and juveniles were removed. Food was replenished on Days 7, 8 and 11.

5. Statistics/Data evaluation

The number of living and dead mites was counted and recorded on the assessment dates. At Day 7 of the study, the number of dead and escaped mites was summed up for each replicate and calculated as percentage. A mean value of the five replicates was calculated. Mites that could not be found on the test units or which stuck in the glue barrier were recorded as escapees and added to those which had died.

The corrected mortality was obtained by comparing the values observed in the treated groups with those in the control group, according to the formula of [redacted] (1947).

The number of eggs per female was determined by counting the number of females and eggs at the assessment days from Day 7 to Day 14.

The mortality data were analysed for significance using the Fisher Exact test (one-sided with Bonferroni-Holm adjustment; $\alpha = 0.05$), which is a distribution-free test method and does not require testing for normality or homogeneity of variance prior analysis.

The reproduction data were tested for normal distribution using the Shapiro-Wilk test and for homogeneity of variance using the Levene test. As the reproduction data in this study were normally distributed and homogenous one-way ANOVA and the Williams test (one-sided; $\alpha = 0.05$) were used.

The LR_{50} value was calculated using the Trimmed Spearman-Karber method.

The ER_{50} value was calculated using Probit analysis.

The computer program SAS (Version 9.4) was used to perform the statistical analyses.

II. RESULTS AND DISCUSSION

Analytical verification

Analytical verification was not required.

Biological data

Mortality

The mortality / escaping rate in the control group up to day 7 after treatment was 15%.

At the test item rates of 240, 427, 759 and 1350 g a.s./ha, a corrected mortality of 8.2%, -2.4%, 15.3% and 13.2% was observed, respectively. At the highest rate of 2400 g a.s./ha, a corrected mortality of 51.8% occurred. Only the highest test item rate showed a statistically significantly different mortality compared to the control.

The NOER (no observed effect rate) for mortality was 1350 g a.s./ha. The LR_{50} was calculated to be 2336 g a.s./ha.

In the reference item group a corrected mortality of 92.9% occurred.

Reproduction

The mean number of offspring produced per female in the control group was 5.20. This compared to 4.00 eggs/female in the 240 g a.s./ha rate of the test item, 4.00 eggs/female in the 427 g a.s./ha rate, 2.56 eggs/female in the 759 g a.s./ha rate, 2.27 eggs/female in the 1350 and 0.52 eggs/female in the 2400 g a.s./ha rate.

Statistically significant reduction in reproductive success occurred at all test item rates.

At 240 and 427 g a.s./ha the reproduction was reduced by 23.0% and 23.1%, respectively. At 759 and 1350 g a.s./ha the reduction was 50.8% and 56.3% respectively. At the highest rate of 2400 g a.s./ha, a reduction of 89.9% has been observed.

The NOER (no observed effect rate) for reproduction was < 240 g a.s./ha. The ER_{50} was calculated to be 601 g a.s./ha.

Table: Effects of aclonifen SC 600 g/L on mortality and reproduction of adult *Typhlodromus pyri* exposed to fresh dried residue in an extended laboratory study

Nominal application rate (g a.s./ha)	Mortality after 7 days (%)	Corrected mortality after 7 days[2] (%)	Reproduction (eggs per female)	Effect on reproduction (%)
Control	15.0	-	5.2	-
240	22.0	8.2	4.0	23.0**
427	13.0	-2.4	4.0	23.1**
759	28.0	15.3	2.6	50.8**
1350	26.3	13.2	2.3	56.3**
2400	59.0	51.8*	0.5	89.9**
Reference item	94.0	92.9	n.a.	n.a.

* Fisher`s Exact test (one-sided, a=0.05)
**one-way ANOVA, Williams test (one-sided, a=0.05)
n.a. not assessed

C. Validity Criteria

Validity criterion	Required	Achieved
Control mortality	≤20%	15.0%
Reference item mortality	≥50%	92.9%
Control reproduction	>4 eggs per female	5.20

All validity criteria were satisfied and therefore this study can be considered to be valid.

III. CONCLUSION

A study was conducted to investigate the lethal and sub lethal toxicity of aclonifen SC 600 g/L to the predatory mite *Typhlodromus pyri* when exposed to treated leaf surfaces. The LR_{50} was calculated to be 2336 g a.s./ha. The NOER for mortality was 1350 g a.s./ha. The ER_{50} was calculated to be 601 g a.s./ha. The NOER for reproduction was < 240 g a.s./ha.

[redacted] *(2017)*

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

Following exposure of *Typhlodromus pyri* to aclonifen SC 600 g/L via treated leaf surfaces, The LR_{50} was calculated to be 2336 g a.s./ha. The NOER for mortality was 1350 g a.s./ha. The ER_{50} was calculated to be 601 g a.s./ha. The NOER for reproduction was < 240 g a.s./ha.

Assessment and conclusion by RMS:

Data Point:	KCP 10.3.2.2/06
Report Author:	[redacted]
Report Year:	2016
Report Title:	Toxicity to the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) using an extended laboratory test with aged residues on potato aclonifen SC 600 g/L
Report No:	M-574023-01-1
Document No:	M-574023-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable
Deviations from current test guideline:	Current guideline: [redacted] (2000) modified and [redacted] (2001) No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of aclonifen, 600 g/L on the survival of the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) using an extended laboratory test with aged residues on potato. Aging of the spray deposits of the test item on the potted potato plants took place under semi-field conditions with UV permeable rain protection. Nominal test exposure concentration was 600 g a.s./ha in 400 L water/ha, plus a control and reference item.

Predatory mites (*Typhlodromus pyri)* were exposed to test item residues on treated leaf surfaces. Mortality of protonymphs was assessed up to 14 days after exposure by counting the number of living and dead mites. The reproduction rate of surviving mites was evaluated over the period of 7 - 14 days after exposure by counting the total number of offspring (eggs and larvae) produced.

In the first bioassay started on the day of the application of the test item, a corrected mortality of 9.0% was observed which was statistically significantly different compared to the control. In the bioassay started on day 14 (two weeks after application of the test item), the corrected mortality was 4.5% which was not statistically significant.

The assessment of the reproductive performance in the first bioassay resulted in 19.5% reduction of reproduction compared to the control. No reduction (-12.8%) was found in the second bioassay.

Both bioassays (started on day 0 and day 14) resulted in a corrected mortality of <50% and a reduction of reproduction of <50%. Therefore, the ER_{50} was estimated to be above the single tested concentration, >600 g a.s./ha. The NOER (no observed effect rate) for mortality and reproduction was estimated to be ≥600 g a.s./ha.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Item:** Aclonifen SC 600 g/L
   Batch no.: EV56005993
   Active Ingredient / Purity: Aclonifen, 596.7 g/L (49.5% w/w)
   Appearance: Yellow liquid
   Storage: Room temperature (between +2 and +30 °C), dark
   Expiry date: 9 February 2017

2. **Reference item:** Dimethoate EC 400 g/L
   Batch no.: FRE-001226
   Active Ingredient / Purity: Dimethoate, 420.3 g/L (analytical)

3. **Test Organism:** Predatory mite *Typhlodromus pyri*
   Age: Protonymph
   Source: [redacted]
   Acclimatisation: Until the start of each bioassay the test organisms were maintained at a temperature range of 24.5 - 25.0°C and a relative humidity range of 60 - 73%
   Feeding: pollen mixture (one part birch : one part pine)

A. STUDY DESIGN AND METHODS

1. In-life phase: 29 April to 27 May 2016

2. Exposure conditions

Test vessels: After application or the appropriate aging period, one intact leaf randomly selected from different potato plants was cut for each test unit. One unit consisted of the upper side of a leaf disc, which was laid after application on a layer of wet filter paper on top of a water soaked floral foam. A circle of insect glue (approx. 40 mm) was formed on the leaves. Sets of units were placed on a plastic tray such that the filter paper was constantly provided with deionised water

Experimental design: 3 experimental groups: control, test item and toxic (reference) standard.

Replicates: 5 replicates
20 per replicate (10 male + 10 female)

Temperature: Daily mean: 9.0 – 21.12ºC
Min.: 0.46 ºC
Max.: 29.46 ºC

Relative humidity: Daily mean: 42.6 – 70.6%
Min: 16.4%
Max.: 91.4%C

Photoperiod : 16h light:8h dark

Light intensity: Daily mean: 3389 – 9131 lux

Min: 10.7 lux
Max.: 58542 lux

3. Administration of the test item

Dose preparation

The required amounts of test item were mixed with deionized water without the addition of solubility mediators. The spray equipment was calibrated before use to deliver 200 L/ha ±10%.

Prior to the first application, potato leaves were individually tagged to make sure that only treated leaves are taken in the subsequent bioassays.

Deionised water was used as diluent for the test item and for the reference item. The application of the test treatments was done under laboratory conditions on whole potato plants.

For the bioassay that started 2 weeks after the application of the test item, the reference item was freshly applied in the laboratory on leaves taken from untreated plants, which were stored until this time at outdoor conditions.

Test organism assignment and exposure

Untreated potato plants (*Solanum tuberosum*; variety: Zorba) were provided by the horticultural group of [redacted] These plants received no additional pesticide treatments (besides the test treatment) before or during the study.

Aging of the spray residues on the potted potato plants took place under semi-field conditions with rain protection (Plexi-glass, UV permeable). The climatic conditions (temperature, relative humidity and light intensity) in the outdoor area were continuously recorded using a data logger ([redacted]. The temperature ranged from 0.5 to 30.5°C and the relative humidity from 16% to 91% during the aging time of the potato plants.

The laboratory phase for each exposure date was performed in a controlled environment room (target range 25 ± 2 °C and 60 - 90% relative humidity.

Day 0: After the test units were set up the protonymphs were placed onto the exposure units by test group. The mites were transferred with a fine brush under a stereomicroscope and immediately afterwards examined to ensure they were undamaged and in good condition. Then pollen (birch - pine mixture) was supplied as food and the units were maintained under the climatic conditions of the test. The water supply for the mites was ensured by sticking a pin into each of the leaves.

Day 4: The number of dead and living mites was counted. Dead mites were removed with a fine brush. The number of escaped mites was calculated. Food was replenished.

Day 7, 10, 12 and 14: The number of dead and living mites was counted, the dead mites were removed and the number of escaped mites was calculated. The number of females, males, eggs and juveniles was counted. Eggs and juveniles were removed. Food was replenished

4. Measurements and observations

The number of living and dead mites was counted and recorded on the assessment dates.

At day +7 of each bioassay, the number of dead and escaped mites was summed up for each replicate and calculated as percentage. A mean value of the five replicates per test group was calculated.

Mites that could not be found on the test units or which stuck in the glue barrier were recorded as escapees and added to those which had died. The corrected mortality was obtained by comparing the values observed in the treated groups with those in the control group, according to the formula of [redacted] (1947).

The number of eggs per female was determined by counting the number of females and eggs at the assessment days from day 7 to day 14 for both bioassays.

5. Statistics/Data evaluation

The mortality data were analysed for significance using the Fisher Exact test (one-sided with Bonferroni-Holm adjustment; $\alpha = 0.05$), which is a distribution-free test method and does not require testing for normality or homogeneity of variance prior analysis.

The reproduction data were tested for normal distribution using the Shapiro-Wilk test and for homogeneity of variance using the Levene test. As the reproduction data in the first bioassay were normally distributed but not homogenous the Welch test ($\alpha = 0.05$) was used. As the reproduction data in the second bioassay were normally distributed and homogenous one-way ANOVA and the Williams test (one-sided; $\alpha = 0.05$) were used.

The computer program SAS (Version 9.4) was used to perform the statistical analyses.

II. RESULTS AND DISCUSSION

A: ANALYTICAL VERIFICATION

Analytical verification was not required.

B: BIOLOGICAL DATA

The effects of aclonifen SC 600 g/L applied once at a rate of 600 g a.s./ha in 400 L deionised water/ha on potted potato plants (*Solanum tuberosum*) were tested after exposure of the predatory mites to freshly applied and aged spray residues on excised potato leaf discs.

The data for mortality from each test bioassay:

Table: Mortality of predatory mite, *Typhlodromus pyri* exposed to aclonifen 600 g/L

Test treatment	Day 0 (after treatment)	Day 14 (after treatment)
	Mortality after 7 days (%)	
Control	0	12
Test item (aclonifen 600g/L)	9	16
Reference item	99	100
	Corrected mortality (%)	
Test item (aclonifen 600g/L)	9.0	4.5
Reference item	99	100

*: Significant difference from the control (Fisher's Exact test (one-sided, $\alpha = 0.05$), p-values adjusted according to Bonferroni-Holm)

A statistically significant corrected mortality of 9.0% was found in the first bioassay started on the application day of the test item. In the second bioassay two weeks later, the corrected mortality was 4.5% which was not statistically significantly different compared to the control.

The exposure to the reference item resulted in 99% and 100% corrected mortality of the test organisms in the first and second bioassay, respectively.

The data for mortality from each test bioassay:

Table: Reproduction of predatory mite, *Typhlodromus pyri* exposed to aclonifen 600 g/L

Test treatment	Day 0 (after treatment)	Day 14 (after treatment)
	No. eggs per female	
Control	5.5	4.5
Test item (aclonifen 600g/L)	4.5	5.1
	Reduction (%)	
Test item (aclonifen 600g/L)	19.5[a]	-12.8[b]

*: Significant difference from the control
a: Welch test, $\alpha = 0.05$
b: one-way ANOVA, Williams test (one-sided, $\alpha = 0.05$)

In both bioassays, the reproduction was assessed. In the first bioassay started directly after application of the test item a reduction of reproduction by 19.5% occurred which was not statistically significantly different compared to the control. In the second bioassay after two weeks, no reduction (-12.8%) was found.

C. VALIDITY CRITERIA

Validity criterion	Required	Achieved	
		Day 0 (Bioassay 1)	Day 14 (Bioassay 2)
Mortality Escape rate in control (Day 7)	≤20%	0%	12%
Average corrected mortality in control	≥50%	99%	100%

Average no. eggs/female (sum of 4 assessment dates from day 7) in control	≥4	5.5	4.5

Validity criteria according to Blümel *et al* (2000) were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Nominal Application Rate (g a.s./ha)
ER_{50} reproduction	>600
NOER	≥600

III. CONCLUSION

Both bioassays (started on day 0 and day 14) resulted in a corrected mortality of <50% as well a reduction of reproduction of <50%.

The study met the validity criteria according to Blümel *et al*. (2000).

[redacted] *(2016)*

Assessment and conclusion by applicant:

Validity criteria according to [redacted] (2000) were satisfied and therefore this study can be considered to be valid.

Both bioassays (started on day 0 and day 14) resulted in a corrected mortality of <50% and a reduction of reproduction of <50%. Therefore, the ER_{50} was estimated to be above the single tested concentration, >600 g a.s./ha. The NOER (no observed effect rate) for mortality and reproduction was estimated to be ≥600 g a.s./ha.

Assessment and conclusion by RMS:

Data Point:	KCP 10.3.2.2/07
Report Author:	[redacted]
Report Year:	2018
Report Title:	Toxicity to the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) using an extended laboratory test with aged residues on potato; aclonifen SC 600 g/L
Report No:	CW18/020
Document No:	M-639666-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP Not Applicable BLÜMEL ET AL. (2000) modified CANDOLFI ET AL. (2001)
Deviations from current test guideline:	Current guideline: Blümel et al., 2000 No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of aclonifen, 600 g/L on the survival of the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) using an extended laboratory test with aged residues on potato. Aging of the spray deposits of the test item on the potted potato plants took place under semi-field conditions with UV permeable rain protection. Nominal test exposure concentration was 1.8 kg a.s./ha in 400 L water/ha, plus a control and reference item.

Predatory mites (*Typhlodromus pyri*) were exposed to test item residues on treated leaf surfaces. Mortality of protonymphs was assessed up to 14 days after exposure by counting the number of living and dead mites. The reproduction rate of surviving mites was evaluated over the period of 7 - 14 days after exposure by counting the total number of offspring (eggs and larvae) produced.

The first bioassay was started on the application day of the test item (0DAT1) and the second bioassay 14 days later (14DAT1). In both bioassays a statistically significant mortality occurred (Fisher`s Exact test, one-sided, $\alpha = 0.05$). The corrected mortality was 47.4% and 18.2%, respectively.

The exposure to the reference item resulted in 95.8% corrected mortality of the test organisms in the first and 100% corrected mortality in the second bioassay.

The reproduction was assessed in both bioassays. In the first bioassay started on the application day of the test item, a reduction of reproduction of 25.5% occurred which was statistically significantly different compared to the control (Dunnett test, one-sided, $\alpha = 0.05$). In the second bioassay started 14 days after application, the reduction was 13.8% which was not statistically significant (Welch test, one-sided, $\alpha = 0.05$).

Both bioassays started on 0DAT1 and 14DAT1 resulted in a corrected mortality of < 50% as well a reduction of reproduction of < 50%.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	Aclonifen SC 600 g/L
	Batch no.:	EV56007828
	Active Ingredient / Purity:	Aclonifen, 50.3% w/w (607.1 g/L)
	Appearance:	Yellow dispersion
	Storage:	+2°C to +30°C
	Expiry date:	29 November 2019
2.	**Reference item:**	Dimethoate EC 400 g/L
	Batch no.:	BAS 152 11 I
	Active Ingredient / Purity:	Dimethoate, 429.0 g/L (analytical)
3.	**Test Organism:**	Predatory mite *Typhlodromus pyri*
	Age:	Protonymph, less than 24 hours old at each bioassay start
	Source:	[redacted]
	Acclimatisation:	Until the start of each bioassay the test organisms were maintained at a temperature range of 20 - 25°C and a relative humidity range of 60 - 80%
	Feeding:	Pollen mixture (one part birch:one part pine)

A. STUDY DESIGN AND METHODS

1.	In-life phase:	18 May – 15 June 2018

2. Exposure conditions

Test vessels:	After application or the appropriate aging period, one intact leaf randomly selected from different potato plants was cut for each test unit. One unit consisted of the upper side of a leaf disc, which was laid after application on a layer of wet filter paper on top of a water soaked floral foam. A circle of insect glue (approx. 40 mm) was formed on the leaves. Sets of units were placed on a plastic tray such that the filter paper was constantly provided with deionised water CA 1
Experimental design:	3 experimental groups: control, test item and toxic (reference) standard.
Replicates:	5 replicates 20 per replicate
Temperature:	24.0 – 25.0°C
Relative humidity:	60 - 73%
Photoperiod :	16h light:8h dark
Light intensity:	290 - 995 lux

3. Administration of the test item

Dose preparation

The required amounts of test item were mixed with deionized water without the addition of solubility mediators. The spray equipment was calibrated before use to deliver 400 L/ha ±10%.

Prior to the first application, potato leaves were individually tagged to make sure that only treated leaves are taken in the subsequent bioassays.

Deionised water was used as diluent for the test item and for the reference item. The application of the test treatments was done under laboratory conditions on whole potato plants.

For the bioassay that started 2 weeks after the application of the test item, the reference item was freshly applied in the laboratory on leaves taken from untreated plants, which were stored until this time at outdoor conditions.

Test organism assignment and exposure

Untreated potato plants (*Solanum tuberosum*; variety: Zorba) were provided by the horticultural group of [redacted].

Aging of the spray residues on the potted potato plants took place under semi-field conditions with rain protection (Plexi-glass, UV permeable). The climatic conditions (temperature, relative humidity and light intensity) in the outdoor area were continuously recorded using a data logger ([redacted]). The temperature ranged from 7.5 to 37.5°C and the relative humidity from 20% to 99% during the aging time of the potato plants.

The laboratory phase for each exposure date was performed in a controlled environment room (target range 25 ± 2 °C and 60 - 90% relative humidity.

Day 0: After the test units were set up the protonymphs were placed onto the exposure units by test group. The mites were transferred with a fine brush under a stereomicroscope and immediately afterwards examined to ensure they were undamaged and in good condition. Then pollen (birch - pine mixture) was supplied as food and the units were maintained under the climatic conditions of the test. The water supply for the mites was ensured by sticking a pin into each of the leaves.

Day 4: The number of dead and living mites was counted. Dead mites were removed with a fine brush. The number of escaped mites was calculated. Food was replenished.

Day 7: The number of dead and living mites was counted, the dead mites were removed and the number of escaped mites was calculated. The number of females, males, eggs and juveniles was counted. Eggs and juveniles were removed. The sex-ratio (number of females per exposure unit divided by the total number of males and females) was assessed.

The reproduction phase of the second bioassay was done on glass plates as the treated leaves showed a very high phytotoxicity and could not be used anymore. Therefore all living mites were transferred to glass plates on Day 7 of this bioassay, separated for each replicate.

In order to achieve the appropriate sex ratio of at least 5 females : 1 male, males originating from another replicate of the same treatment group were transferred where necessary. Food was replenished.

Day 10, 12 and 14: The number of dead and living mites was counted, the dead mites were removed and the number of escaped mites was calculated. The number of females, males, eggs and juveniles was counted. Eggs and juveniles were removed. Food was replenished on Day 10 and 12.

4. Measurements and observations

The number of living and dead mites was counted and recorded on the assessment dates.

At Day 4 and 7 of each bioassay, the number of dead and escaped mites was summed up for each replicate and calculated as percentage. A mean value of the five replicates was calculated.

Mites that could not be found on the test units or which stuck in the glue barrier were recorded as escapees and added to those which had died. The corrected mortality was obtained by comparing the values observed in the treated groups with those in the control group, according to the formula of [redacted] (1947).

Reproductive performance was calculated for each replicate and expressed as eggs per female.

5. Statistics/Data evaluation

The mortality data were analysed for significance using the Fisher Exact test (one-sided with Bonferroni-Holm adjustment; $\alpha = 0.05$), which is a distribution-free test method and does not require testing for normality or homogeneity of variance prior analysis.

The reproduction data were tested for normal distribution using the Shapiro-Wilk test ($\alpha = 0.05$) and for homogeneity of variance using the Levene test ($\alpha = 0.05$).

As the reproduction data in the first bioassay were normally distributed and homogenous the Dunnett test (one-sided; $\alpha = 0.05$) was used. As the reproduction data in the second bioassay were normally distributed but not homogenous the Welch test ($\alpha = 0.05$) was used.

The computer program SAS (Version 9.4) was used to perform the statistical analyses.

II. RESULTS AND DISCUSSION

A: ANALYTICAL VERIFICATION

Analytical verification was not required.

B: BIOLOGICAL DATA

The effects of aclonifen SC 600 g/L applied once at a rate of 1.8 kg a.s./ha in 400 L deionised water/ha on potted potato plants (*Solanum tuberosum*) were tested after exposure of the predatory mites to freshly applied and aged spray residues on excised potato leaf discs.

The data for mortality from each test bioassay:

Table: Mortality of predatory mite, *Typhlodromus pyri* exposed to aclonifen 600 g/L

Test treatment	Day 0 (after treatment)	Day 14 (after treatment)
	Mortality after 7 days (%)	
Control	5.0	12.0
Test item (aclonifen 600g/L)	50.0	28.0
Reference item	96.0	100.0
	Corrected mortality (%)	
Test item (aclonifen 600g/L)	47.4 (p-value < 0.001, significant*)	18.2 (p-value 0.004, significant*)
Reference item	95.8	100.0

*: Fisher´s Exact test (one-sided, α = 0.05), p-values adjusted according to Bonferrom-Holm)

In both bioassays a statistically significant mortality occurred (Fisher`s Exact test, one-sided, α = 0.05). The corrected mortality was 47.4% and 18.2% for the first and second bioassay's, respectively.

The exposure to the reference item resulted in 95.8% corrected mortality of the test organisms in the first and 100% corrected mortality in the second bioassay.

Table: Reproduction of predatory mite, *Typhlodromus pyri* exposed to aclonifen 600 g/L

Test treatment	Day 0 (after treatment)	Day 14 (after treatment)
	No. eggs per female	
Control	9.0	9.5
Test item (aclonifen 600g/L)	6.7	8.2
	Reduction (%)	
Test item (aclonifen 600g/L)	25.5 (p-value 0.028, significant[a])	13.8 (p-value 0.075, not significant[b])

[a] Dunnett test (one-sided, α = 0.05)
[b] Welch test (one-sided, α = 0.05)

The reproduction was assessed in both bioassays. In the first bioassay started on the application day of the test item, a reduction of reproduction of 25.5% occurred which was statistically significantly different compared to the control (Dunnett test, one-sided, α = 0.05). In the second bioassay started 14 days after application, the reduction was 13.8% which was not statistically significant (Welch test, one-sided, α = 0.05).

C. VALIDITY CRITERIA

Validity criterion	Required	Achieved	
		Day 0 (Bioassay 1)	Day 14 (Bioassay 2)
Mortality Escape rate in control (Day 7)	≤20%	5.0%	12.0%

Average corrected mortality in control	≥50%	95.8%	100.0%
Average no. eggs/female (sum of 4 assessment dates from day 7) in control	≥4	9.0	9.5

Validity criteria according to Blümel *et al* (2000) were satisfied and therefore this study can be considered to be valid.

III. CONCLUSION

Both bioassays started on 0DAT1 and 14DAT1 resulted in a corrected mortality of < 50% as well a reduction of reproduction of < 50%.

The study met the validity criteria according to [redacted] (2000).

[redacted] (2018)

Assessment and conclusion by applicant:
Validity criteria according to [redacted] (2000) were satisfied and therefore this study can be considered to be valid.
Both bioassays (started on day 0 and day 14) resulted in a corrected mortality of <50% and a reduction of reproduction of <50%. Therefore, by non-statistical estimation the ER_{50} was estimated to be above the single tested concentration, 1.8 kg a.s./ha. The NOER (no observed effect rate) for mortality and reproduction was ≤1.8 kg a.s./ha.

Assessment and conclusion by RMS:

CP 10.3.2.3 Semi-field studies with non-target arthropods

No studies available.

CP 10.3.2.4 Field studies with non-target arthropods

No studies available.

CP 10.3.2.5 Other routes of exposure for non-target arthropods

No studies available.

CP 10.4 Effects on non-target soil meso- and macrofauna

CP 10.4.1 Earthworms

A summary of the relevant endpoints for the effects of Aclonifen SC600 G on earthworms is provided in the following table.

Table 10.4-1: Earthworm endpoints for aclonifen and Aclonifen SC600 G

Test item	Test species	Time-scale Test type / Application method	Endpoint	Reference

Aclonifen	*Eisenia andrei*	Acute 14 days	NOEC = 100 mg a.s./kg d.w. EC_{50} = 300 mg a.s./kg d.w.[1]	KCA 8.4/01 M-174306-01-1 [redacted] 1990
Aclonifen SC600 G	*Eisenia andrei*	Acute 14 days	NOEC = 180 mg a.s./kg d.w. EC_{50} = 390 mg a.s./kg d.w.	KCP 10.4.1/01 M-175857-01-1 [redacted] 1992
Aclonifen SC 600 G	*Eisenia andrei*	56-d Reproduction Application via spray onto soil surface	$NOEC_{corr}$ = 20 mg product/L[2, 4]	KCA 8.4.1/01 KCP 10.4.1.1/01 M-174912-01-1 [redacted], 1995
Aclonifen SC 600 G	*Eisenia fetida*	56-d Reproduction Application via spray onto soil surface	$NOEC_{corr}$ = 65.7 mg a.s./kg[3, 4]	KCP 10.4.1.1/02 M-200225-01-1 [redacted] 2001 & KCP 10.4.1.1/03 M-229240-01-1 [redacted] 2004
Aclonifen SC 600 G	*Eisenia fetida*	56-d Reproduction Application via incorporation into soil	**$NOEC_{corr}$ = 15.6 mg a.s./kg[4]**	KCP 10.4.1.1/04 M-580432-02-1 [redacted], 2019
Aclonifen SC 600 G	Earthworm field populations	1 year Field study Application via spray onto soil surface	No unacceptable adverse effect on the population of earthworms at an application rate of 3.5 kg a.s./ha	KCP 10.4.1.2/03 M-441991-02-1 [redacted], 2012

Values in **bold** used in risk assessment
[1]: This study design and endpoint is no longer required for the registration of active ingredients in the EU
[2]: Study not valid. Juvenile production in control was too low
[3]: Study not used as method of test substance application does not meet current data requirements for plant protection products
[4]: Corrected value derived by dividing the endpoint by a factor of 2 in accordance with SANCO/10329/2002

Summary of the risk assessment for Aclonifen SC 600 G and earthworms

The chronic toxicity endpoint for earthworms exposed to Aclonifen SC 600 G was used to calculate the toxicity exposure ratio (TER) value in accordance with the Terrestrial Guidance Document (SANCO/10329/2002)[17] and EFSA Journal 2017; 15(2):4690[18]. The TER_{LT} value for aclonifen was above the trigger value of 5 in accordance with the proposed uses and therefore, the risk was considered to be acceptable.

Risk assessment for earthworms

The risk assessment for earthworms has been conducted in line with the Terrestrial Guidance Document (SANCO/10329/2002) and EFSA Journal 2017; 15(2):4690.

[17] European Commission (EC), 2002. Guidance document on terrestrial ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002) revision 2, final. 1–39.
[18] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), [redacted] 2017. Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms. EFSA Journal 2017;15(2):4690, 225 pp. doi:10.2903/j. efsa.2017.4690

Details on the predicted environmental concentrations (standard field calculations) in soil (PEC_{soil}) for aclonifen are presented in Document M-CP9, Section CP 9.1.3.

The relevant earthworm reproduction study performed on Aclonifen SC 600 G ([redacted], 2017, K-CP 10.4.1.1/03) determined both EC_{10} and NOEC values. In accordance with EFSA's Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EFSA, 2019)[19], as the NOEC was lower than the EC_{10}, the NOEC was used in the risk assessment.

The log P_{ow} for aclonifen is greater than 2 and the organic carbon content of the artificial soil used in the earthworm reproduction study was high (10% peat content) and hence, in line with the EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) the NOEC has to be divided by 2 for use in the risk assessment. This gives a $NOEC_{corr}$ of 31.5 mg test item/kg. In terms of the active ingredient content, assuming an active ingredient content of 49.5% w/w, the $NOEC_{corr}$ was estimated to be 15.6 mg aclonifen/kg.

Table 10.4-2: Earthworm toxicity exposure ratios for the proposed uses of Aclonifen SC 600 G

Test item	Maximum PEC_{soil} (mg/kg soil dw)	Corrected Endpoint (mg/kg soil dw)	TER_{LT}	Trigger value
Aclonifen	0.5697	15.6	27	5

The long-term TER value was above the risk assessment trigger value of 5. It is therefore concluded that the risks to earthworms are acceptable when Aclonifen SC 600 G is used according to the recommended GAP.

Earthworm studies performed on the formulation, Aclonifen SC 600 G are presented below:

Data Point:	KCP 10.4.1/01
Report Author:	[redacted]; [redacted]
Report Year:	1992
Report Title:	The acute toxicity of EXP04209 to earthworms (Eisenia foetida).
Report No:	R007910
Document No:	M-175857-01-1
Guideline(s) followed in study:	EU (=EEC): 87/302/EEC; OECD: 207, (1984)
Deviations from current test guideline:	Current Guideline: OECD 207 (1984) None
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

[19] EFSA (European Food Safety Authority), 2019. Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117 pp. doi:10.2903/sp.efsa.2019.EN-1673

In the previous submission (DAR, 2006), this study was evaluated and accepted as valid for risk assessment purposes. This study design and endpoint is no longer required for the registration of active ingredients in the EU and hence a summary of this study is not presented in this dossier.

CP 10.4.1.1 Earthworms sub-lethal effects

Data Point:	KCP 10.4.1.1/01
Report Author:	[redacted]
Report Year:	1995
Report Title:	Assessment of Sublethal Effects of EXP4209 - (Official German Registration Name: Bandur) - on Eisenia foetida in artificial soil - (Determination of Effects on Reproduction)
Report No:	R007431
Document No:	M-174912-01-1
Guideline(s) followed in study:	BBA: VI, 2-2; ISO: 11268-2
Deviations from current test guideline:	Current Guideline: ISO Guideline 11268-2 and BBA Guideline VI, 2-2 None
Previous evaluation:	yes, evaluated, not accepted Source: DAR, Vol. 3-B9 (9.6.2), August 2006 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Now is no longer acceptable

In the previous submission (DAR, 2006), this study was evaluated and not accepted as valid for risk assessment purposes. Therefore a summary of this study is not presented in this dossier.

Data Point:	KCP 10.4.1.1/02
Report Author:	[redacted]
Report Year:	2001
Report Title:	Effects of EXP04209E on reproduction and growth of earthworms Eisenia fetida in artificial soil
Report No:	C015297
Document No:	M-200223-01-1
Guideline(s) followed in study:	BBA: VI 2-2 (1994); ISO: 11268-2 (1998)
Deviations from current test guideline:	Current Guideline: ISO Guideline 11268-2 and BBA Guideline VI, 2-2 None
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Data Point:	KCP 10.4.1.1/03
Report Author:	[redacted]
Report Year:	2004
Report Title:	Effects of EXP04209E on reproduction and growth of earthworms Eisenia fetida in artificial soil Calculation to convert rates in L/ha into mg/kg artificial soil
Report No:	M-229240-01-1
Document No:	M-229240-01-1
Guideline(s) followed in study:	BBA: VI 2-2 (1994); ISO: 11268-2 (1998)
Deviations from current test guideline:	Not applicable
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

In the previous submission (DAR, 2006), this study and the subsequent conversion of application rates was evaluated and accepted as valid for risk assessment purposes. EU Regulation 284/2013 setting out the data requirements for plant protection products requires that for earthworm sub-lethal effects studies the test substance is incorporated into the soil rather than sprayed onto the surface of the soil as was performed for this study.

Therefore, as this study does not meet current data requirements, it should be considered as supportive only and hence no summary for this study is provided.

Assessment and conclusion by RMS:

Data Point:	KCP 10.4.1.1/04
Report Author:	[redacted]
Report Year:	2019
Report Title:	Amendment no. 1: Aclonifen SC 600 G: Effects on survival, growth and reproduction of the earthworm Eisenia andrei tested in artificial soil
Report No:	16 10 48 169 S
Document No:	M-580432-02-1
Guideline(s) followed in study:	OECD 222 (2004), ISO 11268-2 (1998)
Deviations from current test guideline:	Current guideline: OECD 222, 2016 No Deviation
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary:

The effects of Aclonifen SC 600 G on the mortality, body weight and reproduction of adult *Eisenia fetida* were investigated in a laboratory study lasting eight weeks.

Adult earthworms (*Eisenia andrei*, about 3 months old) were exposed to 20, 35, 63, 112, 200, 355, 631 and 1122 mg test item/kg dry weight mixed into artificial soil. Mortality and biomass change were determined after 4 weeks and reproduction was determined after 8 weeks.

Aclonifen SC 600 G showed statistically significant adverse effects on survival of the earthworm *Eisenia andrei* in artificial soil at 1122 mg test item/kg soil dry weight, i.e. the highest concentration tested.

Statistically significantly adverse effects on biomass were determined at 355, 631 and 1122 mg test item/kg soil dry weight. The test item showed statistically significant adverse effects on reproduction at 112, 200, 355, 631 and 1122 mg test item/kg d.w. Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction was determined to be 63 mg test item/kg d.w., and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction was determined to be 112 mg test item/kg d.w.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	Aclonifen SC 600 G
	Batch no.:	EV56005993
	Active Ingredient / Purity:	Aclonifen 600 g/L (nominal); 49.5% w/w, 596.7 g/L (analysed)
	Appearance:	Yellow suspension
	Expiry date:	09 February 2017
	Storage:	25 ± 5 °C
2.	**Reference item:**	Maypon Flow
	Batch no:	Not reported
	Active Ingredient / Purity:	Carbendazim, SC 500
3.	**Test Species:**	earthworm *Eisenia andrei* (BOUCHÉ, 1972)
	Source:	[redacted]
	Age:	Adult worms (approximately 3 months old with clitellum)
	Acclimatisation:	Approximately 24 hours in the artificial substrate (with food)
	Feeding:	Air-dried and finely ground horse manure

B. STUDY DESIGN AND METHODS

1. In-life phase: 27 July – 21 September 2016

2. Exposure conditions

Test vessels:	Plastic vessel of Bellaplast (inside dimensions: about 16.5 cm x 12 cm x 6 cm) with a lid pervious to air and light
Test soil:	Artificial soil 10% sphagnum peat; origin: Torfwerk Moorkultur Ramsloh, 26683 Saterland, Germany, classified according to DIN 11540 (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 > 30%); type: Kaolin W, origin: [redacted], Germany 0.5% calcium carbonate; origin: [redacted] Germany 69.5% industrial quartz sand; type: Millisil W3, origin: [redacted] Germany (fine sand is dominant with more than 50% of the particles between 50 and 200 µm) deionised water
Treatment:	Control 20, 35, 63, 112, 200, 355, 631, 1122 mg test item/kg soil dry weight Reference item, 5 and 10 mg product/kg soil dry weight (tested in a separate study)
Number of replicates:	8 for the control and 4 per treatment group
Number of individuals:	10 per replicate, i.e. 80 individuals for the control and 40 individuals per treatment group
Test duration:	8 weeks
Temperature:	18.7 – 21.8 °C
Light regime:	16 h light: 8 h dark
Light intensity:	550 lux
pH:	5.62 – 6.03
Water content:	34.2 – 35.0% (54.5 – 55.7% of the water holding capacity)

3. Administration of the test item

Dose preparation and dosing

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.

Test organism exposure

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. The control substrate contained the corresponding amount of deionised water only. Each test vessel was then filled with the treated soil. 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.

4. Measurements and observations

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 of the experiment. The test was then continued for another four weeks. The final assessment included counting of 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.

5. Statistics/Data evaluation

The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between 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 3.2.1 ([redacted] 2015). The EC_{10} and EC_{20} values (number of juveniles) were calculated by Weibull analysis using linear max. likelihood regression. Confidence limits (95%) of the ECx values were computed by normal approximation. The Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, Welch-t-test after Bonferroni-Holm 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.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

No analytical verification of the dose solutions was performed.

B. BIOLOGICAL DATA

The mortality of adult worms was 0 – 60% in the treated groups and 0% in the control group. Statistically significant mortality compared to the control was observed at 1122 mg test item/kg d.w. (Multiple Sequentially-rejective Fisher Test After [redacted] $\alpha = 0.05$, one-sided greater). No pathological symptoms and no further effects on behaviour of the worms were observed. The feeding activity of adult worms was reduced at 355, 631 and 1122 mg test item/kg soil d.w.

The weight change of adult worms ranged between -31.8 and 20.5% in the treated groups and 19.8% in the control group. The test item caused statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control groups were recorded at concentrations of 355, 631 and 1122 mg test item/kg d.w. (Welch-t-test after [redacted] $\alpha = 0.05$, two-sided).

Statistically significant effects (Williams-t-test, α = 0.05, one-sided smaller) on number of juveniles compared to the control group were recorded at concentrations of 112, 200, 355, 631 and 1122 mg test item/kg d.w.

Table: Effect of Aclonifen SC 600 G on earthworms (*Eisenia fetida*) in a 56-day reproduction study

Treatment (mg/kg d.w)	Mortality after 4 weeks (%)	Mean biomass change after 4 weeks (mg)	Reproduction (no. of juveniles / replicate after 8 weeks)
Control	0.0	88.7	141.5
20	0.0	92.8	129.8
35	0.0	87.0	140.5
63	0.0	85.9	121.8
112	0.0	89.2	108.0[3]
200	0.0	78.8	116.5[3]
355	0.0	47.8[2]	109.5[3]
631	0.0	-34.4[2]	62.5[3]
1122	60.0[1]	-144.0[2]	0.0[3]

[1]: Significantly different compared to the control (Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, α = 0.05, one-sided greater)
[2]: Significantly different compared to the control (Welch-t-test after Bonferroni-Holm, α = 0.05, two-sided)
[3]: Significantly different compared to the control (Williams-t-test, α = 0.05, one-sided smaller)

Based on the statistical evaluation of these results, the No-Observed-Effect-Concentration (NOEC) for reproduction was determined to be 63 mg test item/kg soil d.w., and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction was determined to be 112 mg test item/kg soil d.w. The EC_{10} and EC_{20} values for reproduction were calculated to be 144 and 240 mg test item/kg soil d.w., respectively.

In the reference test, the number of juveniles was reduced by 39 and 96% by the toxic standard Maypon Flow (Carbendazim, SC 500) at concentrations of 5 and 10 mg product/kg d.w. in comparison to the control. Therefore, the observed effects assure a high sensitivity of the test system.

C. VALIDITY CRITERIA

Validity criterion	Required (OECD 222, 2016)	Achieved
Mortality	≤ 10%	0%
Reproduction (worms per container)	≥ 30	141.5
Reproduction (coefficient of variation)	≤ 10%	14.7%

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Nominal Concentration (mg/kg d.w.)
$NOEC_{mortality}$	631
$NOEC_{biomass\ change}$	200
$NOEC_{reproduction}$	63
$LOEC_{reproduction}$	112
EC_{10} [95% confidence limits]	144 [59 – 352]
EC_{20} [95% confidence limits]	240 [130 – 442]

III. CONCLUSION

Aclonifen SC 600 G showed statistically significant adverse effects on survival of the earthworm *Eisenia andrei* in artificial soil at 1122 mg test item/kg soil dry weight, i.e. the highest concentration tested.

Statistically significantly adverse effects on biomass were determined at 355, 631 and 1122 mg test item/kg soil dry weight. The test item showed statistically significant adverse effects on reproduction at 112, 200, 355, 631 and 1122 mg test item/kg d.w. Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction was determined to be 63 mg test item/kg d.w., and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction was determined to be 112 mg test item/kg d.w.

[redacted] *(2017)*

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

Based on the most relevant biological endpoint of reproduction, the NOEC was determined to be 63 mg test item/kg soil and the EC_{10} was determined to be 144 mg test item/kg soil.

EFSA's Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EFSA, 2019)[20] recommends that the lowest of the EC_{10} and NOEC values be used for risk assessment purposes. In this study, as the NOEC was lower than the EC_{10}, the NOEC of 63 mg test item/kg soil should be used for risk assessment.

For use in the risk assessment, as the log P_{ow} for aclonifen is greater than 2 and the organic carbon content of the artificial soil was high (10% peat content), in line with the EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) the endpoints have to be divided by 2.

Results have also been calculated in terms of the active ingredient content, assuming an active ingredient content of 49.5% w/w.

Table: Summary of endpoints

Endpoint	Concentration (mg/kg d.w.)	Concentration corrected for log P_{ow}	Concentration corrected for log P_{ow}

[20] EFSA (European Food Safety Authority), 2019. Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117 pp. doi:10.2903/sp.efsa.2019.EN-1673

		(mg/kg d.w.)	and active ingredient content (mg a.s./kg d.w.)
$NOEC_{mortality}$	631	316	156
$NOEC_{biomass\ change}$	200	100	49.5
$NOEC_{reproduction}$	63	31.5	15.6
$LOEC_{reproduction}$	112	56	27.7
EC_{10} [95% confidence limits]	144 [59 – 352]	72 [29.5 - 176]	35.6 [14.6 – 87.1]
EC_{20} [95% confidence limits]	240 [130 – 442]	120 [65 – 221]	59.4 [32.2 – 109]

Assessment and conclusion by RMS:

CP 10.4.1.2 Earthworms field studies

Data Point:	KCP 10.4.1.2/01
Report Author:	[redacted]
Report Year:	2000
Report Title:	Toxicity testing of BANDUR (EXP04209) to earthworms in the field
Report No:	C010320
Document No:	M-198806-01-1
Guideline(s) followed in study:	BBA: VI, 2-3 (1994)
Deviations from current test guideline:	Current Guideline: BBA Guideline VI, 2-3, 1994 None
Previous evaluation:	yes, evaluated, not accepted Source: DAR, Vol. 3 B9 (9.6.3), August 2006 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Now is no longer acceptable

In the previous submission (DAR, 2006), this study was evaluated and not accepted as valid for risk assessment purposes. Therefore a summary of this study is not presented in this dossier.

Data Point:	KCP 10.4.1.2/02
Report Author:	[redacted]
Report Year:	2001
Report Title:	Evaluation of the earthworm population in a field treated with EXP04209E
Report No:	C020613
Document No:	M-209888-01-1
Guideline(s) followed in study:	BBA: 1994, part VI, No. 2-3; ISO: 11268-3/1999
Deviations from current test guideline:	Current Guideline: BBA Guideline VI, 2-3, 1994 None
Previous evaluation:	yes, evaluated, not accepted Source: DAR, Vol. 3 B9 (table 9.6-7), August 2006 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Now is no longer acceptable

In the previous submission (DAR, 2006), this study was evaluated and not accepted as valid for risk assessment purposes. Therefore a summary of this study is not presented in this dossier.

Data Point:	KCP 10.4.1.2/03
Report Author:	[redacted]
Report Year:	2012
Report Title:	Amendment to study report - Aclonifen SC 600 G: Effect on the earthworm fauna within one year under field conditions
Report No:	M-441991-02-1
Document No:	M-441991-02-1
Guideline(s) followed in study:	BBA (Federal Biological Research Centre for Agriculture and Forestry, Germany): Guidelines for the Testing of Plant Protection Products within Registration, Part VI, 2 – 3 (January 1994): Effects of Plant Protection Products on Earthworms in the Field ISO (International Standard Organisation): Draft Guideline CD 11268-3 (E), Soil Quality – Effects of pollutants on Earthworms, Part 3: Guidance on the determination of effects in field situations (1999) OPPTS 850.supp
Deviations from current test guideline:	Current guideline: BBA Guideline VI, 2-3, 1994 No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The effects of Aclonifen SC 600 G on earthworm populations under field conditions were studied. To ensure an abundant earthworm population, an area was selected which was used as grassland for four years, located in [redacted] (Germany).

Aclonifen SC 600 G was applied once at rate of 5.83 L/ha (3.5 kg a.s./ha) on four treatment plots (10 x 10 m) within a total, a test area of 30 x 40 m in size. Four untreated plots served as controls. Four plots were used as positive controls and were treated with Carbendazim SC 500 with an application rate of 10 kg a.s./ha. Within ten days after application 29.9 mm of precipitation was measured. One, two and three days after the application, all plots were screened for alive and dead earthworms on the soil surface.

The earthworm abundance and biomass was sampled four weeks, five months and eleven months after the application, respectively. Earthworms were collected by a combination of hand-sorting and formalin extraction. At each sampling time point 16 samples per treatment (4 plots, 4 samples per plot) were collected.

Soil samples from the control and from the treated plots were taken on the day of application. For the determination of the residues of Aclonifen soil samples were analysed by HPLC-MS/MS using a suitable method.

No statistically significant reductions in total earthworm abundance and biomass could be observed in the test item treatment neither one, five and eleven months after application. Dominant earthworm species found in the field site at pre-sampling were the endogeic species *Apporectodea caliginosa* (65.1% of total earthworms) and the anecic species *Lumbricus terrestris* (13.6% of total earthworms). No statistically significant reductions in the abundance and biomass of single earthworm species could be observed in the test item treatment group compared to the control throughout the whole test period, except for *L. terrestris* juvenile. At the 3rd sampling the abundance of juvenile *L. terrestris* was significantly reduced by 29%. This lead to a significant reduction of the abundance of total *L. terrestris* by 22% and of anecic juvenile earthworms by 26%.

However, the biological meaning of this statistical finding is considered to be limited. Effects were less than 30% and within the range of natural variability. 1 and 5 months after application no effects on abundance and biomass were observed. 1, 5 and 11 months after application the biomass of adult, juvenile, and total anecic earthworms and of *L. terrestris* were not significantly affected by the test item.

The earthworm field study shows, that Aclonifen SC 600 G applied with application rate of 3.5 kg a.s./ha (5.83 L product/ha) has no unacceptable adverse effect on the population of earthworms one, five and eleven months after the application. Thus, it can be concluded, that Aclonifen SC 600 G has no unacceptable ecologically adverse long-term effects on earthworm population, if applied at rates of 3.5 kg a.s./ha.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	Aclonifen SC 600 G
	Batch no.:	EV54001166

Active Ingredient / Purity: Aclonifen, 595.0 g/L (49.4%w/w)
Expiry date: 16 February 2013
Appearance: Yellow suspension

B. STUDY DESIGN AND METHODS

1. In-life phase: 06 June 2011 – 09 August 2012

2. Exposure conditions

Test field: The test field has been used as a grassland area for many years and is located close to [redacted]

The study area has not been treated with pesticides for at least 4 years (in the year 2007); it was thus assumed that a natural, roughly uniform, and dense earthworm population exists.

The test site was treated with Glyphos ultra max at a rate of 5 L/ha to reduce the grass mass seven weeks before the application of the test item. Four weeks later, the test site was harrowed and the soil surface was carefully crushed and flattened.

In this pasture, a plain area measuring 50 x 30 m was used for the study (test site). The minimum distance from the edge of the pasture was approximately 2 m, so that effects from adjacent fields were precluded. Plots of 10 x 10 m were laid out in this area and identified by marking stakes at the edge of the pasture

Test soil: On October 13, 2011 soil samples were taken to characterize the essential properties of the soil. Samples of the top 0 - 10 cm soil were taken at randomly selected places of the study area. The samples were combined, air dried and homogeneously mixed after removal of stones, roots and larger particles and subsequently sieved at 2 mm (= mixed sample).

Parameter	October 2011
pH	5.83
Water holding capacity (%)	34.8
Total C (%)	1.30
N (%)	0.14
P (mg/kg)	536.13
% sand	66.2
% silt	22.7
% clay	11.1
Characterization (DIN)	Loamy sand

Sowing:	Field beans (*Vicia faba*) Sort: Fuego TKW (Thousand Corn Weight): 365.2 g Germination: 89% Seeding: 146.1 kg beans/ha The seeds were drilled by help of a harrow with sowing equipment according to normal agricultural practice. 400000 seeds/ha were drilled onto the test plots (distance between rows 12 cm).
Experimental design:	Control, reference substance and single application rate of 3.5 kg a.s./ha
Replicates:	4 plots for the control, test item and reference substance treatment.
Loading:	The abundance of earthworms at the study site was determined 13 days before the application of the test substance by pre-sampling
Climatic conditions:	Since the activity of earthworms (and consequently, their potential exposure to the product tested) depends to a high degree on climatic parameters, records of mean daily air temperature, sunshine duration and precipitation at the nearby [redacted] were used to characterize climatic conditions from June 2011 to May 2012.

3. Administration of the test item

Aclonifen SC 600 G was applied at a nominal rate of 3.5 kg a.s. Aclonifen/ha. Application of 3.5 kg a.s./ha corresponds to 70.824 g of the formulated product per plot (10 x 10 m = 100 m^2). Four plots were treated with 283.29 g Aclonifen SC 600 G/12 L water. The formulated product was applied once in 300 L water/ha (= 12 L water/400 m^2). A total of four plots were served as control.

The test substance was applied by means of a compressed air sprayer. The spray boom (12 m long) was fitted with 24 nozzles so that a strip of 12 m width was sprayed at a pressure of 2.5 bar.

In the study Carbendazim SC 500 (a.s.-content: 500 g/L carbendazim) was tested simultaneously serving as the reference substance, as this substance is known to cause toxic effects on earthworm populations. Four plots (out of 12) were treated with a nominal application rate of 10 kg a.s./ha. The reference substance was applied in 300 L water / ha (= 3 L water / plot of 100 m^2).

The test item and reference substance were applied according to normal agricultural practice.

Test organism assignment and exposure

Directly after application the 11-12 days old collembolans from the synchronised cohort were collected with an exhauster into small glass tubes. They were counted to ensure that 10 non-damaged individuals were introduced. Then the 10 collembolans were placed on the soil surface of the treated soil and the

untreated control respectively. The sequence of inserting the test organisms into the test vessels followed a computer-generated random design.

4. Measurements and observations

During a few days after the application, the soil surface of the test plots was thoroughly searched for alive and dead earthworms. For this purpose, each plot was carefully scored on the surface of the soil for 3 minutes by one person. During this time, attention was also paid to earthworms with noticeable behavioral changes or injuries.

Sampling of earthworms

Earthworm abundance and biomass in soil were determined four weeks, five months and eleven months after application, respectively by sampling using by hand-sorting.

Earthworm numbers and biomass were determined by extracting earthworms using the "hand-sorting". Four samples were obtained from 4 randomly chosen sampling positions covering an area of 50 x 50 cm each and located in the inner 6 x 6 m core of each plot in a regular scheme. Steel frames, 50 x 50 cm in size, were pressed into the ground of the sampling positions confining an area of 0.25 m^2. The soils of the 0.25 m^2 were dug and hand-sorted to a depth of about 40 cm. 5 litres of 0.2% formalin solution were poured in the hole. Earthworms crawling out within at least 30 minutes after formalin treatment were sampled.

Analytical verification

Soil samples from the treated plots were taken within 24 hours after the application of the test item. Soil samples were analysed for the determination of the residues of Aclonifen in soil by HPLC-MS/MS according to a suitable method.

Soil samples of 0 - 10 cm depth were taken by using a manual sampling system ("Piercer", Ø 5 cm) by [redacted] ([redacted]). Samples were drawn outside from the inner 6 x 6 m core of each plot, in a zone of about 2 m width from the edge of each plot. Each sample was consisted of 20 soil cores per plot of Aclonifen SC 600. The samples were stored frozen until they were analysed.

A soil sample consisting of 20 soil cores was drawn on the verge of the study site before the first application of the test item, to serve as untreated control.

5. Statistics/Data evaluation

The results of the sampling were statistically evaluated by the Student t-test ([redacted] Angewandte [redacted] 1978; probability level P = 0.05, one-sided smaller). The statistic software used was ToxRatPro Version 2.10. No statistical comparisons between treatment groups were made in case of < 10 ind./m^2, according to the recommendation of [redacted] (2006).

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Soil samples were taken after application of the annual rate in 0 - 10 cm soil depth. The results of analysis of these samples are summarised below.

The results were calculated by the corrected recovery rate of 97.3% of Aclonifen. The limit of quantification (LOQ) for Aclonifen was 50 µg/kg.

All conversions from µg a s./kg soil to g a.s./ha and vice versa assume a soil depth of 10 cm and a soil density of 1.5 g/mL. Considering these assumptions and conversions it cannot be expected that exactly 100% of the nominal amount of Aclonifen can be analytically verified in the samples especially under field conditions.

Table: Concentrations of Aclonifen

Sample name	Calculated Concentration dry Sediment (mg/kg)			
	Sample a	Sample b	Sample c	Mean
Aclonifen C Laacher Hof	<LOQ	<LOQ	<LOQ	<LOQ
Aclonifen SC 600 Control	<LOQ	<LOQ	<LOQ	<LOQ
Aclonifen SC 600 Probe 1	2423	2345	2306	2.358
Aclonifen SC 600 Probe 2	2536	2278	2435	2.416
Aclonifen SC 600 Probe 3	2109	2253	2298	2.220
Aclonifen SC 600 Probe 4	2188	2085	1974	2083

C: Control
LOQ: Limit of Quantitation, 50.0 µg aclonifen/kg

The validated method is summarised in Document M-CP5 (CP 5.1.2/19).

B. BIOLOGICAL DATA

Alive and dead earthworms on the soil surface within a few days after application of Aclonifen SC 600 G
Some earthworms could be detected on the soil surface of treated and control plots. Overall the abundance of earthworms found was very low and the data do not indicate any difference between treated and control plots.

Abundance and biomass of earthworms sampled in soil
In the control plots, the total earthworm abundance amounted to 86.5, 130,5 and 171 ind./m^2 for the 1st, 2nd and 3rd sampling, respectively. The corresponding total biomass in the control amounted to 31.2, 90.3 and 156.13 g/m^2.

The treatment with the reference substance Carbendazim showed effects four weeks after the application on the earthworm community in comparison to the control. The reference substance applied at a rate of 10 kg a.s./ha did decrease the abundance of earthworms by 57%. Therefore, the reference item treatment confirmed the sensitivity of the earthworm population under the specific experimental conditions and the validity of the study.

The application of the test item tested at 3.5 kg a.s./ha revealed no statistical significant reduction in total earthworm abundance and biomass at any sampling dates.

No statistically significant reductions in total earthworm abundance and biomass could be observed in the test item treatment neither one, five and eleven months after application. Dominant earthworm species found in the field site at pre-sampling were the endogeic species *Apporectodea caliginosa*

(65.1% of total earthworms) and the anecic species *Lumbricus terrestris* (13.6% of total earthworms). No statistically significant reductions in the abundance and biomass of single earthworm species could be observed in the test item treatment group compared to the control throughout the whole test period, except for *L. terrestris* juvenile. At the 3rd sampling the abundance of juvenile *L. terrestris* was significantly reduced by 29%. This lead to a significant reduction of the abundance of total *L. terrestris* by 22% and of anecic juvenile earthworms by 26%.

However, the biological meaning of this statistical finding is considered to be limited. Effects were less than 30% and within the range of natural variability. 1 and 5 months after application no effects on abundance and biomass were observed. 1, 5 and 11 months after application the biomass of adult, juvenile, and total anecic earthworms and of *L. terrestris* were not significantly affected by the test item.

Thus, it can be concluded, that Aclonifen SC 600 G has no unacceptable ecologically adverse long-term effects on earthworm population, if applied at rates of 3.5 kg a.s./ha.

Table: Changes in abundance for total earthworm, total juveniles & total adults and the dominant species *L. terrestris* and *A. caliginosa* earthworms, summary

Treatment group		Abundance (ind./m²) Sampling No. 1	2	3
Total	Control	86.50 ± 27.18 (100%)	130.50 ± 48.97 (100%)	171.00 ± 45.85 (100%)
	Aclonifen SC 600 (3.5 kg a.s./ha)	96.25 ± 7.46 (111%)	111.25 ± 29.57 (85%)	147.00 ± 40.36 (86%)
	Carbendazim (10 kg a.s./ha)	34.75 ± 4.11 *) (40%)	133.75 ± 37.44 (102%)	148.75 ± 12.76 (87%)
Total adults	Control	17.75 ± 5.19 (100%)	40.50 ± 17.54 (100%)	62.50 ± 23.87 (100%)
	Aclonifen SC 600 (3.5 kg a.s./ha)	22.25 ± 2.22 (125%)	32.75 ± 12.82 (81%)	59.25 ± 13.52 (95%)
	Carbendazim (10 kg a.s./ha)	10.00 ± 2.45 *) (56%)	70.75 ± 22.29 (175%)	61.75 ± 16.64 (99%)
Total juveniles	Control	68.75 ± 22.77 (100%)	90.00 ± 38.70 (100%)	108.50 ± 22.46 (100%)
	Aclonifen SC 600 (3.5 kg a.s./ha)	74.00 ± 9.38 (108%)	78.50 ± 19.47 (87%)	87.75 ± 27.28 (81%)
	Carbendazim (10 kg a.s./ha)	24.75 ± 3.86 *) (36%)	63.00 ± 17.68 (70%)	87.00 ± 10.23 (80%)
Lumbriculus terrestris	Control	11.25 ± 6.70 (100%)	27.50 ± 4.93 (100%)	39.25 ± 3.59 (100%)
	Aclonifen SC 600 (3.5 kg a.s./ha)	12.75 ± 5.06 (113%)	29.00 ± 13.44 (105%)	30.50 ± 7.19 *) (78%)
	Carbendazim (10 kg a.s./ha)	4.25 ± 2.06 *) (38%)	21.75 ± 9.18 (79%)	31.50 ± 3.00 *) (80%)
Aporrectodea caliginosa	Control	69.25 ± 24.70 (100%)	62.25 ± 29.81 (100%)	111.00 ± 37.19 (100%)
	Aclonifen SC 600 (3.5 kg a.s./ha)	74.00 ± 7.87 (107%)	49.50 ± 13.38 (80%)	96.25 ± 29.65 (87%)
	Carbendazim (10 kg a.s./ha)	29.50 ± 4.80 *) (43%)	46.50 ± 16.44 (75%)	87.75 ± 4.03 (79%)

1st sampling on July 4-7, 2011 (4 weeks after application)
2nd sampling on October 13-19, 2011 (5 months after application)

3rd sampling on May 8-16, 2012 (11 months after application)
*) Significant difference from control according to the Student-t test one-sided smaller at the significance level alpha = 0.05.

Table: Changes in biomass for total earthworm, total juveniles & total adults and the dominant species *L. terrestris* and *A. caliginosa* earthworms, summary

Biomass (ind./m²)				
Treatment group		**Sampling No.**		
		1	**2**	**3**
Total	Control	31.20 ± 9.66 (100%)	90.30 ± 28.97 (100%)	156.13 ± 44.79 (100%)
	Aclonifen SC 600 (3.5 kg a.s./ha)	33.54 ± 5.29 (108%)	101.09 ± 35.17 (112%)	135.34 ± 38.58 (87%)
	Carbendazim (10 kg a.s./ha)	15.79 ± 2.83 *) (51%)	79.19 ± 26.22 (88%)	118.00 ± 17.40 (76%)
Total adults	Control	15.55 ± 4.04 (100%)	51.87 ± 16.45 (100%)	95.11 ± 30.16 (100%)
	Aclonifen SC 600 (3.5 kg a.s./ha)	16.20 ± 3.03 (104%)	55.02 ± 16.78 (106%)	85.65 ± 26.14 (90%)
	Carbendazim (10 kg a.s./ha)	9.09 ± 1.51 *) (58%)	47.44 ± 15.60 (91%)	66.00 ± 16.67 (69%)
Total juveniles	Control	15.65 ± 6.17 (100%)	38.43 ± 14.26 (100%)	61.02 ± 14.98 (100%)
	Aclonifen SC 600 (3.5 kg a.s./ha)	17.34 ± 7.56 (111%)	46.08 ± 20.67 (120%)	49.69 ± 14.23 (81%)
	Carbendazim (10 kg a.s./ha)	6.70 ± 1.44 *) (43%)	31.75 ± 12.18 (83%)	52.00 ± 3.96 (85%)
Lumbriculus terrestris	Control	13.47 ± 8.05 (100%)	51.91 ± 15.05 (100%)	93.45 ± 22.78 (100%)
	Aclonifen SC 600 (3.5 kg a.s./ha)	11.74 ± 5.13 (87%)	60.19 ± 33.17 (116%)	78.39 ± 30.09 (84%)
	Carbendazim (10 kg a.s./ha)	5.59 ± 3.83 *) (42%)	26.08 ± 9.48 (50%)	61.80 ± 8.95 *) (66%)
Aporrectodea caliginosa	Control	16.97 ± 7.49 (100%)	20.67 ± 7.73 (100%)	47.83 ± 22.89 (100%)
	Aclonifen SC 600 (3.5 kg a.s./ha)	20.82 ± 4.44 (123%)	20.62 ± 7.79 (100%)	43.72 ± 12.48 (91%)
	Carbendazim (10 kg a.s./ha)	9.62 ± 1.56 *) (57%)	28.64 ± 7.48 (139%)	37.26 ± 6.51 (78%)

1st sampling on July 4-7, 2011 (4 weeks after application)
2nd sampling on October 13-19, 2011 (5 months after application)
3rd sampling on May 8-16, 2012 (11 months after application)
*) Significant difference from control according to the Student-t test one-sided smaller at the significance level alpha = 0.05.

III. CONCLUSION

The earthworm field study shows, that Aclonifen SC 600 G applied with application rate of 3.5 kg a.s./ha (5.83 L product/ha) has no unacceptable adverse effect on the population of earthworms one, five and eleven months after the application. Thus, it can be concluded, that Aclonifen SC 600 G has no unacceptable ecologically adverse long-term effects on earthworm population, if applied at rates of 3.5 kg a.s./ha.

[redacted] *(2012)*

Assessment and conclusion by applicant:

The study followed current accepted practices for conducting field studies and was performed under GLP. The study is therefore considered to be valid.

The study shows, that Aclonifen SC 600 G applied at an application rate of 3.5 kg a.s./ha (5.83 L product/ha) has no unacceptable adverse effect on the population of earthworms one, five and eleven months after the application. Thus, it can be concluded, that Aclonifen SC 600 G has no unacceptable ecologically adverse long-term effects on earthworm population, if applied at rates of up to 3.5 kg a.s./ha.

Assessment and conclusion by RMS:

CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A summary of the relevant endpoints for the effects of Aclonifen SC 600 G on non-target soil meso- and macrofauna (other than earthworms) is provided in the following table.

Table 10.4-3: Non-target soil meso- and macrofauna (other than earthworms) endpoints used in risk assessment

Test item	Test species	Time-scale Test type	Endpoint	Reference
Aclonifen SC 600 G	*Hypoaspis aculeifer*	14-d Reproduction	$NOEC_{repro}$ = 133 mg a.s./kg[1] $NOEC_{mort}$ = 13.3 mg a.s./kg[1]	KCA 8.4.2.1/01 KCP 10.4.2.1/01 M-217404-01-1 [redacted], 2002
Aclonifen SC 600 G	*Hypoaspis aculeifer*	14-d Reproduction	NOEC = 562 mg prod./kg dw EC_{10} = N.D. **$NOEC_{corr}$ = 139 mg a.s./kg dw[2]** $EC_{10\ corr}$ = N.D.	KCP 10.4.2.1/02 M-404537-01-1 [redacted]2011
Aclonifen SC 600 G	*Folsomia candida*	28-d Reproduction	NOEC = 316 mg prod./kg dw EC_{10} = 311 mg prod./kg dw $NOEC_{corr}$ = 78 mg a.s./kg dw[2] **$EC_{10\ corr}$ = 77 mg a.s./kg dw[2]**	KCP 10.4.2.1/02 M-404393-01-1 [redacted], 2011 & KCP 10.4.2.1/04 M-675907-01-1 [redacted], 2019
Aclonifen SC 600 G	*Litter bag*	133 days Field study	No adverse effect on litter degradation up to 1010 g a.s./ha[3]	KCP 10.4.2/01 M-209896-02-1 [redacted], 2002
Aclonifen SC 600 G	*Soil mite field populations (Acari)*	1 year Field study	No unacceptable effects on soil mite populations at an application rate of 3.3 kg a.s./ha	~~KCP 10.4.2.2/02~~ KCP 10.4.2.2/01 M-594981-01-1 [redacted]20107 &

				~~KCP 10.4.2.2/03~~ ~~M-595114-01-1~~ [redacted], ~~2017~~ KCP 10.4.2.2/04 M-688800-01-1 [redacted], 2020

Values in **bold** used in risk assessment
N.D.: Not determined
[1]: Study not used in risk assessment as does not meet the requirements of the current OECD guideline
[2]: Corrected value derived by dividing the endpoint by a factor of 2 in accordance with SANCO/10329/2002 and correcting for an active substance content of 49.4% w/w
[3]: Study design and endpoint no longer required for the registration of plant protection products in the EU
N.D.: Not Determined

Summary of the risk assessment for Aclonifen SC 600 G and non-target soil meso- and macrofauna (other than earthworms)

The chronic toxicity endpoints for *Hypoaspis aculeifer* and *Folsomia candida* exposed to Aclonifen SC 600 G was used to calculate the toxicity exposure ratio (TER) values in accordance with the Terrestrial Guidance Document (SANCO/10329/2002)[21] and EFSA Journal 2017; 15(2):4690[22]. The TER_{LT} values for aclonifen were above the trigger value of 5 in accordance with the proposed uses and therefore, the risk was considered to be acceptable.

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

The risk assessment for non-target soil meso- and macrofauna (other than earthworms) has been conducted in line with the Terrestrial Guidance Document (SANCO/10329/2002) and EFSA Journal 2017; 15(2):4690.

Details on the predicted environmental concentration in soil (PEC_{soil}) for aclonifen are presented in Document M-CP9, Section 9.1.3.

The log P_{ow} for aclonifen is greater than 2 and therefore an additional factor of 2 which covers the possible sorption of high log P_{ow} substances to soil was applied to the endpoints determined from the *Hypoaspis aculeifer* study (M-404537-01-1) and the *Folsomia candida* study (M-404393-01-1).

Table 10.4-4: Non-target soil meso- and macrofauna (other than earthworms) toxicity exposure ratios for the proposed uses of Aclonifen SC 600 G

Test species	$Endpoint_{corr}$[1] (mg a.s./kg soil dw)	Maximum PEC_{soil} (mg/kg soil dw)	TER_{LT}	Trigger value
Hypoaspis aculeifer	139	0.5697	244	5
Folsomia candida	77		135	

[1]: Study endpoints divided by a factor of 2 to correct for log P_{ow} >2

The long-term TER values were above the risk assessment trigger value of 5. It is therefore concluded that the risks to non-target soil meso- and macrofauna (other than earthworms) are acceptable when Aclonifen SC 600 G is used according to the recommended GAP.

[21] European Commission (EC), 2002. Guidance document on terrestrial ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002) revision 2, final. 1–39.
[22] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), Ockleford C, *et al.*, 2017. Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms. EFSA Journal 2017;15(2):4690, 225 pp. doi:10.2903/j. efsa.2017.4690

Studies performed on non-target soil meso- and macrofauna (other than earthworms) on the formulation, Aclonifen SC 600 G are presented below:

Data Point:	KCP 10.4.2/01
Report Author:	[redacted]; [redacted]
Report Year:	2002
Report Title:	Effects of EXP04209E on the decomposition of organic material enclosed in litter bags in the field
Report No:	C020883
Document No:	M-209896-02-1
Guideline(s) followed in study:	BBA: WG draft method, march 2001
Deviations from current test guideline:	Current Guideline: BBA draft (2001) Wild boars damaged the field site and some of the bags were pulled out and missing. This influenced the result of the last sampling. Current method guideline: SANCO/3029/99 rev.4 Yes, no recovery experiments were performed during method validation
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

In the previous submission (DAR, 2006), this study was evaluated and accepted as valid for risk assessment purposes. This study design and endpoint is no longer required for the registration of plant protection products in the EU and hence a summary of this study is not presented in this dossier.

CP 10.4.2.1 Species level testing

Data Point:	KCP 10.4.2.1/01
Report Author:	[redacted]
Report Year:	2002
Report Title:	AE F068300 00 SC50 A203 = EXP04209E (Bandur): Laboratory dose-repons test to evaluate effect on survival and reproduction of the predaceous mite Hypoaspis aculeifer Canestrini (Acari. Laelapidae) in standard soil (LUFA 2.1)
Report No:	C029557
Document No:	M-217404-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Current Guideline: OECD 226, 2008 The test was performed to the outdated Bakker test design with 14-day mortality, 7-day mating and 7-day reproduction phases rather than a single 14-day mortality and reproduction phase
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

In the previous submission (DAR, 2006), this study was evaluated and accepted as valid for risk assessment purposes. The study was performed to the outdated Bakker test design with 14-day mortality, 7-day mating and 7-day reproduction phases rather than a single 14-day mortality and reproduction phase as per the current OECD 226 (2008) test guideline.

Therefore, as this study does not meet the requirements of the current OECD guideline, it should be considered as supportive only and hence no summary for this study is provided.

Assessment and conclusion by RMS:

Data Point:	KCP 10.4.2.1/02
Report Author:	[redacted]
Report Year:	2011
Report Title:	Aclonifen SC 600 G: Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	KRA-HR-45/11
Document No:	M-404537-01-1
Guideline(s) followed in study:	OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil
Deviations from current test guideline:	Current guideline: OECD 226, 2008 Transfer of the test animals was finished within three hours after the application of the test item rather than within two hours due to technical reasons. This has no impact on this study.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of Aclonifen SC 600 G on mortality and reproduction of the predaceous mite *Hypoapsis aculeifer*.

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 4 replicates for each test item concentration) were exposed to control and treatments. Concentrations of 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil were tested.

After a period of 14 days, the surviving adults and the living juveniles were counted.

In the control group 3.8% of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤20% mortality. The LC_{50} could not be calculated and is considered to be > 1000 mg test item/kg dry weight artificial soil.

Concerning the number of juveniles, statistical analysis (Williams t-test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and all concentrations up to 562 mg test item/kg dry weight artificial soil. Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction is 562 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 1000 mg test item/kg dry weight artificial soil.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Aclonifen SC 600 G

Batch no.: EV54001166

Active Ingredient / Purity: Aclonifen 595.0 g/L (49.4%w/w)

Expiry date: 16 February 2013

Appearance: Yellow suspension

Storage: 25 ± 5 °C

2. Test Organism: Hypoaspis aculeifer (Acari: Laelapidae)

Age: Adult, fertilized, females were used as test organisms in the study 29 days after start of egg laying

Source: [redacted]

Feeding: Tyrophagus putrescentiae (cheese mites)

B. STUDY DESIGN AND METHODS

1. In-life phase: 22 February – 21 March 2011

2. Exposure conditions

Test vessels:	Glass vessels (Weck Mini-Sturzglas, volume 140 mL, diameter 5 cm at the bottom, height 7 cm). The test vessels were covered with glass lids to prevent *Hypoaspis aculeifer* from escaping but allowing aeration during the test period
Test soil:	Artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74.8% fine quartz sand (sort F36, particle size 0.2 – 0.05 mm = 91.35%) 5% Sphagnum peat, air dried and finely ground 20% Kaolin clay (content of Kaolinite: $Al_2Si_2O_5(OH)_4$) = 30.2%) approximately 0.2% Calcium carbonate ($CaCO_3$) (for the adjustment to pH to 6.0 ± 0.5)
Experimental design:	Control and five test item groups (100, 178, 316, 562 and 1000 mg a.s./kg dry soil)
Replicates:	Control: 8 replicates; Test item treatments: 4 replicates
Loading:	10 adult, fertilized female *Hypoaspis aculeifer* per replicate
Temperature:	20 ± 2 °C
Photoperiod:	16-hour light, 8-hour darkness
Light intensity:	400 – 800 lux

3. Administration of the test item

Dose preparation

All test item solutions were prepared freshly on the day of the application.

- Stock solution (= solution 1) 2.4991 g test item filled up to a volume of 250 mL with deionised water (1000 mg test item/kg dry weight artificial soil).
- Solution 2: 141 mL solution 1 was filled up to 250 mL with deionised water (562 mg test item/kg dry weight artificial soil)
- Solution 3: 141 mL solution 2 was filled up to 250 mL with deionised water (316 mg test item/kg dry weight artificial soil)
- Solution 4: 141 mL solution 3 was filled up to 250 mL with deionised water (178 mg test item/kg dry weight artificial soil)
- Solution 5: 140 mL solution 4 was filled up to 250 mL with deionised water (100 mg test item/kg dry weight artificial soil)

A uniform volume of 50 mL was used for all application solutions (starting with the lowest application rate and ending with the highest application rate). The test item was thoroughly mixed into 500 g artificial dry weight artificial soil of each application rate using a laboratory mixer (Krefft). The control group was treated first in the same way as described above but with 50 mL deionised water only. Afterwards the treated artificial soil of each application rate and the control was portioned out. Each test vessel of the 8 control replicates and the 4 treatment replicates of each concentration plus the one for

measurement purpose was filled up with 20 g dry weight artificial soil avoiding compression of the artificial soil. The remaining artificial soil was disposed.

Test organism assignment and exposure

Directly after application of the test item, the adult, fertilized, female (29 days after start of egg laying for three days) were exposed to the control and treatment vessels. This was achieved by putting 10 females individually onto the surface of the artificial soil using a fine brush.

4. Measurements and observations

Directly after the addition of the *Hypoaspis aculeifer*, they were fed with cheese mites (*Tyrophagus putrescentiae*). During the test the soil mites were fed 3, 7 and 10 days after test start with the cheese mites.

Each test vessel was weighed for the determination of water loss. Seven days after test start water loss was determined and replenished.

After a period of 14 days, the surviving adults and the living juveniles per test vessel were extracted, applying a temperature gradient. All *Hypoaspis aculeifer* (adult, females and juveniles) were counted under a binocular.

5. Statistics/Data evaluation

The calculation of mean, standard deviation and % mortality of the control and treatment groups with Excel sheets (Microsoft Excel 2003) were documented in the raw data.

For the reproduction, for normal distribution and homogeneity of variance using Kolmogorroff-Smirnov Test and Cochran-Test (α = 0.05), respectively were used. Data of reproduction were normally distributed and homogeneity of variances was given. Therefore Williams t-test (one-sided smaller, α = 0.05) was used to determine NOEC and LOEC values. Probit analysis was used to determine the EC_{50} value.

The software used to perform the statistical analysis was ToxRat Pro 2.10 (released February 19, 2009); ([redacted] 2001-2009).

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Analytical verification was not required.

B. BIOLOGICAL DATA

In the control group 3.8% of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤20% mortality. The LC_{50} could not be calculated and is considered to be >1000 mg test item/kg dry weight artificial soil.

Concerning the number of juveniles, statistical analysis (Williams t-test, one-sided smaller, α = 0.05) revealed no significant differences between control and the all concentrations tested. Therefore, the No Observed-Effect-Concentration (NOEC) for reproduction is 562 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect- Concentration (LOEC) for reproduction is 1000 mg test item/kg dry weight artificial soil.

Table: Effects of Aclonifen SC 600 G on mortality and reproduction of *Hypoaspis aculeifer*

Nominal concentration (mg/kg)	Mortality (%)	Reproduction (juveniles/vessel)	Reproduction (% of control)
Control	3.8	333.0	
100	7.5	331.5	99.5
178	0.0	335.0	100.6
316	5.0	335.8	100.8
562	2.5	343.5	103.2
1000	2.5	301.8[1]	90.6

[1]: statistical significance (Williams t-test, one-sided smaller, $\alpha = 0.05$)

In a separate non-GLP reference item test, Dimethoate showed a LC_{50} of 4.051 mg a.s./kg and a $NOEC_{reproduction}$ of 3.156 mg a.s./kg. The EC_{50} for reproduction was 6.445 mg a.s./kg which was within the recommended range of the guideline of 3.0 – 7.0 mg a.s./kg dry weight artificial soil. This shows that the test organisms are sufficiently sensitive

C. VALIDITY CRITERIA

Validity criterion	Required (OECD 226, 2016)	Achieved
Control mortality	≤20%	3.8%
Mean number of juveniles per control replicate	≥50	333
Coefficient of variation for juveniles/control replicate	≤30%	4.4%

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Nominal Concentration (mg test item/kg)
LC_{50} mortality	> 1000
$NOEC_{reproduction}$	562
$LOEC_{reproduction}$	1000

III. CONCLUSION

There were no significant differences in reproduction between control and all concentrations up to 562 mg test item/kg dry weight artificial soil.

Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction is 562 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect- Concentration (LOEC) for reproduction is 1000 mg test item/kg dry weight artificial soil.

[redacted] *(2011)*

Assessment and conclusion by applicant:
All validity criteria were satisfied and therefore this study can be considered to be valid. The No-Observed-Effect-Concentration (NOEC) for reproduction is 562 mg product/kg dry weight artificial soil. Effects on reproduction at the highest test concentration of 1000 mg product/kg dry weight artificial soil were less than 10%. It was not therefore possible to determine EC_{10}, EC_{20} or EC_{50} values. For use in the risk assessment, as the log P_{ow} for aclonifen is greater than 2, in line with the EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002), endpoints have to be divided by 2. The $NOEC_{corr}$ was therefore 281 product/kg dry weight artificial soil. In terms of the active ingredient content, based on an active ingredient content of 49.4%, the $NOEC_{corr}$ was estimated to be 139 mg aclonifen/kg.

Assessment and conclusion by RMS:

Data Point:	KCP 10.4.2.1/03
Report Author:	[redacted]
Report Year:	2011
Report Title:	Aclonifen SC 600 G: Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	FRM-COLL-112/11
Document No:	M-404393-01-1
Guideline(s) followed in study:	OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil
Deviations from current test guideline:	Current Guideline: OECD 232, 2016 Due to technical reasons the soil was premoistened at test start instead of 2 to 7 days before start of the test. No influence on the study
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of Aclonifen SC 600 G on the mortality and reproduction of the collembolan species *Folsomia candida*. 10 collembolans (11-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated), 100, 178, 316, 562 and 1000 mg test item/kg artificial soil dry weight at 20 ± 2°C, 400 – 800 lux, 16h light : 8h dark. During the study, they were fed with granulated dry yeast.

Mortality and reproduction were determined after 28 days. In the control group 8.8% of the adult *Folsomia candida* died which is below the allowed maximum of ≤20% mortality.

Concerning the number of juveniles, statistical analysis (William's-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and the treatment groups with 100 to 316 mg test item/kg artificial soil dry weight. Therefore, the No Observed Effect Concentration (NOEC) for reproduction is 316 mg test item/kg artificial soil dry weight. The Lowest Observed Effect Concentration (LOEC) for reproduction is 562 mg test item/kg artificial soil dry weight.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Aclonifen SC 600 G

Batch no.: EV54001166

Active Ingredient / Purity: Aclonifen, 595.0 g/L (49.4%w/w)

Expiry date: 16 February 2013

Appearance: Yellow suspension

Storage: 25 ± 5 °C

2. Test Organism: *Folsomia candida* (Collembolan, Isotomidae)

Age: Collembolans from a synchronised culture at an age of 11-12 days

Source: [redacted]

Feeding: Bakers dry yeast

B. STUDY DESIGN AND METHODS

1. In-life phase: 22 February – 23 March 2011

2. Exposure conditions

Test vessels: Glass vessels (volume 140 mL, diameter 5 cm at the bottom, height 7 cm). The test vessels were covered with glass lids to prevent the collembolans from escaping but allowing aeration during the test period

Test soil: Artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis):

74.8% fine quartz sand (sort F 36, particle size 0.2 – 0.05 mm = 91.35%)

5% Sphagnum peat, air dried and finely ground

20% Kaolin clay (content of Kaolinite: $Al_2Si_2O_5(OH)_4$) = 30.2%)

	approximately 0.2% Calcium carbonate ($CaCO_3$) (for the adjustment to pH to 6.0 ± 0.5)
Experimental design:	Control and five test item groups (100, 178, 316, 562 and 1000 mg a.s./kg dry soil)
Replicates:	Control – 8 replicates; Test item treatments – 4 replicates
Loading:	10 collembolans per replicate
Temperature:	20 ± 2 °C
Photoperiod:	16-hour light: 8-hour darkness
Light intensity:	400 – 800 lux

3. Administration of the test item

Dose preparation

All test item solutions were prepared freshly on the day of the application.

- Stock solution (= solution 1) 2.4991 g test item filled up to a volume of 250 mL with deionised water (1000 mg test item/kg dry weight artificial soil).
- Solution 2: 141 mL solution 1 was filled up to 250 mL with deionised water (562 mg test item/kg dry weight artificial soil)
- Solution 3: 141 mL solution 2 was filled up to 250 mL with deionised water (316 mg test item/kg dry weight artificial soil)
- Solution 4: 141 mL solution 3 was filled up to 250 mL with deionised water (178 mg test item/kg dry weight artificial soil)
- Solution 5: 140 mL solution 4 was filled up to 250 mL with deionised water (100 mg test item/kg dry weight artificial soil)

A uniform volume of 50 mL was used for all application solutions (starting with the lowest application rate and ending with the highest application rate). The test item was thoroughly mixed into 500 g artificial dry weight artificial soil of each application rate using a laboratory mixer (Krefft). The control group was treated first in the same way as described above but with 50 mL deionised water only. Afterwards the treated artificial soil of each application rate and the control was portioned out. Each test vessel of the 8 control replicates and the 4 treatment replicates of each concentration plus the one for measurement purpose was filled up with 30 g dry weight artificial soil avoiding compression of the artificial soil. The remaining artificial soil was disposed.

Test organism assignment and exposure

Directly after application the 11-12 days old collembolans from the synchronised cohort were collected with an exhauster into small glass tubes. They were counted to ensure that 10 non-damaged individuals were introduced. Then the 10 collembolans were placed on the soil surface of the treated soil and the untreated control respectively. The sequence of inserting the test organisms into the test vessels followed a computer-generated random design.

4. Measurements and observations

Directly after the addition of the collembolans, they were fed with granulated dry yeast. Feeding was also done 14 days after test start. Approximately 2 mg (one spatula tip) per test vessel was added per feeding date.

At test start each test vessel was weighed for the determination of water loss. After 14 days the loss of water was determined by reweighing the test vessels. The vessels were re-wetted with the approximately 2-fold amount of the missing water. The test vessels were set up randomised in a climatic test room. After 7, 14 and 21 days the test vessels were re-randomised.

After 28 days, the soil of each replicate was transferred into a plastic vessel (volume: 200 mL; surface: 75 cm^2). Each portion was stirred up with 80 mL of deionised water and the collembolans drifted to the surface. The water was coloured with 10 mL black ink in order to increase the contrast between the water and the white collembolans. From each vessel a digital image was taken. Each digital image was checked by visual inspection. In a first step the adult collembolans were visually counted and marked on the digital image. In a second step the automatically counted juveniles were checked for mistakes and the counting was manually corrected if necessary. These procedures were carried out with the LemnaTec Scanalyzer, Software: LemnaTec Launcher ([redacted] Germany).

5. Statistics/Data evaluation

Endpoints of the test were mortality of the adult collembolans in comparison to the initially placed test organisms expressed in % and the number of offspring hatched from the eggs and surviving until the end of the test period per test vessel (reproduction). Missing adults (compared to the number of initially placed test organisms) were considered to be dead, since dead collembolans cannot be extracted.

Data of reproduction were tested for normal distribution and homogeneity of variance using Kolmogorov - Smirnov -Test and Cochran's -Test (α = 0.05) respectively. Data of reproduction were normally distributed and homogeneity of variances was given. Therefore William's-t test (one-sided-smaller, α = 0.05) was used to determine NOEC and LOEC values.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Analytical verification was not required.

B. BIOLOGICAL DATA

In the control group 8.8% of the adult collembolans died which is below the allowed maximum of ≤20% mortality.

Concerning the number of juveniles, a statistically significant effect (William's-t test, one-sided-smaller, α = 0.05) was found in the treatment groups with 562 and 1000 mg test item/kg artificial soil dry weight. The No Observed Effect Concentration ($NOEC_{reproduction}$) is 316 mg test item/kg artificial soil dry weight. The Lowest Observed Effect Concentration ($LOEC_{reproduction}$) is 562 mg test item/kg artificial soil dry weight.

Table: Effects of Aclonifen SC 600 G on mortality and reproduction of *Folsomia candida*

Nominal concentration (mg/kg)	Mortality (%)	Reproduction (juveniles/vessel)	Reproduction (% of control)
Control	8.8	1053.3	-
100	7.5	1154.3	109.6
178	10.0	1086.8	103.2
316	7.5	1056.5	100.3
562	15.0	657.5	62.4[1]
1000	25.0	455.0	43.2[1]

1 Statistical significance (Williams t-test, one-sided smaller, α = 0.05)

In a separate, non-GLP reference item test, boric acid showed an EC_{50} for reproduction of 91 mg/kg which was within the recommended range of the guideline of about 100 mg/kg dry weight artificial soil. This shows that the test organisms are sufficiently sensitive.

C. VALIDITY CRITERIA

Validity criterion	Required (OECD 232, 2016)	Achieved
Control mortality	≤20%	8.8%

The validity criterion was satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Nominal concentration (mg test item/kg)
$LC_{50\ mortality}$	> 1000
$NOEC_{reproduction}$	316
$LOEC_{reproduction}$	562

III. CONCLUSION

There were no significant differences in reproduction between control and the treatment groups with 100 to 316 mg test item/kg artificial soil dry weight.

Therefore, the No Observed-Effect-Concentration (NOEC) for reproduction is 316 mg test item/kg dry weight artificial soil. The Lowest Observed-Effect- Concentration (LOEC) for reproduction is 562 mg test item/kg dry weight artificial soil.

[redacted] *(2011)*

Data Point:	KCP 10.4.2.1/04
Report Author:	[redacted]
Report Year:	2019
Report Title:	Aclonifen SC 600 G: Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil - Statistical re-analysis of [redacted] 2011 (M-404393-01-1) study
Report No:	VC/19/027/003
Document No:	M-675907-01-1
Guideline(s) followed in study:	Not applicable. Report is a re-evaluation of previously generated study data
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The report for the collembolan reproduction test of Aclonifen SC 600 G to Folsomia candida (M-404393-01-1, [redacted], 2011) only provided LOEC and NOEC values for the test item. Data from the study has been re-analysed in order to provide L/EC$_{10}$, L/EC$_{20}$ and L/EC$_{50}$ values.

Statistical analyses of the available data resulted in the calculation of the following L/EC$_x$ values:

Parameter	Reproduction			Survival		
	EC$_{10}$	EC$_{20}$	EC$_{50}$	LC$_{10}$	LC$_{20}$	LC$_{50}$
Value (mg/kg)	311.268	429.338	794.310	736.116	1058.61	n.d
Lower 95%-cl	228.355	315.847	529.884	482.58	788.82	n.d
Upper 95%-cl	424.286	584.331	1168.703	1062.64	1238.95	n.d

n.d.: not determined due to mathematical reasons (inappropriate data) or value is beyond the tested concentrations

All computations were carried out in ToxRat Professional version 3.3.0 (ToxRat Solutions GmbH, 2018).

[redacted] *(2019)*

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

The No-Observed-Effect-Concentration (NOEC) for reproduction is 316 mg product/kg dry weight artificial soil.

L/EC$_{10}$, L/EC$_{20}$ and L/EC$_{50}$ values were determined following re-analysis of the original study data and are summarised below:

Parameter	Reproduction			Survival		
	EC$_{10}$	EC$_{20}$	EC$_{50}$	LC$_{10}$	LC$_{20}$	LC$_{50}$

Value (mg prod./kg)	311.268	429.338	794.310	736.11	1058.61	n.d
Lower 95%-cl	228.355	315.847	529.884	482.58	788.82	n.d
Upper 95%-cl	424.286	584.331	1168.703	1062.64	238.95	n.d

n.d.: not determined due to mathematical reasons (inappropriate data) or value is beyond the tested concentrations

EFSA's Outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology (EFSA, 2019)[23] recommends that the lowest of the EC_{10} and NOEC values be used for risk assessment purposes. In this study, as the EC_{10} was lower than the NOEC, the EC_{10} should be used for risk assessment.

For use in the risk assessment, as the log P_{ow} for aclonifen is greater than 2, in line with the EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002), endpoints have to be divided by 2. The $NOEC_{corr}$ and $EC_{10\ corr}$ were therefore 158 and 156 mg product /kg dry weight artificial soil respectively..

In terms of the active ingredient content, based on an active ingredient content of 49.4%, the $NOEC_{corr}$ and $EC_{10\ corr}$ were estimated to be 78 and 77 mg a.s./kg respectively

Assessment and conclusion by RMS:

CP 10.4.2.2 Higher tier testing

Data Point:	KCP 10.4.2.2/01
Report Author:	[redacted]
Report Year:	2017
Report Title:	Aclonifen SC 600 G: Effects on acari under field conditions
Report No:	15 10 48 001 F
Document No:	M-594981-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC, Regulation (EC) No 1107/2009 (2009); US EPA OCSPP Not Applicable
Deviations from current test guideline:	Current guideline: Not applicable No deviations from study plan were recorded
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

[23] EFSA (European Food Safety Authority), 2019. Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117 pp. doi:10.2903/sp.efsa.2019.EN-1673

Executive Summary

The objective of this field study was to investigate potential effects and the potential recovery of field populations of soil Acari after application of the test item. Therefore, a field experiment lasting about one year was performed and the effects of the test item with regard to species composition and abundance were compared to an untreated control and to a reference item ([redacted]).

The study was performed following the guideline ISO 23611-2 (2006) - Soil quality - Sampling of soil invertebrates, Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina) and taking into account the recommendations of [redacted] (2006): Technical Recommendations for the Update of the ISO Earthworm Field Test Guideline (ISO 11268-3) and [redacted] (2010): Guidance for summarising and evaluating field studies with non-target arthropods.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	Aclonifen SC 600 G
	Batch no.:	EV56905993
	Active Ingredient / Purity:	Aclonifen, 596.7 g/L (49.5% w/w)
	Expiry date:	2 April 2018
	Appearance:	Yellow crystalline powder
	Storage:	5 ± 5 °C
2.	**Test Organism:**	Acari (extracted from upper 5 cm of soil)
	Source:	Collected from field study area

B. STUDY DESIGN AND METHODS

1.	**In-life phase**	10 April 2015 (pre-sampling) to 24 February 2017
2. Exposure conditions		
	Test field description	Arable land, located near Machern, Saxony, Germany
	Test soil:	Sandy loamy silt soil (DIN 4220 / loam USDA)
	Suitability:	Preliminary sampling (non-GLP) of Acari before test start found sufficient abundance and diversity of Acari species. Acari of 2 orders, 3 suborders, 1 super cohort, 4 cohorts, 21 families, 16 genera and 7 species were found
	Site history:	Cultural practices performed on the test field during 2012 till 2015 followed the usual agricultural practice. The only cultivated crop within this time span was *Phacelia tanacetifolia*. No further plant protection products others than the test item and the reference item were applied on the test field. No mineral or organic fertilisers were applied to the test field
	Treatment:	5 treatment groups arranged in a randomised block design. Each treatment group comprised 4 replicates each on 10 x 10 m (100 m²) plots and surrounded by 2 m wide paths between plots

3. Administration of the test item

Dose preparation

The application was performed on bare soil. The application of the control tap water as well as of the test item/reference item was carried out for each plot separately. Water (control) was applied first followed by the test item at increasing application rates. The reference item was applied last. The sprayer was thoroughly cleaned and flushed with fresh water before applying the reference item. Verification of the application rate was achieved by determining the remaining spray liquid after application of each plot, taking the amount for technical loss into account. For all treatment groups the applied rates were within the tolerated deviation of ±10%. The application was performed on a day with low wind and no rain forecast.

Test exposure

The trial took place on arable land [redacted] Germany.

The test item Aclonifen SC 600 G was applied once on bare soil at application rates of 5.55 L/ha, 11.1 L/ha and 16.64 L/ha corresponding to 3.31 kg a.s./ha, 6.62 kg a.s./ha and 9.93 kg a.s./ha. Clarnet (chlorpyrifos 48.0% w/v (nominal)) was applied once to the plots as a reference item at a rate of 2.5 L/ha (nominally equivalent to 1200 g a.s./ha). Tap water (600 L/ha) was applied once as a control.

Twenty plots, each 10 m x 10 m, were arranged in a 5 x 4 formation, each plot surrounded by a 2 m wide path between the plots. The set-up was a randomised block design. Defined areas were sampled to assess the Acari population before application and seven times after application, i.e. 14, 27, 62, 151, 214, 330 and 365 days after application.

Acari were extracted from soil cores taken in the upper 5 cm of the soil (6 per plot) using a MacFadyen high-gradient extractor.

Maintenance of the field during the present study was according to general agricultural practice. Due to observed differences in the vegetation cover between the control and the plots treated with the different test item treatment applications the soil of the test field was mechanically mixed at the top 5 cm about one month after application by means of a grubber. Thereby, the complete plant material was incorporated into the soil, i.e. in the control and the reference item treatment group. A clover grass mixture "Landsberger Gemenge" was sown on all plots about three months after application, which stayed on the field until the end of the test. No further plant protection products others than the test item and the reference item were applied on the test field. No mineral or organic fertilisers were applied to the test field.

Irrigation of the test field was required after application to support the exposure of the test organisms if no or little rainfall occurred. The test field was irrigated with 10 mm tap water on day 3 after application.

4. Measurements and observations

Environmental data (air temperature at 2 m height, soil temperature at 10 cm depth, and rainfall) were recorded by the Thies weather station of the [redacted], Germany, about 5 km distance to the test field. Weather data were recorded continuously starting from April 2015 and ending with the last sampling for Acari (7th sampling).

Soil for water content analyses was taken in 0 to 5 cm soil depth, one soil core per plot, and transferred into tightly sealed glass bottles for transport to the laboratory for further analyses. Following application, rainfall was monitored on site with a rain gauge [redacted]

On each sampling occasion for Acari, soil moisture (water content on a mass basis with the gravimetric method) and soil temperature (with a digital soil thermometer Testo 925) were observed. Soil specimens for water content analyses were taken in 0 to 5 cm soil depth (one soil core per plot) and transferred into tightly sealed glass bottles for transport to the laboratory for further analyses. Soil temperature was assessed in 10 cm soil depth for each plot. Soil specimens were analysed for residues of aclonifenonifen as part of this study. Therefore, immediately after application, soil specimens were taken from the control plots and the test item plots. Plots were sampled in the sequence control, followed by the test item at increasing application rates. The plots treated with the reference item were not sampled. On each test item and control plot, 10 sub-specimens (soil cores) were taken in an "X" shape sampling scheme across the plot, which were pooled to one specimen per plot.

The following population samplings for Acari were performed:

pre-sampling	6 days before application (DAA* -6)
1st	14 days after application (DAA 14)
2nd	27 days after application (DAA 27)
3rd	62 days after application (DAA 62)
4th	151 days after application (DAA 151)
5th	214 days after application (DAA 214)
6th	330 days after application (DAA 330)
7th	365 days after application (DAA 365)

DAA = Days After Application (of test item)

5. Statistics/Data evaluation

For the statistical analysis taxa are grouped as follows:

- Total abundance: comprising all identified and unidentified Acari
- Abundance on order level: comprising all Acari of the identified orders
- Abundance on suborder level: comprising all Acari of the identified suborders
- Abundance on cohort level: comprising all Acari of the identified cohort
- Abundance on family level: comprising all Acari of the identified families
- Abundance on genus level: comprising all Acari of the identified genera
- Abundance on species level: comprising all Acari of the identified species

Only taxa with a minimum total abundance of 10 individuals of at least one control plot on at least two sampling dates were taken into account. However, only a small part of the Acari community was not statistically analysed. A negative impact on the outcome of the study can, therefore, be excluded.

No Observed Effect Level (NOEL)

Abundances in the plots of the control and the toxic reference item were tested by the one-sided Student t-test for significant declines of abundances. The one-sided multiple t-test by Williams ([redacted] 1972) was used to test for differences between control and each treatment level of the test item. The No Observed Effect Level (NOEL) as the highest treatment level without a significant difference to the control was determined for each sampling occasion. Abundance data were ln(a x n +1) transformed for the tests to achieve normal distribution and variance homogeneity of variance and thus, using a

parametric test ([redacted] 1995). In this study the factor *a* was set to 12 which results in a transformed value of approximately 1 for the lowest abundance value (above zero) in the data set (i.e. 0.167 representing 1 animal found in the 6 soil core samples per plot and date). All tests were conducted with an error level $\alpha = 0.05$.

The calculation of NOELs, diversity and similarity analysis was done using the Community Analysis (CA) software Version V4.3. An earlier version is described in [redacted] (1994).

Ordination analysis

Principal Response Curves (PRC) is a multivariate approach developed to analyse and visualise effects on the community level time, originally suggested to evaluate aquatic mesocosm studies ([redacted] 1998, 1999, [redacted] 2003). It focuses on the relative difference between species composition in controls and treatments over time. PRCs are calculated via the ordination technique Redundancy Analysis (RDA), which can be seen as a canonical (or constrained) form of a Principal Component Analysis (PCA) because RDA uses only the variance, which can be attributed to the explanatory variables. For PRCs the combination of time and treatment level is used as an explanatory dummy variable while the time is considered as a co-variable. It cannot be assumed *a priori* that the toxic reference item acts in the same way as the test item, i.e. that the species are ranked in a similar way according to their sensitivity to both compounds. Therefore, the ordination analysis was restricted to the data of control and treatment with the test item. For the ordination analysis, all data were used (including the rare taxa).

Ordinations were conducted with the program CANOCO 4.5 (Ter Braak & Šmilauer 2002).

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

In the plots treated with the test item, mean residue values of 118 - 142% of the nominal application rates were found in the soil specimens taken immediately after application. Since the mean residue levels of the active substance of the test item in the soil specimens taken immediately after application were within the recommended range of 50% to 150% of the nominal values, the correct application was verified.

B. BIOLOGICAL DATA

The pre-sampling confirmed high abundance and diversity of Acari and therefore the test field was considered as suitable for the trial. Acari of 2 orders, 3 suborders, 1 super cohort, 4 cohorts, 21 families, 16 genera and 7 species were found.

The mean Acari abundance in the upper 5 cm of the control plots was 14451.3 ind./m² at pre-sampling (6 days before application), 26504.6 ind./m² at 1st sampling (14 days after application), 17591.9 ind./m² at 2nd sampling (27 days after application), 11395.5 ind./m² at 3rd sampling (62 days after application), 14514.9 ind./m² at 4th sampling (151 days after application), 12414.1 ind./m² at 5th sampling (214 days after application), 12965.8 ind./m² at 6th sampling (330 days after application) and 12605.1 ind./m² at 7th sampling (365 days after application).

The dominant Acari order present in the upper 5 cm of the soil of the test field was Sarcoptiformes with 10096.8 ind./m² (68% of the total Acari abundance at pre-sampling). Dominant suborders of the Acari

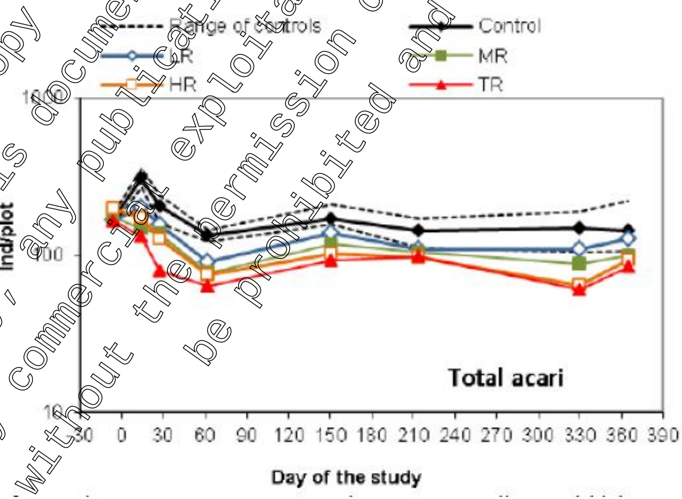
order Sarcoptiformes were Oribatida (46% of the total abundance at pre-sampling) and Actinedida (22% of the total abundance at pre-sampling). A super cohort of the suborder Oribatida was Enarthronotides (4% of the total abundance at pre-sampling) represented by the family Hypochthoniidae, the genus Hypochthonius and the species *Hypochthonius rufulus* (4% of the total abundance at pre-sampling). Dominant cohorts of the suborder Oribatida were Brachypylina (18% of the total abundance at pre-sampling) and Astigmatina (21% of the total abundance at pre-sampling). Dominant families of the cohort Brachypylina were Oppiidae (5% of the total abundance at pre-sampling) with the genus Oppiella represented by the species *Oppiella nova* (4% of the total abundance at pre-sampling) and Tectocepheidae (10% of the total abundance at pre-sampling) with the genus *Tectocepheus* (10% of the total abundance at pre-sampling) and the species *Tectocepheus velatus* (9% of the total abundance at pre-sampling).

The second dominant Acari order present in the upper 5 cm of the soil of the test field was Mesostigmata with 4770.4 ind./m² (32% of the total Acari abundance at pre-sampling) represented by the suborder Monogynaspida with the cohorts Gamasina (31% of the total abundance at pre-sampling) and Uropodina (1% of the total abundance at pre-sampling). Dominant families of the cohort Gamasina were Ascidae (5% of the total abundance at pre-sampling), Laelapidae (6% of the total abundance at pre-sampling) represented by the genus *Hypoaspis* and the species *Hypoaspis aculeifer* (6% of the total abundance at presampling) and Rhodacaridae (11% of the total abundance at pre-sampling) with the genus Rhodacarellus (10% of the total abundance at pre-sampling).

The reference item reduced total Acari abundance (statistically significant) in the upper 5 cm of the soil of the test field by 57% at 1st sampling (14 days after application (DAA)), 62% at 2nd sampling (27 DAA), 52% at 3rd sampling (62 DAA), 43% at 4th sampling (151 DAA), 33% at 5th sampling (214 DAA), 60% at 6th sampling (330 DAA) and 43% at 7th sampling (365 DAA).

The clear effects of the reference item on Acari in the upper 5 cm of the soil confirmed the sensitivity of the test system.

Figure: Abundance of total Acari in soil cores



Where:
LR = Lower rate treatment (application rate of 5.55 L/ha, corresponding to 3.31 kg a.s./ha)
MR = Medium rate treatment (application rate of 11.1 L/ha, corresponding to 6.62 kg a.s./ha)

HR = Higher rate treatment (application rate of 16.64 L/ha, corresponding to 9.93 kg a.s./ha)
TR = Reference item treatment

Due to the strong initial herbicidal activity of the test item remarkable differences in the vegetation coverage were observed in the first month after application between controls and the plots treated with the different test item rates. Increasing herbicidal effects with increasing rates were observed (control and reference item: about 50% vegetation coverage; at 5.55 L/ha: 80% herbicidal effect; 11.1 L/ha: 100% herbicidal effect; 16.64 L/ha: 100% herbicidal effect). In order to achieve more homogenous conditions between the control and the test item treatment groups the soil was mechanically mixed at the top 5 cm one month after application in all plots. Due to this measure the plant material present was incorporated into the top 5 cm soil layer, i.e. in the control plots, by means of a grubber. A clover grass mixture was sown on all plots about three months after application. In summer the vegetation coverage in the test item treated plots equalled to the control plots. However, the observed strong initial vegetation differences and the resulting higher organic matter incorporation into soil (i.e. in the control) could have affected a different population growth of i.e. *Hypochthonius rufulus* in the controls compared to the test item treated plots.

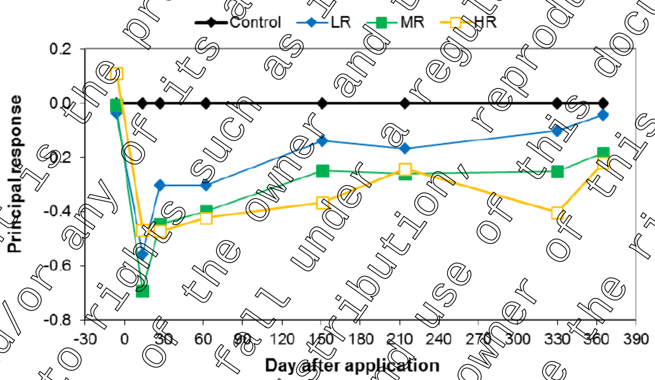
The control abundances of *Hypochthonius rufulus* are characterized by a strong increase after the mechanical mixing until the 6th sampling date, whereas abundances of other taxa remained constant or even decreased. This species could have benefited from the higher organic matter content and an associated different water and nutrition regime in the control plots i.e. after mechanical soil mixing one month after application. *Hypochthonius rufulus* is a detritivorous and thermophilic species potentially profiting on an increase in organic matter content and higher temperatures in soil compared to other taxa[24]. The water content in the control and reference item treatment was considerably lower over the summer period compared to the herbicide treatment groups leading to higher temperatures in soil. This might be the result of the initially higher plant coverage and the stronger water demand by plants in both treatment groups. Especially *Hypochthonius rufulus* seemed to benefit on the lower soil moisture (which is usually associated with higher temperatures) and the higher organic matter content in soil. Due to the different nutrition conditions as well as water and temperature regime in the control and the herbicide treatments a straightforward interpretation of this statistical finding (*Hypochthonius rufulus*) is problematic. It is unclear whether the statistically significant reduction in abundance of *Hypochthonius rufulus* one year after application would have been observed when the recommended crop (potato) would have been planted and such strong initial differences in vegetation coverage and the resulting differences in soil organic matter content at 0-5 cm depth would not have been created. The beneficial conditions for *Hypochthonius rufulus* in the soils of the control, only, and its strong artificially created population growth over the summer seem to impede recovery of this species in the test item treatment groups until the end of the test.

24 [redacted] (1971): Ecologie et Biocenotique de quelquespeuplements dárthropodes edaphiques. Institut Royal des Sciences naturelles de Belgique, Memoire 165
[redacted] (1951): Analysis of the animal community in a beech forest floor. Tijdschr. Ent.
[redacted] (1960): Comparaison de l'efficacité de différentes variants de l'appareil de Berlese-Tullgren. - Z. angew. Ent. 24: 216-247

The Oribatida mite species *Hypochthonius rufulus* is a widely distributed, ubiquitous occurring species in European agricultural, forest, and marsh areas[25]. Its wide distribution in different habitats indicates that is should be able to migrate between different habitats including agricultural fields. Therefore, it is considered unlikely that the observed reduction in abundance of this species would seriously affect biodiversity in agricultural landscapes. As all other taxa (e.g. total Acari and the orders Mesostigmata and Sarcoptiformes) were not affected up to 11.1 L/ha (equivalent to 6.62 kg a.s./ha) at the end of the test period a negative impact on soil functions and fertility are not expected.

Ordination analysis revealed short-term effects of the test item on the Acari community structure at all rates tested down to an application rate of 5.55 L/ha (corresponding to 3.31 kg a.s./ha). However, in the treatment groups treated with 5.55 L/ha and 11.1 L/ha (corresponding to 3.31 kg a.s./ha and 6.62 kg a.s./ha) recovery of the community was shown until day 151 after application (4th sampling). At an application rate of 16.64 L/ha (corresponding to 9.93 kg a.s./ha) recovery of the Acari community took place until day 330 day after application (6th sampling).

Figure: Principal Response Curve for Acari taxa



Where:
LR = Lower rate treatment (application rate of 5.55 L/ha, corresponding to 3.31 kg a.s./ha)
MR = Medium rate treatment (application rate of 11.1 L/ha, corresponding to 6.62 kg a.s./ha)
HR = Higher rate treatment (application rate of 16.64 L/ha, corresponding to 9.93 kg a.s./ha)

Statistical analysis

The test item caused initial statistically significant reductions (Williams t-test; α = 0.05) in total Acari abundance as well as in Acari abundance of the dominant taxa on order, suborder, supercohort, cohort, family, genus and species level (i.e. Sarcoptiformes, Oribatida, *Hypochthonius rufulus*, Rhodacaridae, and Rhodacarellus) at all application rates up to and including day 330 after application (6th sampling).

The statistical findings (at day 330) after application down to the lowest test item rate of 5.55 L/ha were driven by effects on two species, *Hypochthonius rufulus* and *Rhodacarellus sp.*, which strongly impact the results of the respective higher taxonomic levels. However, most of these taxa show full recovery

[25] [redacted] (2015): Acarofauna Germanica – Oribatida. [redacted]
[redacted] (1971): Ecologie et Biocenotique de quelquespeuplements dárthropodes edaphiques [redacted]

one year after application at 5.55 L/ha (3.31 kg a.s./ha) and 11.1 L/ha (6.62 kg a.s./ha). For one species statistically significant reductions continued up to day 365 after application (7th sampling). At the end of the test, only Acari of the supercohort Enarthronotides (4% of the total abundance at pre-sampling) represented by the family Hypochthoniidae, the genus *Hypochthonius* and the species *Hypochthonius rufulus* were statistically significantly reduced in all test item treatment groups i.e. 5.55, 11.1 and 16.64 L/ha (corresponding to 3.31, 6.62, and 9.93 kg a.s./ha).

Acari of the order Mesostigmata (32% of the total Acari abundance at pre-sampling) represented by the suborder Monogynaspida with the cohorts Gamasina (31% of the total abundance at pre-sampling), the family Laelapidae (6% of the total abundance at pre-sampling) represented by the genus *Hypoaspis* and the species *Hypoaspis aculeifer* (6% of the total abundance at pre-sampling) were statistically significantly reduced in the test item treatment group treated with 16.64 L/ha (corresponding to 9.93 kg a.s./ha) at the end of the test. However, no statistically significant effects were observed at 5.55 and 11.1 L/ha (3.31 and 6.62 kg a.s./ha, respectively) at the end of the test.

C. VALIDITY CRITERIA

No validity criteria were identified. However, there were no deviations from the study protocol, therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of NOEL based on one-sided Williams t-test for reduction of abundance to relevant Acari taxa

Taxon	Application rate (kg a.s./ha)							
	Day after application							
	-6	14	27	62	151	214	330	365
Total Acari	≥9.93	<3.31	<3.31	<3.31	3.31	3.31	<3.31	≥9.93
Order Mesostigmata (suborder Monogynsaspida)	≥9.93	<3.31	<3.31	<3.31	6.62	6.62	6.62	6.62
Order Sarcoptiformes	≥9.93	<3.31	≥9.93	<3.31	6.62	<3.31	<3.31	≥9.93
Suborder Oribatida	≥9.93	<3.31	≥9.93	<3.31	6.62	<3.31	<3.31	≥9.93
Suborder Actinedida	≥9.93	3.31	3.31	≥9.93	6.62	≥9.93	6.62	≥9.93
Cohort Gamasina	≥9.93	<3.31	<3.31	<3.31	6.62	≥9.93	6.62	6.62
Cohort Brachypylina	≥9.93	6.62	≥9.93	3.31	6.62	≥9.93	≥9.93	≥9.93
Cohort Astigmatina	≥9.93	≥9.93	<3.31	<3.31	6.62	≥9.93	6.62	≥9.93
Cohort Enarthronotides (species *Hypochthonius rufulus*)	≥9.93	<3.31	<3.31	<3.31	≥9.93	<3.31	<3.31	<3.31
Family Ascidae	≥9.93	<3.31	3.31	<3.31	<3.31	≥9.93	3.31	≥9.93
Family Laelapidae (species *Hypoaspis aculeifer*)	≥9.93	3.31	6.62	3.31	3.31	3.31	≥9.93	6.62
Family Rhodacaridae	≥9.93	<3.31	<3.31	<3.31	≥9.93	≥9.93	<3.31	≥9.93
Family Oppiidae	≥9.93	≥9.93	≥9.93	≥9.93	6.62	≥9.93	≥9.93	≥9.93
Family Tectocepheidae	≥9.93	<3.31	3.31	≥9.93	≥9.93	3.31	≥9.93	≥9.93
Genus Rhodacarellus	≥9.93	<3.31	6.62	<3.31	≥9.93	≥9.93	<3.31	≥9.93
Species *Oppiella nova*	≥9.93	≥9.93	≥9.93	≥9.93	6.62	≥9.93	≥9.93	≥9.93

Species *Tectocepheus velatus*	≥9.93	<3.31	≥9.93	≥9.93	≥9.93	3.31	≥9.93	≥9.93

LR (Lower Rate) = 5.55 L/ha (corresponding to 3.31 kg a.s./ha)
MR (Medium Rate) = 11.1 L/ha (corresponding to 6.62 kg a.s./ha)
HR (Higher Rate) = 16.64 L/ha (corresponding to 9.93 kg a.s./ha)

III. CONCLUSION

The application of Aclonifen SC 600 G tested at application rates of 5.55 L/ha, 11.1 L/ha and 16.64 L/ha (corresponding to 3.31 kg a.s./ha, 6.62 kg a.s./ha and 9.93 kg a.s./ha) caused no long-term effects on the Acari community structure. Acari populations initially impacted at 5.55 L/ha (3.31 kg a.s./ha) and 11.1 L/ha (6.62 kg a.s./ha) showed full recovery after one year, except for *Hypochthonius rufulus*.

Statistically significant reductions in abundance of Acari of the super cohort Enarthronotides (*Hypochthonius rufulus)* were observed one year after application. However, as the observed initial herbicide effects seemed to influence the water, temperature, and nutrition regime in soil this statistical finding should be interpreted with care. The beneficial conditions for *Hypochthonius rufulus* in the soils of the control, only, and its strong artificially created population growth over the summer seem to impede recovery of this species in the test item treatment groups until the end of the study.

[redacted] *(2017)*

~~Data Point:~~	~~KCP 10.4.2.2/02~~
~~Report Author:~~	[redacted]
~~Report Year:~~	~~2017~~
~~Report Title:~~	~~Statement: Evaluation of effects of Aclonifen SC600 on Acari populations under field conditions~~
~~Report No:~~	~~M-595114-01-1~~
~~Document No:~~	~~M-595114-01-1~~
~~Guideline(s) followed in study:~~	~~--~~
~~Deviations from current test guideline:~~	~~Not applicable~~
~~Previous evaluation:~~	~~No, not previously submitted~~
~~GLP/Officially recognised testing facilities:~~	~~No, not conducted under GLP/Officially recognised testing facilities~~
~~Acceptability/Reliability:~~	~~Yes~~

~~**Executive Summary**~~

~~The study design was discussed with the German authorities and was comparable to the principle setup for an earthworm field study (ISO 11268-3, taking into account the recommendations of~~ [redacted] ~~(2006) with adaptations as required for soil mites. The study followed a dose response design: 4 replicate plots (10 x 10 m) served as replicates for each of three rates of aclonifen SC 600 applied onto bare soil.~~

The application rate of 5.55 L/ha corresponds to a rate of 3.31 kg a.s./ha and reflects the maximum single application rate of 2.4 kg a.s./ha plus 0.91 kg a.s./ha to address a worst-case long-term plateau within the topsoil layer of 20 cm depth. This worst-case plateau was calculated based on the highest available DT_{50} value for aclonifen (DT_{50} = 195 d).

The rates of 11.1 L/ha (6.62 kg aclonifen/ha) and 16.64 L/ha (9.93 kg aclonifen/ha) reflect two times and three times the amount of the low rate. The total amounts of aclonifen were applied in a single spray application onto the soil surface. In addition, water treated controls and a toxic reference (Chlorpyrifos dosed at 1200 g a.s./ha) were included.

A pre-sampling, 6 days before application, was followed by seven post-application samplings throughout a twelve-month period (14, 27, 62, 151, 214, 330 and 365 days after application (DAA)). Soil cores of 5 cm soil depth were taken at each sampling date. Acari were extracted from soil cores (6 per plot) using a MacFadyen high-gradient extractor. After taxonomic identification, statistical evaluations were performed as univariate comparisons (Williams-test) for the mite populations and by generating multivariate Principle Response Curves for the community analysis.

Analytical investigations of soil concentrations after application on day 0 were performed by extraction of soil cores and analysis by HPLC/MS-MS.

I. RESULTS AND DISCUSSION

A. Analytical Verification

In the plots treated with the test item, mean residue values of 118 - 142% of the nominal application rates were found in the soil specimens taken immediately after application.

B. Biological Data

Effect on soil mite community

Ordination analysis revealed short-term effects of the test item on the Acari community structure at all three test rates. However, in the plots treated with 3.31 kg aclonifen/ha (LR) and 6.62 kg aclonifen/ha (MR) recovery of the community was shown until DAA 151 (4th sampling). At an application rate of 9.93 kg a.s./ha (HR) recovery of the Acari community took place until day DAA 330 (see Table).

Table: Effects on Acari community structure

Sampling day	NOEL (kg a.s./ha)
-6	≥9.93
14	≥9.93
27	<3.31
62	>3.31
151	6.62
214	6.62
330	≥9.93
365	≥9.93

Effect on soil mite population

Treatment with aclonifen SC 600 caused transient statistically significant differences (Williams t-test; α = 0.05) in total Acari abundance, as well as in abundance of several taxa, at all application rates.

At the end of the study, 12 month after application, Acari of the dominant order Mesostigmata (32% of the total Acari abundance at pre-sampling) represented by the suborder Monogynaspida with the cohorts Gamasina (31% of the total abundance at pre-sampling), the family Laelapidae (6% of the total abundance at pre-sampling) represented by the genus *Hypoaspis* and the species *Hypoaspis aculeifer* (6% of the total abundance at pre-sampling) showed a statistically significant reduction only in the plots treated with the high-rate (9.93 kg aclonifen/ha), whereas in mid-rate and low-rate no statistical differences were detected at study end.

Within the other dominant order Sarcoptiformes aclonifen SC 600 caused transient statistically significant reductions in abundance of certain Acari in all test item treatment groups up to and including day DAA 330 . However, recovery took place in low and mid rate for nearly all Sarcoptiformes (except a single species in the cohort Enarthronoides so that no statistically significant reductions could be observed for this taxon at the end of the test (DAA 365).

Effects on Hypochthonius rufulus

Hypochthonius rufulus was present at pre-sampling with 446 ind./m² representing 3% of the total Acari. At the end of the study *Hypochthonius rufulus* represented 26% of the total mite population sampled from the 5 cm top soil of the control. The abundance of *Hypochthonius rufulus* stayed stable in the control from mean numbers of around 500 ind./m² in the period of the first month. From DAA 27 to DAA 62 its numbers were strongly increasing to 1974 ind./m² and further to numbers of up to 5390 ind./m² until DAA 330 after which the abundance decreased to 3268 ind./m².

The overall same trend of an increase between DAA 27 and DAA 62 and a further increase until DAA 330 followed by a decrease was also seen in the test item treatment groups. In the first two samplings after the application the levels of *Hypochthonius rufulus* individuals were statistically significant lower in the treatments. Considering the overall effect on the mite community this difference could be treatment related. From the third sampling onwards the numbers in all treatments increased but with a lower rate than in the control. The difference between control and all treatments steadily increased until DAA 330.

During the study the presence of *Hypochthonius rufulus* increased to a dominance level of 26% in the control, which is an order of magnitude higher than in the pre-sampling and, based on literature, such a dominance level is unexpected for this species in Germany. Generally, *Hypochthonius rufulus* has been detected with low maximum mean dominance in Germany being close to 2% and classified as a recedent species (UBA, 2012). It is considered that the specific ecology of the affected species (*Hypochthonius rufulus*) and the environmental factors of the treatment groups contributed to the outcome observed in the field study.

The population dynamics of *Hypochthonius rufulus* can be influenced by vegetation, soil organic matter and soil temperature. The application of aclonifen was performed onto bare soil. During the 27 days until the second sampling a natural seed germination of weeds from the seedbank took place on the control plots and on the toxic reference plots. This resulted in a vegetative cover of approx. 50% of the soil surface with a vegetation height of ca. 20 cm in the control plots. In contrast, in the plots treated with aclonifen SC 600, strong herbicidal effects led to inhibition of vegetation growth and thus only 10% of vegetative cover in the low rate and no cover (100% herbicidal effect) in the mid and the high rate treatment groups.

~~The higher vegetative cover during the period until DAA 29 resulted in a lower soil moisture content in the corresponding plots (control, toxic reference) as a result of the increased transpiration losses through the plants. This was confirmed by the results of the soil moisture measurements. The largest difference was observed during the third sampling. The decrease in soil moisture content resulting from the vegetation in turn has led to an increase in topsoil temperature. Higher soil temperatures favour population growth of thermophilic species such as *Hypochthonius rufulus* in the control group plots and have thus led to artificially higher populations.~~

~~In order to control the emerging vegetation and to achieve a more homogenous vegetation cover between the control and the test item treatment groups, at DAA 29 the vegetation present on the plots was mechanically incorporated by means of a harrow. Due to this soil tillage measure the plant material present was incorporated into the top soil layer (approximately 5 cm). As a result of the incorporation the amount of plant material in the top soil layer of the control plots was significantly increased compared to the treatment plots. To further support growth of a homogenous vegetation cover a clover and grass mixture was sown over all the plots at DAA 82. During summer the vegetation cover in the test item treated plots equalled the control plots and by autumn a difference in vegetative cover was no longer visible. This was reflected in the more homogenous soil moisture values from this time forward. However, due to the high amount of plant material that had been incorporated on DAA 29 into the control plots, it is suggested that a higher amount of biomass in the topsoil of the control group was present for a longer period, even after establishment of equal vegetative cover by the clover and grass mixture, thereby providing an additional food source for detritivorous species. Therefore, the population development of *Hypochthonius rufulus* was not only favoured by an increased temperature in the control plots due to the initial vegetation cover but also by an increase in plant foliage which served as a food source following its incorporation at DAA 27.~~

~~II. CONCLUSION~~

~~After initial effects, the mite community fully recovered even at the high rate (9.93 kg aclonifen/ha) within a year after the application.~~

~~It can be concluded that in mid and low rate all initially affected taxa recovered within a year after the application of the test substance with the exception of one species: The Acari species *Hypochthonius rufulus* (supercohort Enarthronotides represented by the family Hypochthoniidae, the genus *Hypochthonius*) showed a statistically significant difference to control for all three test rates.~~

~~In conclusion, the beneficial conditions for *Hypochthonius rufulus* in the control group led to an artificially larger population in the control plots over summer which could not be compensated in the treatment groups. The differences are thus likely due to an artefact of the incorporation treatment providing more feeding material and to the initially higher soil temperatures together leading to inflated *Hypochthonius rufulus* populations in the control plots.~~

~~Furthermore, the Oribatid mite species *Hypochthonius rufulus* is a widely distributed, ubiquitous occurring species in European agricultural, forest, and marsh areas. *Hypochthonius rufulus* is able to live in the litter layer, prefers the layers in vicinity to the soil surface and is capable of withstanding higher temperatures. Its wide distribution in different habitats strongly suggests that it is able to migrate between different habitats, including agricultural fields. Due to the wide distribution and ubiquitous occurrence of *Hypochthonius rufulus*, it is considered unlikely that the observed difference in abundance~~

would seriously affect biodiversity in agricultural landscapes. As total Acari and all other taxa (e.g. the orders Mesostigmata and Sarcoptiformes) were not affected up to and including 6.62 kg a.s./ha at the end of the study, a negative impact on soil functions and fertility is not to be expected.

[redacted] (2017)

Data Point:	KCP 10.4.2.2/03
Report Author:	[redacted]; [redacted] T.
Report Year:	2018
Report Title:	Aclonifen SC 600: Statement answering questions of UK CRD with regard to population recovery of the suborder oribatida and the family Rhodacaridae
Report No:	M-630669-01-1
Document No:	M-630669-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In the CRD answers to Bayer concerning the risk assessment for soil mite field test, a concern was raised by CRD regarding the recovery of the Acari suborder Oribatida and the family Rhodacaridae in particular. Based on the observed pattern of population recovery, CRD concluded that there is uncertainty whether these taxa will be affected again under harsh environmental conditions after 365 DAT (e.g. drop in temperature).

This statement provides additional information on these taxa in the context of the field study.

The observed statistical significant effect on the suborder Oribatida at the low rate, is only driven by the species *Hypochthonius rufulus*. Effects on this species are, however, considered not to be treatment-related, as discussed by [redacted] (2017; M-595114-01-1).

In the field study with Aclonifen SC 600 ([redacted] M-594981-01-1) the family Rhodacaridae is most likely represented by *Rhodacarellus silesiacus*.

Due to its ecology and behavior *R. silesiacus* can deal well with harsher environmental conditions. Long lasting treatment-related population effects are therefore not to be expected in case of reoccurrence of harsh environmental conditions (e.g. lower temperatures after DAT 365).

I. RESULTS AND DISCUSSION

Evaluation of effects on the suborder Oribatida

In the soil mite field study the abundance of Acari in the suborder Oribatida showed significant differences to control at the low test rate of 3.31 kg a.s./ha in the sampling 330 days after application (DAT 330), and no differences to control anymore even at the highest rate of 9.93 kg a.s./ha at the last sampling date (DAT 365).

Analyzing the composition of the suborder Oribatida in this study it becomes evident that the species *Hypochthonius rufulus* is a dominant taxon of the suborder Oribatida at the sampling dates DAT 330 and DAT 365, particularly in the control group.

In order to investigate the influence of the abundance of *H. rufulus* on the statistical performance of the overall mite population, an additional statistical analysis of the suborder Oribatida excluding the species *Hypochthonius rufulus* was conducted.

Table: Abundance of total Oribatida excluding *Hypochthonius rufulus* (mean of four replicate plots)

Treatment group		Sampling	
		6th (DAT 330)	**7th (DAT 365)**
Control	ind./m²	3798	3310
	%	100	100
Low rate (3.31 kg/ha)	ind./m²	3714	4096
	%	**98**	**124**
Mid rate (6.62 kg/ha)	ind./m²	2525	2759
	%	**66**	**83**
High rate (9.93 kg/ha)	ind./m²	2398	2610
	%	**63**	**79**

Excluding *Hypochthonius rufulus*, the abundance of 'remaining' Oribatida at DAT 330 in the low rate is only 2% lower than the control. On DAT 365 'remaining' Oribatida in the low rate even exceed the abundance in the control. For the mid and the high rate, reductions of 34% to 37% were observed on DAT 330 for the 'remaining' Oribatida, which decreased on DAT 365 to 17% to 21%, respectively.

A statistical evaluation of the underlying data for DAT 330 and DAT 365, indicates for the 'remaining' Oribatida no statistically significant differences for the low rate compared to the control. For the mid and the high rate treatments the difference of 'remaining' Oribatida to the control was statistically significant only on DAT 330, and not significant on DAT 365.

For the low rate treatment group it can therefore be concluded that the observed effects on the 'total' Oribatida as observed on DAT 330 are exclusively caused by the species *Hypochthonius rufulus* and the 'remaining' Oribatida were not affected on the last two sampling dates of the field study. This indicates that in the soil mite field study ([redacted] M-594981-01-1) there were no long lasting adverse effects on the 'remaining' Oribatida ('total' Oribatida excluding *H. rufulus*) at the low test rate.

As discussed earlier by [redacted] (2017; M-595114-01-1), it is considered likely that the observed differences of the *H. rufulus* abundance values in the control compared to the Aclonifen SC 600 treated plots are caused by more favorable habitat conditions of the control plot. The higher initial vegetation, the higher moistures, temperatures and organic matter content in the top soil layer of the control plots steadily supported a better population growth of *Hypochthonius rufulus* in the control group until the end of the study.

Evaluation of effects on family Rhodacaridae

In the soil mite field study of [redacted] with Aclonifen SC 600 (2017; M-594981-01-1), the family Rhodacaridae showed no statistical significant difference to control on DAT 151, after an initial significant reduction at all three test rates. A further statistically significant reduction of this taxon was observed at DAT 330, which was going along with a drop in soil temperature to 4.8 °C. At DAT 365, again, no statistical significant difference to control was observed. CRD pointed out uncertainty whether the family Rhodacaridae will be affected again under harsh environmental conditions after DAT 365 (e.g. another drop in temperature).

In the soil mite field study ([redacted] M-594981-01-1), the family Rhodacaridae is almost exclusively represented by the genus *Rhodacarellus* sp. [redacted] 1935 (about 90%). Within the genus *Rhodacarellus* the euedaphic (in soil living) species *Rhodacarellus silesiacus*, [redacted] 1935 is widely distributed (Europe, Asia, Northern Africa, Northern America, and Australia) and shows a continuous frequency and high abundance in agricultural soils ([redacted] 1993; [redacted] 1999; [redacted] 2006; [redacted] 2012). In contrast, the other six European species of this genus are not very frequent or abundant and prefer forest, meadow and floodplain habitats ([redacted] 1993).

Rhodacarellus silesiacus prefers moderate humidity and was also detected in extreme habitats (e.g. sandy coast soils, heavy loamy soils, calcareous soils ([redacted], 1993)) indicating its high resilience towards more extreme environmental conditions. The preferred prey of this species are nematodes, collembola (e.g. *Mesaphorura* sp.) and small insect larvae, which *R. silesiacus* can forage also in deeper soil layers and small soil pores due to its small and slender shape ([redacted] 2012). *R. silesiacus* is therefore considered to be a robust species occurring in intensively managed agricultural soils and is tolerating harsh environmental conditions like drought or cold events through migration into deeper soil layers ([redacted] 1989; [redacted] 2008; [redacted] 2012).

This conclusion is confirmed by a 13-year long-term study from [redacted] (2000) in Northern Germany, where the succession of the cohort Gamasina was investigated after transferring a dump partly into a natural succession site and partly into a lawn site (the lawn site was regularly mown for the first seven years and consecutively ruderalized for the last five years). The early Gamasina communities of both sites are very similar with two dominant species, the 'survivor' species *Rhodacarellus silesiacus* and the 'phoretic' species *Arctoseius cetratus* ([redacted] 1940). Both species can be considered as early pioneers, but only *R. silesiacus* was able to persist throughout the 13-year study with high frequency and abundance indicating the ability of *R. silesiacus* to adapt to changing habitat conditions, whereas *A. cetratus* was not found any more on neither site after 3 years of succession ([redacted] 2000).

Thus, when considering the available information regarding the family of Rhodacaridae, it can be assumed with a high level of certainty that the observed genus Rhodacarellus sp. in the soil mite field study with Aclonifen SC 600 ([redacted] M-594981-01-1) is mainly represented by the species *R. silesiacus*.

Based on the behavioural properties of *Rhodacarellus silesiacus* the observed changes of abundance of family Rhodacaridae in the soil mite field study for Aclonifen SC 600 ([redacted] M-594981-01-1), can be explained as follows:

Due to the high amount of plant material that has been incorporated on DAT 29 into the control plots.it is expected that in these plots a higher amount of biomass in the top soil was present for a longer period of time, providing a higher amount of food for detritivorous species. The subsequent increase of microbiological species (microbes and fungi) in the second and third decomposition phase of this additional biomass in the control group also served as valuable food for specialized micro arthropods and nematodes in the top soil layer. It can be expected that this leads to a longer lasting, higher abundance of detritivorous (e.g. *Hypochthonius rufulus*), bacterivorous and fungivorous microarthropod and nematode species in the control plots, which subsequently served as food source for *R. silesiacus*. In contrast, in the absence of this initial organic matter input in the treatment groups (due to the herbicidal activity of the test item), the amount of these potential food source species for *R. silesiacus* can be expected to be lower, forcing *R. silesiacus* to search for food in deeper layers . It can therefore be expected that the additionally available food allowed *R. silesiacus* to stay and to forage longer in the upper layer of the control plots as compared to the treatment plots, even when a part of the prey species have migrated to deeper layers due to harsh environmental conditions (e.g. drop in temperature at DAT 330). This conclusion is also in line with the observed fast recovery of Rhodacaridae till DAT 365, which might be caused by a remigration of *R. silesiacus* from the deeper soil layers.

II. CONCLUSION

The observed statistical significant effect on the suborder Oribatida at the low rate, is only driven by the species *Hypochthonius rufulus*. Effects on this species are, however, considered not to be treatment-related, as discussed by [redacted] (2017; M-595114-01-1).

In the field study with Aclonifen SC 600 ([redacted] 2017; M-594981-01-1) the family Rhodacaridae is most likely represented by *Rhodacarellus silesiacus*.

Due to its ecology and behavior *R. silesiacus* can deal well with harsher environmental conditions. Long lasting treatment-related population effects are therefore not to be expected in case of reoccurrence of harsh environmental conditions (e.g. lower temperatures after DAT 365).

[redacted] *(2018)*

Assessment and conclusion by applicant:

No validity criteria are available for this study, however, no deviations from the study protocol were recorded, and therefore this study can be considered to be valid.

The application of aclonifen SC 600 tested at rates of 3.31, 6.62 and 9.93 kg aclonifen/ha caused initial statistically significant reductions in abundance of several Acari taxa. The Acari community and all populations recovered within one year after application of 3.31 kg a.s./ha (maximum single application rate of 2.4 kg a.s./ha plus 0.91 kg a.s./ha to address a worst-case long-term plateau within the topsoil layer of 20 cm depth). Only one species (*Hypochthonius rufulus*) showed a statistically significant reduction in the abundance one year after application.

It is considered likely that the differences in the control vs. the aclonifen treated plots, such as strong initial vegetation differences, higher topsoil moisture content and temperature as well as the higher organic matter content in the topsoil layer in the control had created a more favourable habitat in the

~~control plots that was steadily supporting a population growth, especially of *Hypochthonius rufulus*, until the end of the study.~~

~~*Hypochthonius rufulus* is a widely distributed and ubiquitously occurring soil mite species in European agricultural, forest, and marsh areas and able to migrate between different habitats in agricultural areas. Therefore, it is considered unlikely that the observed reduction in abundance of this species would seriously affect biodiversity in agricultural landscapes.~~

~~As total Acari and all other taxa were not affected up to and including 6.62 kg a.s./ha at the end of the study, a negative impact on soil functions and fertility is not to be expected.~~

~~Assessment and conclusion by RMS:~~

Data Point:	KCP 10.4.2.2/04
Report Author:	[redacted]
Report Year:	2020
Report Title:	Evaluation of effects of aclonifen SC 600 on Acari (soil mite) populations under field conditions – Interpretation of all major taxa
Report No:	M-688800-01-1
Document No:	M-688800-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	--
Previous evaluation:	
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	

Executive summary

There have been two previous statements evaluating the results of certain species of this study (M-595114-01-1 and M-630669-01-1). These two statements have been superseded by this combined statement covering all major taxa.

The study design for the soil mite field study was discussed with the German authorities and was comparable to the principle setup for an earthworm field study (ISO 11268-3) with adaptations as required for soil mites. The study followed a dose response design: 3 application rates with 4 replicate plots (10 x 10 m) served as replicates for each of three rates of Aclonifen SC 600: 5.55 L/ha corresponding to a rate of 3.31 kg a.s./ha (= low rate) and reflecting the maximum single application rate of 2.4 kg a.s./ha plus 0.91 kg a.s./ha to address a worst-case long-term plateau within the topsoil

layer of 20 cm depth calculated based on the highest available DT_{50} value for aclonifen (DT_{50} = 195 d). The rates of 11.1 L/ha (6.62 kg aclonifen/ha, = mid rate) and 16.64 L/ha (9.93 kg aclonifen/ha, = high rate) reflect two-times and three-times the amount of the low rate. The total amounts of aclonifen (including additions accounting for plateau) were applied in a single spray application onto the soil surface. In addition, water treated controls and a toxic reference (Chlorpyrifos dosed at 1200 g a.s./ha) were included.

Analytical investigations of soil concentrations after application on day 0 were performed by extraction of soil cores and analysis by HPLC/MS-MS.

A pre-sampling, 6 days before application, was followed by seven post-application samplings throughout a twelve-month period (14, 27, 62, 151, 214, 330 and 365 days after application (DAA)). Soil cores of 5 cm soil depth (6 per plot) were taken at each sampling date, the mites extracted and taxonomically identified. Statistical evaluations were performed as univariate comparisons (Williams-test) for the mite populations and by generating multivariate Principle Response Curves for the community analysis.

At DAA 29 the weeds covering approx. 50% of the soil surface and growing exclusively on the control (and about 10% on the low rate plots) were incorporated into the top 5 cm soil layer providing a constant source of organic matter that steadily decomposed during the course of the study and influenced the soil mite species differently. In the plots treated with Aclonifen SC 600 there was no additional biomass included in the top soil. A clover grass mixture sawn at DAA 82 started to cover the different plots equalling at DAA 151 on all plots.

Based on their ecology and behaviour, this statement provides an interpretation of the population development in the field study for all major soil mite taxa as influenced by the differences in soil moisture and temperature, vegetation cover and organic matter in the top soil.

The application of Aclonifen SC 600 tested at rates of 3.31, 6.62 and 9.93 kg aclonifen/ha caused initial statistically significant reductions in abundance of several Acari taxa. The Acari community and all populations recovered within one year after application of 3.31 kg a.s./ha (maximum single application rate of 2.4 kg a.s./ha plus 0.91 kg a.s./ha to address a worst-case long-term plateau within the topsoil layer of 20 cm depth). Only one species (*Hypochthonius rufulus*) showed a statistically significant reduction in the abundance one year after application.

It is considered likely that the differences in the control vs. the aclonifen treated plots, such as strong initial vegetation differences, higher topsoil moisture content and temperature as well as the higher organic matter content in the topsoil layer in the control had created a more favourable habitat in the control plots that was steadily supporting a population growth, especially of *Hypochthonius rufulus*, until the end of the study.

Hypochthonius rufulus is a widely distributed and ubiquitously occurring soil mite species in European agricultural, forest, and marsh areas and able to migrate between different habitats in agricultural areas. Therefore, it is considered unlikely that the observed reduction in abundance of this species would seriously affect biodiversity in agricultural landscapes.

For the other major taxa (*Rhodacarellus silesiacus*, *Hypoaspis aculeifer*, *Tectocepheus velatus*, *Oppiella nova*) only transiently significant differences to control were observed. These also were to a major extent

explainable by direct or indirect influences of the differences in the vegetation above and below the soil surface.

As total Acari and all other taxa were not affected up to and including 6.62 kg a.s./ha (mid rate) at the end of the study, a negative impact on soil functions and fertility is not to be expected.

I. RESULTS AND DISCUSSION

A Analytical Verification

In the plots treated with the test item, mean residue values of 118 - 142% of the nominal application rates were found in the soil specimens taken immediately after application verifying the intended rates.

B Biological Data

Effects on soil mite community

Ordination analysis revealed short-term effects of the test item on the Acari community structure at all three test rates. However, in the plots treated with 3.31 kg aclonifen/ha (low rate) and 6.62 kg aclonifen/ha (mid rate) recovery of the community was shown until DAA 151 (4th sampling). At an application rate of 9.93 kg a.s./ha (high rate) recovery of the Acari community took place until day DAA 330 (see Table).

Table: Effects on Acari community structure

Sampling day	NOEL (kg a.s./ha)
-6	≥9.93
14	≥9.93
27	<3.31
62	>3.31
151	6.62
214	6.62
330	≥9.93
365	≥9.93

Effects on soil mite population

Treatment with Aclonifen SC 600 caused transient statistically significant differences (Williams t-test; α = 0.05) in total Acari abundance, as well as in abundance of several taxa, at all application rates.

At the end of the study, 12 month after application, Acari of the dominant order Mesostigmata (32% of the total Acari abundance at pre-sampling) represented by the suborder Monogynaspida with the cohorts Gamasina (31% of the total abundance at pre-sampling), the family Laelapidae (6% of the total abundance at pre-sampling) represented by the genus *Hypoaspis* and the species *Hypoaspis aculeifer* (6% of the total abundance at pre-sampling) showed a statistically significant reduction only in the plots treated with the high-rate (9.93 kg aclonifen/ha), whereas in mid-rate and low-rate no statistical differences were detected at study end.

Within the other dominant order Sarcoptiformes, Aclonifen SC 600 caused transient statistically significant reductions in abundance of certain Acari in all test item treatment groups up to and including day DAA 330. However, recovery took place in low and mid-rate for nearly all Sarcoptiformes - except

for the single species *Hypochthonius rufulus* in the cohort Enarthronoides - so that no statistically significant reductions could be observed for this taxon at the end of the study (DAA 365).

Effects on Hypochthonius rufulus

Population development and observed effects on Hypochthonius rufulus in the context of its biology

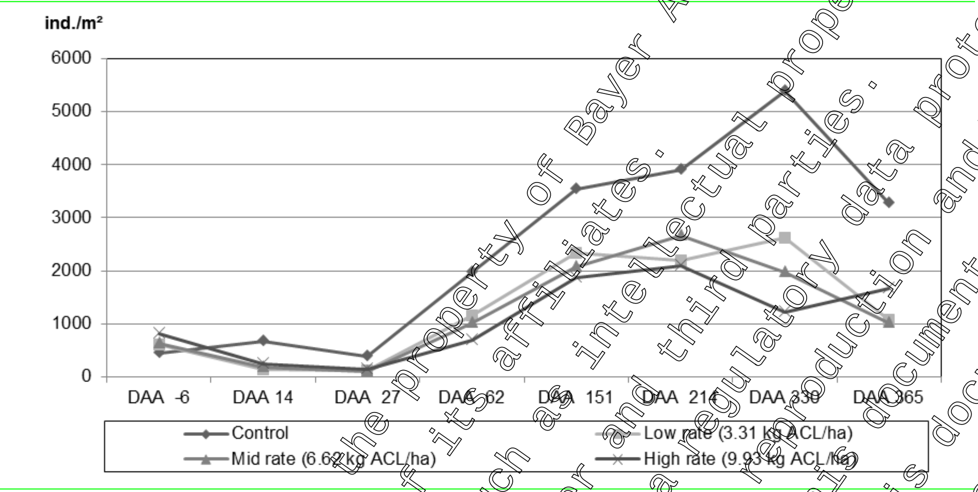


Figure: Population development of *Hypochthonius rufulus* (ind./m^2)

Hypochthonius rufulus was present at pre-sampling with 446 ind./m^2 representing 3% of the total Acari. At the end of the study *H. rufulus* represented 26% of the total mite population sampled from the 5 cm top soil of the control. The abundance of *H. rufulus* stayed stable in the control from mean numbers of around 500 ind./m^2 in the period of the first month. From DAA 27 to DAA 62 its numbers were strongly increasing to 1974 ind./m^2 and further to numbers of up to 5390 ind./m^2 until DAA 330. Aferwards the abundance decreased to 3268 ind./m^2.

The overall same trend of an increase between DAA 27 and DAA 62 and a further increase until DAA 330 followed by a decrease was also seen in the test item treatment groups. In the first two samplings after the application (DAA 14 and 27) the levels of *H. rufulus* were statistically significant lower in the treatments. Considering the application of Aclonifen SC 600 onto the surface and low mobility of Aclonifen in combination with the preference of *H. rufulus* to exclusively populate the litter and the top 5 cm layer, this difference could be treatment-related.

From the third sampling onwards the numbers in all treatments increased but with a lower rate than in the control. The difference between control and all treatments steadily increased until DAA 330.

During the study the presence of *H. rufulus* increased to a dominance level of 26% in the control, which is an order of magnitude higher than in the pre-sampling. Based on literature, such a dominance level is unexpected for this species in Germany. Generally, *H. rufulus* has been detected with low maximum mean dominance in Germany being close to 2% and classified as a recedent species. It is considered that the specific ecology of the affected species (*H. rufulus*) and the environmental factors of the treatment groups contributed to the outcome observed in the field study.

The population dynamics of *H. rufulus* can be influenced by vegetation, soil organic matter and soil temperature. *H. rufulus* has its habitat in the litter and the top 4 cm soil layer and is a thermophilic species. The application of aclonifen was performed onto bare soil. During the 27 days until the second sampling a natural seed germination of weeds from the seedbank took place on the control plots and on the toxic reference plots. This resulted in a vegetative cover of approx. 50% of the soil surface with a vegetation height of ca. 20 cm in the control plots. In contrast, in the plots treated with Aclonifen SC 600, strong herbicidal effects led to inhibition of vegetation growth and thus only 10% of vegetative cover in the low rate and no cover (100% herbicidal effect) in the mid and the high rate treatment groups.

The higher vegetative cover during the period until DAA 29 resulted in a lower soil moisture content in the corresponding plots (control, toxic reference) as a result of the increased transpiration losses through the plants. This was confirmed by the results of the soil moisture measurements. The largest difference was observed during the third sampling. The decrease in soil moisture content resulting from the vegetation in turn leads to an increase in topsoil temperature. Higher soil temperatures favour population growth of thermophilic species such as *H. rufulus* in the control group plots and have thus led to artificially higher populations in this first period of the study.

In order to control the emerging vegetation and to achieve a more homogenous vegetation cover between the control and the test item treatment groups, at DAA 29 the vegetation present on the plots was mechanically incorporated by means of a harrow. Due to this soil tillage measure the plant material present was incorporated into the top soil layer (approximately 5 cm). As a result of the incorporation the amount of plant material in the top soil layer of the control plots was significantly increased compared to the treatment plots. To further support growth of a homogenous vegetation cover a clover and grass mixture was sown over all the plots at DAA 82. During summer the vegetation cover in the test item treated plots started to equal the control plots and by autumn a difference in vegetative cover was no longer visible. This was reflected in more homogenous soil moisture values from this time onwards. However, due to the high amount of plant material that had been incorporated on DAA 29 into the control plots, it is exected that a clearly higher amount of biomass in the topsoil of the control group was present for a longer period, even after establishment of equal vegetative cover by the clover and grass mixture, thereby providing an additional food source for detritivorous species such as *H. rufulus*. Therefore, the population development of *H. rufulus* was not only favoured by an increased temperature in the control plots due to the initial weed vegetation cover. The growth of *H. rufulus* was also constantly supported by the increase in decomposing plant foliage which served as a food source following its incorporation into the top soil at DAA 29.

Evaluation of abundance effects of H. rufulus on the suborder Oribatida

In the soil mite field study the abundance of Acari in the suborder Oribatida showed significant differences to control at the low test rate of 3.31 kg a.s./ha in the sampling 330 days after application (DAA 330), and no differences to control anymore even at the highest rate of 9.93 kg a.s./ha at the last sampling date (DAA 365).

Analyzing the composition of the suborder Oribatida in this study it becomes evident that the species *H. rufulus* is a dominant taxon of the suborder Oribatida at the sampling dates DAA 330 and DAA 365, particularly in the control group.

In order to investigate the influence of the abundance of *H. rufulus* on the statistical performance of the overall mite population, an additional statistical analysis of the suborder Oribatida excluding the species *H. rufulus* was conducted.

Table: Abundance of total Oribatida excluding *Hypochthonius rufulus* (mean of four replicate plots)

Treatment group		Sampling 6th (DAA 330)	Sampling 7th (DAA 365)
Control	ind./m²	3798	3310
	%	100	100
Low rate (3.31 kg/ha)	ind./m²	3714	4096
	%	98	124
Mid rate (6.62 kg/ha)	ind./m²	2525	2759
	%	**66**	83
High rate (9.93 kg/ha)	ind./m²	2398	2610
	%	**63**	79

Excluding *H. rufulus*, the abundance of 'remaining' Oribatida at DAA 330 in the low rate is only 2% lower than the control. On DAA 365 'remaining' Oribatida in the low rate even exceed the abundance in the control. For the mid and the high rate, reductions of 34% to 37% were observed on DAA 330 for the 'remaining' Oribatida, which decreased on DAA 365 to 17% to 21%, respectively.

A statistical evaluation of the underlying data for DAA 330 and DAA 365, indicates for the 'remaining' Oribatida no statistically significant differences (Williams Multiple Sequential t-test) for the low rate compared to the control. For the mid and the high rate treatments the difference of 'remaining' Oribatida to the control was statistically significant only on DAA 330, and not significant on DAA 365.

For the low rate treatment group it can therefore be concluded that the observed effects on the 'total' Oribatida as observed on DAA 330 are exclusively caused by the species *H. rufulus* and the 'remaining' Oribatida were not affected on the last two sampling dates of the field study. This indicates that in the soil mite field study there were no long-lasting adverse effects on the 'remaining' Oribatida ('total' Oribatida excluding *H. rufulus*) at the low test rate.

As discussed above it is considered likely that the observed differences of the *H. rufulus* abundance values in the control compared to the Aclonifen SC 600 treated plots are caused by more favorable habitat conditions in the control plots until the end of the study.

Evaluation of effects on Rhodacarellus silesiacus (family Rhodacaridae)

In the soil mite field study, the family Rhodacaridae showed no statistically significant difference to control on DAA 151, after an initial (DAA 14 till DAA 62) significant reduction partially at all three test rates. A further statistically significant reduction of this taxon was observed at DAA 330, which was going along with a drop in soil temperature from 10.4 to 4.7 °C (measured on site, but not separately on the different plots). At DAA 365, again, no statistically significant difference to control was observed. In the context of the national registration of Aclonifen and Aclonifen SC 600 (2018), the UK authority

(CRD) raised the question whether the family Rhodacaridae will be affected again under harsh environmental conditions after DAA 365 (e.g. in case of another drop in temperature).

Taxonomic assignment as Rhodacarellus silesiacus in the study

In the soil mite field study, the family Rhodacaridae is almost exclusively represented by the genus *Rhodacarellus* sp. Willmann, 1935 (about 90%). Within the genus *Rhodacarellus* the euedaphic (in soil living) species *Rhodacarellus silesiacus*, Willmann 1935 is widely distributed (Europe, Asia, Northern Africa, Northern America, and Australia) and shows a continuous frequency and high abundance in agricultural soils. In contrast, the other six European species of this genus are not very frequent or abundant and they prefer forest, meadow and floodplain habitats.

In order to gain certainty about the assignment of genus *Rhodacarellus* sp. as *R. silesiacus,* additional soil core samples (non-GLP) were taken in 2019 from the same site on which the GLP study took place from 2015 to 2016 and the soil mites present determined by a taxonomic expert. The species *R. silesiacus* was the only member detected from the family Rhodacaridae and could unequivocally be determined as *R. silesiacus* at nymph and adult stage. Thus, when considering the available information regarding the family of Rhodacaridae and the exclusive occurrence in the new samples, it can be assumed with a high level of certainty that the observed genus *Rhodacarellus* sp. in the soil mite field study with Aclonifen SC 600 is represented by the species *Rhodacarellus silesiacus*.

Population development of Rhodacarellus silesiacus

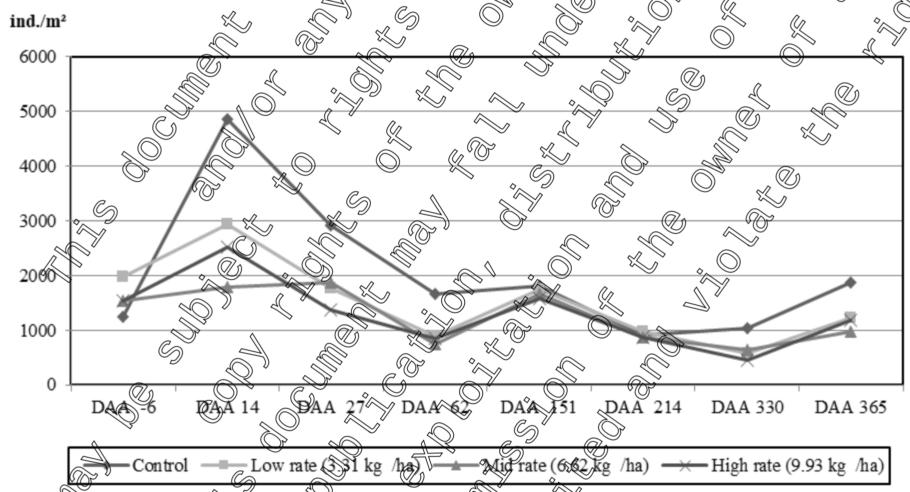


Figure: Population development of *Rhodacarellus silesiacus* (ind./m^2)

After application at DAA 14, the abundance of *R. silesiacus* increased four-fold in the control. The abundance increased significantly less in the treatments with Aclonifen. After DAA 14 the levels in general decreased till DAA 151. In the period from DAA 14 to DAA 151 the mean levels in the treatments were always higher in control than in all treatments. Then at DAA 151 and DAA 214 the levels in control and treatments were nearly equal. Only at DAA 330 *R. silesiacus* was slightly more abundant in the control than in all treatments but this difference was statistically significant. From DAA 330 to DAA 365 (along with increasing temperatures) the levels were increasing again on control and all treatment plots and there was no statistically significant difference of the treatments to control anymore.

Observed effects on R. silesiacus in the context of its biology

R. silesiacus is a predatory species preferring nematodes, Collembola and small insect larvae as prey, which *R. silesiacus* can forage also in small soil pores and soil layers as deep as 10-15 cm depth due to its small and slender shape. By migration into deeper soil layers *R. silesiacus* tolerates harsh environmental conditions like drought or cold events and even becomes eudominant in intensively managed agricultural soils. However, *R. silesiacus* prefers moderate humidity.

Based on the known behavioural properties of *R. silesiacus* the observed changes of abundance in the soil mite field study for Aclonifen SC 600 are interpreted as two parallel processes:
- The initial sharp increase in abundance (from pre-treatment to DAA 14) followed by a clear decrease (till DAA 27) in the control and less pronounced in the plots treated with Aclonifen SC 600 were most likely the result of upward movement from deeper soil layers followed by downward movement to deeper layers. Reproduction or the development from eggs would be too slow at field conditions (8 °C at pre-sampling increasing to 17 °C in soil at DAA 27) to alone trigger these large and fast shifts. Under optimal laboratory conditions of food (nematodes available in excess) and temperatures of 25 °C, a development period from eggs to adults of about 24 days was determined for *R. silesiacus*.

The upward movement could have been triggered by increasing amounts of humidity in the top soil caused by the irrigation of 10 L/m^2 applied within an hour at DAA 0 (as part of the study agricultural measures), and by a general trend for increasing temperatures. It is expected that the rapidly growing weeds on the control plots (and a bit on the low rate plots) initially created with their roots a favorable environment for mobile food organisms of *R. silesiacus* (e.g. microarthropod species) which then in the controls (and a bit in the low rate) enabled a steady development of adults from eggs that supported the population till DAA 151. The decrease of the population of *R. silesiacus* in the control from DAA 14 to DAA 27 and further till DAA 62 and the plateau till DAA 151 could have been caused by downward movement triggered by the suddenly decreased soil moisture that was measured for DAA 27 and that was further going down at DAA 62 (and was still slightly reduced at DAA 151) only in the controls (see section above about *H. rufulus*).

The decrease of the population of *R. silesiacus* in the control from DAA 14 to DAA 27 and further till DAA 62 and the plateau till DAA 151 could have been caused by downward movement triggered by the decrease in soil moisture that was measured for DAA 27 and at DAA 62 only in the controls.

- At DAA 151 the population levels in the control and in the plots treated with Aclonifen were then very similar and also the measured soil moisture was now more comparable between control and treatments equaling fully at DAA 214 where the population values were also nearly identical.

- After DAA 214 again a period (DAA 330 and DAA 365) with values higher in the control than in all the plots treated with Aclonifen followed. This period can also be explained by differences in the soil below the surface. Although on the surface the grass clover mixture sown on DAA 82 was equaling more and more till it was similar at DAA 214 it is expected that below the surface (top soil layer 0-5 cm) the situation was still very different between control and treatments. It is expected that the high amount of weed plant material in the control plots that has been incorporated into the top 5 cm layer on DAA 29 has been present for the whole study period breaking down more and more. The second and third decomposition phase is assumed to be more intensely starting in autumn (DAA 151) supported by clearly higher moisture values (ca. 20% as compared to ca. 10% before) in the top soil. The increase of

microbial organisms decomposing the residues of this additional biomass in the control group then served as valuable food in particular for nematodes in the top soil layer. The nematode themselves were serving as food source especially for *R. silesiacus* (and also for *Hypoaspis aculeifer*, see section below) even towards the end of the study.

In contrast, in the absence of this initial organic matter input in the treatment groups (due to the herbicidal activity of the test item), the amount of these potential food source species for *R. silesiacus* can be expected to be lower at DAA 330 and DAA 365, forcing *R. silesiacus* to search for food in deeper layers. It can be expected that the additionally available food allowed *R. silesiacus* to stay and to forage longer in the upper layer of the control plots as compared to the treatment plots, even when a part of the prey species have migrated to deeper layers due to harsh environmental conditions (e.g. drop in temperature at DAA 330).

Evaluation of effects on Hypoaspis aculeifer

Population development and observed effects on Hypoaspis aculeifer in the context of its biology

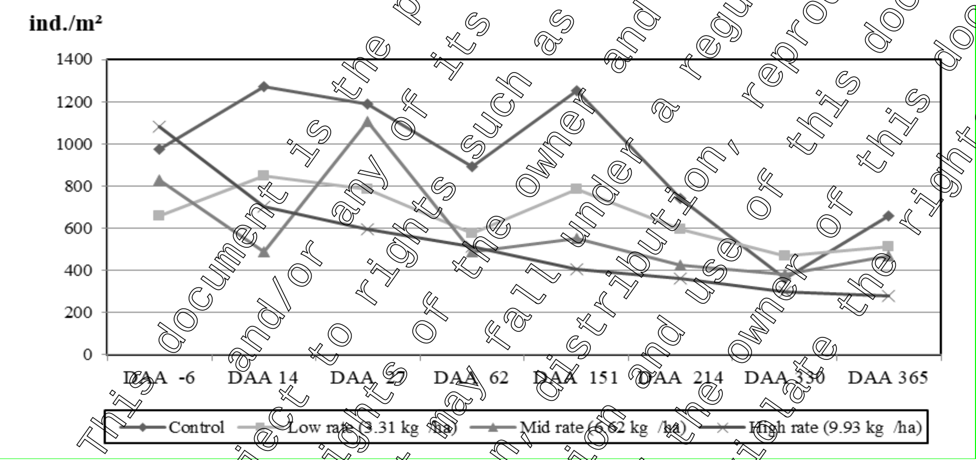


Figure: Population development of *Hypoaspis aculeifer* (ind./m²)

In the present field study performed with Aclonifen, the predatory mite *H. aculeifer* seemed not to profit from the weeds growing on the soil surface or incorporated into soil in the controls. In the period between pre-treatment (DAA -6) and DAA 151 its levels stayed on a plateau level of around 1100 ind./m². The fluctuations of +/- ca. 170 ind./m² (standard occurring within the period DAA 0 and DAA 151 are however minor compared to the ability of *H. aculeifer* for double digit growth within few weeks from fertilized females under optimal laboratory conditions (suggested by OECD 226). A comparable and nearly parallel population development took place in the low rate but starting from a clearly lower level of 658 ind./m². Obviously, the conditions provided on the field did not allow *H. aculeifer* to compete in a way with other predatory species that a sustainable increase in population was possible. Therefore, even the small differences measured between the treatment groups at the pre-sampling stayed very stable.

The situation on the test field at the start of the study is comparable with a situation in spring on a field left fellow after several seasons of cropping. [redacted] and colleagues (2012, M-630982-01-1) compared many soil mite species regarding their performance on three different fellows in a long-term study over

a period of twelve years. *H. aculeifer* was present but not dominant (3.50%, 7.79% and 3.76% in the three sites, equivalent to recendent after Engelmann 1978) in the first samplings after 2-3 years (comparable to the 6% of the total abundance at pre-sampling in the soil mite field study). On the long run, during the following years [redacted] and colleagues measured a slow increase in dominance to finally reach double digit portions (7.01%, 21.57% and 25.51%) of the total soil mite population after 12-15 years and to become one of the most dominant species. The population development of the above-mentioned predatory mite species *Rhodacarellus silesiacus* however was invers in the study by Wissuwa and colleagues. *R. silesiacus* obviously strongly profited from the situation on fields freshly left fallow rapidly reaching the highest dominance level of all species on two of the three sites (29.22%, 23.25% and 3.43%). This shows that *H. aculeifer* seems to be less tolerant than *R. silesiacus* to the harsh conditions present in a field freshly left fellow and not able to grow significantly. This is in line with the findings in the field study. Notably, due to its bigger size (Idiosoma: 520-685 µm) and in contrast to *R. silesiacus*, *H. aculeifer* is not able to avoid the surface layer by migration into deeper layers.

H. aculeifer profits on the long run from more stable conditions of a site on which a dense plant cover and a constant plant community is established. This again is in contrast to *R. silesiacus*.

In the present field study at DAA 214 the abundance of *H. aculeifer* in the control had decreased from 1252 to 743 followed by an abundance of 361 and 658 ind./m² at DAA 330 and DAA 365. The decrease from September (DAA 151) onwards and the low abundance in March (DAA 330) is seen in many mite species in the study. It is the period with the lowest soil temperatures dropping from ca. 10.5 to 4.7 °C creating a temporary stress also to *H. aculeifer*. Its population then starts to grow in the controls, the low and the mid-rate plots in April (DAA 365) when the temperatures have increased again to above 10 °C. In contrast to the detritus feeding species *Hypochthonius rufulus* which clearly profits from the incorporated weeds and the decaying material in the top 5 cm soil layer, *H. aculeifer* did not grow further between DAA 151 and 330. This is in line with *H. aculeifer* known to be only sparsely occurring in compost (decaying plant material).

Evaluation of effects on Tectocepheus velatus

Population development and observed effects on Tectocepheus velatus in the context of its biology

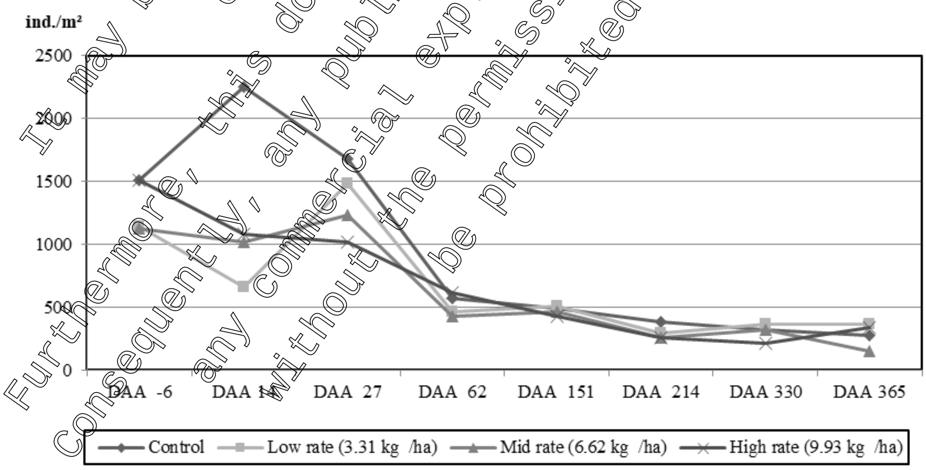


Figure: Population development of *Tectocepheus velatus* (ind./m²)

Initially, from pre-treatment till DAA 14, the population of *T. velatus* increased in the control from 1507 to 2249 ind./m² followed by a decrease to about the initial level (1676 ind./m²). The increase at this point in time is in line with *T. velatus* being known as parthenogenetically reproducing opportunistic mite species that can competitively profit very early from the harsh conditions of bare soils or freshly growing plants, as they are also present in fresh fellows, and to become dominant. In case of *T. velatus*, the increase in the control is likely the cause of a mixture of upward movement and development of earlier stages to more adult stages since in the study at pre-sampling considerable numbers of *Tectocepheus* larvae (in control: 297 larvae/m²) were detected. In the low rate the phase of strong population growth and occurrence in the sampling layer as in the control, i.e. an increase by ca. 700 ind./m², seemed to be delayed by two weeks indicating the beginning of the population recovery at the low rate in this period. In the high and mid-rate plots such an increase seemed not to be possible presumably because the bare soil situation persisted and there were no freshly growing plants or because the toxicity of Aclonifen did not allow for.

Between DAA 27 and DAA 62 in control and all treatment groups the populations of *T. velatus* sharply decreased and then stayed at a lower level of ca. 400-600 ind./m². The decrease occurred just after the incorporation of vegetation into the soil exactly when *H. rufulus* abundance was sharply increasing. *T. velatus* shares the preference for the same soil layer with *H. rufulus*. The ongoing strong increase in the population of *H. rufulus* may have either inhibited the growth of *T. velatus* during the following about 300 days of the study - both species have partially overlapping food preferences (use of decaying plants as food among others). Typically preferring the top 4 cm, *T. velatus* might have partially migrated to deeper layers (as known from literature and in line with its small body size of 280-320 µm) thus avoiding the very dense population of *H. rufulus* or the predator pressure (by e.g. *H. aculeifer* and *R. silesiacus*).

During the period from DAA 62 till the end of the study the population levels of *T. velatus* in control and all treatment groups were very similar (fluctuating around a level of 300 ind./m²) indicating a recovery of the population for all rates, with the exception of a transient and small (however statistically significant) difference to control of 127 ind./m² only at DAA 214 in mid and high rate. Afterwards there were no reduced levels of *T. velatus* observed or there were even higher levels in the treatments than in the control.

Evaluation of effects on Oppiella nova

Population development and observed effects on Oppiella nova in the context of its biology

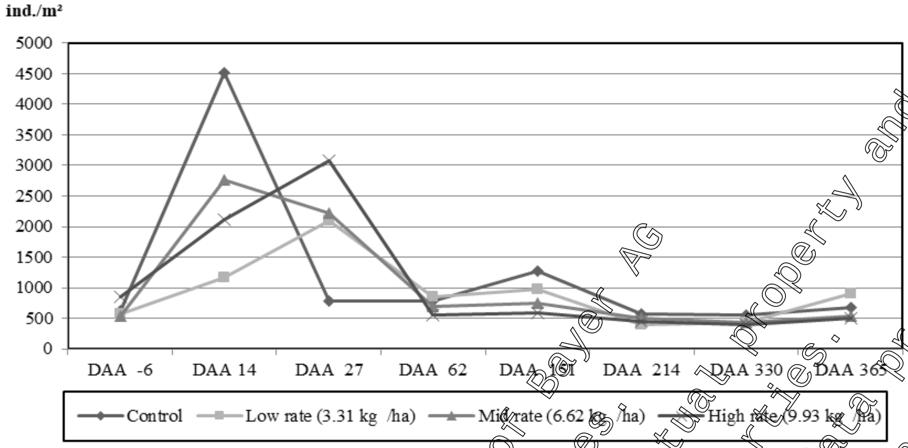


Figure: Population development of *Oppiella nova* (ind./m²)

In general, *O. nova* is very flexible regarding its presence in different soil depth layers. *O. nova* is described as typically being absent in the litter layer (or soil surface) but able to withstand the more extreme conditions in the top layer (0-4 cm) during summer. It also populates the deeper layers (4-8 cm) and even moves down to the 8-12 cm horizon which it clearly prefers during winter.

Within the study the initial sharp increase in abundance of *O. nova* in the control is unexpected. Although *O. nova* reproduces parthenogenetically it needs at least a few month from egg to adult stage. The initial strong (7.3 fold) increase within 3 weeks from mean values of 615 ind./m² to 4520 ind./m² (although driven strongly by a single replicate plot) can only be explained by an upward movement of already present stages from deeper layers to the top 0-5 cm layer. Likewise, the sudden decrease in abundance in the control within the next two weeks down to 785 ind./m² is expected to be a downward movement to deeper soil layers. There was no dose-response for the upward movement from pre-sampling to DAA 14 and DAA 27. The observed figures indicate that the differences in abundance between all treatments and control in the period from pre-sampling till DAA 27 are likely not due to toxicity of Aclonifen SC 600 but due to a high variability in movement between layers.

Between DAA 27 and DAA 62 in control and all treatment groups the populations of *O. nova* clearly decreased and then fluctuated at a lower level till the end of the study. In the control the decrease had already occurred before DAA 27. The soil tillage performed to incorporate the weed vegetation (present on control and low rate plots) into the soil has been done on all plots. It is known that in agricultural fields *O. nova* shows a preference for deeper soil layers especially if the fields are regularly tilled. Based on this, it can be assumed that in the study, after the tillage (incorporation of plants into the top 5 cm layer) on DAA 29, *O. nova* moved to deeper soil layers and therefore only a small portion of the population was present in the top 5 cm (sampling) layer after DAA 62.

At DAA 82 the clover grass mixture was sown which in control and low rate germinated rapidly, in the mid and the high rate, only slowly. In control and low rate plots, the growing plants and their roots as well as the incorporated weed plant residues from DAA 29 provided a good sink and barrier for the high amounts of water that were present on the soil surface after the two pronounced rain events on DAA 97 (37 L/m²) and DAA 123/124 (in total 54 L/m²). In contrast, in the mid and the high rate, the plants were not developed so far and in the top layer there were no incorporated weed plant residues present. Thus, in mid and high rate there was just a weak barrier for the high amounts of water, enabling a higher

portion of water to drench downwards and keep the top layer infiltrated for a longer period. Since it is known that *O. nova* avoids very humid situations, it can be assumed that after these two rain events and soil temperatures decreasing to more moderate values of around 25 - 20 °C only in the control and the low rate a certain portion of the population was moving upwards again whereas in mid and high rate, the major part of the population stayed in the deeper layers. The significantly higher abundance of *O. nova* in control and low rate at DAA 151 can therefore be explained by this indirect effect of the above described difference in vegetation cover (clover grass mixture).

At DAA 214 (November) and DAA 330 (March) the abundances of *O. nova* in the study were very similar in control and all treatment groups and stayed in the upper 5 cm layer at lower numbers of around 500 ind./m^2. These lower abundances are in line with the typical presence in deeper soil layers of *O. nova* during winter. At DAA 365, along with an increase in temperatures, the levels of *O. nova* had increased similarly in control and all treatments (in the low rate even showing the highest value) indicating a full recovery.

Regarding a potential influence of *H. rufulus*, sharing partially a similar food source (i.e. decaying plant material) with *O. nova* it is expected that the two species are only weakly competing due to differential habitat preference. As described in the literature, when comparing both species within the same site, *O. nova* was absent in the litter layer (or soil surface) but living in lower soil layers of 3-6 cm (especially the gravid females) and only part time in 0-3 cm whereas *H. rufulus* was preferring the litter layer (or soil surface) and the top soil layer 0-3 cm.

II. CONCLUSION

After initial effects, the mite community fully recovered even at the high rate (9.93 kg aclonifen/ha) within a year after the application.

It can be concluded that in mid and low rate all initially affected taxa recovered within a year after the application of the test substance with the exception of one species: The Acari species *Hypochthonius rufulus* (supercohort Enarthronotides represented by the family Hypochthoniidae, the genus *Hypochthonius*) showed a statistically significant difference to control for all three test rates.

The beneficial conditions for *Hypochthonius rufulus* in the control group led to an artificially larger population in the control plots over summer which could not be compensated in the treatment groups. The differences are thus likely due to an artefact of the incorporation treatment providing more feeding material and to the initially higher soil temperatures together leading to inflated *Hypochthonius rufulus* populations in the control plots.

Furthermore, the Oribatid mite species *Hypochthonius rufulus* is a widely distributed, ubiquitous occurring species in European agricultural, forest, and marsh areas. *Hypochthonius rufulus* is able to live in the litter layer, prefers the layers in vicinity to the soil surface and is capable of withstanding higher temperatures. Its wide distribution in different habitats strongly suggests that it is able to migrate between different habitats, including agricultural fields. Due to the wide distribution and ubiquitous occurrence of *Hypochthonius rufulus*, it is considered unlikely that the observed difference in abundance would seriously affect biodiversity in agricultural landscapes.

The observed statistically significant effect on the suborder Oribatida at the low rate, is only driven by the species *Hypochthonius rufulus*. For the low rate treatment group it can therefore be concluded that

the observed effects on the 'total' Oribatida as observed on DAA 330 are exclusively caused by the species *Hypochthonius rufulus* and the 'remaining' Oribatida were not affected on the last two sampling dates of the field study. This indicates that in the soil mite field study there were no long-lasting adverse effects on the 'remaining' Oribatida ('total' Oribatida excluding *H. rufulus*) at the low test rate.

The abundances of *Rhodacarellus silesiacus* in control plots and in plots treated with Aclonifen SC 600 can be explained by the differences in weed growth (initially) and in steadily decomposing plant material (after incorporation into top soil) that were still present and influencing until the end of the study. Dependent on climate, soil moisture and the availability of food organisms *R. silesiacus* was differently moving up and down between the top and deeper soil layers within the field study in the different treatment groups. Since *R. silesiacus*, due to its ecology and behavior, can deal well also with harsher environmental conditions and agricultural measures and is known for its high resilience), long-lasting treatment-related population effects of Aclonifen are not to be expected.

In the present study *H. aculeifer* behaves in the control as it is expected from other studies regarding its slow growth on a field freshly left fallow, also after some time of growth in soil on which a mixture of weed plants or a homogenous clover grass are growing, and as compared to the other dominant predatory mite species *R. silesiacus*. A parallel behavior is observed in the low rate indicating a no effect situation. In the mid-rate the population had recovered from DAA 330 onwards. Overall, due to the widespread occurrence and the ability for a rapid growth in case of favorable conditions, no long-term effects of Aclonifen on *H. aculeifer* are to be expected.

In the present study there were transient significant differences to control only at two individual sampling time points (DAA 14 and DAA 214) for *Tectocepheus velatus*. Since is known to be a eurytopic species and has a high colonisation ability in many habitats. Especially in agricultural fields it represents often a dominant species being tolerant to ploughing and pesticide application. In the present study, after initial (DAA 14) differences, there was no significant difference to control at the low rate and (with exception of a small difference at DAA 214) at mid and high rate anymore. Any decrease due to agricultural measures or pesticide applications is therefore expected to be transient - as in the present study - and as not having any long-term effect on the populations of *T. velatus*.

In the present study *Oppiella nova* behaves as it is expected from other studies regarding its ecology and behavior and its responds to the differential conditions observed in the study. With the exception of DAA 151 in the high rate, no significant differences to control were observed. *O. nova* is known to be an extremely eurytopic species in many habitats, often present dominantly, also in agricultural fields. It is tolerant to drought. Due to its soil depth flexibility it is also tolerant to tillage and can avoid extremely humid situations by downward movement to deeper soil layers. If decreased due to agricultural measures or pesticide applications it is expected that this will be short-term and transient. - as in the present study. Therefore, no long-lasting treatment-related population effects of Aclonifen on *O. nova* are to be expected.

Overall, in the present study the differences to control can be explained by differences in vegetation, top soil moistures, temperatures and organic matter contents in the top soil layer driven by the herbicidal activity of Aclonifen SC 600. *Hypochthonius rufulus* benefited strongly from these conditions leading to an artificially high population in the controls. As total Acari and all taxa were not affected up to and including 6.62 kg a.s./ha at the end of the study, a negative impact on soil functions and fertility is not to be expected from applications of Aclonifen SC 600 according to GAP.

[redacted] *and* [redacted] *(2020)*

<u>Assessment and conclusion by applicant</u>:

No validity criteria are available for this study, however, no deviations from the study protocol were recorded, and therefore this study can be considered to be valid.

The application of Aclonifen SC 600 tested at rates of 3.31, 6.62 and 9.93 kg aclonifen/ha caused initial statistically significant reductions in abundance of several Acari taxa. The Acari community and all populations recovered within one year after application of 3.31 kg a.s./ha (maximum single application rate of 2.4 kg a.s./ha plus 0.91 kg a.s./ha to address a worst-case long-term plateau within the topsoil layer of 20 cm depth). Only one species (*Hypochthonius rufulus*) showed a statistically significant reduction in the abundance one year after application.

It is considered likely that the differences in the control vs. the aclonifen treated plots, such as strong initial vegetation differences, higher topsoil moisture content and temperature as well as the higher organic matter content in the topsoil layer in the control had created a more favourable habitat in the control plots that was steadily supporting a population growth, especially of *Hypochthonius rufulus*, until the end of the study.

Hypochthonius rufulus is a widely distributed and ubiquitously occurring soil mite species in European agricultural, forest, and marsh areas and able to migrate between different habitats in agricultural areas. Therefore, it is considered unlikely that the observed reduction in abundance of this species would seriously affect biodiversity in agricultural landscapes.

For the other major taxa (*Rhodacarellus silesiacus*, *Hypoaspis aculeifer*, *Tectocepheus velatus*, *Oppiella nova*) only transiently significant differences to control were observed that also were mostly explainable by the differences in vegetation above and below the soil surface.

As total Acari and all other taxa were not affected up to and including 6.62 kg a.s./ha at the end of the study, a negative impact on soil functions and fertility is not to be expected.

<u>Assessment and conclusion by RMS</u>:

CP 10.5 Effects on soil nitrogen transformation

A summary of the endpoints related to the effects on soil nitrogen transformation is provided in the following table. Details and a full description of the studies performed on the active substance, aclonifen, used in this risk assessment can be found in Document M-CA 8 of this dossier.

Table 10.5-1: Summary of data on the effects of aclonifen and Aclonifen SC 600 G to soil nitrogen transformation

Test item	Time scale	Endpoint	Reference
Aclonifen	28 days	**No adverse effect after 28 days at a maximum tested concentration of 15 kg a.s./ha (20 mg a.s./ha)**	KCA 8.5/01 M-218214-01-2 [redacted], 1984
Aclonifen	5 days	No adverse effect after 5 days at a maximum tested concentration of 13.5 kg a.s./ha (18 mg a.s./kg)	KCA 8.5/02 M-174171-01-1 [redacted], 1994
Aclonifen SC 600 G	28 days	Up to and including 15 kg a.s./ha (20 mg a.s./kg) <25% deviation from control by the study end	KCP 10.5/01 M-174595-01-1 [redacted], 1993
Aclonifen SC 600 G	28 days	**Up to and including 16 kg a.s./ha (21.3 mg a.s./kg) <25% deviation from control by the study end[1]**	KCP 10.5/02 M-571069-01-1 [redacted] 2016

When more than one endpoint is available for a substance for the same study type, the endpoint in **bold** is the one used in the risk assessment

[1]: This study was used in the risk assessment as it was performed according to current guideline requirements (OECD 216, 2000)

Risk assessment for Soil Nitrogen Transformation

The risk to soil microbial processes has been assessed in accordance with the Terrestrial Guidance Document (SANCO/10329/2002).

To assess the risk to soil microbial processes, the PEC_{soil} is compared to the No Effect Concentration determined from a suitable laboratory study. If the PEC_{soil} is lower than the No Effect Concentration then the risks to soil micro-organisms is considered acceptable.

Table 10.5-2: Assessment of effects on soil microbial processes

Test item	Concentration where effects <25% are seen, (mg a.s./kg)	PEC_{soil} (g/kg)	PEC_{soil} < Endpoint
Aclonifen	20	0.5697	Yes
Aclonifen SC 600 G	21.3		Yes

The PEC_{soil} was lower than the no effect concentration indicating the risks to soil organisms from the proposed uses of Aclonifen SC 600 G are acceptable.

Studies on the effect of the formulation Aclonifen SC 600 G on soil nitrogen transformation have been conducted and presented below.

Data Point:	KCP 10.5/01
Report Author:	[redacted]
Report Year:	1993
Report Title:	A laboratory assessment of the effects of EXP4209 on soil microflora respiration and nitrogen turnover
Report No:	R007278
Document No:	M-174595-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The effect of aclonifen on nitrogen turnover was investigated by determining ammonium, nitrate and nitrite-nitrogen concentrations in soil amended with Lucerne meal. The test item was applied at 2 rates to 2 soils to give 8 mg formulation/kg soil (6 kg formulation/ha) or 40 mg formulation/kg soil (30 kg formulation/ha). A reference substance dinoseb acetate was used.

Aliquots of soil were extracted with 2M KCl within 3 hours of treatment and after 14, 28 for sandy loam and 64 days (for clay loam soil). The concentrations of mineral nitrogen species in extracts were determined colorimetrically.

EXP4209 did not adversely affect the nitrogen transformation process of ammonification and nitrification in either soil.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	Aclonifen (EXP 4209)
	Batch no.:	OP910750
	Active Ingredient / Purity:	Aclonifen, 610 g/L
	Appearance:	Yellow opaque liquid
	Expiry date:	6 May 1994
	Storage:	At room temperature in original container
2.	**Reference item:**	Aretit Flussig
	Batch no.:	1/M 9754
	Active Ingredient / Purity:	Dinoseb acetate - 49.2% (w/v)
3.	**Test Soil:**	Clay loam and sandy loam
	Source:	[redacted]

Pre-treatment: The soils used in the study were obtained from [redacted] They had been sieved to pass a 2 mm screen prior to despatch. On receipt, the soils were stored at 4°C prior to conditioning. The soils were conditioned for at least 7 days at a moisture content of 14.62% (clay loam) and 10.43% (sandy loam) respectively.

B. STUDY DESIGN AND METHODS

1. In-life phase: 23 November 1992 – 26 January 1993

2. Exposure conditions

Experimental design: Two test concentrations (6.0 kg formulation/ha (field rate) and 30 kg formulation/ha (5 times field rate) plus one control; three replicates of each

Temperature: 22 ± 1°C

Moisture content: 40 ± 5% of water holding capacity (WHC)

3. Administration of the test item

The target application concentrations were achieved by diluting 400.2 mg (field rate) and 1.999 g (5 times field rate) of the test item to 500 mL of distilled water. 10 mL of each dilution was distributed per kg soil (dry weight). This was equivalent to 8 mg/kg and 40 mg/kg (6.0 kg formulation/ha and 30 kg formulation/ha).

4. Measurements and observations

The conditioned soils (at least 7 days) were amended in bulk with ground Lucerne (0.5% w/w) and treated with the field rate and 5 times the field rate of application of the test substance. One level of the reference substance was added to both soils. Control untreated soils were also prepared. Quantities of the treated soils were placed in containers and incubated under aerobic conditions at 22 ± 1°C. At 0 (within 3 hours of addition of the test substance), 14, 28 and 64 days (for the clay loam soil), samples were removed for determination of ammonium, nitrate and nitrite-nitrogen.

The nitrogen was extracted in 2M KCI by shaking for 1 hour, decanting off the supernatant and centrifuging to remove fine particles. The analyses were carried out colorimetrically.

5. Statistics/Data evaluation

No statistical analysis of the generated data was performed.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Analytical verification was not required.

B. BIOLOGICAL DATA

EXP 4209 had no significant effect on nitrogen turnover in the sandy loam soil when applied at either the field rate or 5 times the field rate after 28 days. At the maximum and 5 times maximum field rate in

the clay loam soil, levels of mineralised nitrogen were within 4% of the controls after 64 days. Nitrite concentrations were below the level of detection for both soils. The reference substance had a significant effect on nitrogen mineralisation in both soils.

Table: The effect of EXP4209 on ammonium-nitrogen and nitrate-nitrogen concentrations (mg/kg soil) in a clay loam soil:

Treatment	Time (days)				Time (days)			
	0	14	28	64	0	14	28	64
	Ammonium-N				Nitrate-N			
Control	4.33	<0.1	<0.1	<0.1	31.33	46.00	54.67	79.33
6 kg formulation/ha	5.33 (23.1)	<0.1	<0.1	<0.1	30.67 (-2.1)	48.33 (5.1)	57.33 (4.9)	81.00 (2.1)
30 kg formulation/ha	5.33 (23.1)	<0.1	<0.1	<0.1	30.33 (-3.2)	50.00 (8.7)	64.33 (17.7)	82.33 (3.8)
Dinoseb acetate	5.33 (23.1)	1.0 (100)	<0.1	<0.1	30.33 (-3.2)	50.33 (9.4)	62.67 (14.7)	89.00 (12.2)

( ) variation in % from control

Table: The effect of EXP4209 on ammonium-nitrogen and nitrate-nitrogen concentrations (mg/kg soil) in a sandy loam soil:

Treatment	Time (days)			Time (days)		
	0	14	28	0	14	28
	Ammonium-N			Nitrate-N		
Control	4.67	<0.1	<0.1	14.00	32.33	50.00
6 kg formulation/ha	4.67	<0.1	<0.1	15.00 (7.1)	33.00 (2.1)	52.00 (4.0)
30 kg formulation/ha	4.67	<0.1	<0.1	14.00	36.00 (11.4)	52.33 (4.7)
Dinoseb acetate	6.00 (28.5)	50.00 (>100)	40.50 (>100)	14.00	11.00 (-66.0)	23.00 (-54.0)

( ) variation in % from control

C. VALIDITY CRITERIA

Validity criterion	Required (OECD 216, 2010)	Achieved
Variation between controls	≤15%	≤5%

The validity criterion was satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Effect
Nitrogen transformation	No adverse effect (<25%) after 28 days at a maximum tested concentration of 30 kg formulation/ha (equivalent to field application rate of 18.3 kg a.s./ha)

III. CONCLUSION

EXP4209 did not adversely affect the nitrogen transformation process of ammonification and nitrification in either soil at a maximum concentration of 30 kg formulation/ha.

[redacted] *(1993)*

Assessment and conclusion by applicant: All validity criteria were satisfied and therefore this study can be considered to be valid. EXP4209 did not adversely affect the nitrogen transformation process of ammonification and nitrification in either soil at a maximum concentration of 30 kg formulation/ha. The maximum concentration tested, 30 kg formulation/ha was equivalent to 15 kg a.s./ha. (20 mg a.s./kg)

Assessment and conclusion by RMS:

Data Point:	KCP 10.5/02
Report Author:	[redacted]
Report Year:	2016
Report Title:	Aclonifen SC 600: Effects on the activity of soil microflora (nitrogen transformation test)
Report No:	16 10 48 085 N
Document No:	M-571069-01-1
Guideline(s) followed in study:	OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation. US EPA OCSPP not applicable
Deviations from current test guideline:	Current guideline: OECD 216, 2000 No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The effect of Aclonifen SC 600 G on nitrogen transformation was investigated by determining ammonium, nitrate and nitrite-nitrogen concentrations in soil amended with Lucerne meal.

A loamy sand soil (DIN 4220) was exposed for 28 days to 6.43 mg test item/kg soil dry weight and 32.13 mg test item/kg soil dry weight. Application rates were equivalent to 4 L test item/ha and 20 L test item/ha. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5%). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an Autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment).

The coefficients of variation in the control (NO_3-N) were maximum 3.7% and thus fulfilled the demanded range (≤ 15%).

No adverse effects of Aclonifen SC 600 G on nitrogen transformation in soil could be observed at both test concentrations (6.43 mg test item/kg soil dry weight and 32.13 mg test item/kg soil dry weight) during the 28-day experiment. Differences from the control of +9.1% (test concentration 6.43 mg test item/kg soil dry weight) and -1.0% (test concentration 32.13 mg test item/kg soil dry weight) were measured at the end of the 28-day incubation period (time interval 14-28).

Aclonifen SC 600 G caused no adverse effects (difference to control < 25%, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 32.13 mg test item/kg soil dry weight, which are equivalent to application rates up to 20 L test item/ha.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	Aclonifen SC 600 G
	Batch no.:	EV56005993
	Active Ingredient / Purity:	Aclonifen, 49.5% w/w, 596.7 g/L
	Appearance:	Yellow suspension
	Expiry date:	09 February 2017
	Storage:	25 ± 5 °C
2.	**Test Soil:**	Agriculturally utilised soil
	Source:	[redacted]
	Pre-treatment:	The soil was removed to a depth of 20 cm as mixed sample. Afterwards, the soil was carefully dried at room temperature, passed through a 2 mm mesh sieve and then stored at a temperature of approx. 4 °C in containers under aerobic conditions in the dark. Before application, the soil was adapted to test conditions.

B. STUDY DESIGN AND METHODS

1. In-life phase: 29 August – 29 September 2016

2. Exposure conditions

Experimental design:	Two test concentrations (4 and 20 L test item/ha) plus one control; three replicates of each
Temperature:	19.8 – 20.9 ºC
Moisture content:	41.77 – 43.84% of water holding capacity (WHC)
Photoperiod:	In darkness

3. Administration of the test item

200 g soil dry weight (= one sub-sample) per test vessel was weighed. The soil was mixed with 0.5% (i.e. 1.0 g/200 g soil d.w.) lucerne meal by means of a hand-stirrer (the C/N ratio of the lucerne meal was 13.2/1). One additional soil sample (without Lucerne meal) was used for determination of the initial NO_3-N-content. The initial NO_3-N-content was 2.18 mg /100 g soil d.w.

The test item was mixed with deionised water and the test solution was subsequently mixed with the soil by means of a hand-stirrer. Water was added to the soil to achieve a water content of approximately 45% of WHC.

4. Measurements and observations

The incubation of the prepared soil was carried out in wide mouth glass flasks (500 mL). The screw caps of the flasks used permitted an air exchange.

The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40 - 50% of WHC. The pH-values of the soil used in the test were measured at test start (after application) and at the final sampling on day 28.

Soil samples (10 g soil d.w. per replicate) were taken at intervals of 3 hours, 7, 14 and 28 days after application and the NH_4-N-, NO_3-N- and NO_2-N-contents were determined. Soil was extracted by adding 50 mL 1 M KCl solution to the equivalent of 10 g soil d.w. and mixing on a rotator at 150 rpm for 60 minutes. The mixtures were centrifuged and stored deep-frozen prior to analysis at -20 ± 5 °C.

5. Statistics/Data evaluation

The mean nitrogen-content, standard deviation and coefficient of variation were calculated for each treatment group and sampling date. Furthermore the nitrogen transformation rate per time interval and the nitrogen transformation rate/time interval/day were calculated for each treatment group.

The % differences in the quantities of nitrogen formed between the control and the test item treatment groups were determined as follows:

% difference to control = ((test item rate - control rate)/control rate) x 100%

A statistical evaluation of the test results was performed by means of a 2-sided Student-t-test (for homogeneous variances at 5% significance level).

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Analytical verification was not required.

B. BIOLOGICAL DATA

No adverse effects of Aclonifen SC 600 G on nitrogen transformation in soil could be observed at both test concentrations (6.43 mg test item/kg soil dry weight and 32.13 mg test item/kg soil dry weight) during the 28-day experiment. Differences from the control of +9.1% (test concentration 6.43 mg test item/kg soil dry weight) and -1.0% (test concentration 32.13 mg test item/kg soil dry weight) were measured at the end of the 28-day incubation period (time interval 14-28).

Table: Effects on nitrogen transformation in soil after treatment with aclonifen SC 600 G

Time interval (Days)	Control	6.43 mg test item/kg soil d.w. equivalent to 4 L test item/ha	32.13 mg test item/kg soil d.w. equivalent to 20 L test item/ha

	Nitrate-N[1]	Nitrate-N[1]	% difference to control	Nitrate-N[1]	% difference to control
0 – 7	4.17	4.50	+7.8	4.42	+6.1
7 – 14	1.52	1.35	-11.2	1.87	+22.5
14 - 28	1.15	1.25	+9.1	1.14	-1.0

[1]: Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates

C. VALIDITY CRITERIA

Validity criterion	Required (OECD 216, 2010)	Achieved
Variation between controls	≤15%	≤3.7%

The validity criterion was satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	Effect
Nitrogen transformation	No adverse effect (<25%) after 28 days at a maximum tested concentration of 32.13 mg test item/kg soil dry weight

III. CONCLUSION

Aclonifen SC 600 G caused no adverse effects (difference to control < 25%, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 32.13 mg test item/kg soil dry weight, which are equivalent to application rates up to 20 L test item/ha.

[redacted] *(2016)*

<u>Assessment and conclusion by applicant:</u>

All validity criteria were satisfied and therefore this study can be considered to be valid.

Aclonifen SC 600 G caused no adverse effects (difference to control < 25%, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 32.13 mg test item/kg soil dry weight, which are equivalent to application rates up to 20 L test item/ha.

The maximum concentration tested, 32.13 mg test item/kg was equivalent to 16 kg a.s./ha. (21.3 mg a.s./kg)

<u>Assessment and conclusion by RMS:</u>

The following publication was identified as being relevant for soil nitrogen transformation processes during the literature review performed, see Document M-CA9, Section CA9/05.

Data Point:	KCP 10.5/03
Report Author:	[redacted]; [redacted]
Report Year:	2012
Report Title:	Influence of aclonifen on the growth of rhizobium phaseoli and the yield of green beans (phaseolus vulgaris l.)
Report No:	M-670494-01-1
Document No:	M-670494-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In this study, the degree of the negative effect of Aclonifen containing herbicide on *Rhizobium phaseolii*, total mesophilic bacteria (TAMB), yeast and molds (YM), the yield and correlation among parameters of bean under natural field conditions were investigated. *Rhizobium phaseolii* stock culture (8.71 log cfu/g), was mixed with the media in pots homogenously at a dose of 0, 1 and 2 g. When the young bean plants reached the 5-6 true leaf stage, 600 g/L Aclonifen containing Challenge 600, was applied as a herbicide at the dose of 0, 625, 1250, 1875 and 2500 mL/ha, respectively. The effect of the *Rhizobium* and herbicide treatments on *Rhizobium phaseolii*, TAMB, YM and the bean yield were tested. The results obtained from the trial revealed that the number of *Rhizobium* bacteria, TAMB, YM and also the yield were reduced by the increased herbicide dose. The number of TAMB and YM were not affected by *Rhizobium* treatments but yield was.

I. MATERIALS AND METHODS

The study was carried out at [redacted] The 18 liter capacity plastic bags were filled with the media prepared as a mixture of manure, loamy soil and sand at a ratio of 1:1:1 at a total of 15 liters of each. In order to provide the natural environment, the filled bags were buried in the field to keep both the soil and plastic bags surface at the same level.

Before the application of *Rhizobium phaseolii*, the microbiological properties of the media were determined as total mesophilic bacteria, (TAMB), 6.63 log cfu/g, yeast and molds (YM): 4.30 log cfu/g and *Rhizobium phaseolii* 1.40 log cfu/g.

Rhizobium phaseolii stock culture (8.71 log cfu/g), produced by [redacted], was mixed with the media in pots homogenously at a dose of 0, 1 and 2 g. Two seeds of the Romano bean variety were sown in each pot on June 5th. This variety of beans is widely produced in Turkey for fresh bean consumption. The planting distance was adjusted to 50 cm between rows and 25 cm between plants.

When the young bean plants reached the 5-6 true leaf stage, 600 g/L Aclonifen containing Challenge 600 were applied as a herbicide at the dose of 0, 625, 1250, 1875 and 2500 mL/ha, for the first time. The surface area of the pots was 962 cm^2. The generally recommended dose should have been 1250 mL/ha. The field trial was designed and carried out according to split plot design with four replications. Each replication consisted of four pots and 8 plants.

During the growing period with 30 days interval three times 20 g soil samples were taken from each pot starting from 17th of July in order to determine the quantity of *Rhizobium*, TAMB and YM. Each time the samples were taken in the morning 24 hours after each irrigation.

The sterilized jars were used for the soil samples. The 10 g of soil samples mixed with various concentrations of *Rhizobium phaseolii* and herbicide were added in 90 mL NaCl solution (0.85%) and diluted up to 10^{-5}.

The total aerobic mesophilic bacteria (TAMB) were enumerated on Plate Count agar ([redacted], Germany). The number of yeasts and molds (YM) was determined in Potato Dextrose agar ([redacted], Germany) reduced to pH 3.5 with tartaric acid. *Rhizobium phaseolii* counts of the soil samples were enumerated on Mannitol Yeast Extract agar and incubated at 25°C for 3-4 days. The microbial counts were determined as colony forming units (cfu) in gram of the samples. The results of microbiological analyses shown as log cfu/g. All analyses were formed in duplicate.

II. RESULTS AND DISCUSSION

Effects of Aclonifen Treatments on *R. Phaseolii*

Rates of Aclonifen adversely affected the *R. phaselii* counts. It was determined that the number of *Rhizobium* bacteria in the media were reduced with the increased doses of herbicides. The interactions of Herbicide x *Rhizobium* were significant at the 0.01 level. At the same time the two doses of *Rhizobium* were not significant between themselves but the control was significantly different.

Table: Rhizobium phaseolii counts (log cfu/g) of the soil samples

Herbicide rates	*Rhizobium* sp			Means
	0 g/pot *Rhizobium*	1 g/pot *Rhizobium*	2 g/pot *Rhizobium*	
0 mL/ha	246.3	702	711	5.53 a*
625 mL/ha	99.6	448.3	436	3.27 b
1250 mL/ha	96.3	349.3	352	2.65 c
1875 mL/ha	67.3	183	195.6	1.48 d
2500 mL/ha	67.6	92.6	93	0.84 e
Average	1.15 b	3.55 a	3.57 a	

* Means with different letters within columns are significantly different ($p<0.05$)

Effects of Treatments on TAMB

The number of TAMB was not affected by the *Rhizobium* treatments. On the other hand, the herbicide treatments were statistically effective on the number of TAMB. Increased herbicide doses reduced the number of the TAMB. Herbicide x *Rhizobium* interaction was not significantly important at the level of 0.05.

Table: TAMB counts (log cfu/g) of the soil samples

Herbicide rates	TAMB	Means

	0 g/pot *Rhizobium*	1 g/pot *Rhizobium*	2 g/pot *Rhizobium*	
0 mL/ha	690	666	629	6.61 a *
625 mL/ha	409.6	404	363.6	3.92 b
1250 mL/ha	363.3	351.6	345	3.53 c
1875 mL/ha	295.3	273	252	2.73 d
2500 mL/ha	220.3	207.6	195.3	2.07 e
Average	3.95 a	3.80 a	3.56 a	

* Means with different letters within columns are significantly different (p<0.05)

Effect of Treatments on YM

It is statistically determined that the number of YM was not affected by the *Rhizobium* treatments. However the effect of the interreaction of the Herbicide x *Rhizobium* on YM numbers in media was statistically significant at the 0.01 level. The number of YM was high on non-treated check pots but it was the lowest on the fourth dose of the herbicide application. It is clear that increased herbicide dose reduced the number of YM in the media.

Table: YM counts (log cfu/g) of the soil samples

Herbicide rates	YM			Means
	0 g/pot *Rhizobium*	1 g/pot *Rhizobium*	2 g/pot *Rhizobium*	
0 mL/ha	488.6	511.3	497	4.98 a *
625 mL/ha	309.6	302.3	286	2.99 b
1250 mL/ha	275.6	262	245.6	2.61 c
1875 mL/ha	216	194.3	191	2.00 d
2500 mL/ha	163	132.3	105.3	1.33 e
Average	2.90 a	2.80 a	2.64 a	

* Means with different letters within columns are significantly different (p<0.05)

Effects of Treatments on Yield and Correlation among Parameters

The highest yield was obtained from the 1 g/pot *Rhizobium* treatment. This was followed by the 2 g/pot treatment and the lowest yield was obtained from the check pots. As can be clearly seen from the table that the herbicide application reduced the yield. The highest yield was obtained from the check pots. The differences of either herbicides or *Rhizobium* application were significant at 0.05 level. Also the effect of interreaction between Herbicide x *Rhizobium* was significant on the yield.

Table: The effect of herbicide and *Rhizobium* treatments on yield (g/plant)

Herbicide rates	Yield			Means
	0 g/pot *Rhizobium*	1 g/pot *Rhizobium*	2 g/pot *Rhizobium*	
0 mL/ha	681	827	814	774 a *
625 mL/ha	574	777	783	711 b
1250 mL/ha	493	709	653	618 c
1875 mL/ha	462	643	572	559 d
2500 mL/ha	416	562	559	512 e
Average	525 c	703 a	676 b	

* Means with different letters within columns are significantly different (p<0.05)

Table: Correlation among parameters

	Yield	TAMB	*Rhizobium*	YM
Yield	1.00			
TAMB	0.65 ± 0.12 ***	1.00		
Rhizobium	0.91 ± 0.06 ***	0.71 ± 0.11 ***	1.00	
YM	0.66 ± 0.11 ***	0.98 + 0.03 ***	0.73 ± 0.10 ***	1.00

*** All of the correlations among the parameters taken into account in the trial were significant at 0.01 level

Based on the results obtained from this trial, it was found that the application of aclonifen had a reducing effect on *Rhizobium* bacteria together with TAMB and YM. There is a probability that Aclonifen may have a toxic effect on *Rhizobium*, TAMB and YM population existing in the bean producing field. Toxicity of Aclonifen to *Rhizobium*, TAMB and YM increased progressively with increase in rates of herbicide.

Compared to check pots, the population of *Rhizobium* increased in the *Rhizobium* applied pots. However there were no differences in terms of *Rhizobium* population between 1 g/pot and 2 g/pot *Rhizobium* application. Also *Rhizobium* application had no effect neither on the TAMB nor YM populations.

III. CONCLUSION

Both the *Rhizobium* and herbicide application considerably affected the bean yield. The highest yield was obtained from 1 g/pot *Rhizobium* application. Increased herbicide application reduced the yield. Very important correlations were determined among all the parameters evaluated in this research. These findings are probably due to the quick inactivation of Aclonifen in growing media.

As a result of this research, it was found out that the application of aclonifen for controlling weeds in bean production had a negative effect on the soil microbiology. The application of Aclonifen may only be recommended as a last resort for weed control in bean production. The trial was carried out under natural field conditions. The same type of trial may also be carried out in a laboratory to find out the effect of Aclonifen on the soil microbial activity.

[redacted] *(2012)*

<u>Assessment and conclusion by applicant</u>:

The study described in this publication does not follow a standard test guideline and was not conducted according to GLP, never less the methodology followed is sufficiently well described and hence the study results are considered acceptable.

<u>Assessment and conclusion by RMS</u>:

CP 10.6 Effects on terrestrial non-target higher plants

The effects of Aclonifen SC 600 G on non-target plants has been studied under laboratory conditions and in higher tier semi-field tests. A summary of the endpoints from the most sensitive plant species from each study is provided in the following table.

Table 10.6-1: Summary of data on the effects of Aclonifen SC 600 G on non-target plants

Test design	Test species	ER$_{50}$ (g a.s./ha)	Reference
Seedling emergence (tests under laboratory conditions)	*Brassica napus*	1.42[1] / > 19.77	KCP 10.6.2/02 M-203247-01-1 [redacted], 2001
	Brassica napus	**25.70**	KCP 10.6.2/04 M-215787-01-1 [redacted], 2002
Seedling emergence (higher tier tests)	*Lactuca sativa* (28 DAA)	74.04	KCP 10.6.4/01 M-229238-01-1 [redacted], 2003
	Lactuca sativa (49 DAA)	198.91	KCP 10.6.4/01 M-229238-01-1 [redacted], 2003
	Brassica napus (21 DAA)	157.19	KCP 10.6.4/02 M-229242-01-1 [redacted], 2004
Vegetative vigour (tests under laboratory conditions)	*Brassica napus*	**13.7**	KCP 10.6.2/01 M-203241-01-1 [redacted], 2001
	Brassica napus	306.72	KCP 10.6.2/03 M-215783-01-1 [redacted], 2002
	Cucumis sativus	17[2]	[redacted], 2019 M-671392-01-1 KCP 10.6.2/05
Vegetative vigour (higher tier tests)	*Lactuca sativa* (21 DAA)	237.01	KCP 10.6.4/01 M-229238-01-1 [redacted], 2003
	Lactuca sativa (33 DAA)	740.89	KCP 10.6.4/01 M-229238-01-1 [redacted], 2003
	Brassica napus (21 DAA)	6650.96	KCP 10.6.4/02 M-229242-01-1 [redacted], 2004

DAA = days after application
Endpoints in **bold** were used in the risk assessment
[1]: Endpoint not used in risk assessment as determined under stress conditions of high temperature & high soil moisture. Second experiment performed under medium temperatue and medium soil moisture conditions showed an ER$_{50}$ of >19.8 g a.s./ha.
[2]: Endpoint expressed as 28.54 mL product/ha in study report. Result re-calculated in terms of g a.s./ha based on an active substance concentration of 607.1 g a.s./L in the formulated product

Summary of the Risk assessment for Terrestrial Non-Target Higher Plants

The risk assessment for effects of Aclonifen SC 600 G on non-target terrestrial plants was performed in accordance with the EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002).

Unacceptable risk to non-target terrestrial plants following the application of Aclonifen SC 600 G to peas according to the GAP was shown when using both a deterministic or probabilistic risk assessment approach and therefore risk mitigation measures were required.

Acceptable risk was demonstrated when a 5 m in-crop buffer was applied to the probabilistic risk assessment. Alternatively, 50% drift reducing nozzles without buffer could be applied.

Risk assessment for Terrestrial Non-Target Higher Plants

The potential risk to non-target terrestrial plants from the proposed uses of Aclonifen SC 600 G has been evaluated using the recommendations presented in the EU Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 of October 2002).

Deterministic risk assessment

According to the Terrestrial Guidance Document the risk to non-target plants is evaluated by comparing the lowest ER_{50} from the laboratory studies with the calculated Predicted Environmental Rates ($PER_{off-field}$). A trigger of 5 can be accepted if at least 6 plant species have been tested.

For Aclonifen SC 600 G, a broad database is available for non-target terrestrial plants.

A series of vegetative vigour tests and/or seedling emergence tests with a variety of plants have been performed: *Allium* cepa (Onion), *Avena sativa* (Oat), *Beta vulgaris* (Sugar beet), *Brassica napus* (Oilseed rape), *Cucumis sativus* (Cucumber), *Daucus carota* (Carrot), *Glycine max* (Soybean), *Gossypium hirsutum* (Cotton), *Hordeum vulgare* (Barley), *Lactuca sativa* (Lettuce), *Linum usitatissimum* (Linseed), *Lolium perenne* (Perennial ryegrass), *Lycopersicon esculentum* (Tomato), *Raphanus sativa* (Radish), *Triticum aestivum* (Wheat), *Vicia faba* (Broad bean) and *Zea mays* (Corn). Thus, data on sensitivity towards the product are available for a total of 17 plant species. Most species were tested under laboratory conditions. Studies with *Brassica napus* (Oilseed rape), *Lactuca sativa* (Lettuce) and *Lolium perenne* (Perennial ryegrass) were also performed under field exposure conditions.

Off-field predicted environmental rates (PER) were calculated according to SANCO 10329/2002 considering a distance of 1 m from the field edge, and TER values compared to a trigger value of 2.

Table 10.6-2: Assessment of the risk for non-target plants due to the use of Aclonifen SC 600 G in peas (1 x 600 g a.s./ha) – Deterministic risk assessment

Test species	ER_{50} (g a.s./ha)	Drift rate (%)	$PER_{off-field}$ (g/ha)	TER	Trigger value
Brassica napus - seedling emergence	25.7	2.77	16.62	**0.82**	5
Brassica napus - vegetative vigour	13.7			**1.55**	

TER values shown in bold fall below the relevant trigger

TER values for both seedling emergence and vegetative vigour are below the trigger value of 5 and hence risk mitigation measures are required.

Probabilistic risk assessment

Due to the richness of data available for the effects of Aclonifen SC 600 G, a probabilistic risk assessment approach is also presented. SSD calculation was performed using the DEFRA webfram tool (https://webfram.com/home.aspx).

Seedling emergence

The following table shows the most reliable endpoints for a total of 11 species determined from the two seedling emergence studies performed under laboratory conditions ([redacted], 2001, M-203247-01-1, KCP 10.6.2/02 and [redacted], 2002, M-203247-01-1, KCP 10.6.2/04) that have been used to calculate

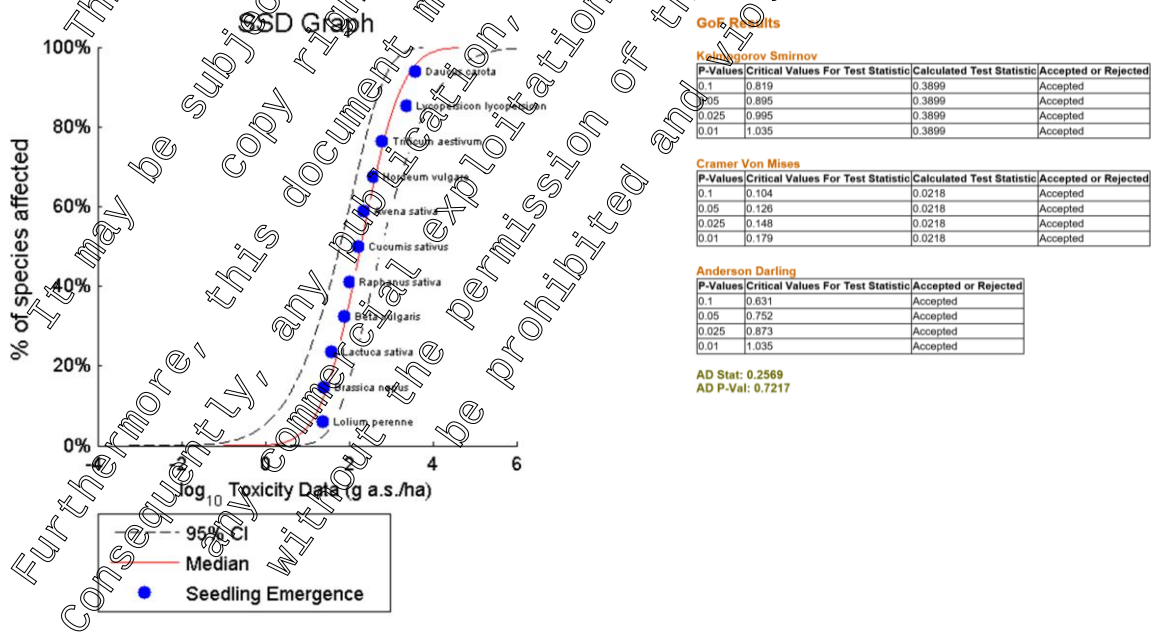
an HR_5. Unbound values (i.e. *Gossypium hirsutum* and *Glycine max*) and an endpoint generated under non-guideline conform stress conditions (*Brassica napus*) were excluded from the calculation.

Table 10.6-3: Seedling emergence endpoints and applicability to SSD calculation

Reference	Species	ER_{50} (g a.s./ha)
KCP 10.6.2/02	*Brassica napus*	1.12[1]
KCP 10.6.2/02	*Lolium perenne*	23.41
KCP 10.6.2/04	*Brassica napus*	25.70
KCP 10.6.2/02	*Lactuca sativa*	37.40
KCP 10.6.2/04	*Beta vulgaris*	77.84
KCP 10.6.2/02	*Raphanus sativus*	100.18
KCP 10.6.2/04	*Cucumis sativus*	163.67
KCP 10.6.2/02	*Avena sativa*	225.81
KCP 10.6.2/04	*Hordeum vulgare*	370.43
KCP 10.6.2/04	*Triticum aestivum*	601.77
KCP 10.6.2/02	*Lycopersicon esculentum*	2305.10
KCP 10.6.2/04	*Daucus carota*	3743.80
KCP 10.6.2/04	*Gossypium hirsutum*	>2400[2]
KCP 10.6.2/02	*Glycine max*	>2700[2]
HR_5		**10.5**

[1]: Unreliable endpoint determined under stress conditions of high temperature & high soil moisture, not used in SSD calculation
[2]: Unbound value, not used in SSD calculation

Figure: SSD curve based on seedling emergence endpoints (HR_5 = 10.5 g a.s./ha)



Vegetative vigour

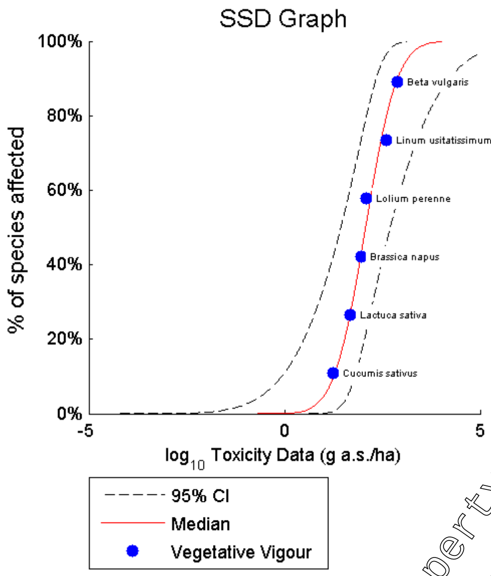
The following table shows the most reliable endpoints for a total of 6 species determined from the three vegetative vigour studies performed under laboratory conditions ([redacted], 2001, M-203241-01-1, KCP 10.6.2/01, [redacted], 2002, M-215783-01-1, KCP 10.6.2/03 and [redacted], 2019, M-671392-01-1, KCP 10.6.2/05) that have been used to calculate an HR_5. Unbound values and an endpoint generated under non-guideline conform stress conditions (*Raphanus sativa* and *Lycopersicon esculentum*) were excluded from the calculation.

Table 10.6-4: Vegetative vigour endpoints and applicability to SSD calculation

Reference	Species	ER_{50} (g a.s./ha)
KCP 10.6.2/05	*Cucumis sativus*	17.33
KCP 10.6.2/05	*Lactuca sativa*	47.79
KCP 10.6.2/05	*Brassica napus*	89.16
KCP 10.6.2/05	*Lolium perenne*	122
KCP 10.6.2/05	*Linum usitatissimum*	398.03
KCP 10.6.2/05	*Beta vulgaris*	773.79
KCP 10.6.2/01	*Raphanus sativa*	2747.5[1]
KCP 10.6.2/01	*Lycopersicon esculentum*	16830[1]
KCP 10.6.2/05	*Glycine max*	>1033[2]
KCP 10.6.2/05	*Avena sativa*	>1033[2]
KCP 10.6.2/03	*Gossypium hirsutum*	>2400[2]
KCP 10.6.2/03	*Hordeum vulgare*	>2400[2]
KCP 10.6.2/03	*Triticum aestivum*	>2400[2]
KCP 10.6.2/03	*Daucus carota*	>2400[2]
KCP 10.6.2/01	*Vicia faba*	>4800[2]
KCP 10.6.2/05	*Zea mays*	>5468[2]
KCP 10.6.2/05	*Allium cepa*	>5468[2]
HR_5		**10.4**

[1]: Unreliable endpoint determined under stress conditions of high temperature & high soil moisture, not used in SSD calculation
[2]: Unbound value, not used in SSD calculation

Figure 10.6-1: SSD curve based on vegetative vigour endpoints (HR_5 = 10.4 g a.s./ha)



GoF Results

Kolmogorov Smirnov

P-Values	Critical Values For Test Statistic	Calculated Test Statistic	Accepted or Rejected
0.1	0.819	0.4413	Accepted
0.05	0.895	0.4413	Accepted
0.025	0.995	0.4413	Accepted
0.01	1.035	0.4413	Accepted

Cramer Von Mises

P-Values	Critical Values For Test Statistic	Calculated Test Statistic	Accepted or Rejected
0.1	0.104	0.0099	Accepted
0.05	0.126	0.0099	Accepted
0.025	0.148	0.0099	Accepted
0.01	0.179	0.0099	Accepted

Anderson Darling

P-Values	Critical Values For Test Statistic	Accepted or Rejected
0.1	0.631	Accepted
0.05	0.752	Accepted
0.025	0.873	Accepted
0.01	1.035	Accepted

AD Stat: 0.1872
AD P-Val: 0.9039

Off-field predicted environmental rates (PER) were calculated according to SANCO 10329/2002 considering a distance of 1 m from the field edge, and TER values compared to a trigger value of 1.

Table 10.6-5: Assessment of the risk for non-target plants due to the use of Aclonifen SC 600 G in peas (1 x 600 g a.s./ha) – Probabilistic risk assessment

Test species	HR_5 (g a.s./ha)	Drift rate (%)	$PER_{off\text{-}field}$ (g/ha)	TER	Trigger value
Seedling emergence	10.5	2.77	16.62	**0.63**	1
Vegetative vigour	10.40			**0.63**	

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

TER values for both seedling emergence and vegetative vigour are below the probabilistic risk assessment trigger value of 1 and hence risk mitigation measures are required.

Risk mitigation measures

Both the deterministic and the probabilistic risk assessment did not pass the trigger, indicating a need for further assessment under consideration of risk mitigation measures in order to reduce the off-field exposure. These mitigation measures correspond to unsprayed in-field buffer strips of a given width and/or the usage of drift reducing nozzles. The results of the risk assessment using typical mitigation measures (no-spray buffer zones of 5 or 10 m; drift-reducing nozzles with reduction by 50%, 75%, or 90%) are summarised in the following table.

Table 10.6-6: Assessment of the risk for non-target plants due to the use of Aclonifen SC 600 G in peas (1 x 600 g a.s./ha) – Probabilistic risk assessment considering risk mitigation

Buffer strip (m)	Drift rate (%)	$PER_{off\text{-}field}$ (g/ha)	$PER_{off\text{-}field}$ 50% drift red. (g/ha)	$PER_{off\text{-}field}$ 75% drift red. (g/ha)	$PER_{off\text{-}field}$ 90% drift red. (g/ha)
1	2.77	16.62	8.31	4.16	1.66
5	0.57	3.42	1.71	0.86	0.34
10	0.29	1.74	0.87	0.44	0.17

Seedling emergence HR5 = 10.5 g a.s./ha	**TER** **Trigger: TER ≥ 1**			
1	**0.63**	1.26	2.53	6.32
5	3.07	6.14	12.28	30.70
10	6.03	12.07	24.14	60.34
Vegetative vigour HR5 = 10.4 g a.s./ha	**TER** **Trigger: TER ≥ 2**			
1	**0.63**	1.25	2.50	6.26
5	3.04	6.08	12.16	30.41
10	5.98	11.95	23.91	59.77

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

The probabilistic risk assessment based on both HR_5 from seedling emergence and vegetative vigour studies resulted in an acceptable risk provided that a 5 m in-crop buffer is kept. Alternatively, 50% drift reducing nozzles without buffer could be applied.

Non-target terrestrial plant studies performed on the formulation, Aclonifen SC 600 G are presented below:

Data Point:	KCP 10.6/01
Report Author:	[redacted]
Report Year:	2000
Report Title:	Ecotoxicity studies & environmental risk assessment for non-target plants BANDUR (EXP 04209) Code: AE F068300 00 SC50 A2
Report No:	C010049
Document No:	M-198290-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable
Previous evaluation:	yes, evaluated, not accepted Source: DAR, Vol. 3 B9 (9.9.1), August 2006 (RMS: DE)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Now is no longer acceptable

In the previous submission (DAR, 2006), this study was evaluated and not accepted as valid for risk assessment purposes. Therefore, a summary of this study is not presented in this dossier.

CP 10.6.1 Summary of screening data

Not required as the formulated product has been evaluated for phytotoxicity (see point CP 10.6.2 below).

CP 10.6.2 Testing on non-target plants

Studies on the effect of the formulation Aclonifen SC 600 G on non-target plants have been conducted and presented below.

Data Point:	KCP 10.6.2/01
Report Author:	[redacted]
Report Year:	2001
Report Title:	Effects of EXP 04209E on terrestrial (non-target) plants: vegetative Vigour test
Report No:	C016889
Document No:	M-203241-01-1
Guideline(s) followed in study:	OECD: 208 (update proposal)
Deviations from current test guideline:	Current Guideline: OECD 227 (2006) On a number of occasions the environmental conditions were not recorded or exceeded the permitted range. These environmental deviations may have caused more sever effects on the plants. Current method guideline: SANCO/3029/99 rev.4 Yes, not all requirements for precision fulfilled
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of EXP04209E on vegetative vigour in eight terrestrial non-target plant species representing five plant families.

The test was run over 21 days using five application rates applied as a foliar spray per test species. Visual phytotoxicity ratings (e.g. chlorosis, necrosis, abnormal growth) were recorded on Days 7, 14 and 21. Mortality and growth (fresh weight) was determined on Day 21.

The most sensitive species were *Brassica napus* with an EC_{50} of 13.66 g a.s./ha, *Lactuca sativa* (41.27 g a.s./ha) and *Lolium perenne* (135.3 g a.s./ha). The least sensitive species was *Vicia faha* with a NOEC > 4800 g a.s./ha. In spite of showing significant effects in all tested application rates, reduction of fresh weight in *Glycine max* did not exceed 30% compared to control.

Lactuca sativa showed the most sensitive reaction in mortality (59.26 g a.s./ha and higher) followed by *Brassica napus* (177.78 g a.s./ha and higher). Significant mortality for the remaining species was not observed in 2400 g a.s./ha and below.

Main phytotoxic effects observed in different species at different dose levels were chlorosis which resulted in necrotic leaves.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: EXP 04209E

Batch no.: OP 200622

Active Ingredient / Purity: Aclonifen: 589g/L

Expiry date: 11 December 2002

Appearance: Luminous yellow liquid

Storage: In original container, at room temperature (+2 to +30 °C) in the dark

2. Test species: 6 dicotyledoneae and 2 monocotyledoneae species were chosen representing 5 plant families

	Family	Species	Common name
Dicotyledonae	Asteraceae	*Lactuca sativa*	Lettuce
Dicotyledonae	Brassicaceae	*Brassica napus*	Oilseed Rape
Dicotyledonae	Brassicaceae	*Raphanus sativus*	Radish
Dicotyledonae	Leguminosae	*Glycine max*	Soybean
Dicotyledonae	Leguminosae	*Vicia faba*	Broad Bean
Dicotyledonae	Solanaceae	*Lycopersicon lycopersicum = L. esculentum*	Tomato
Monocotyledonae	Gramineae	*Avena sativa*	Oat
Monocotyledonae	Gramineae	*Lolium perenne*	Perennial Ryegrass

B. STUDY DESIGN AND METHODS

1. In-life phase: 02 May – 19 July 2001

2. Exposure conditions

Test vessels: Commercial plastic flower pots of diameter 16 cm and 9 x 9 cm

Soil: Lufa 2.3 (sandy loam). All particles under 0.2 cm, 1.32 ± 0.1% organic matter, pH 6.5 ± 0.1

Experimental design: Control, test item (5 applications within the range 6.58 g a.s./ha and 4800 g a.s./ha)

Replicates: Dicotyledonae: 10 pots each containing 3 plants
Monocotyledonae: 6 pots each containing 5 plants

Temperature: Day 23 °C ± 4 °C, night 18 °C ± 4 °C (intended)
The temperatures during the tests were in the upper part of the range, sometimes the maximum temperature was exceeded.
The mean temperature in the run with the species *Lactuca sativa, Lolium perenne, Avena sativa, Raphanus sativus, Lycopersicon esculentum, Viciafaba* was 24.23 °C.
The mean temperature in the run with *Brassica napus* and *Glycine max* was21.62°C

Relative humidity: Day: approximately 70%, night: approximately 85%

Photoperiod: 16h light:8h dark

Light intensity: 15446 Lux (mean); Range 4019 – 19514 Lux

Irrigation: The irrigation with tap water was done automatically with fibreglass-wicks through the bottom which results in a constant maximum water saturation

Nutrient media: Flory 9 (Euflor) 1 g/L with Sequestren (Ciba-Geigy) 0.1 g/L supplied once a week in the watering system

3. Administration of the test item

The test item was applied as a singular application according to agricultural practice with a laboratory spraying equipment.

Species	Application Rates (g a.s./ha)	
	Minimum	Maximum
Lactuca sativa	19.75	1600.00
Brassica napus	6.58	533.33
Raphanus sativus	59.26	4800.00
Glycine max	59.26	4800.00
Vicia faba	59.26	4800.00
Lycopersicon lycopersicum	59.26	4800.00
Avena sativa	59.26	4800.00
Lolium perenne	61.44	2400.00

The range included 5 increasing concentrations from the indicated minimum to the maximum

At application, all species were in 2 to 4 leaf stage (BBCH 12 – 14).

4. Measurements and observations

At least duplicate samples from the freshly prepared and continuously stirred stock solution were taken before application for verification of test item concentrations.

Visual phytotoxicity ratings (e.g. chlorosis, necrosis, abnormal growth) were recorded on Days 7, 14 and 21 according to EPPO Standard 135.

The fresh weight was determined on Day 21. The plants of one pot represented one replicate. The number of plants died after application were recorded at Day 21. Dead plants were weighed if it was practicable.

5. Statistics/Data evaluation

Fresh weight data were tested for normality by using Kolmogoroff-Smirmov-Test. Homogeneity was tested with Cochran-Test if data were not normally distributed. If the normal distribution was accepted Bartlett Test was used for all data with $n > 10$ and Cochran Test for data with $n < 10$. If the data were normally distributed and homogeneous Williams Test (monotonously increasing or decreasing) or Dunnett Test (not monotonously increasing or decreasing) were used for comparing treatment groups and control. If the data were not homogeneous Bonferroni U-Test was used.

In order to determine the EC_{25} and EC_{50} values, a regression analysis (Probit-analysis) was performed. For the mortality data Fischer Exact Test was used.

The significance level for all tests was α=0.05. The decision on weight (one-sided, two-sided) was made dependent on the data. Computer program used to perform the statistical analyses was ToxRat® SPiRiT Solutions (1999-2001), Version 1.08 and SYSTAT Version 9.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Mean recovery of active ingredient aclonifen in the stock solutions for preparation of the spray dilutions was 101 and 102% of the nominal concentration. The validated method is summarised in Document M-CP5 (CP 5.1.2/10).

B. BIOLOGICAL DATA

High light intensity produced temperatures exceeding the intended temperature range and constant wick watering was leading to high soil moisture. The overall mean temperature during the HTHSM runs (HTHSM: high temperature & high soil moisture) was 24.2 °C. Two species were run at lower mean temperatures of 21.6 °C but with continuous wick watering (MTHSM = medium temperature & high soil moisture).

Table: Effects of EXP04209E on vegetative vigour

Species	Family	Test conditions	NOEC (g a.s./ha)	EC_{50} (g a.s./ha)
Brassica napus (Oilseed rape)	Brassicaceae	MTHSM	< 6.6	13.7
Lactuca sativa (Lettuce)	Asteraceae	HTHSM	< 19.8	41.3
Lolium perenne (Perennial ryegrass)	Gramineae	HTHSM	< 61.4	135.3
Avena sativa (Oat)	Gramineae	HTHSM	< 59.3	546.5
Raphanus sativa (Radish)	Brassicaceae	HTHSM	177.8	2747.5
Lycopersicon esculentum (Tomato)	Solanaceae	HTHSM	1600	16830
Glycine max (Soybean)	Leguminosae	MTHSM	< 59.3	174606
Vicia faba (Broad bean)	Leguminosae	HTHSM	≥ 4800	> 4800

HTHSM: high temperature & high soil moisture
MTHSM: medium temperature & high soil moisture

Fresh weight: The most sensitive species identified was *Brassica napus* (EC_{50} of 13.7 g a.s./ha). The least sensitive species was *Vicia faba* (no significant effect up to 4800 g a.s./ha).

The most sensitive species with regard to treatment related plant mortality was *Lactuca sativa* (59.3 g as/ha and above), followed by *Brassica napus* (177.81 g as/ha and above).

Phytotoxic effects were observed in form of chlorosis which later resulted in necrosis at different dose levels in different species.

C. VALIDITY CRITERIA

The test was performed according to OECD 208 (updated proposal; draft July 2000), however validity has been assessed against the current vegetative vigour test guideline (OECD 227, 2006).

It was not possible to determine from the reported data whether the validity criterion for seedling emergence (≥ 70% required) was satisfied, however as sufficient plants were available for the application of the test item this omission is considered not to have affected the validity of the study.

Control plant survival was 100% and therefore satisfied the validity criterion of being ≥ 90%.

The control plants did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants only exhibited normal variation in growth and morphology for each particular species.

In addition, environmental conditions for each species were identical and the growing media contained the same amount of soil matrix, support media, or substrate from the same source.

Based on the above assessment, this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Species	Endpoint (g a.s./ha)	
	NOEC	EC_{50}
Brassica napus (Oilseed rape)	< 6.6	13.7
Lactuca sativa (Lettuce)	< 19.8	41.3
Lolium perenne (Perennial ryegrass)	< 61.4	135.3
Avena sativa (Oat)	< 59.3	546.5
Raphanus sativa (Radish)	177.8	2747.5
Lycopersicon esculentum (Tomato)	1600	16830
Glycine max (Soybean)	< 59.3	174606
Vicia faba (Broad bean)	≥ 4800	> 4800

III. CONCLUSION

After post-emergence application of aclonifen as EXP04209E in the laboratory, the most sensitive plant species identified was *Brassica napus* with a 21-day EC_{50} based on freshweight of 13.7 g a.s./ha.

[redacted] *(2001)*

Assessment and conclusion by applicant:

All validity criteria were considered to have been satisfied and therefore this study can be considered to be valid.

The most sensitive plant species identified was *Brassica napus* with a 21-day ER_{50} based on freshweight of 13.7 g a.s./ha. Care should be taken in the interpretation of the results as due to high temperatures exceeding the intended temperature range and constant wick watering leading to high soil moisture. These stress conditions may have affected the derived endpoints.

Assessment and conclusion by RMS:

Data Point:	KCP 10.6.2/02
Report Author:	[redacted]
Report Year:	2001
Report Title:	Effects of EXP04209E on terrestrial (non-target) plants: seedling emergence and seedling growth test
Report No:	C016892
Document No:	M-203247-01-1
Guideline(s) followed in study:	OECD: 208 (update proposal)
Deviations from current test guideline:	Current Guideline: OECD 208, 2006 The temperature exceeded the recommended maximum on a number of occasions, these high temperatures may have caused more severe effects on the plants. Current method guideline: SANCO/3029/99 rev.4 Yes, not all requirements for precision fulfilled
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of EXP04209E on seedling emergence in seven terrestrial non-target plant species representing five plant families.

The test was run over 21 days using five application rates applied as a soil spray per test species. Percentage of emergence and visual phytotoxicity ratings (e.g. chlorosis, necrosis, abnormal growth) were recorded on Days 7, 14 and 21. Mortality and growth (fresh weight) was determined on Day 21.

Due to technical problems the temperatures during the initial experimental part of the study (with *Brassica napus, Lactuca saliva, Raphanus sativus, Lycopersicon lycopersicon* and *Glycine max*) were higher than expected. This part is coded HTHSM (High Temperatures & High Soil Moisture).

The second part of the test was conducted under conditions of medium temperatures and high soil moisture (*Lolium perenne* MTHSM and *Avena saliva* MTHSM). An additional run was performed with medium temperature and medium soil moisture in *Brassica napus* (MTMSM).

The most sensitive species was *Brassica napus* with an EC_{50} of 1.12 g a.s./ha when test was performed under conditions of elevated temperatures and high soil moisture (HTHSM). The second test of *Brassica napus* with medium temperatures and soil moisture (MTMSM) showed no fresh weight reduction up to 19.77 g a.s./ha. (NOEC >19.77 g a.s./ha). This decrease in sensitivity appears to be a result of the changed test conditions with regard to moisture and temperature.

Based on the results of *Brassica napus* (MTMSM) *Lolium perenne* would be the most sensitive species with an EC_{50} of 23.41 g a.s./ha and a NOEC of < 19.77 g a.s./ha. The least sensitive species were *Lycopersicon esculentum* (EC 50 2305.10) and *Glycine max* with a NOEC > 2700 g a.s./ha.

Germination was only affected in *Raphanus sativus* at the top dose of 300 g a.s./ha.

Most sensitive species in mortality was *Brassica napus* (HTHSM) with 32% at 33.33 g a.s./ha followed by *Lactuca sativa* with 31%at 100 g a.s./ha.

Phytotoxic effects observed in different species at different dose levels were chlorosis which later resulted in necrosis.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: EXP 04209E
Batch no.: OP 200622
Active Ingredient: Aclonifen: 589g/L
Purity:
Expiry date: 11 December 2002
Appearance: Luminous yellow liquid
Storage: In original container, at room temperature (+2 to +30 °C) in the dark

2. Test species: 5 dicotyledoneae and 2 monocotyledoneae species were chosen representing 5 plant families

Family	Species	Common name
Dicotyledonae	Asteraceae	*Lactuca sativa*	Lettuce
Dicotyledonae	Brassicaceae	*Brassica napus*	Oilseed Rape
Dicotyledonae	Brassicaceae	*Raphanus sativus*	Radish
Dicotyledonae	Leguminosae	*Glycine max*	Soybean
Dicotyledonae	Solanaceae	*Lycopersicon lycopersicum*	Tomato
Monocotyledonae	Gramineae	*Avena sativa*	Oat
Monocotyledonae	Gramineae	*Lolium perenne*	Perennial Ryegrass

B. STUDY DESIGN AND METHODS

1. In-life phase: 16 March – 10 August 2001

2. Exposure conditions

Test vessels: Commercial plastic flower pots of diameter 16 cm and 9 x 9 cm

Soil: Lufa 2.3 (sandy loam). All particles under 0.2 cm, 1.32 ± 0.1% organic matter, pH 6.5 ± 0.1

Experimental design: Control, test item (5 applications within the range 0.24 g a.s./ha and 2700 g a.s./ha)

Replicates: 6 pots each containing 5 seeds per treatment group

Temperature: Day 23°C ± 4°C, night 18°C ± 4°C (intended)
During the first test period the high light intensity caused high temperatures which could not be compensated by the air condition because of high temperatures outsides. The overall mean temperature in the HT runs was approximately 25 °C. In order to come into the desired temperature range, light intensity with the negative side effect 'heating' had to be reduced. The overall mean temperature in the MTMSM run with *Brassica napus* was approx. 22 C

Relative humidity: Day: 70% ± 20%, night: 85% ± 15%

Photoperiod: 16h light:8h dark

Light intensity: 11000 - 23000 lux (mean light intensity 15413 lux, maximum 20600, minimum 12100 lux during the first test period.
For the repetition of *Brassica napus, Lolium perenne* and *Avena sativa* light intensity was changed. Mean light intensity for *Brassica napus* MTMSM was 8767 lux; minimum 7100, maximum 10700 lux

Irrigation: Irrigation with tap water was done automatically with fibreglass-wicks through the bottom (high soil moisture, HSM).
For the repetition of *Brassica napus* (medium soil moisture MSM) the irrigation system was changed. Irrigation with tap water was also done automatically with fibreglass-wicks through the bottom but to avoid supersaturation of the soil with water, the wicks were removed from the water as soon as the moisture content of the soil was saturated. At the time the soil started drying out on the surface, the wicks were dipped in the reserves again. In order to control the water content and to avoid water deficit five pots were weighted once a week

3. Administration of the test item

The test item was applied as a singular application according to agricultural practice with a laboratory-spraying equipment.

Species	Application Rates (g a.s./ha)	
	Minimum	Maximum
Lactuca sativa	3.7	300.0
Brassica napus	0.41	33.33

Raphanus sativus	3.7	300.0
Glycine max	33.3	2700.0
Lycopersicon lycopersicum	33.3	2700.0
The concentrations for the second run with *Brassica napus, Lolium perenne* and *Avena sativa* were different from the protocol dosages. For *Lolium perenne* and *Avena sativa* the concentrations were increased in order to determine an EC_{50}. As the results from the first run were not appropriate to assess effects on these species, the results of the first run were not reported. For *Brassica napus* the determination of EC_{50} was possible but *Brassica napus* was repeated under more realistic environmental conditions to verify the results of the first test. Therefore *Brassica napus* was reported twice.		
Brassica napus	0.24	19.77
Avena sativa	19.77	1601.42
Lolium perenne	19.77	1601.42

The range included 5 increasing concentrations from the indicated minimum to the maximum

In total 30 seeds per species and treatment group were sown. The day before the application the seeds were introduced manually in the soil. After sowing the pots were placed on the watering system.

4. Measurements and observations

Duplicate samples from the freshly prepared and continuously stirred stock solution were taken before application for verification of test item concentrations.

The percentage of emerged seedlings and visual phytotoxicity ratings (e.g. chlorosis, necrosis, abnormal growth) was recorded on Days 7, 14 and 21 after application.

The fresh weight was determined on Day 21. The plants of one pot represented one replicate. The number of plants died after application were recorded on Day 21. Dead plants were weighed if it was practicable. Growth stages at Day 21 were also reported.

5. Statistics/Data evaluation

Fresh weight data were tested for normality by using Kolmogoroff-Smirmov-Test. Homogeneity was tested with Cochran-Test if data were not normally distributed. If the normal distribution was accepted Bartlett Test was used for all data with $n > 10$ and Cochran Test for data with $n < 10$. If the data were normally distributed and homogeneous Williams Test (monotonously increasing or decreasing) or Dunnett Test (not monotonously increasing or decreasing) were used for comparing treatment groups and control. If the data were not homogeneous Bonferroni U-Test was used.

In order to determine the EC_x values, a regression analysis (Probit-analysis) was performed. For the germination and mortality data Fischer Exact Test was used.

The significance level for all tests was α=0.05. The decision on weight (one-sided, two-sided) was made dependent on the data. Computer program used to perform the statistical analyses was ToxRat® SPiRiT Solutions (1999-2001), Version 1.08 and SYSTAT Version 9.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

The analytically determined mean of aclonifen concentrations in the analysed stock solutions was 94.25%. The validated method is summarised in Document M-CP5 (CP 5.1.2/11).

B. BIOLOGICAL DATA

Due to technical problems the temperatures during the initial experimental part of the study (with *Brassica napus, Lactuca sativa, Raphanus sativus, Lycopersicon lycopersicon* and *Glycine max*) were higher than expected. This part is coded HTHSM (High Temperatures & High Soil Moisture). The second part of the test was conducted under conditions of medium temperatures and high soil moisture (*Lolium perenne* MTHSM and *Avena sativa* MTHSM). An additional run was performed with medium temperature and medium soil moisture in *Brassica napus* (MTMSM).

Table: Effects of EXP04209E on seedling emergence and growth

Species	Family	Test conditions	NOEC (g a.s./ha)	EC_{50} (g a.s./ha)
Brassica napus (Oilseed rape)	Brassicaceae	HTHSM	0.41	1.12
Brassica napus (Oilseed rape)	Brassicaceae	MTMSM	≥ 19.77	> 19.77
Lolium perenne (Perennial ryegrass)	Gramineae	MTHSM	< 19.77	23.41
Lactuca sativa (Lettuce)	Asteraceae	HTHSM	11.11	37.40
Raphanus sativa (Radish)	Brassicaceae	HTHSM	33.33	100.18
Avena sativa (Oat)	Gramineae	MTHSM	59.31	225.81
Lycopersicon lycopersicon (Tomato)	Solanaceae	HTHSM	< 33.33	2305.10
Glycine max (Soybean)	Leguminosae	HTHSM	>2700	> 2700

HTHSM: high temperature & high soil moisture
MTHSM: medium temperature & high soil moisture
MTMSM: medium temperature and medium soil moisture

Fresh weight: The most sensitive species identified under HTHSM conditions was *Brassica napus*, with the EC_{50} of 1.12 g a.s./ha. However, under conditions of the MTMSM run, no effects on freshweight of *B. napus* were detected up to 19.8 g a.s./ha. Analysis of all observed parameter indicated that the conditions of the HTHSM runs may have contributed to very fast growing but vulnerable *B. napus* seedlings whilst MTMSM conditions resulted in slower growing but more robust seedlings. With regard to the environmental conditions in spring when EXP04209E is applied mainly as pre-emergence herbicide in agriculture, the HTHSM run was considered less relevant to field conditions than the MTMSM run.

Treatment related plant mortality was observed in *B. napus* (HTHSM) with 32% at 33.3 g a.s./ha, followed by *Lactuca sativa* (31%) at 100 g a.s./ha.

Germination was affected by the test item only in *Raphanus sativus* at the top dose of 300 g a.s./ha.

Phytotoxic effects were observed in form of chlorosis which later resulted in necrosis at different dose levels in different species.

C. VALIDITY CRITERIA

The test was performed according to OECD 208 (updated proposal; draft July 2000), however validity has been assessed against the current test guideline (OECD 208, 2006).

Control seedling emergence ranged from 70 – 100% and therefore satisfied the validity criterion of being ≥ 70%.

Control plant survival was 100% and therefore satisfied the validity criterion of being ≥ 90%.

The control plants did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants only exhibited normal variation in growth and morphology for each particular species.

In addition, environmental conditions for each species were identical and the growing media contained the same amount of soil matrix, support media, or substrate from the same source.

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Species	Test conditions	Endpoint	
		NOEC (g a.s./ha)	EC_{50} (g a.s./ha)
Brassica napus (Oilseed rape)	HTHSM	0.41	1.12
Brassica napus (Oilseed rape)	MTMSM	≥ 19.77	> 19.77
Lolium perenne (Perennial ryegrass)	MTHSM	< 19.77	23.41
Lactuca sativa (Lettuce)	HTHSM	11.11	37.40
Raphanus sativa (Radish)	HTHSM	33.33	100.18
Avena sativa (Oat)	MTHSM	59.31	225.81
Lycopersicon lycopersicon (Tomato)	HTHSM	< 33.33	2305.10
Glycine max (Soybean)	HTHSM	>2700	> 2700

HTHSM: high temperature & high soil moisture
MTHSM: medium temperature & high soil moisture
MTMSM: medium temperature and medium soil moisture

III. CONCLUSION

The most sensitive species was *Brassica napus* with an EC_{50} of 1.12 g a.s./ha when test was performed under conditions of elevated temperatures and high soil moisture (HTHSM). The second test of *Brassica napus* with medium temperatures and soil moisture (MTMSM) showed no fresh weight reduction up to 19.77 g a.s./ha. (NOEC >19.77 g a.s./ha). This decrease in sensitivity appears to be a result of the changed test conditions with regard to moisture and temperature.

Based on the results of *Brassica napus* (MTMSM), *Lolium perenne* would be the most sensitive species with an EC_{50} of 23.41 g a.s./ha and a NOEC of < 19.77 g a.s./ha. The least sensitive species were *Lycopersicon esculentum* (EC 50 2305.10) and *Glycine max* with a NOEC > 2700 g a.s./ha.

[redacted] *(2001)*

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

Under conditions of elevated temperatures and high soil moisture (HTHSM), the most sensitive plant species identified was *Brassica napus* with a 21-day ER_{50} based on freshweight of 1.12 g a.s./ha. Under conditions of medium temperatures and soil moisture (MTMSM), no freshweight reduction was observed for *Brassica napus* up to a maximum tested concentration of 19.77 g a.s./ha.

Care should therefore be taken in the interpretation of the results obtained under HTHSM as these stress conditions may have affected the derived endpoints.

Based on the results obtained under medium temperature & high soil moisture (MTHSM), *Lolium perenne* would be the most sensitive species with an ER_{50} of 23.41 g a.s./ha and a NOEC of < 19.77 g a.s./ha.

The lowest possible endpoint for seeding emergence, ER_{50} = 1.12 g a.s./ha, was derived for *Brassica napus* under stress conditions. This endpoint is listed in the EFSA LoEP (2008) but is superseded by higher endpoints determined under "normal" conditions. The non-relevance of this "stress" endpoint was confirmed by NL in Evaluation of Challenge SC 600 (2012): "*However, this value was obtained under high temperature and high soil moisture conditions, which is not very realistic for the Netherlands. Another test was performed with the same plant species under medium temperature and high soil moisture conditions resulting in an ER_{50} of 25.7 g a.s./ha (seedling emergence test). This value is considered to be more realistic for risk assessment.*".

The lowest endpoint to be used in NTTP deterministic RA is therefore an **ER_{50} = 13.7 g a.s./ha** for *Brassica napus* obtained in a vegetative vigour study performed under normal conditions (KCP 10.6.2/01).

Assessment and conclusion by RMS:

Data Point:	KCP 10.6.2/03
Report Author:	[redacted]
Report Year:	2002
Report Title:	Effects of EXP 04209E on terrestrial (non-target) plants: vegetative vigour test
Report No:	C023845
Document No:	M-215783-01-1
Guideline(s) followed in study:	OECD: 208 (Draft Document July 2000)
Deviations from current test guideline:	Current Guideline: OECD 227, 2006 Temperatures for some species exceeded the intended range for a short period. This deviation might have led to a slightly increased effect of the product. Current method guideline: SANCO/3029/99 rev.4 Yes, not all requirements for precision fulfilled
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of EXP04209E on vegetative vigour in seven terrestrial non-target plant species representing six plant families.

The test was run over 21 days using five application rates applied as a foliar spray per test species. Visual phytotoxicity ratings (e.g. chlorosis, necrosis, abnormal growth) were recorded on Days 7, 14 and 21. Mortality and growth (fresh weight) was determined on Day 21.

The most sensitive species was the dicotyledoneae *Brassica napus* with an EC_{50} of 306.72 g a.s./ha.

Mortality was not observed during the study.

Typical phytotoxic effects appeared as chlorosis, necrosis and wilting. The most sensitive species was *Brassica napus*. In this species, effects rated at ≥ 25% phytotoxicity were observed at treatment rates of 61.44 g a.s./ha and above after 21 days. No other species showed this level of phytotoxicity below 153.6 g a.s./ha.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Item:** EXP 04209E

Batch no.: OP 200622

Active Ingredient / Purity: Aclonifen: 589g/L

Expiry date: 11 December 2002

Appearance: Luminous yellow liquid

Storage: In original container, at room temperature (+2 to +30 °C) in the dark

2. Test species: 5 dicotyledoneae and 2 monocotyledoneae species were chosen representing 6 plant families

	Family	Species	Common name
Dicotyledonae	Brassicaceae	*Brassica napus*	Oilseed Rape
Dicotyledonae	Curcubitaceae	*Cucumis sativus*	Cucumber
Dicotyledonae	Chenopodiaceae	*Beta vulgaris*	Sugar Beet
Dicotyledonae	Malvaceae	*Gossypium hirsutum*	Cotton
Dicotyledonae	Umbellifereae	*Daucus carota*	Carrot
Monocotyledonae	Gramineae	*Hordeum vulgare*	Barley
Monocotyledonae	Gramineae	*Triticum aestivum*	Wheat

B. STUDY DESIGN AND METHODS

1. In-life phase: 15 February – 08 April 2002

2. Exposure conditions

Test vessels: Commercial plastic flower pots of diameter 16 cm and 9 x 9 cm

Soil: Lufa 2.3 (sandy loam). All particles under 0.2 cm, 1.32 ± 0.1% organic matter, pH 6.5 ± 0.1

Experimental design: Control, test item (5 to 6 applications within the range 9.83 g a.s./ha and 2400 g a.s./ha)

Replicates: *Brassica napus, Cucumis sativus, Beta vulgaris* and *Gossypium hirsutum*: 10 pots each containing 3 plants
Daucus carota, Hordeum vulgare and *Triticum aestivum*: 6 pots each containing 5 plants

Temperature: Day 23 °C ± 4 °C, night 18 °C ± 4 °C (intended)
Temperature for all species with the exception of *Gossypium hirsutum*: day 25 °C (21-30 °C), night 19 °C (18-19 °C), mean day temperature (24 h) during the test period: 23 °C
Temperature for *Gossypium hirsutum*: day 24 °C (24-25 °C), night 19 °C (18-19 °C), mean day temperature (24 h) during the test period: 22 °C

Relative humidity: Day: approximately 70%, night: approximately 85%

Photoperiod: 16h light:8h dark

Light intensity: 8503 Lux (mean); Range 5190 – 15800 Lux

Irrigation: Irrigation with tap water was done automatically with fibreglass-wicks connecting soil and water supply (bowl standing below each pot and containing maximum 500 mL water)

Nutrient media: Flory 9 (Euflor) Ig/L with Sequestren (Ciba-Geigy) 0.05 g/L was given one to three times a week after development of the first true leaves.

3. Administration of the test item

The test item was applied as a singular application according to agricultural practice with a laboratory-spraying equipment.

Species	Application Rates (g a.s./ha)	
	Minimum	Maximum
Brassica napus	9.83	960
Cucumis sativus	61.44	2400
Beta vulgaris	24.58	2400
Gossypium hirsutum	61.44	2400
Daucus carota	61.44	2400
Hordeum vulgare	61.44	2400
Triticum aestivum	61.44	2400

The range included 5 to 6 increasing concentrations from the indicated minimum to the maximum

At application, all species were in 2 to 4 leaf stage.

4. Measurements and observations

Duplicate samples from the freshly prepared and continuously stirred stock solutions were taken before application for verification of test item concentrations.

Visual phytotoxicity ratings (e.g. chlorosis, necrosis, abnormal growth) were recorded on Days 7, 14 and 21 according to EPPO Standard 135.

The fresh weight was determined on Day 21. The plants of one pot represented one replicate. The number of plants died after application were recorded at Day 21. Dead plants were weighed if it was practicable.

5. Statistics/Data evaluation

Fresh weight data were tested for normality by using Kolmogoroff-Smirmov-Test. Homogeneity was tested with Cochran-Test if data were not normally distributed. If the normal distribution was accepted Bartlett Test was used for all data with $n > 10$ and Cochran Test for data with $n < 10$. If the data were normally distributed and homogeneous Williams Test (monotonously increasing or decreasing) or Dunnett Test (not monotonously increasing or decreasing) were used for comparing treatment groups and control. If the data were not homogeneous Bonferroni U-Test was used.

In order to determine the EC_{25} and EC_{50} values, a regression analysis (Probit-analysis) was performed. For the mortality data Fischer Exact Test was used.

The significance level for all tests was $\alpha=0.05$. The decision on weight (one-sided, two-sided) was made dependent on the data. Computer program used to perform the statistical analyses was ToxRat® SPiRiT Solutions (1999-2001), Version 1.08 and SYSTAT Version 9.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Mean recovery of active ingredient aclonifen in the stock solutions for preparation of the spray dilutions was 98% of the nominal concentration. The validated method is summarised in Document M-CP5 (CP 5.1.2/12).

B. BIOLOGICAL DATA

Table: Effects of EXP04209E on vegetative vigour

Species	Family	NOEC (g a.s./ha)	EC_{50} (g a.s./ha)
Brassica napus (Oilseed Rape)	Brassicaceae	9.83	306.72
Cucumis sativus (Cucumber)	Curcubitaceae	153.6	1470.27
Beta vulgaris (Sugar Beet)	Chenopodiaceae	61.44	>2400
Gossypium hirsutum (Cotton)	Malvaceae	61.44	>2400
Daucus carota (Carrot)	Umbellifereae	960	>2400
Hordeum vulgare (Barley)	Gramineae	>2400	>2400
Triticum aestivum (Wheat)	Gramineae	>2400	>2400

The most sensitive species was the dicotyledoneae *Brassica napus* with an EC_{50} of 306.72 g a.s./ha.

Mortality was not observed during the study.

Typical phytotoxic effects appeared as chlorosis, necrosis and wilting. The most sensitive species was *Brassica napus*. In this species, effects rated at > 25% phytotoxicity were observed at treatment rates of 61.44 g a.s./ha and above after 21 days. No other species showed this level of phytotoxicity below 153.6 g a.s./ha.

C. VALIDITY CRITERIA

The test was performed according to OECD 208 (updated proposal; draft July 2000), however validity has been assessed against the current vegetative vigour test guideline (OECD 227, 2006).

It was not possible to determine from the reported data whether the validity criterion for seedling emergence (> 70% required) was satisfied, however as sufficient plants were available for the application of the test item this omission is considered not to have affected the validity of the study.

Control plant survival was 100% and therefore satisfied the validity criterion of being ≥ 90%.

The control plants did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants only exhibited normal variation in growth and morphology for each particular species.

In addition, environmental conditions for each species were identical and the growing media contained the same amount of soil matrix, support media, or substrate from the same source.

Based on the above assessment, this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Species	Endpoint	
	NOEC (g a.s./ha)	EC_{50} (g a.s./ha)
Brassica napus	9.83	306.72
Cucumis sativus	153.6	1470.27
Beta vulgaris	61.44	>2400
Gossypium hirsutum	61.44	>2400
Daucus carota	960	>2400
Hordeum vulgare	>2400	>2400
Triticum aestivum	>2400	>2400

III. CONCLUSION

After post-emergence application of aclonifen as EXP04209E in the laboratory, the most sensitive plant species identified was *Brassica napus* with a 21-day EC_{50} based on freshweight of 306.72 g a.s./ha.

[redacted] *(2002)*

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

The most sensitive plant species identified was *Brassica napus* with a 21-day ER_{50} based on freshweight of 306.72 g a.s./ha.

Assessment and conclusion by RMS:

Data Point:	KCP 10.6.2/04
Report Author:	[redacted]
Report Year:	2002
Report Title:	Effects of EXP 04209E on terrestrial (non-target) plants: seedling emergence and seedling growth test
Report No:	C023847
Document No:	M-215787-01-1
Guideline(s) followed in study:	OECD: 208 (Draft Document July 2000)
Deviations from current test guideline:	Current Guideline: OECD 208, 2006 Minor deviations in light intensity with no impact on study. Current method guideline: SANCO/3029/99 rev.4 Yes, not all requirements for precision fulfilled
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of EXP04209E on seedling emergence in seven terrestrial non-target plant species representing six plant families.

The test was run over 21 days using five application rates applied as a soil spray per test species. Percentage of emergence and visual phytotoxicity ratings (e.g. chlorosis, necrosis, abnormal growth) were recorded on Days 7, 14 and 21. Mortality and growth (fresh weight) was determined on Day 21.

The most sensitive species were the dicotyledoneae *Brassica napus* and *Beta vulgaris* with an EC_{50} for fresh weight of 25.7 and 77.84 g a.s./ha, respectively..

Effects of EXP 04209E on germination rate were only observed in *Hordeum vulgare* in rates of 960 g a.s./ha and higher.

The most sensitive species for the parameter mortality were *Brassica napus* and *Beta vulgaris*, in which significant mortality occurred from 61.44 and 153.6 g a.s./ha. For *Cucumis sativus* significant mortality did not occur at rates lower than 960 g a.s./ha.

Typical phytotoxic effects appeared as stunting, chlorosis and necrosis. Most sensitive species were *Brassica napus* and *Beta vulgaris*. In these species, severe effects were observed at treatment rates of 61.44 and 153.6 g a.s./ha after 21 days.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item:	EXP 04209E
Batch no.:	OP 200622
Active Ingredient / Purity:	Aclonifen: 589g/L
Expiry date:	11 December 2002

Appearance: Luminous yellow liquid

Storage: In original container, at room temperature (+2 to +30 °C) in the dark

2. Test species: 5 dicotyledoneae and 2 monocotyledoneae species were chosen representing 6 plant families

	Family	Species	Common name
Dicotyledonae	Brassicaceae	*Brassica napus*	Oilseed Rape
Dicotyledonae	Curcubitaceae	*Cucumis sativus*	Radish
Dicotyledonae	Chenopodiaceae	*Beta vulgaris*	Soybean
Dicotyledonae	Malvaceae	*Gossypium hirsutum*	Soybean
Dicotyledonae	Umbelliferae	*Daucus carota*	Tomato
Monocotyledonae	Gramineae	*Hordeum vulgare*	Oat
Monocotyledonae	Gramineae	*Triticum aestivum*	Perennial Ryegrass

B. STUDY DESIGN AND METHODS

1. In-life phase: 12 March – 03 April 2002

2. Exposure conditions

Test vessels: Commercial plastic flower pots of diameter 16 cm and 9 x 9 cm

Soil: Lufa 2.3 (sandy loam). All particles under 0.2 cm, 1.32 ± 0.1% organic matter, pH 6.5 ± 0.1

Experimental design: Control, test item (5 applications within the range 24.58 g a.s./ha and 2700 g a.s./ha)

Replicates: 6 pots each containing 5 seeds per treatment group

Temperature: Day 23°C ± 4°C, night 18°C ± 4°C (intended)
Achieved: day 24°C (24-25 °C), night 19°C (18-19°C)
Mean day temperature (24 h) during the test period: 22 °C

Relative humidity: Day: approximately 70%, night: approximately 85% (intended)
Achieved: day 65% (50-90%), night 90% (70-100%)

Photoperiod: 16h light/8h dark

Light intensity: Minimum light intensity 5000 Lux (intended)
Light intensity was measured once a week and recorded in the raw data. Mean light intensity was 8745 Lux, with a maximum of 17470 Lux and minimum of 2990 Lux

Irrigation: Irrigation with tap water was done automatically with fibreglass-wicks connecting soil and water supply (bowl standing below each pot and containing maximum 500 mL water). Water supply was not permanent. Water was given (as described above) as soon as the soil started to dry out.

3. Administration of the test item

The test item was applied as a singular application according to agricultural practice with a laboratory-spraying equipment.

Species	Application Rates (g a.s./ha)	
	Minimum	Maximum
Brassica napus	24.58	960
Cucumis sativus	61.44	2400
Beta vulgaris	24.58	960
Gossypium hirsutum	61.44	2400
Daucus carota	61.44	2400
Hordeum vulgare	61.44	2400
Triticum aestivum	61.44	2400

The range included 5 increasing concentrations from the indicated minimum to the maximum

In total 30 seeds per species and treatment group were sown. The day before the application the seeds were introduced manually in the soil. After sowing the pots were placed on the watering system.

4. Measurements and observations

Duplicate samples from the freshly prepared and continuously stirred stock solution were taken before application for verification of test item concentrations.

The percentage of emerged seedlings and visual phytotoxicity ratings (e.g. chlorosis, necrosis, abnormal growth) was recorded on Days 7, 14 and 21 after application.

The fresh weight was determined on Day 21. The plants of one pot represented one replicate. The number of plants died after application were recorded on Day 21. Dead plants were weighed if it was practicable. Growth stages at Day 21 were also reported.

5. Statistics/Data evaluation

Fresh weight data were tested for normality by using Kolmogoroff-Smirmov-Test. Homogeneity was tested with Cochran-Test if data were not normally distributed. If the normal distribution was accepted Bartlett Test was used for all data with $n > 10$ and Cochran Test for data with $n < 10$. If the data were normally distributed and homogeneous Williams Test (monotonously increasing or decreasing) or Dunnett Test (not monotonously increasing or decreasing) were used for comparing treatment groups and control. If the data were not homogeneous Bonferroni U-Test was used.

In order to determine the EC_x values, a regression analysis (Probit-analysis) was performed. For the germination and mortality data Fischer Exact Test was used.

The significance level for all tests was $\alpha=0.05$. The decision on weight (one-sided, two-sided) was made dependent on the data. Computer program used to perform the statistical analyses was ToxRat® SPiRiT Solutions (1999-2001), Version 1.08 and SYSTAT Version 9.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

The analytically determined mean of aclonifen concentrations in the analysed stock solutions was 94.25%. The validated method is summarised in Document M-CP5 (CP 5.1.2/13).

B. BIOLOGICAL DATA

Table: Effects of EXP04209E on seedling emergence and growth

Species	Family	NOEC (g a.s./ha)	EC_{50} (g a.s./ha)
Brassica napus (Oilseed rape)	Brassicaceae	<24.58	25.70
Beta vulgaris (Sugar beet)	Chenopodiaceae	24.58	77.84
Cucumis sativus (Cucumber)	Curcubitaceae	61.44	163.67
Hordeum vulgare (Barley)	Gramineae	<61.44	370.43
Triticum aestivum (Wheat)	Gramineae	153.60	601.77
Daucus carota (Carrot)	Umbellifereae	960	3743.80
Gossypium hirsutum (Cotton)	Malvaceae	>2400	>2400

Fresh weight: The most sensitive species identified was *Brassica napus*, with the ED_{50} of 25.7 g a.s./ha.

The most sensitive species for the parameter mortality were *B. napus* at 61.44 g a.s./ha, followed by *B. vulgaris* at 153.6 g a.s./ha and *C. sativus* at 960 g a.s./ha.

Germination was affected by the test item only in *Hordeum vulgare* at the highest rates of 960 and 2400 g a.s./ha.

Phytotoxic effects were observed in form of stunting, chlorosis and necrosis at different dose levels in different species.

C. VALIDITY CRITERIA

The test was performed according to OECD 208 (updated proposal; draft July 2000), however validity has been assessed against the current test guideline (OECD 208, 2006).

Control seedling emergence ranged from 87 – 100% and therefore satisfied the validity criterion of being ≥ 70%.

Control plant survival was 100% and therefore satisfied the validity criterion of being ≥ 90%.

The control plants did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants only exhibited normal variation in growth and morphology for each particular species.

In addition, environmental conditions for each species were identical and the growing media contained the same amount of soil matrix, support media, or substrate from the same source.

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Species	Family	Endpoint	
		NOEC (g a.s./ha)	EC_{50} (g a.s./ha)
Brassica napus (Oilseed rape)	Brassicaceae	<24.58	25.70
Beta vulgaris (Sugar beet)	Chenopodiaceae	24.58	77.84
Cucumis sativus (Cucumber)	Curcubitaceae	<61.44	163.67
Hordeum vulgare (Barley)	Gramineae	<61.44	370.43
Triticum aestivum (Wheat)	Gramineae	153.60	601.77
Daucus carota (Carrot)	Umbelliferae	960	3743.80
Gossypium hirsutum (Cotton)	Malvaceae	>2400	>2400

III. CONCLUSION

After pre-emergence application of aclonifen as EXP04209E in the laboratory, the most sensitive plant species identified was *Brassica napus* (21-day EC_{50} based on fresh weight = 25.7 g a.s./ha).

[redacted] *(2002)*

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

The most sensitive plant species identified was *Brassica napus* with a 21-day ER_{50} based on freshweight of 25.7 g a.s./ha.

Assessment and conclusion by RMS:

Data Point:	KCP 10.6.2/05
Report Author:	[redacted]
Report Year:	2019
Report Title:	Effects on the vegetative vigor of ten species of non-target terrestrial plants (Tier 2) aclonifen SC 600 g/L
Report No:	VV18/043
Document No:	M-671392-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP 850.4150 (2012) OECD 227 (2006)
Deviations from current test guideline:	Current guideline: OECD 227 (2006) Minor deviations from climatic and growth conditions which were considered not to have had any negative impact on the outcome and integrity of the study
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was conducted to determine the effect of aclonifen SC 600 g/L on vegetative vigour in ten terrestrial non-target plant species representing eight plant families.

Planting density included 2 or 4 plants per pot with 10 or 5 replicate pots, respectively, for a total of 20 plants per treatment level. The plant species were treated at the 2-4 leaf stage with 5 to 7 test item rates and a water control.

A second run applying lower test item rates was performed for *Cucumis sativus* in order to allow for a robust statistical calculation of effect values.

Serial dilutions of aclonifen SC 600 g/L were sprayed onto the foliage of plants and above-ground portions of plants using a calibrated laboratory track sprayer at a volume rate of 200 L/ha.

Following application, the pots with plants were maintained under greenhouse conditions and natural daylight was supplemented by artificial lighting. Assessments were made 7, 14 and 21 days after application. On day 7 and 14, only plant survival and visual phytotoxicity were recorded. Final assessments (on day 21 after application) were made for plant survival, visual phytotoxicity, plant growth stage, and shoot dry weight.

Statistical analysis of the data were performed to obtain NOER (No Observed Effect Rate), LOER (Lowest Observed Effect Rate) and ER_{25}/ER_{50} (Effect Rate producing 25%/50% effect) for survival and shoot dry weight, using ToxRat statistical software.

All plant species in this study met the validity criteria of at least 70% emergence rate of the seeds sown, and 90% survival in the controls. In accordance with US EPA guideline (OCSPP 850.4150) and OECD guideline (OECD 227), there was no visible phytotoxicity, and normal growth occurred in the controls of the ten species tested.

The analysis of aclonifen content in the initial test item stock solution revealed measured concentrations of 96.0%, 94.6% and 93.9% of nominal.

Phytotoxic symptoms observed at the final assessment (on day 21 after application) in this vegetative

vigor study include chlorosis, necrosis, deformation and stunting of the plants. The severity and occurrence of phytotoxic symptoms differed among species and test item rates.

The most sensitive species was found to be *Cucumis sativus* with the lowest ER_{50} of 28.54 mL product/ha based on shoot dry weight. Based on survival, the most sensitive species was *Lactuca sativa* with an ER_{50} of 344.76 mL product/ha.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: Aclonifen SC 600 g/L
Batch no.: EV56007828
Active Ingredient / Purity: aclonifen (AE F068300): 50.3% w/w (607.1 g/L)
Expiry date: 29 November 2019
Appearance: Yellow liquid
Storage: +2°C to +30°C

2. Test species: 6 dicotyledoneae and 4 monocotyledoneae species were chosen representing 8 plant families

	Family	Species	Common name
Dicotyledonae	Chenopodiaceae	*Beta vulgaris*	Sugar beet
Dicotyledonae	Brassicaceae	*Brassica napus*	Oilseed rape winter
Dicotyledonae	Cucurbitaceae	*Cucumis sativus*	Cucumber
Dicotyledonae	Fabaceae	*Glycine max*	Soybean
Dicotyledonae	Asteraceae	*Lactuca sativa*	Butterhead lettuce
Dicotyledonae	Hugoniaceae Linaceae	*Linum usitatissimum*	Linseed
Monocotyledonae	Amaryllidaceae	*Allium cepa*	Onion
Monocotyledonae	Poaceae	*Avena sativa*	Oat
Monocotyledonae	Poaceae	*Lolium perenne*	Ryegrass
Monocotyledonae	Poaceae	*Zea mays*	Corn

B. STUDY DESIGN AND METHODS

1. In-life phase: 18 January 2018 – 18 July 2019

2. Exposure conditions

Test vessels: Commercial non-porous plastic flower pots (15 cm diameter), filled with approximately 1.2 L soil

Soil: A mixture of standard "Riedberg" soil + washed sand (ratio 9/1) from [redacted] Germany. The soil was sieved to 2 mm.

Composition and particle size (according to USDA): < 0.002 mm clay = 15.4%
0.002 – 0.050 mm silt = 49.8%

	0.050 – 2.000 mm sand = 34.8%
Organic carbon:	0.59% C
pH:	6.60 ($CaCl_2$)
Experimental design:	Control, test item (5 to 7 applications within the range 5 – 9006 mL product/L)
Replicates:	*Beta vulgaris*, *Brassica napus*, *Cucumis sativus*, *Glycine max*, *Lactuca sativa*, *Linum usitatissimum and Zea mays*: 10; each pot contained 2 plants. *Allium cepa*, *Avena sativa* and *Lolium perenne*: 5; each pot contained 4 plants. In total 20 plants per test group were tested.
Temperature:	19.01°C to 25.25°C during light and dark cycle
Relative humidity:	62.77 to 78.86% during light and dark cycle
Photoperiod:	16h light:8h dark
Light intensity:	Natural daylight supplemented by artificial lighting. Measured values were 201.1- 863.9 µmol/m²/sec.

3. Administration of the test item

Test item preparation and application

A test item stock solution, with an equivalent rate of 9006 L product/ha and a volume rate equivalent to 200 L/ha, was prepared by dissolving the test item in deionized water. The test item rates were prepared by dilution of the test item stock solution with deionized water. The amount of deionized water needed to prepare each test item rate was determined gravimetrically.

The test item was applied with a volume rate of 200 L/ha.

The blank control spray solution was 200 L/ha deionized water.

The sprayer was calibrated beforehand to deliver 200 L/ha ±10% by spraying glass plates of known weight and area and weighing them immediately afterwards to determine the actual amount of water applied.

Species	Application Rates (mL product/ha)	
	Minimum	Maximum
Beta vulgaris	61	1702
Brassica napus	5	140
Cucumis sativus 1st run	140	3916
Cucumis sativus 2nd run	5	740
Glycine max	61	1702
Lactuca sativa	12	322
Linum usitatissimum	61	1702
Allium cepa	322	9006
Avena sativa	61	1702
Lolium perenne	61	1702
Zea mays	322	9006

The range included 5 to 7 increasing concentrations from the indicated minimum to the maximum

Assignment of test organisms

At application, all species were in 2 to 4 leaf stage (BBCH 12 – 14).

After application, the pots of each plant species were transferred to the greenhouse and placed on the tables in a randomized design with all plants of one species arranged together in a species plot. During the course of the experimental study part, on day 7 after application, the pots of each plant species were rearranged within each species plot. Up to four days prior to the final assessment, the pots of each plant species were arranged according to their treatment level to facilitate the final assessment.

After application, bottom watering was performed with saucers standing below each pot throughout the study. Water was given and retained within the saucer according to the need of the plants to maintain an optimal water supply for plant growth. Watering was checked daily and documented in the raw data.

During the course of the study, liquid fertilizer (WUXAL Universaldünger) was added into the saucers during watering for all plant species.

4. Measurements and observations

Samples of the initial test item stock solution (9006 L product/ha) and of deionized water were taken directly before application, put in separate vessels and deep frozen until analysis.

The number of plants that survived was recorded per replicate for each test group on day 7, 14 and 21 after application.

Visual phytotoxicity ratings (e.g. chlorosis, necrosis, bleaching, deformation, reddening, stunting) were recorded per replicate for each test group on day 7, 14 and 21 after application. Phytotoxicity was assessed according to EPPO Standard 135.

Growth stages at the final assessment were recorded per replicate for each test group according to BBCH-Monograph - Growth stages

The shoot dry weight was determined at the final assessment. The dry weight was determined on a replicate basis, i.e. all plants of one pot – representing one replicate – were weighed together. Plants were cut directly at the soil surface and put in pre-weighed bags. The bags containing the plants were dried at 60°C until constant weight and reweighed – considering tare weight – to obtain the shoot dry weight of each replicate.

For the statistical analysis and reporting, average plant weight was determined from each replicate by dividing measured weight by the number of plants per pot.

5. Statistics/Data evaluation

Survival:	The number of surviving plants after application in comparison to the untreated control at the end of the assessment period. The calculated mean value is the arithmetic mean. The inhibition of survival was calculated compared to the control group.
Phytotoxicity:	The individual phytotoxicity ratings for each replicate were expressed in summary tables.
Growth stage:	The minimum and maximum BBCH for the test item rates and controls at the end of the assessment period were expressed in summary tables.
Shoot dry weight:	The mean shoot dry weight for each replicate was compared to those of the untreated controls at the end of the assessment period.

Statistical analysis: Survival and shoot dry weight was compared to the untreated control using the ToxRat software for statistical analysis (ToxRatPro version 3.3.0).

Effect levels: ER_{25}/ER_{50} (Effect Rate) for survival and shoot dry weight with the 95 percent confidence limits as well as the LOER (Lowest Observed Effect Rate) and NOER (No Observed Effect Rate) are given if calculation by ToxRat was possible.

If the NOER was calculated as greater than the highest rate tested, it was reported as the highest rate tested (without > or >=) except in the ToxRat calculations.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Mean recovery of the active ingredient aclonifen in the stock solutions for preparation of the spray dilutions was 93.9 – 96.0% of the nominal concentration. The validated method is summarised in Document M-CP5 (CP 5.1.2/17).

B. BIOLOGICAL DATA

Table: Effects of aclonifen SC 600 g/L on survival

Survival								
Plant species	ER_{25} (mL product/ ha)	95% Confidence limits		ER_{50} (mL product /ha)	95% Confidence limits		LOER (mL product /ha)	NOER (mL product /ha)
		lower	upper		lower	upper		
Beta vulgaris	>1702 [d]	n.d.	n.d.	>1702 [d]	n.d.	n.d.	>1702	1702
Brassica napus	>140 [d]	n.d.	n.d.	>140 [d]	n.d.	n.d.	>140	140
Cucumis sativus 1st run	>3916 [a]	n.r.	n.d	>3916 [a]	n.r	n.d.	3916	1702
Cucumis sativus 2nd run	>740	n.d	n.d	>740 [b]	n.d	n.d	740	322
Glycine max	>1702 [d]	n.d.	n.d.	>1702 [d]	n.d.	n.d.	>1702	1702
Lactuca sativa	305.91	n.d.	n.d.	>322 [a]	n.d.	n.d.	322	140
Linum usitatissimum	>1702 [d]	n.d.	n.d.	>1702 [d]	n.d.	n.d.	>1702	1702
Allium cepa	>9006 [b]	n.d.	n.d.	>9006 [b]	n.d.	n.d.	>9006	9006
Avena sativa	>1702 [d]	n.d.	n.d.	>1702 [d]	n.d.	n.d.	>1702	1702
Lolium perenne	280.91	195.45	360.72	438.18	339.17	567.35	140	61
Zea mays	>9006 [d]	n.d.	n.d.	>9006 [d]	n.d.	n.d.	>9006	9006

n.d.: Confidence limits not determined (outside the range tested)
n.r.: Confidence limits not reported
[a]: Calculated values were outside the range tested.
[b]: Not calculated (outside the range tested).
[d]: Not calculated (no effects were observed up to the highest rate tested).

Table: Effects of aclonifen SC 600 g/L on shoot dry weight

Survival								
Plant species	ER_{25} (mL product/	95% Confidence limits		ER_{50} (mL product	95% Confidence limits		LOER (mL product	NOER (mL product
		lower	upper		lower	upper		

	ha)			/ha)			/ha)	/ha)
Beta vulgaris	119.95	n.r.	240.35	1274.56	657.48	n.r.	≤61	<61
Brassica napus	58.73	36.70	78.16	146.87 [e]	108.10	n.r.	61	26
Cucumis sativus 1st run	<140 [a]	n.d.	n.d.	<140 [b]	n.d.	n.r.	≤140	<140
Cucumis sativus 2nd run	<5[a]	n.r.	n.r.	28.54	19.50	39.79	≤5	<5
Glycine max	189.25	133.92	246.76	>1702 [a]	n.r.	n.r.	≤61	<61
Lactuca sativa	18.59	n.r.	27.48	78.72	58.44	108.55	≤12	<12
Linum usitatissimum	261.86	168.64	348.96	655.62	511.15 [c]	864.42	740	322
Allium cepa	>9006 [a]	n.d.	n.d	>9006[b]	n.d.	n.d.	>9006	9006
Avena sativa	1019.27 [c]	689.33[c]	1751.75[c] [e]	>1702 [cb]	n.d. [c]	n.d.[c]	322	140
Lolium perenne	67.64	n.d.	157.70	200.96	n.r.	n.r.	140	61
Zea mays	>9006 [b]	n.d.	n.d	>9006 [b]	n.d.	n.d.	>9006	3916

n.d.: Confidence limits not determined (outside the range tested)
n.r.: Confidence limits not reported
[a]: Calculated values were outside the range tested.
[b]: Not calculated (outside the range tested).
[c]: Probit analysis: Replicates used while fitting
[e]: Extrapolated value (less then 10% out of the range tested)

C. VALIDITY CRITERIA

Seedling emergence was ≥ 82% and therefore satisfied the validity criterion of being ≥ 70%.

Control plant survival was 100% and therefore satisfied the validity criterion of being ≥ 90%.

The control plants did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants only exhibited normal variation in growth and morphology for each particular species.

In addition, environmental conditions for each species were identical and the growing media contained the same amount of soil matrix, support media, or substrate from the same source.

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Plant species	Survival		Shoot dry weight	
	NOER (mL product/ha)	ER_{50} (mL product/ha)	NOER (mL product/ha)	ER_{50} (mL product/ha)
Beta vulgaris	1702	>1702	<61	1274.56
Brassica napus	140	>140	26	146.87
Cucumis sativus 1st run	1702	>3916	<140	<140
Cucumis sativus 2nd run	322	>740	<5	28.54
Glycine max	1702	>1702	<61	>1702
Lactuca sativa	140	>322	<12	78.72
Linum usitatissimum	1702	>1702	322	655.62
Allium cepa	9006	>9006	9006	>9006
Avena sativa	1702	>1702	140	>1702

Lolium perenne	61	438.18	61	200.96
Zea mays	9006	>9006	3916	>9006

III. CONCLUSION

In a vegetative vigor and growth study, aclonifen SC 600 g/L was tested under greenhouse conditions for effects on the survival, growth and shoot dry weight of ten non-target terrestrial plant species following a post-emergence application of the test item onto the foliage of plants at the 2-4 leaf stage. The most sensitive species was found to be *Cucumis sativus* with the lowest ER_{50} of 28.54 mL product/ha based on shoot dry weight. Based on survival, the most sensitive species was *Lactuca sativa* with an ER_{50} of 344.76 mL product/ha.

[redacted] *(2019)*

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

The most sensitive species was found to be *Cucumis sativus* with the lowest ER_{50} of 28.54 mL product/ha based on shoot dry weight. Based on survival, the most sensitive species was *Lactuca sativa* with an ER_{50} of 344.76 mL product/ha.

To enable comparison with other non-target plant studies performed on the product, results were also calculated in terms of g a.s./ha based on an active substance concentration of 607.1 g a.s./L in the formulated product.

Plant species	Survival		Shoot dry weight	
	NOER (g a.s./ha)	ER_{50} (g a.s./ha)	NOER (g a.s./ha)	ER_{50} (g a.s./ha)
Beta vulgaris	1033	>1033	<37.03	773.79
Brassica napus	84.99	>84.99	15.78	89.16
Cucumis sativus 1st run	1033	>2377	<84.99	<84.99
Cucumis sativus 2nd run	195.49	>449.25	<3.04	17.33
Glycine max	1033	>1033	<37.03	>1033
Lactuca sativa	84.99	>195.49	<7.29	47.79
Linum usitatissimum	1033	>1033	195.49	398.03
Allium cepa	5468	>5468	5468	>5468
Avena sativa	1033	>1033	84.99	>1033
Lolium perenne	37.03	266.02	37.03	122
Zea mays	5468	>5468	2377	>5468

Assessment and conclusion by RMS:

CP 10.6.3 Extended laboratory studies on non-target plants

No extended laboratory studies on the formulated product have been performed.

CP 10.6.4 Semi-field and field tests on non-target plants

Data Point:	KCP 10.6.4/01
Report Author:	[redacted]; [redacted]
Report Year:	2003
Report Title:	Effects of AE F068300 00 SC50 A2 (EXP04209E) on terrestrial (non-target) plants Higher tier test with Lactuca sativa
Report No:	C040615
Document No:	M-229238-01-1
Guideline(s) followed in study:	OECD: 208 (Draft, July 2000)
Deviations from current test guideline:	OECD 208 + 227, 2006 No deviation Current method guideline: SANCO/3029/99 rev.4 Yes, not all requirements for precision fulfilled
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

This higher tier Non-Target plant study was performed as a consequence of tier 2 studies. It was to generate phytotoxicity data deriving from a multiple rate level of AE F068300 00 SC50 A2 (aclonifen 600 g/L). The test design was close to OECD 208 (draft July 2000) with the difference of the climate conditions and the duration of exposure.

The plants were grown in pots on the field under relevant weather conditions but protected from hale. *Lactuca sativa* was tested on seedling emergence and vegetative vigour.

For the seedling emergence design fresh weight was taken 28 and 49 days after application. For the vegetative vigour test design it was taken on Days 21 and 33. Parameters measured were phytotoxicity, plant fresh weight, mortality and germination rate.

The lowest EC_{50} based on fresh weight was for *Lactuca sativa* in the seedling emergence design 28 days after application followed by *Lactuca sativa*, seedling emergence design 49 days after application (74 and 199 g a.s./ha, respectively). The vegetative vigour design were less sensitive with ER_{50} values of 237 and 740 g a.s./ha (for the first and the second harvest). Both test designs showed a recovery when cultivated for some more weeks.

The germination rates of the seedling emergence designs were variable, germination occurred scattered, mainly during the first two weeks but continuing over the whole test period. The mortality results were treatment related and not higher than 64%. Control mortality was 2% and probably due to unfavourable germination conditions.

The main symptoms of phytotoxicity in the seedling emergence design were mortality and growth reduction. In the vegetative vigour design the main symptom was growth reduction.

The EC_{50} value based on fresh weight for *Lactuca sativa* was in both test designs higher than in the previous laboratory studies.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	AE F068300 00 SC50 A2 (EXP04209E)
	Trade name:	Bandur SC 600
	Batch no.:	OP 220331
	Active Ingredient / Purity:	Aclonifen: 591 g/L (analysed)
	Expiry date:	17 January 2005
	Appearance:	Luminous yellow liquid
	Storage:	In original container, at room temperature (+2 to +30 °C) in the dark
2.	**Test species:**	*Lactuca sativa* (Lettuce) *Lactuca sativa* was set up in two complete test sets because it was planned to evaluate recovery effects. One test set was to harvest 21 days after application (or about 21 days after 50% germination in the control). The other test set was to harvest 42 days after application (or about 42 days after 50% germination in the control but not before control plants had reached at least the growth stage of the previous studies).

B. STUDY DESIGN AND METHODS

1. In-life phase: 15 July – 03 September 2003

2. Exposure conditions

Test vessels:	Commercial plastic pots with a volume of 3 L were used
Soil:	Soil Type: Silty Loam; according to DIN 19682 (sand 14.2%, silt 65.1%, clay 20.7%) Organic Carbon: 1.19% pH7.4
Experimental design:	Control, test item (5 applications within the range 20 g a.s./ha and 320 g a.s./ha)
Replicates:	Vegetative vigour: 14 pots each containing 3 plants per treatment group Seedling emergence: 10 pots each containing 5 plants per treatment group
Test Environment:	The test was performed under relevant weather conditions on the field which was prepared with a woven tissue on the ground and a hale protection system. The woven tissue allowed the rain to drain through the fabric into the ground but prevented plant

	(weed) growth. The field was localized in [redacted], Germany.
Temperature:	4.9 – 40.5 ºC
Relative humidity:	57.3 – 64.6% (mean)
Precipitation:	25.2 – 50.0 (sum)
Irrigation:	Bottom watering (saucers) was done where necessary after a daily spot check. It was intended to assure optimal water supply.
Fertilizer:	Liquid fertilizer was given one to three times a week
Crop protection measures:	Crop protection was necessary especially because of the climatic conditions which were suitable mainly for the development of insects

3. Administration of the test item

Dose preparation and application

The test item was applied using a plot application system for field trials (PSG-System 2, with a spray boom of 2.5 m including 5 spraying nozzles ([redacted])). An area of 25 m2 (2.5 x 10 m) was prepared for the application on a field next to the test site. For the application the pots were placed on clean saucers with wide intervals in the centre of this area. Then the pots were sprayed with the test item and carried back to the test site.

Sowing details

In order to do the application at the same day for both test designs, seedling emergence and vegetative vigour, *Lactuca sativa* (vegetative vigour) was sown about 4 weeks before the application. Due to the test conditions one could foresee that the germination rate might not be sufficiently high enough. Therefore the pots were over sown and the additional plants were removed before sowing. When the plants had reached the 3 to 4 true leaf stage (BBCH 13-14) the application date was fixed and the sowing for the Seedling Emergence Test was done.

The sowing of the plants for the seedling emergence test was done the day before application. Germination rate was not sure to be high enough for a representative number of plants per pot because of the test conditions. Therefore pots were oversown with 40 seeds (intended to have 5 plants per pot). Germinating seeds were counted. The first 5 seedlings were marked with stickers and allowed to grow, all other seeds were removed after counting. Germinated and removed plants were recorded.

Table: Technical data for introduction of seeds

Test Design	Date of sowing	No of seeds per pot	No of plants per pot	No of pots per treatment group
Vegetative vigour	17/06/03	>40	3	14
Seedling emergence	15/07/03	40	5	10

4. Measurements and observations

Duplicate samples from the stock solution were taken before application for verification of test item concentrations.

Seven days after application the germination in the seedling emergence design had achieved 39 and 19% germination rate in the control. 14 days after application the germination in the seedling emergence design had achieved 99 and 67% germination rate in the control. Therefore it was decided to harvest 28 and 49 days after application.

The vegetative vigour was harvested 21 and 33 days after application.

Visual phytotoxicity ratings (e.g. chlorosis, necrosis, abnormal growth) were done once a week based on EPPO Standard 135.
Germinated plants were determined once a week until the end of the test because *Lactuca sativa* germinated very scattered.

Fresh weight was determined at each harvest date, the plants of one pot represented one replicate. The number of dead plants was recorded at times of harvest. Dead plants were weighed if it was practicable. Plants which were decomposed during duration of exposure were calculated as the difference between emerged plants (seedling emergence) and plants found at the end of the test.

Growth stages were recorded at times of harvest according to BBCH-Monograph - Growth stages.

5. Statistics/Data evaluation
Fresh weight data were tested for normality by using Kolmogoroff-Smirmov-Test. Homogeneity was tested with Cochran-Test if data were not normally distributed. If the normal distribution was accepted Bartlett Test was used for all data with n > 10 and Cochran Test for data with n < 10. If the data were normally distributed and homogeneous Williams Test (monotonously increasing or decreasing) or Dunnett Test (not monotonously increasing or decreasing) were used for comparing treatment groups and control. If the data were not homogeneous Bonferroni U-Test was used.

In order to determine the EC_x values, a regression analysis (Probit-analysis) was performed. For the mortality data Fischer Exact Test was used.

The significance level for all tests was α=0.05. The decision on weight (one-sided, two-sided) was made dependent on the data. Computer program used to perform the statistical analyses was ToxRat® SPiRiT Solutions (1999-2001), Version 1.08 and SYSTAT Version 9.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

The analytically determined mean of aclonifen concentrations in the analysed stock solutions was 96%. The validated method is summarised in Document M-CP5 (CP 5.1.2/14).

B. BIOLOGICAL DATA

The extreme high air temperatures of the 2003 summer necessitated extensive watering of the pots. Especially during the period of germination it was necessary to give a lot of water in the saucer in order

to guarantee a moist soil surface during the whole germination process. A considerable side effect was the over saturation of the lower parts of the soil with water, which led to an oxygen lack in the growing zone of the roots. This affected the healthy development of the smaller seedlings. Once the first true leaves appeared the seedling growth was good.

The watering system for this test was chosen following the guideline. However, for such a study design, especially under these climatic conditions, a top watering system would have been more realistic and more suitable for germination and seedling growth.

Table: Summary of effects of EXP04209E based on fresh weight

Species	Test design	NOEC (g as/ha)	EC_{50} (g as/ha)
Lactuca sativa (Lettuce)	Vegetative vigour 21 DAA	<20	237.01
	Vegetative vigour 33 DAA	40	740.89
	Seedling emergence 28 DAA	20	74.04
	Seedling emergence 49 DAA	80	198.91

DAA: Days after application

The lowest EC_{50} based on fresh weight was for *Lactuca sativa* in the seedling emergence design 28 days after application followed by *Lactuca sativa*, seedling emergence design 49 days after application (74 and 199 g a.s./ha, respectively). The vegetative vigour design were less sensitive with ER_{50} values of 237 and 740 g a.s./ha (for the first and the second harvest). Both test designs showed a recovery when cultivated for some more weeks.

The main symptoms of phytotoxicity in the seedling emergence design were mortality and growth reduction. In the vegetative vigour design the main symptom was growth reduction.

The EC_{50} value based on fresh weight for *Lactuca sativa* was in both test designs higher than in the previous laboratory studies.

C. VALIDITY CRITERIA

The test was performed according to OECD 208 (updated proposal; draft July 2000), however validity has been assessed against the current test guideline (OECD 208 and 227, 2006).

Control seedling emergence ranged from 77 – 88% and therefore satisfied the validity criterion of being ≥ 70%.

The maximum control mortality was 2% and therefore and therefore satisfied the validity criterion of being ≥ 90% survival.

The control plants did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants only exhibited normal variation in growth and morphology for each particular species.

In addition, environmental conditions for each species were identical and the growing media contained the same amount of soil matrix, support media, or substrate from the same source.

All validity criteria were satisfied and therefore this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Species	Test design	Endpoint	
		NOEC (g as/ha)	EC_{50} (g as/ha)
Lactuca sativa (Lettuce)	Vegetative vigour 21 DAA	<20	237.01
	Vegetative vigour 33 DAA	40	740.89
	Seedling emergence 28 DAA	20	74.04
	Seedling emergence 49 DAA	80	198.91

DAA: Days after application

III. CONCLUSION

The EC_{50} value based on fresh weight for *Lactuca sativa* was in both test designs higher than in the previous laboratory studies (pre-emergence application EC_{50} = 74.04 g a.s./ha; post-emergence application EC_{50} = 237.01 g a.s./ha). Furthermore the potential to recover was shown in both test designs.

[redacted] *(2003)*

Assessment and conclusion by applicant:

All validity criteria were satisfied and therefore this study can be considered to be valid.

The ER_{50} value based on fresh weight for *Lactuca sativa* was in both test designs higher than in the previous laboratory studies (pre-emergence application ER_{50} = 74.04 g a.s./ha; post-emergence application ER_{50} = 237.01 g a.s./ha). Furthermore, the potential to recover was shown in both test designs.

Assessment and conclusion by RMS:

Data Point:	KCP 10.6.4/02
Report Author:	[redacted]; [redacted]
Report Year:	2004
Report Title:	Effects of AE F068300 00 SC50 A2 (EXP04209E) on terrestrial (non-target) plants Higher tier test with Brassica napus and Lolium perenne
Report No:	C040617
Document No:	M-229242-01-1
Guideline(s) followed in study:	OECD: 208 (Draft, July 2000)
Deviations from current test guideline:	Current Guideline: OECD 208 + 227, 2006 No deviation Current method guideline: SANCO/3029/99 rev.4 Yes, not all requirements for precision fulfilled
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

This higher tier Non-Target plant study was performed as a consequence of tier 2 studies. It was to generate phytotoxicity data deriving from a multiple rate level of AE F068300 00 SC50 A2 (aclonifen 600 g/L). The test design was close to OECD 208 (draft July 2000) with the difference of the climate conditions and the duration of exposure.

The plants were grown in pots on the field under relevant weather conditions but protected from hale. *Brassica napus* was tested on seedling emergence and vegetative vigour, *Lolium perenne* was tested on seedling emergence. Fresh weight was taken 21 days after application. For *Lolium perenne* it was taken 42 days after application, additionally. Parameters measured were phytotoxicity, plant fresh weight, mortality and germination rate.

The lowest EC_{50} based on fresh weight was for *Brassica napus* in the seedling emergence design 21 days after application (157.19 g a.s./ha). All other EC_{50} values were calculated to be higher than the highest rate tested in this study.

The germination rates of all seedling emergence designs (34 to 77%) were variable and not necessarily treatment related. The mortality results showed a high variability possibly due to the test conditions.

The main symptoms of phytotoxicity in the seedling emergence design were mortality and growth reduction, in the vegetative vigour design symptoms were chlorosis, necrosis and growth reduction.

The EC_{50} value based on fresh weight for *Brassica napus* was in both test designs higher than in the previous laboratory studies (pre-emergence application EC_{50} = 157.19 g a.s./ha; post-emergence

application EC_{50} = 6650.96 g a.s./ha). The same could be shown for *Lolium perenne* (pre-emergence application EC_{50} = 1676.11 g a.s./ha), and the EC_{50} value of the second harvest is clearly higher.

I. MATERIALS AND METHODS

A. MATERIALS

1.	**Test Item:**	AE F068300 00 SC50 A2 (EXP04209E)
	Trade name:	Bandur SC 600
	Batch no.:	OP 220331
	Active Ingredient / Purity:	Aclonifen: 591 g/L (analysed)
	Expiry date:	17 January 2005
	Appearance:	Luminous yellow liquid
	Storage:	In original container, at room temperature (+2 to +30 °C) in the dark
2.	**Test species:**	*Brassica napus* was tested on vegetative vigour and seedling emergence (21 days of exposure). *Lolium perenne* was tested on seedling emergence and set up in two complete test sets because it was planned to evaluate recovery effects. One test set was to harvest 21 days after application (or about 21 days after 50% of the control war germinated) and the other one to harvest 42 days after application (but not before control plants had reached at least the growth stage of the previous studies).

B. STUDY DESIGN AND METHODS

1. In-life phase:	05 June – 18 July 2003
2. Exposure conditions	
Test vessels:	Commercial plastic pots with a volume of 3 L were used
Soil:	Soil Type: Silty Loam, according to DIN 19682 (sand 14.2%, silt 65.1%, clay 20.7%) Organic Carbon 1.19% pH7.4
Experimental design:	Control, test item (5 applications within the range 20 g a.s./ha and 1280 g a.s./ha)
Replicates:	Vegetative vigour: 14 pots each containing 3 plants per treatment group Seedling emergence: 8 pots each containing 5 plants per treatment group
Test Environment:	The test was performed under relevant weather conditions on the field which was prepared with a woven tissue on the ground and a hale protection system. The woven tissue allowed the rain to drain through the fabric into the ground but prevented plant

	(weed) growth. The field was localized in [redacted], Germany.
Temperature:	2 – 36 °C
Relative humidity:	48.2 – 97.1% (mean)
Precipitation:	17.2 – 78 mm (sum)
Irrigation:	Bottom watering (saucers) was done where necessary after a daily spot check. It was intended to assure optimal water supply.
Fertilizer:	Liquid fertilizer was given one to three times a week
Crop protection measures:	Crop protection was necessary especially because of the climatic conditions which were suitable mainly for the development of insects

3. Administration of the test item

Dose preparation and application

The test item was applied using a plot application system for field trials (PSG-System 2, with a spray boom of 2.5 m including 5 spraying nozzles ([redacted])). An area of 25 m2 (2.5 x 10 m) was prepared for the application on a field next to the test site. For the application the pots were placed on clean saucers with wide intervals in the centre of this area. Then the pots were sprayed with the test item and carried back to the test site.

Sowing details

In order to do the application at the same day for both test designs, seedling emergence and vegetative vigour, *Brassica napus* (vegetative vigour) was sown about 3 weeks before the application. When the plants had reached the 3 to 4 true leaf stage (BBCH 13-14) The application date was fixed and the sowing for the Seedling Emergence Test was done. In order to have the aimed number of plants per pot more seeds were sown and the additional plants were removed before application.

The sowing of the plants for the seedling emergence test was done the day before application. Germination rate was not sure to be high enough for a representative number of plants per pot because of the test conditions. Therefore pots were oversown with 20 or 25 seeds (intended to have 5 plants per pot). Germinating seeds were counted. The first 5 seedlings were marked with stickers and allowed to grow, all other seeds were removed after counting. Germinated and removed plants were recorded.

Table: Technical data for introduction of seeds

Species	Test Design	Date of sowing	No of seeds per pot	No of plants per pot	No of pots per treatment group
Brassica napus	Vegetative vigour	15/05/03	>10	3	14
Brassica napus	Seedling emergence	05/06/03	20	5	8
Lolium perenne	Seedling emergence	05/06/03	25	5	8

4. Measurements and observations

Duplicate samples from the stock solution were taken before application for verification of test item concentrations.

Seven days after application the Seedling Emergence Test achieved 62% germination rate. Thus, *Brassica napus* the Seedling Emergence and Vegetative Vigour Design were harvested 21 days after application. The 2nd harvest of *Brassica napus* for both test designs (vegetative vigour and seedling emergence) had to be cancelled due to a high infestation of maggots which lead to a total loss of these plants.

Lolium perenne in the seedling Emergence design was harvested 21 and 42 days after treatment. The first harvest was dated on 21 days after application because germination rate in the control group of both test sets exceeded 50% on day 7 after application (74% for the first and 62% for the second test set). The second harvest was dated 42 days after treatment because plants of the control group had a higher growth stage (BBCH 29) than in the previous study.

Visual phytotoxicity ratings (e.g. chlorosis, necrosis, abnormal growth) were done once a week based on EPPO Standard 135.

The first harvest (fresh weight) for all species and test designs was done 21 days after the application. The plants of one pot represented one replicate. For *Lolium perenne* a second harvest was performed 42 days after the application, additionally. The 2nd harvest of *Brassica napus* for both test designs (vegetative vigour and seedling emergence) had to be cancelled due to a sudden and unexpected high infestation with maggots which lead to a total loss of these plants.

The number of dead plants was recorded at times of harvest. Dead plants were weighed if it was practicable. Plants which were decomposed during duration of exposure were calculated as the difference between emerged plants (seedling emergence) and plants found at the end of the test.

Germinated plants were determined once a week and up to 3 or 4 weeks after sowing (dependent on the test design). After this period germination was not expected any more.

Growth stages were recorded at times of harvest according to BBCH-Monograph - Growth stages.

5. Statistics/Data evaluation

Fresh weight data were tested for normality by using Kolmogoroff-Smirmov-Test. Homogeneity was tested with Cochran-Test if data were not normally distributed. If the normal distribution was accepted Bartlett Test was used for all data with n > 10 and Cochran Test for data with n < 10. If the data were normally distributed and homogeneous Williams Test (monotonously increasing or decreasing) or Dunnett Test (not monotonously increasing or decreasing) were used for comparing treatment groups and control. If the data were not homogeneous Bonferroni U-Test was used.

In order to determine the EC_x values, a regression analysis (Probit-analysis) was performed. For the mortality data Fischer Exact Test was used.

The significance level for all tests was α=0.05. The decision on weight (one-sided, two-sided) was made dependent on the data. Computer program used to perform the statistical analyses was ToxRat® SPiRiT Solutions (1999-2001), Version 1.08 and SYSTAT Version 9.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

The analytically determined mean of aclonifen concentrations in the analysed stock solutions was 94%. The validated method is summarised in Document M-CP5 (CP 5.1.2/15).

B. BIOLOGICAL DATA

The extreme high air temperatures of the 2003 summer necessitated extensive watering of the pots. Especially during the period of germination it was necessary to give a lot of water in the saucer in order to guarantee a moist soil surface during the whole germination process. A considerable side effect was the over saturation of the lower parts of the soil with water, which led to an oxygen lack in the growing zone of the roots. This affected the healthy development of the smaller seedlings. For *Brassica napus* this led to seedling mortality (control mortality 5%). It is likely that in general the constitution of the seedlings (also *Lolium perenne*) was not the best at the start. Once the first true leaves appeared the seedling growth was good.

The watering system for this test was chosen following the guideline. However, for such a study design, especially under these climatic conditions, a top watering system would have been more realistic and more suitable for germination and seedling growth.

Table: Summary of effects of EXP04209E based on fresh weight

Species	Test design	NOEC (g as/ha)	EC_{50} (g as/ha)
Brassica napus (Oilseed rape)	Seedling emergence 21 DAA	80	157.19
	Vegetative vigour 21 DAA	160	6650.96
Lolium perenne (Perennial ryegrass)	Seedling emergence 21DAA	80	1676.11
	Seedling emergence 42 DAA	<20	56701.11

DAA: Days after application

The lowest EC_{50} based on fresh weight was for *Brassica napus* in the seedling emergence design 21 days after application (157.19 g a.s./ha). All other EC_{50} values were calculated to be higher than the highest rate tested in this study.

The germination rates of all seedling emergence designs (34 to 77%) were variable and not necessarily treatment related. The mortality results showed a high variability possibly due to the test conditions.

The main symptoms of phytotoxicity in the seedling emergence design were mortality and growth reduction, in the vegetative vigour design symptoms were chlorosis, necrosis and growth reduction.

C. VALIDITY CRITERIA

The test was performed according to OECD 208 (updated proposal; draft July 2000), however validity has been assessed against the current test guideline (OECD 208 and 227, 2006).

Control seedling emergence ranged from 70 – 77% and therefore satisfied the validity criterion of being ≥ 70% with the exception of the *Lolium perenne* Seedling Emergence Design - Second Harvest where germination in the control was 67%. This slightly lower germination rate was considered to be due to the test conditions (over saturation of the soil with water) and as all plants were grown under the same conditions this was considered not to affect the validity of the study.

The maximum control mortality was 5% and therefore and therefore satisfied the validity criterion of being ≥ 90% survival.

The control plants did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants only exhibited normal variation in growth and morphology for each particular species.

In addition, environmental conditions for each species were identical and the growing media contained the same amount of soil matrix, support media, or substrate from the same source.

Based on the above assessment, this study can be considered to be valid.

D. TOXICITY ENDPOINTS

Table: Summary of endpoints

Species	Test design	Endpoint	
		NOEC (g as/ha)	EC_{50} (g as/ha)
Brassica napus (Oilseed rape)	Seedling emergence 21 DAA	80	157.19
	Vegetative vigour 21 DAA	160	6650.96
Lolium perenne (Perennial ryegrass)	Seedling emergence 21DAA	80	1676.11
	Seedling emergence 42 DAA	<20	56701.11

DAA: Days after application

III. CONCLUSION

The EC_{50} value based on fresh weight for *Brassica napus* was in both test designs higher than in the previous laboratory studies (pre-emergence application EC_{50} = 157.19 g a.s./ha; post-emergence application EC_{50} = 6650.96 g a.s./ha). The same could be shown for *Lolium perenne* (pre-emergence application EC_{50} = 1676.11 g a.s./ha), and the EC_{50} value of the second harvest is clearly higher.

[redacted] *(2004)*

Assessment and conclusion by applicant:

All validity criteria were considered to have been satisfied and therefore this study can be considered to be valid.

The ER_{50} value based on fresh weight for *Brassica napus* was in both test designs higher than in the previous laboratory studies (pre-emergence application ER_{50} = 157.19 g a.s./ha; post-emergence application ER_{50} = 6650.96 g a.s./ha). The same could be shown for *Lolium perenne* (pre-emergence application ER_{50} = 1676.11 g a.s./ha), and the ER_{50} value of the second harvest is clearly higher.

Assessment and conclusion by RMS:

CP 10.7 Effects on other terrestrial organisms (flora and fauna)

No further testing on, or assessment of risk to, other organisms is considered necessary.

The data provided on birds, mammals, aquatic organisms, non-target arthropods, soil dwelling organisms, soil micro-organisms and non-target plants are considered adequate to assess the possible impact of tetraconazole on non-target flora and fauna.

CP 10.8 Effects on biological methods for sewage treatment

A summary of the endpoints related to the effects on biological methods for sewage treatment is provided in the following table. Details and a full description of the studies performed on the active substance, aclonifen, used in this risk assessment can be found in Document M-CA 8 of this dossier.

Table 10.8-1: Summary of data on the effects of aclonifen and Aclonifen SC 600 G on biological methods for sewage treatment

Test item	Test species	Time scale	Endpoint	Reference
Aclonifen SC 600 G	*Pseudomonas putida*	16 hours	EC_{10} = 0.04 mg a.s./L EC_{50} = 0.54 mg a.s./L	KCA 8.8/01 & KCP 10.8/01 M-175842-02-1 [redacted], S.D., 1993
Aclonifen	Activated sewage sludge micro-organisms	3 hours	EC_{50} > 1000 mg a.s./L	KCA 8.8/02 M-177356-01-1 [redacted], 1999
Aclonifen	Activated sewage sludge micro-organisms	3 hours	**EC_{50} > 100 mg a.s./L[1]**	KCA 8.8/03 M-664091-01-1 [redacted], 2019

When more than one endpoint is available for a substance for the same study type, the endpoint in **bold** is the one used in the risk assessment

[1]: This study was used in the risk assessment as it was performed according to current guideline requirements (OECD 209, 2010)

Risk assessment for biological methods for sewage treatment

The risk to biological methods for sewage treatment has been assessed.

The most recent study performed ([redacted], 2019, KCA 8.8/03) was conducted according to the latest update to an internationally accepted test design and hence it is considered to be the most appropriate study for the risk assessment.

Based on the maximum predicted surface water PEC (28.7 µg aclonifen/L, FOCUS Step 1) given in Document M-CP 9, Section CP 9.2.5, the effects reported in the Spoo-Klöppel study indicate that adverse effects on biological sewage treatment plants are not to be expected.

Studies on the effect of the formulation Aclonifen SC 600 G on biological methods for sewage treatment have been conducted and presented below.

Data Point:	KCP 10.8/01
Report Author:	[redacted]
Report Year:	1994
Report Title:	Bandur EXP04209 - Acute toxicity in bacteria (Pseudomonas putida).
Report No:	R007904
Document No:	M-175842-02-1
Guideline(s) followed in study:	DIN: 38/412
Deviations from current test guideline:	Current Guideline: DIN 38412-16, 1985 None
Previous evaluation:	yes, evaluated and accepted Source: Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A study was performed to determine the acute toxicity of the test item, Bandur EXP04209, in bacteria (Pseudomonas putida). The method used was designed to meet the requirements of the German Water Hazard Classification ("Bewertung wassergefahrdender Stoffe" - Herausgegeben vom Umweltbundesamt, September 1979 LTwS - Nr.10), the 'International Organisation for Standardisation' (ISO) procedure for "Determination of the inhibitory effect of water constituents on bacteria (Pseudomonas cell multiplication inhibition test)", the 'Deutsches Institut for Normung' (DIN) procedure for "Determination of the inhibitory effect of water constituents on bacteria by the Pseudomonas cell multiplication inhibition test".

Following a dose range-finding study, three identical dilution series of test item were inoculated with the test organism *Pseudomonas putida* NCIMB 12708 to give triplicate test cultures at concentrations of 4 mg/L to 0.0039 mg/L of test item. The cultures were incubated at 25°C ± 1°C for 16 ± 1 hours.

The EC_{10} was found to be 0.07 mg/L. This gave an EC_{10} evaluation number of 7.2.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Item: BANDUR EXP 04209

Batch no.:	OP920521
Active Ingredient / Purity:	Not reported
Appearance:	Bright yellow opaque liquid
Expiry date:	Not reported
Storage:	Room temperature, in the dark

2. Test Species: *Pseudomonas putida* migula strain designation Berlin 33/2 strain number DMS 50026 NCIMB 12708

Pre-treatment: Cultures of the test organism were prepared by inoculating "Nutrient medium for pre-cultures" not more than one day before commencing the test. The growth was harvested from a 1-7 day old stock culture. This bacterial suspension was diluted with further amounts of pre-culture nutrient medium to give a turbidity of about 100 FTU.
10 mL of this suspension was added to 90 mL of the pre-culture nutrient medium (giving a turbidity of about 10 FTU). After incubation at 25°C ± 1°C for 5-7 hours, the bacterial suspension was diluted further with test-culture nutrient medium to give a turbidity of 50 FTU.

3. Test water: Sterile distilled water

B. STUDY DESIGN AND METHODS

1. In-life phase: 05 April – 05 July 1993

2. Exposure conditions

Test vessels:	300 mL Erlenmeyer flasks
Experimental design:	0.0039, 0.0078, 0.016, 0.031, 0.063, 0.13, 0.25, 0.50, 1.0, 2.0, 3.0 and 4.0 mg/L (three replicates) plus a control (10 replicates)
Loading:	Approximately 5 FTU initial turbidity
Temperature:	25 ± 1 °C
Aeration:	None
Test duration:	16 hours

3. Administration of the test item

Dose preparation and dosing

A four dilution series of the test item were prepared from an initial concentration of 4 mg/L. The concentrations tested were as follows:-

initial concentration	= 4 mg/L
1 in 1.333	= 3 mg/L
1 in 2	= 2 mg/L
1 in 4	=1 mg/L

1 in 8	=0.5 mg/L
1 in 16	= 0.25 mg/L
1 in 32	= 0.13 mg/L
1 in 64	=0.063 mg/L
1 in 128	= 0.031 mg/L
1 in 256	= 0.016 mg/L
1 in 512	= 0.0078 mg/L
1 in 1024	= 0.0039 mg/L

Preparation of test system

To each flask in three of the four test item dilution series, and to ten positive control flasks, 10 mL of nutrient solutions, and 10 mL of the 50 FTU bacterial suspension were added. This gave test cultures at each concentration and ten control cultures.

To the fourth test item dilution series, 10 mL of nutrient solutions, and 10 mL of sterile distilled water were added. This series acted as the uninoculated dilution series.

4. Measurements and observations

After incubation at 25 ± 1°C for 16 ± 1 hours the extinction at 436 nm of each of the test and control cultures was determined using a Pye Unicam PU8600 UV/VIS spectrophotometer. The spectrophotometer was blanked against distilled water for the control cultures and the corresponding uninoculated dilution for the test cultures.

5. Statistics/Data evaluation

Percentage inhibitory effect values were plotted against the test item concentration using logarithmic probit paper. From the graph EC_{10} and EC_{50} values were determined from the intersection of the straight line with lines parallel to the x-axis at 10% and 50% inhibitory effect. The EC_{10} value in kg/litre was converted to the exponential form. The negative logarithm to the base ten of this figure gave the evaluation number.

II. RESULTS AND DISCUSSION

A. ANALYTICAL VERIFICATION

Analytical verification was not required.

B. BIOLOGICAL DATA

The results obtained are summarised in the following table:

Table: Mean extinction and Inhibitory effect after exposure of *Pseudomonas putida* to BANDUR EXP 04209 for 16 hours

Nominal Concentration (mg/L)	Mean Extinction after 16 Hours (436 nm)	Inhibitory Effect (%)
4.0	0.345	44.2
3.0	0.370	40.03
2.0	0.388	37.04

1.0	0.395	35.9
0.50	0.452	26.4
0.25	0.426	30.7
0.13	0.484	21.1
0.063	0.483	21.3
0.031	0.478	22.1
0.016	0.474	22.8
0.0078	0.520	15.1
0.0039	0.594	2.8
Control	0.611	

The concentration of the test item which began to inhibit growth of the test organism was 0.07 mg/L.

This gives an EC_{10} evaluation number of 7.2.

C. TOXICITY ENDPOINTS

Table: Summary of endpoints

Endpoint	EC_x (mg/L)
EC_{10}	0.07
EC_{50}	0.9

III. CONCLUSION

The concentration of BANDUR EXP 04209 which begins to inhibit the growth of the test organism *Pseudomonas putida* migula strain designation Berlin 33/2 strain Number DMS 50026 NCIMB 12708 was found to be 0.07 mg/L.

This gives an EC_{10} evaluation number of 7.2.

[redacted] *(1993)*

Assessment and conclusion by applicant:

No validity criteria were specified for the test and hence it cannot be determined whether the results are valid. However, the test was performed according to GLP and there were no known circumstances which may have affected the quality or integrity of the study.

Exposure of *Pseudomonas* putida to BANDUR EXP 04209 gave EC_{10} and EC_{50} values of 0.07 and 0.9 mg/L respectively. Based on a nominal active substance concentration of 600 g/L, the EC_{10} and EC_{50} values were equivalent to 0.04 and 0.54 mg a.s./L.

Assessment and conclusion by RMS:

CP 10.9 Monitoring data

No data available.