Document Title

Summary of the ecotoxicological studies for Amidosulfuron

Data Requirements

EU Regulation 1107/2009 & EU Regulation 283/2013
Document MCA
Section 8: Ecotoxicological studies

According to the guidance document SANCO/10181/2013 for preparing dossiers for the approval of a chemical active substance

Date

2016-05-31

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M-557067-01-3

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Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[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

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

INTRODUCTION

This document provides detailed summaries of new ecotoxicological studies which were not available at the time of the first EU review of amidosulfuron and were therefore not evaluated for the Annex I inclusion of this active substance. Existing studies already submitted for the first EU review are found evaluated in the Draft assessment report (DAR) or its Addenda; in the present document these studies are therefore only briefly referenced, marked in grey shade. In exemption from this, upon specific request by the RMS expressed at the pre-application meeting, studies that have been submitted as part of the confirmatory data post Annex I are summarised and discussed as 'new information', even though they have undergone review for the EU by former RMS AGES Austria and are found summarised in the 'Addendum to monograph prepared in the context of post Annex I procedure (new Annex II data)', December 2010 (rev. 1 Feb. 2011) and are reflected in the updated EU List of Endpoints of December 2010.

Complete reports to all studies are found included in the electronic dossier provided by Bayer CropScience. The numbering and the headlines correspond to latest EU requirements.

For transparent overall data interpretation and risk assessment, key endpoints derived from both, old and new studies, are listed in the overview tables. For easy discrimination, new information is printed black, whilst existing information is marked in grey shaded font.

Due to new data concerning the fate and behaviour of amidosulfuron in the environment, additional metabolites are proposed to be included in the residue definition for the risk assessment (see Table CA 8-1). Accordingly, studies have been prepared to describe the ecotoxicological profile of these metabolites in the relevant environmental compartment.

Table CA 8- 1: Definition of the residue relevant for risk assessment*

Compartment	Residue Definition	Major Metabolite in
Soil	Amidosulfuron	*(parent substance)*
	A.-Desmethyl (AE F101630)	Aerobic soil, anaerobic soil
	A.-Desmethyl-chloropyrimidine (BCS-CO41838)	Aerobic soil
	A.-Guanidine (BCS-CO41839)	Aerobic soil
	A.-Biuret (BCS-CQ51287)	Aerobic soil
	A.-ADMP (AE F092944)	Aerobic soil
Groundwater	Amidosulfuron	*(parent substance)*
	A.-Desmethyl (AE F101630)	Aerobic soil, anaerobic soil
	A.-Desmethyl-chloropyrimidine (BCS-CO41838)	Aerobic soil
	A.-Guanidine (BCS-CO41839)	Aerobic soil
	A.-Biuret (BCS-CQ51287)	Aerobic soil
	A.-ADMP (AE F092944)	Aerobic soil
	A.-ADHP (AE F094206)	Lysimeter leachate, anaerobic soil
Surface Water	Amidosulfuron	*(parent substance)*
	A.-Desmethyl (AE F101630)	Aerobic water/sediment Aerobic soil, anaerobic soil
	A.-Desmethyl-chloropyrimidine (BCS-CO41838)	Aerobic soil
	A.-Guanidine (BCS-CO41839)	Aerobic soil
	A.-Biuret (BCS-CQ51287)	Aerobic soil
	A.-ADMP (AE F092944)	Aerobic soil
	A.- (Guanidinocarbonyl)sulfamic acid (BCS-BI49539)	Aerobic water/sediment
Air	Amidosulfuron	*(parent substance)*

*Justification for the residue definition for risk assessment see provided in MCA Sec.7, Point CA 7.4.

Substance coding

For historic reason, different coding or naming systems have been used for the designation of metabolites in study reports and associated documents. For better transparency and readability, a consistent naming strategy will be followed in the present document, identifying each component by a numeric code, and a "report name".

To maintain comparability to documents from the first submission for Annex I inclusion, numeric identifier will be
the AgrEvo/Aventis CropScience alphanumeric substance code (AE xxxxxx), or
where none assigned, the Bayer CropScience alphanumeric substance code (BCS-XXxxxxx).
with an associated "report name" as shown below.

Where applicable, the components will be addressed in a constant order of appearance. This applies for tabulated information, as well as for the sorting of study summaries in the document main text.

1) **Amidosulfuron** (parent substance) AE F075032
2) Amidosulfuron-**desmethyl** AE F101630
3) Amidosulfuron-**desmethyl-chloropyrimidine** BCS-CO41838
4) Amidosulfuron-**guanidine** BCS-CO41839
5) Amidosulfuron-**biuret** BCS-CQ51287
6) Amidosulfuron-**ADMP** AE F092944
7) Amidosulfuron-**ADHP** AE F094206

8) Amidosulfuron-**sulfamic acid** BCS-AW41401
9) A.-**(Guanidinocarbonyl)sulfamic acid** BCS-BI49539 (A. = Amidosulfuron)

A full overview of chemical structures, names and synonyms of all components is found in Document N3.

CA 8.1 Effects on birds and other terrestrial vertebrates

CA 8.1.1 Effects on Birds

CA 8.1.1.1 Acute oral toxicity to birds

Acute oral studies were performed on Japanese quail, bobwhite quail and mallard duck. The highest tested dose level in these studies was 2000 mg/kg body weight. No mortality occurred. Details of the studies are provided in the following table.

Table CA 8.1.1.1- 1: Avian acute oral toxicity data of amidosulfuron presented in this chapter

Test species	Test design	Ecotoxicological endpoint	Reference
Amidosulfuron			
Japanese quail *(Coturnix coturnix japonica)*	acute, oral	LD_{50} **> 2000** mg a.s./kg bw	[redacted]; 1987; M-120936-01-1 KCA 8.1.1.1 /01
Bobwhite quail *(Colinus virginianus)*	acute, oral	LD_{50} > 2000 mg a.s./kg bw	[redacted]; 1989; M-123940-01-1 KCA 8.1.1.1 /02
Mallard duck *(Anas platyrhynchos)*	acute, oral	LD_{50} > 2000 mg a.s./kg bw	[redacted]; 1988; M-121564-01-1 KCA 8.1.1.1 /03

a.s. = active substance; bw = body weight
Bold letters: Values considered relevant for risk assessment in the MCP document

Report: KCA 8.1.1.1/01; [redacted]; 1987; M-120936-01-1
Title: Hoe 075032 - active ingredient technical (code: Hoe 075032 OH ZC96 0001) Testing for acute oral toxicity in the male and female Japanese quail (Coturnix coturnix japonica)
Report No.: A39349
Document No.: M-120936-01-1
Guideline(s): **USEPA (EPA) FS 71-1 (1982)**
Guideline deviation(s): **Deviation not specified**
GLP/GEP: **yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on an acute oral toxicity test on Japanese quail with amidosulfuron technical a.s. No mortalities occurred and no macroscopically visible finding were seen at necropsy at all tested dose levels up to 2000 mg/kg bw. Observation period was 15 days after treatment, LD_{50} > 2000 mg/kg bw was reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. An EU agreed endpoint of LD_{50} > 2000 mg a.s./kg bw for *Coturnix c. japonica* was derived based on this test.

Report:	KCA 8.1.1.1/02; [redacted]; [redacted]; 1989; M-123940-01-1
Title:	Hoe 075032 - substance technical (Code: Hoe 075032 OH ZC97 0001) Testing for acute oral toxicity in the male and female Bobwhite quail (Colinus virginianus)
Report No.:	A40991
Document No.:	M-123940-01-1
Guideline(s):	**USEPA (=EPA): § 71-1 (1982)**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on an acute oral toxicity test on bobwhite quail with amidosulfuron technical a.s. No mortalities occurred, no intoxication symptoms were observed and no macroscopically visible findings were seen at necropsy at all tested dose levels up to 2000 mg/kg bw. Observation period was 14 days after treatment, LD_{50} > 2000 mg/kg bw was reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. The LD_{50} for bobwhite quail is identical to that of Japanese quail (KCA 8.1.1.1/01).

Report:	KCA 8.1.1.1/03; [redacted]; [redacted]; 1988; M-121564-01-1
Title:	Hoe 075032 - active ingredient technical (Code: Hoe 075032 OH ZC97 0001) Testing for acute oral toxicity in the male and female mallard duck (Anas platyrhynchos)
Report No.:	A39994
Document No.:	M-121564-01-1
Guideline(s):	**USEPA (=EPA): § 71-1 (1982)**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on an acute oral toxicity test on mallard duck with amidosulfuron technical a.s. No mortalities occurred and no macroscopically visible finding were seen at necropsy at all tested dose levels up to 2000 mg/kg bw. Food consumption was slightly reduced during the first three days at the highest dosage. Observation period was 14 days after treatment, LD_{50} > 2000 mg/kg bw. was reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. The LD_{50} for mallard is identical to that of Japanese quail (KCA 8.1.1.1/01).

CA 8.1.1.2 Short-term dietary toxicity to birds

Two short-term dietary studies on non-related bird species, japanese quail and mallard duck, were performed. The lowest LC_{50} was determined to be > 5000 ppm corresponding to an LDD_{50} of 1170 mg a.s./kg bw/d. Details of the studies are provided in the following table.

Table CA 8.1.1.2- 1: Avian short-term dietary toxicity data of amidosulfuron presented in this chapter

Test species	Test design	Ecotoxicological endpoint			Reference
Amidosulfuron					
Japanese quail *(Coturnix coturnix japonica)*	8-day dietary	LC_{50} LDD_{50}	> 5000 > 1170	ppm mg a.s./kg bw/d	[redacted]; 1988; M-120881-01-1 KCA 8.1.1.2 /01
Mallard duck *(Anas platyrhynchos)*	8-day dietary	LC_{50} LDD_{50}	> 5000 > 1687	ppm mg a.s./kg bw/d	[redacted]; [redacted]; 1988; M-120883-01-1 KCA 8.1.1.2 /02

ppm = parts per million; a.s. = active substance; bw = body weight; d = day

Report: KCA 8.1.1.2/01; [redacted]; 1988; M-120881-01-1
Title: Hoe 075032 -active ingredient technical (code: Hoe 075032 OH ZC97 0001) 8-day dietary LC50 test in the Japanese quail (Coturnix coturnix japonica)
Report No.: A39291
Document No.: M-120881-01-1
Guideline(s): **OECD: 205 (1984); USEPA (=EPA): § 71-2 (1982)**
Guideline deviation(s): **Deviation not specified**
GLP/GEP: **yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on an 8-day dietary toxicity test on japanese quail with amidosulfuron technical a.s. No intoxication symptoms were observed and no macroscopically visible findings were seen at necropsy at all tested dose levels up to 5000 ppm. A dietary LC_{50} > 5000 ppm equivalent to a mean daily substance intake of approximately 1170 mg a.s./kg body weight was reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. EU agreed endpoints of LC_{50} > 500 mg a.s./kg feed and LDD_{50} > 1170 mg a.s./kg bw/day for *Coturnix c. japonica* were derived based on this test.

Report: KCA 8.1.1.2/02; [redacted]; [redacted]; 1988; M-120883-01-1
Title: Hoe 075032 -active ingredient technical (Code: Hoe 075032 OH ZC97 0001) 8-day dietary LC50 test in the mallard duck (Anas platyrhynchos)
Report No.: A39293
Document No.: M-120883-01-1
Guideline(s): **OECD: 205 (1984); USEPA (=EPA): § 71-2 (1982)**
Guideline deviation(s): **Deviation not specified**
GLP/GEP: **yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on an 8-day dietary toxicity test on mallard duck with amidosulfuron technical a.s. No intoxication symptoms were observed and no macroscopically visible findings were seen at necropsy at all tested dose levels up to 5000 ppm. A dietary LC_{50} > 5000 ppm equivalent to a mean daily substance intake of approximately 1687 mg a.s./kg body weight was reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. No EU agreed endpoint was derived from this test.

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

A reproductive study on japanese quail was performed. The NOEL was determined to be 100 mg a.s./kg bw/d. Details of the study are provided in the following table.

Table CA 8.1.1.3- 1: Avian reproductive toxicity data of amidosulfuron presented in this chapter

Test species	Test design	Ecotoxicological endpoint			Reference
Japanese quail *(Coturnix coturnix japonica)*	22 weeks feeding, chronic, reproduction	NOEC NOEL	1000 **100**	ppm mg a.s./kg bw/d	[redacted], 1994; M-133167-01-1 KCA 8.1.1.3/01

ppm = parts per million; a.s. = active substance; bw = body weight; d = day
Bold letters: Values considered relevant for risk assessment in the MCP document

Report: KCA 8.1.1.3/01; [redacted]; 1994; M-133167-01-1
Title: Amidosulfuron; substance, technical (Code: HOE 075032 00 ZD97 0002) Avian subchronic toxicity test - oral toxicity (including effects on reproduction) in the Japanese Quail (Coturnix coturnix japonica, Temminck und Schlegel, 1849) following a 6-week administration in the diet
Report No.: A52329
Document No.: M-133167-01-1
Guideline(s): OECD: 206 (1984) Draft guideline of Nov. 1992
Guideline deviation(s): --
GLP/GEP: **yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a reproductive toxicity test on Japanese quail with amidosulfuron technical a.s. No adverse effects of amidosulfuron on reproductive functions were seen in Japanese quail including reproductive capacity and viability of the offspring up to the highest tested dietary level of 1000 ppm. A NOEC of 1000 mg/kg diet was reported. This dietary concentration corresponds to a mean daily intake of approximately 100 mg/kg body weight.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

Although the treatment phase of the quails is with 6 weeks significantly reduced compared to the common test design of OECD guideline 206, the study was considered acceptable. The EU agreed endpoint of NOEC 1000 mg a.s./kg diet for *Coturnix c. japonica* (corresponding to a mean daily intake of 100 mg a.s./kg body weight) was derived based on this test.

Comment on EC_{10} and EC_{20} estimations of the avian reproduction study
This study is aimed to reveal a NOAEL. According EFSA Praper meeting 133 (Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, 2015) the relevant test guidelines for this study has serious limitations for the derivation of reliable EC_{10} estimations. The design of this study implicates that EC_{10} and EC_{20} and their confidence intervals should not be routinely provided. The NOAEC/NOAEL should be retained as primary endpoint.
In the avian reproduction study with amidosulfuron, deviations to the control in all test groups were without statistical relevance. The NOAEL was at the highest test concentration A dose response was not identified. The missing of a dose-response relationship is an acceptable reason not to perform an EC_{10} and EC_{20} calculation.

CA 8.1.2 Effects on terrestrial vertebrates other than birds

Three acute oral toxicity studies and one reproduction toxicity study on mammals have been conducted with the metabolites amidosulfuron-desmethyl, amidosulfuron-ADHP, amidosulfuron-ADMP and amidosulfuron-guanidine. The results of these studies show that the amidosulfuron metabolites are not more toxic to the tested mammals than the active substance. Therefore, no mammal risk assessments for these metabolites are presented in this document.

CA 8.1.2.1 Acute oral toxicity to mammals

Two acute oral toxicity studies were performed on male and female rats and mice. The LD_{50} was greater than 5000 mg/kg bodyweight. Details of the study are provided in the following table.

Table CA 8.1.2.1- 1: Mammalian acute oral toxicity data of amidosulfuron presented in this chapter

Test species	Test design	Ecotoxicological endpoint	Reference
Rat	acute, oral	LD_{50} > 5000 [1)] mg a.s./kg bw	[redacted]; [redacted]; 1987; M-121349-01-1 KCA 5.2.1/01
Mouse	acute, oral	$LD_{50\ (female)}$ **5000** mg a.s./kg bw $LD_{50\ (male)}$ > 5000 mg a.s./kg bw	[redacted]; [redacted]; 1988; M-120196-01-1 KCA 5.2.1/02

a.s. = active substance; bw = body weight
Bold letters: Values considered relevant for risk assessment in the MCP document
[1)] 10 rats per group, no mortality occurred

Endpoint according to the EFSA Scientific Report (2007) 116, 1 – 86, conclusion on the peer review of amidosulfuron and the updated EU List of Endpoints of December 2010:

LD_{50} = 5000 mg as/kg bw*

** corrected for falsely reported unit "mg/kg bw/day" in the previous EU List of Endpoints of December 2010.*

CA 8.1.2.2 Long-term and reproduction toxicity to mammals

During the first EU review (EFSA Scientific Report (2007) 116, 1 – 86 and the updated EU List of Endpoints of December 2010) the NOAEL for reproductive performance of 400 ppm (22.5 mg/kg bw/d) in the rat two generation reproduction study had been identified for use in the risk assessment under the previous EU guidance document on risk assessment for birds and wild mammals (SANCO/4145/2000, 2002).

Meanwhile, the Guidance of EFSA – Risk assessment for birds and mammals (2009) was published which give more guidance how to determine toxicity enpoints from mammalian reproductive toxicity studies (§ 2.3.1). In the Table CA 8.1.2.2- 1 below three different enpoints are presented ([redacted]; [redacted]; [redacted]; 1992; M-135662-01-1).

The ecological endpoint for pups were determined according § 2.3.1 (c). As an example how to distinguish relevant from non-relevant effects, the Guidance Document discusses pup weight as an endpoint: "In order to determine the biological relevance of an effect it should be considered whether the effect could lead to a functional deficit later in the study, e.g. if a reduction in the weight of pups leads to a decrease in level of survival. If not then the effect may not be biologically relevant, however if there is a carry over of effects into the number of survivors, it can be considered biologically relevant."
In the 2-generation study at 2000 ppm the body weight reduction of F1 pups amounted to -3.5%, that of the F2 pups to -4.6%. Both findings were not statistically significant. No impact on any reproduction parameter was indentified. Therefore the body weigth reduction was considered not to be biologically relevant at that dose. The NOAEL was therefore 2000 ppm or 153 mg/kg b.w./day.

In addition, several further subchronic and chronic studies were performed with amidosulfuron. As the 90-day dietary studies, the long-term carcinogenesis studies as well as the developmental toxicity studies resulted in endpoints higher than the relevant endpoint of the two generation reproduction study, 153 mg/kg bw/day, respectively, there is no reason to deviate from the reproduction study as endpoint for risk assessment. Details of all studies are provided in Table CA 8.1.2.2- 1.

Comment on EC_{10} and EC_{20} estimations of the 2-generation reproduction study with rats:
This study is aimed to reveal a NOAEL. According EFSA (Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology, 2015) the relevant test guidelines for this study has serious limitations for the derivation of reliable EC10 estimations. The design of this study implicates that EC_{10} and EC_{20} and their confidence intervals should not be routinely provided. The NOAEC/NOAEL should be retained as primary endpoint. In the two-generation study with rats and amidosulfuron the relevant effects (pup weights) were lower than 10% even at the highest test concentrations (10000 ppm). Therefore an EC_{10} or EC_{20} cannot be calculated.

In overall conclusion, applying current guidance, it is proposed to reconsider the List of Endpoint definition of mammalian reproductive endpoint for amidosulfuron risk assessment, with a proposed updated value of NOAEL = 2000 ppm or 153 mg/kg b.w./day.

Table CA 8.1.2.2- 2: Mammalian reproductive toxicity data of amidosulfuron presented in this chapter

Test species	Test design	Ecotoxicological endpoint			Reference
Rat	2-generation dietary reproduction study	$NOAEL_{parental}$	10 000	ppm	[redacted]; [redacted]; [redacted]; 1992; M-135662-01-1 KCA 5.6.1 /02
		$NOAEL_{repro}$	10 000	ppm	
			570	mg a.s./kg bw/d	
		$NOEL_{pup}$	2000	ppm	
			153*	mg a.s./kg bw/d	
	28-day study	NOEL	2000	ppm	[redacted]; [redacted]; 1988; M-123241-01-1 KCA 5.3.1/01
		≡ $NOEL_{male}$	215	mg a.s./kg bw/d	
		≡ $NOEL_{female}$	199	mg a.s./kg bw/d	
	13-week study	NOEL	5000	ppm	[redacted]; [redacted]; 1989; M-123312-01-1 KCA 5.3.2/01
		≡ $NOEL_{male}$	398	mg a.s./kg bw/d	
		≡ $NOEL_{female}$	434	mg a.s./kg bw/d	
	21-day embryotoxicity study	$NOEL_{maternal,}$ embryonic, foetal	1000	mg a.s./kg bw/d	[redacted]; 1988; M-123121-01-1 KCA 5.6.2/01
	23-day embryotoxicity study	$NOEL_{maternal,}$ embryonic, foetal, perinatal	1000	mg a.s./kg bw/d	[redacted]; [redacted]; 1991; M-130678-01-1 KCA 5.6.2/02
Mouse	28-day study	NOEL	8000	ppm	[redacted]; [redacted]; 1988; M-123311-01-1 KCA 5.3.1/02
		≡ $NOEL_{male}$	1738	mg a.s./kg bw/d	
		≡ $NOEL_{female}$	1846	mg a.s./kg bw/d	
	13-week study	NOEL	4000	ppm	[redacted]; [redacted]; 1989; M-123328-01-1 KCA 5.3.2/02
		≡ $NOEL_{male}$	649	mg a.s./kg bw/d	
		≡ $NOEL_{female}$	698	mg a.s./kg bw/d	
Rabbit	29-day embryotoxicity study	$NOEL_{maternal,}$ embryonic, foetal	1000	mg a.s./kg bw/d	[redacted]; 1988; M-123111-01-1 KCA 5.6.2/03

ppm = parts per million; a.s. = active substance; bw = body weight; d = day
Bold letters: Values considered relevant for risk assessment in the MCP document
* Group mean intake of amidosulfuron (mg/kg bw/day) of F0-females during gestation period at the dose level of 2000 ppm (Table B.6.6.1-4 in the Annex B.6 of the DAR)

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

As the log P_{ow} of the active substance amidosulfuron is below the trigger (< 3), no evaluation of secondary poisoning is needed.

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

Since amidosulfuron is of low toxicity to birds and laboratory rodents, no risk for reptiles and amphibians is to be expected.

CA 8.1.5 Endocrine disrupting properties

Birds
The population relevant effects of Amidosulfuron on birds were studied in a reproductive toxicity study on bobwhite quail. There were no effects on reproductive parameters up to and including the highest tested dietary concentration of 1000 ppm a.s.

As reproduction was not affected in birds up to the highest tested concentration of 1000 mg/kg diet, it is concluded that there are no population relevant adverse effects of Amidosulfuron.

Wild Mammals
There is no evidence from the existing database that amidosulfuron has an effect on the endocrine system. No primary endocrine disrupting effects were observed in vivo and it is considered unlikely that any mechanistic study would add any relevant information.

Based on the absence of any indication of relevant effects it can be concluded that Amidosulfuron is not an endocrine disrupter.

Amphibians and Reptiles
Currently no test methods are established to assess the population relevant effects of chemicals to amphibians or reptiles. While an amphibian metamorphosis test protocol is available, this test was developed to evaluate to potential effect on the thyroid system and not to measure population relevant effects.Therefore no further studies can be suggested at this time for these groups of organisms.

As a conclusion, no further testing for endocrine disrupting properties is warranted.

CA 8.2 Effects on aquatic organisms

Aquatic organisms have been tested with the active substance, and considering the metabolites included in the residue definition for aquatic risk assessment (see MCA Section CA 7.4.1). As typical for sulfonylurea-class herbicides, aquatic vascular plants were identified as the most sensitive organism group, whereas endpoints for fish, daphnids and algae were found orders of magnitude higher.

Accordingly, metabolite testing for all primary and secondary degradates included *Lemna*, the standard aquatic organism species by far the most sensitive to the parent active substance. Rainbow trout (*Oncorhynchus mykiss*), *Daphnia magna* and green algae (*Scenedesmus subspicatus*) were tested in addition for the first generation metabolites in the degradation pathway in water/sediment, i.e.

amidosulfuron-desmethyl and amidosulfuron-ADMP, as well as for one further component, amidosulfuron-ADHP[1].

Guanidinocarbonyl sulfamic acid, a tertiary metabolite in the aquatic environment, was not tested. Based on structural considerations and data on its metabolic predecessor components, risk assessment can be established via non-testing approaches.

Consideration of ionization of amidosulfuron:
According to the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013), the impact of ionization on the aquatic assessment has to be considered. Amidosulfuron is an ionisable substance with a pKa of 3.58 (MCA section 2.8). Hence, both dissociated and undissociated forms will co-exist in water in significant amount up to a pH of ca 4.5. As this pH is below the pH range in ecotoxicological testing and below the range of relevant pH of surface water bodies in agricultural areas of EU ([redacted]; [redacted]; [redacted]; [redacted]; [redacted]; 2015; M-424756-02-1), it is not necessary to consider the effects of the undissociated form of amidosulfuron on aquatic organisms.

CA 8.2.1 Acute toxicity to fish

Some metabolites relevant for risk assessment have been tested for their acute toxicity on fish, these are the primary metabolites A-desmethyl and ADMP. In addition, component ADHP was also tested.

Despite the presence of the structural group responsible for sulfonylureas herbicidal activity (Sinclair, 2009[2]), tests demonstrated a lack of herbicidal activity for both A-desmethyl (KCA 8.6.1 /02) and A-desmethyl-chloropyrimidine (KCA 8.6.1 /06). Moreover, the same results were obtained with ADMP (KCA 8.6.1 /08), ADHP (KCA 8.6.1 /03), A-guanidine (KCA 8.6.1 /06) and A-biuret (KCA 8.6.1 /07). Therefore all tested metabolites lost the toxophore responsible for the biological target activity (i.e. herbicidal activity). According to the risk assessment scheme for metabolites (pp 143-144) of the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013[3]), risk assessment for metabolites without toxophore can be based on active substance data. Consequently further tests on fish with A-desmethyl-chloropyrimidine, A-guanidine and A-biuret were not deemed necessary.

Guanidinocarbonyl sulfamic acid is also a major metabolite for the aquatic environment. No tests were performed to characterize neither its toxicity nor its herbicidal activity. It is a tertiary metabolite resulting from the degradation of amidosulfuron-guanidine which does not show any herbicidal activity. Consequently, it is assumed that the toxophore is no longer present in this metabolite and the risk assessment can be addressed using information from the parent substance.

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[1] note: in consequence of new metabolic pathway information for water/sediment systems generated in study KCA 7.2.2.3 /02, component a.-ADHP is no longer part of the residue definition for aquatic risk assessment. A set of data on a.-ADHP is nevertheless provided for comparative purposes, and as present in the baseline dossier.

[2] CJ Sinclair PhD Thesis University of York Predicting the environmental fate and ecotoxicological and toxicological effects of pesticide transformation products
https://www.researchgate.net/publication/235934684_Predicting_the_environmental_fate_and_ecotoxicological_and_toxicological_effects_of_pesticide_transformation_products

[3] 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 CA 8.2.1- 1: Acute toxicity data of amidosulfuron and metabolite to fish presented in this chapter

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
Amidosulfuron				
Oncorhynchus mykiss [former Salmo gairdneri] (rainbow trout)	static acute	96 h	LC_{50} **> 320**$_{(nom)}$	[redacted]; 1987; M-117660-01-1 KCA 8.2.1 /01
Lepomis macrochirus (bluegill sunfish)	static acute	96 h	LC_{50} **> 100**$_{(nom)}$	[redacted]; 1987; M-119377-01-1 KCA 8.2.1 /02 [redacted]; 1988; M-120514-01-1 KCA 8.2.1 /03
Cyprinodon variegatus (sheepshead minnow)	static acute	96 h	LC_{50} **> 100**$_{(nom)}$	[redacted]; 1989; M-123929-01-1 KCA 8.2.1 /04
Amidosulfuron-desmethyl				
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	LC_{50} **>100**$_{(nom)}$	[redacted]; 1993; M-131849-01-1 KCA 8.2.1 /05
Amidosulfuron-ADMP				
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	LC_{50} **169.2**$_{(mm)}$	[redacted]; 1993; M-131422-01-1 KCA 8.2.1 /06 [redacted]; 2016; M-549001-01-1 KCA 8.2.1 /11
Amidosulfuron-ADHP				
Oncorhynchus mykiss (rainbow trout)	static acute	96 h	LC_{50} >100$_{(nom)}$	[redacted]; 1993; M-138953-01-1 KCA 8.2.1 /07
Amidosulfuron-Lysimeter-leachate				
Brachydanio rerio (zebra fish)	static acute	96 h	LC_{50} **no effects**	[redacted]; 1993; M-138536-01-1 KCA 8.2.1 /08 [redacted]; 1993; M-138604-01-1 KCA 8.2.1 /09

Bold letters: Values considered relevant for risk assessment in the MCP document
$_{(nom)}$ nominal concentration; $_{(mm)}$ mean measured concentration

Studies on amidosulfuron:

Report:	KCA 8.2.1/01; [redacted]; 1987; M-117660-01-1
Title:	The Effect of Hoe 075032 - substance, technical (Identification code : Hoe 075032 OH ZC98 0001) to Salmo gairdneri (Rainbow trout) in a Static-Acute Toxicity Test (Sg365/b, method BBA)
Report No.:	A35829
Document No.:	M-117660-01-1
Guideline(s):	BBA: Leaflet No. 33; USEPA (=EPA): EPA-660/3-75-009
Guideline deviation(s):	Yes, see report
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a static acute toxicity test on rainbow trout with amidosulfuron technical a.s. Based on the test results the LC_{50} could not be calculated. The LC_{50} was assumed as >320 mg/L (visual solubility limit exceeded).
Analytical measurements were performed on a concentration (1 mg a.s./L) outside the range of the tested concentrations (32 to 1000 mg a.s./L). Recoveries at 0, 2, 48 and 96 h show that the substance is stable in the medium, however precipitate is reported at 100 mg a.s./L and above. These analytical deficiencies could invalidate the study; nevertheless the study was not repeated for animal welfare reasons. Moreover, a new study would not provide a better estimate of the acute toxicity of amidosulfuron on Rainbow trout, as it would be limited to concentrations up to 100 mg a.s./L, which would result in a LC_{50} > 100 mg a.s./L.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. An EU agreed endpoint of LC_{50} > 320 mg a.s./L for *Oncorhynchus mykiss* was derived based on this test.

Report:	KCA 8.2.1/02; [redacted]; 1987; M-119377-01-1
Title:	The Effect of Hoe 075032 - substance, technical (Identification code: Hoe 075032 OH ZZ96 0001) to Lepomis macrochirus (Bluegill sunfish) in a Static-Acute Toxicity Test (method EPA)
Report No.:	A37087
Document No.:	M-119377-01-1
Guideline(s):	**USEPA (=EPA): EPA-660/3-75-009; Deviation not specified**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a static acute toxicity test on bluegill sunfish with amidosulfuron technical a.s. No mortality or signs of intoxications were observed at the test concentration of 100 mg a.s./L. A 96 h LC_{50} >100 mg a.s./L and a 96 h NOEC = 100 mg a.s./L were reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be used only as confirmatory data because the test was conducted as a limit test.

Report:	KCA 8.2.1/03; [redacted]; 1988; M-120514-01-1
Title:	The Effect of Hoe 075032 - substance, technical (Identification code : Hoe 075032 OH ZC96 0001) to Lepomis macrochirus (Bluegill sunfish) in a Static-Acute Toxicity Test (method EPA)
Report No.:	A38908
Document No.:	M-120514-01-1
Guideline(s):	**USEPA (=EPA): EPA-660/3-75-009**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a static acute toxicity test on bluegill sunfish with amidosulfuron technical a.s. No mortality or signs of intoxications were observed at the tested concentrations up to 100 mg a.s./L. A 96 h LC_{50} >100 mg a.s./L and a 96 h NOEC = 100 mg a.s./L were reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment. An EU agreed endpoint of LC_{50} > 100 mg/L was derived from this test.

Report:	KCA 8.2.1/04; [redacted]; 1989; M-123929-01-1
Title:	Static acute toxicity of sample number Hoe 075032 to the sheepshead minnow, Cyprinodon variegatus
Report No.:	A40984
Document No.:	M-123929-01-1
Guideline(s):	**USEPA (=EPA): 72-3**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a static acute toxicity test on sheepshead minnow with amidosulfuron technical a.s. No mortality occurred at the tested concentrations. A 96 h LC_{50} >94 mg a.s./L and a 96 h NOEC = 94 mg a.s./L were reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study review discussed that precipitation occurred at the two highest nominal test concentrations and actual concentrations in the test solution were not analytically measured. Nevertheless, since the results were in agreement with the results of the other fish tests, the study was accepted and no new study was considered necessary. No EU agreed endpoint was derived from this test.

For the risk assessment in the MCP document the re-calculated LC_{50} > 100 mg a.s./L based on the content of the active substance and by-products is used.

Studies on the metabolites of amidosulfuron:

Amidosulfuron-desmethyl:

Report:	KCA 8.2.1/05; [redacted]; 1993; M-131849-01-1
Title:	Hoe 101630 - substance, technical (Hoe 101630 00 ZC93 0001) Effect to Oncorhynchus mykiss (Rainbow trout) in a Static-Acute Toxicity Test (method OECD)
Report No.:	A50849
Document No.:	M-131849-01-1
Guideline(s):	**OECD: 203 (1992)**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a static acute toxicity test on rainbow trout with the metabolite amidosulfuron-desmethyl. No mortality or signs of intoxication were observed at the tested concentration of 100 mg metabolite/L. A 96 h LC_{50} >100 mg metabolite/L and a 96 h NOEC = 100 mg metabolite/L were reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment. An EU agreed endpoint of LC_{50} > 100 mg/L was derived from this test.

Amidosulfuron-ADMP:

Report:	KCA 8.2.1/06; [redacted]; 1993; M-131422-01-1
Title:	Hoe 092944 - substance, technical (Hoe 092944 00 ZD99 0001) Effect to Oncorhynchus mykiss (Rainbow trout) in a Static-Acute Toxicity Test (method OECD)
Report No.:	A50396
Document No.:	M-131422-01-1
Guideline(s):	OECD: 203 (1984) **US EPA OCSPP Guideline 850.1075**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

Report:	KCA 8.2.1/11; [redacted]; 2016; M-549001-01-1
Title:	Re-evaluation of acute fish study with metabolite AE F092944 (M-131422-01-1) in context of mesosulfuron approval renewal (EFSA request, Point 33)
Report No.:	M-549001-01-1
Document No.:	M-549001-01-1
Guideline(s):	**Not relevant**
Guideline deviation(s):	**none**
GLP/GEP:	**no**

The study reports on a static acute toxicity test on rainbow trout with the metabolite amidosulfuron-ADMP. Signs of intoxication were observed at concentrations of 180 mg/L and higher. No fish died at concentrations up to 100 mg/L. 100 % mortality was observed at the test concentrations of 560 mg/L

and 1000 mg/L within 24 hours. At the end of test 10 % and 80 % of fish were dead at concentrations of 180 and 320 mg/L. The 96 h LC_{50} value was calculated to be 254 mg metabolite/L. A 96 h NOEC = 100 mg metabolite/L was reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The oxygen concentration in one sample was lower than 60 % of air saturation. As all other measured values were higher, this single value can be classified as erroneous and does not invalidate the test. Therefore, the study was considered to be acceptable and was used for the risk assessment. An EU agreed endpoint of LC_{50} =254 mg/L was derived from this test.

As measured concentration below 80% were obtained at t=0 for some concentrations, the endpoint has been recalculated on the basis of mean measured concentrations. That new endpoint is LC_{50} =169.2 mg/L (M-549001-01-1).

Amidosulfuron-ADHP:

Report:	KCA 8.2.1/07; [redacted]; 1993; M-138953-01-1
Title:	Hoe 094206 - substance technical (Hoe 094206 00 ZC99 0002) Effect to Oncorhynchus mykiss (Rainbow trout) in a Static-Acute Toxicity Test (method OECD)
Report No.:	A49949
Document No.:	M-138953-01-1
Guideline(s):	**OECD: 203 (1984)**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a static acute toxicity test on rainbow trout with the metabolite amidosulfuron-ADHP. No mortality or signs of intoxication were observed at the tested concentrations up to 100 mg/L. A 96 h LC_{50} >100 mg metabolite/L and a 96 h NOEC = 100 mg metabolite/L were reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment. An EU agreed endpoint of LC_{50} > 100 mg/L was derived from this test.

Amidosulfuron-Lysimeter leachate:

Report:	KCA 8.2.1/08; [redacted]; 1993; M-138536-01-1
Title:	Hoe 075032 The Effect of lysimeter percolates (Lysimeter no. IX) to Brachydanio rerio (zebrafish) in a static-acute toxicity test (method DIN)
Report No.:	A49498
Document No.:	M-138536-01-1
Guideline(s):	**Guidelines of Deutsche Normung DIN 38412 - L15 (1982)**
Guideline deviation(s):	**For deviation see Point 4.1**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The static acute study reports on the effect of lysimeter percolates treated with amidosulfuron technical a.s. to zebrafish. No mortality or signs of intoxication were observed until the end of the test after 96 h. The percolates from a lysimeter treated with 49 g a.s./ha were not toxic to zebrafish.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable. An EU agreed endpoint of "no effects" was derived from this test.

Report:	KCA 8.2.1/09; [redacted]; 1993; M-138604-01-1
Title:	Hoe 075032: The Effect of lysimeter percolates (Lysimeter no. XY) to Brachydanio rerio (zebrafish) in a static-acute toxicity test (method DIN)
Report No.:	A49568
Document No.:	M-138604-01-1
Guideline(s):	**Guidelines of Deutsche Normung DIN 38412 - L15 (1982)**
Guideline deviation(s):	**For deviation see Point 4.1**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The static acute study reports on the effect of lysimeter percolates treated with amidosulfuron technical a.s. to zebrafish. No mortality or symptoms of intoxication were observed until the end of the test after 96 h. The percolates from a lysimeter treated with 54 g a.s./ha were not toxic to zebrafish.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable. An EU agreed endpoint of "no effects" was derived from this test.

AE F118772 [not a soil or aquatic metabolite of amidosulfuron]

Report:	KCA 8.2.1/10; [redacted]; 1993; M-132471-01-1
Title:	Hoe 118772 - Substance, technical (Hoe 118772 00 ZC98 0001) Effect to Oncorhynchus mykiss (Rainbow trout) in a Static-Acute Toxicity Test (method OECD)
Report No.:	A51537
Document No.:	M-132471-01-1
Guideline(s):	**OECD: 203 (1992)**
Guideline deviation(s):	**For deviation see Point 4.6**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a static acute toxicity test on rainbow trout for component AE F118772[4]. AE F118772 was not observed to be formed as an environmental degradate of the active substance amidosulfuron. The study is listed only for formal completeness reason, as it was erroneously included in the previous (baseline) dossier.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is available in the previous DAR (2006).

The study was considered to be acceptable, however since the tested component was not found formed in fate studies the endpoint of this test is not of relevance for risk assessments on the active substance amidosulfuron. No EU agreed endpoint was derived from this test.

CA 8.2.2 Long-term and chronic toxicity to fish

In the new European dossier format/data requirements there is no data point that corresponds to fish prolonged toxicity tests. Nevertheless, one study (on the active substance) is mentioned here as supportive information, since it is contained in the baseline dossier and in the List of Endpoints from the first EU review.

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Table CA 8.2.2- 2: Fish prolonged toxicity test data of amidosulfuron presented in this chapter

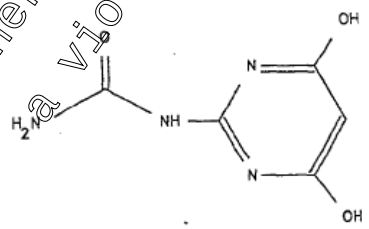
Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
Amidosulfuron				
Oncorhynchus mykiss (rainbow trout)	Juvenile growth test flow-through	21 d	NOEC[1] 0.41(mm)	[redacted]; 1991; M-129610-01-1 KCA 8.2.2 /01

[1] According to DAR, the endpoint was based on mean measured concentrations
(mm) mean measured concentration

[4] **) chemical identity and structure of AE F118772 (synonym Hoe 118772):**

Chemical name: 4,6-dihydroxypyrimidin-2-yl-urea

Structure:



Empirical formula: $C_5H_6N_4O_3$

Molar mass: 170.15 g/mole

Report:	KCA 8.2.2/01; [redacted]; 1991; M-129610-01-1
Title:	Hoe 075032 - substance, technical (Hoe 075032 00 ZC94 0001) Effect to Salmo gairdneri (Rainbow trout) in a 21-day Prolonged Toxicity Test (method OECD)
Report No.:	A45487
Document No.:	M-129610-01-1
Guideline(s):	**OECD: 204 (1984)**
Guideline deviation(s):	**For deviation see Point 4.1**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a 21-day prolonged toxicity test on rainbow trout with amidosulfuron technical a.s.. Fish exposed to the solvent control showed slower movement than the untreated control group. No other visual signs of intoxication or abnormal swimming behaviour were observed in the treated or in the untreated control groups. One fish died at day 18 at 6.41 mg a.s./L. This was regarded as not being related to the test substance since no mortality was observed in the higher test concentrations. Effects on the growth of the test fish were observed. The increase in size and weight declined with increasing concentration of the test substance. A loss in weight was observed at a concentration of 37.59 mg a.s./L and higher. At a concentration of 37.59 mg a.s./L the difference to the controls was significant. A 21 d NOEC = 6.41 mg a.s./L was concluded. (Values expressed as mean measured concentrations as per previous EU review.)

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

CA 8.2.2.1 Fish early life stage toxicity test

Table CA 8.2.2- 3: Fish early life stage toxicity test data of amidosulfuron presented in this chapter

Test species	Test system	Test duration	Endpoint [mg as/L]		Reference
Amidosulfuron					
Pimephales promelas (fathead minnow)	Early Life Stage flow-through	35 d	NOEC	**9.72**[1)]	[redacted]; [redacted]; 2015; M-538454-01-1 KCA 8.2.2.1 /01
			EC_{10}	**> 9.72**(mm)	

[1)] The NOEC of 9.72 mg a.s./L was the highest tested concentration of the study
(mm) mean measured concentration

Studies on amidosulfuron:

Report:	KCA 8.2.2.1/01; [redacted]; [redacted]; 2015; M-538454-01-1
Title:	Early life stage toxicity of amidosulfuron technical to the fathead minnow (Pimephales promelas) under flow-through conditions
Report No.:	EBBEN004
Document No.:	M-538454-01-1
Guideline(s):	**OCSPP Guideline 850.1400; OECD Guideline 210 (2013)**
Guideline deviation(s):	**Deviation none**
GLP/GEP:	**yes**

Executive Summary

The aim of the flow-through early life stage toxicity test was to determine the effects of amidosulfuron (purity 99.0%) on fathead minnow (*Pimephales promelas*). The primary objective of this study was to estimate the No Observed Effect Concentration (NOEC) and the Lowest Observed Effect Concentration (LOEC) for amidosulfuron.

Eggs and fry of *Pimephales promelas* were exposed in a flow through system over a period of 35 days to nominal concentrations of 0.0954, 0.305, 0.977, 3.13 and 10.0 mg a.s./L (corresponding to mean measured concentrations of 0.0809, 0.297, 0.862, 3.89 and 9.72 mg a.s./L (85 to 124% of nominal). In addition a control (corresponding to a mean measured concentration of < 0.01 mg a.s./L) was tested. Sublethal effects, fish hatchability, survival and growth (length and dry weight – all surviving fish on Day 35) were observed. Based on analytical findings the biological endpoints are reported as mean measured figures. The overall NOEC was determined to be 9.72 mg a.s./L, the highest tested concentration.

Materials and Methods:
Test material: Amidosulfuron; Batch No.: ELIR000195; Specification No.: 102000000551-03; CAS No.: 120923-37-7; purity: 99.0%.

Fathead Minnow (*Pimephales promelas*) eggs starting at < 24 hours old were exposed to amidosulfuron (purity 99.0%) in a flow through system over a period of 35 days. Test vessels were dosed via a proportional diluter with a renewal rate of 7 volume turnovers per 24-hour period; Nominal concentrations were 0.0954, 0.305, 0.977, 3.13 and 10.0 mg a.s./L. In addition a dilution water control was tested. Each vessel (glass aquaria; 21.6 x 12.7 x 30.5 cm) served as one replicate containing one oscillating egg cup and filled with approximately 7 L soft processed water (dechlorinated municipal water blended with reverse osmosis water). 35 organisms were used per replicate. Thinning to 20 alevin per replicate took place at day 5, the post-hatch phase started after thinning (when at least 90% of all viable control eggs had hatched). The mean wet weight was 0.16 g/fish (based on controls). The dynamic biological loading was 0.065 g/L/day (mean biomass based on controls). The test was conducted with 4 replicates per treatment level.

Observations for sublethal effects and survival were made daily, hatching observations were made daily during hatching phase; growth determinations were made at the end of the exposure. For analytical verification of the test item concentrations samples were taken at experimental start, weekly (± 2 days) thereafter including experimental finish from all concentrations. The limit of quantification (LOQ) was 0.01 mg a.s./L. The analysis was performed using a Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS).

Dates of experimental work: October 15, 2014 – November 19, 2014

Results:
Validity Criteria
Validity criteria for this study were met. Regarding hatching rate, the test is considered to be valid if the mean hatching success is >66%. The post hatch average survival of controls must be >80% and each control replicate must have at least 70 percent survival. Dissolved oxygen must be 60-100%.

Analytical findings:
Analytical verification of test solutions revealed mean measured concentrations of 0.0809, 0.297, 0.862, 3.89 and 9.72 mg a.s./L. Mean measured recoveries ranged from 85 to 124% of the nominal. Biological results are based on mean measured concentrations. Detailed analytical results are presented in the following table:

Table CA 8.2.2.1- 1: nominal and measured concentrations of amidosulfuron

Nominal Concentration (mg a.s./L)	Mean measured concentration (mg a.s./L)						Mean	SD	Percent of Nominal
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35			
Control	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	NA	NA	NA
0.0954	0.0826	0.0751	0.0989	0.0680	0.0799	0.0811	0.0809	0.01	85
0.305	0.301	0.295	0.302	0.286	0.302	0.300	0.297	0.01	97
0.977	0.847	0.854	0.876	0.875	0.837	0.882	0.862	0.04	88
3.13	3.72	3.63	3.99	4.11	3.83	4.08	3.89	0.20	124
10.0	9.82	9.33	10.30	9.65	9.30	9.94	9.72	0.40	97

Biological findings:
Biological parameters were observed as listed below.

Table CA 8.2.2.1- 2: Effect of amidosulfuron on hatching success and mortality of *Pimephales promelas*

mean measured concentration (mg a.s./L)	mean hatch by study day (%)[A]			mean fry survival (%)		mean wet length (g) at day 35	mean standard length (mm) at day 35	mean dry weight (mg) at day 35
	Day 3	Day 4	Day 5	Day 5[B]	Day 35[C]			
control	5.7	65.7	90.0	87.1	96.3	0.16	25.4	32.0
0.0809	5.0	61.4	87.9	84.3	97.5	NA	25.8	31.1
0.297	3.6	60.0	91.4	90.0	96.3	NA	26.2	33.6
0.862	0.7	64.3	94.3	92.9	96.3	NA	25.9	32.8
3.89	2.1	65.7	93.0	88.6	97.5	NA	26.1	32.3
9.72	4.3	60.0	88.6	86.4	100.0	NA	25.8	31.1

[A] Percent Hatch = (# of alevin / # of eggs on day 0) * 100
[B] Percent Survivorship before thinning = (# of alevin and eggs on Day 5/# of eggs on Day 0) * 100
[C] Percent Survivorship after thinning = (# of fish on day 35/# of fish at thinning) * 100
NA = not applicable
Calculations done in Excel using unrounded numbers; manual calculations may vary

Observations of fish were recorded daily throughout the study. Fish throughout all test levels, including the control, appeared normal during the course of the study with the exception of one alevin in the control and one alevin in the 0.0809 mg a.i./L test level. These fish were small and showed an abnormal development of the spine (spina bifida).

Biological endpoints derived:
From the results presented above the following biological endpoints can be derived:

Alevin survival (Day 5):	NOEC	9.72 mg a.s./L*	LOEC	> 9.72 mg a.s./L
Fry survival (day 35):	NOEC	9.72 mg a.s./L*	LOEC	> 9.72 mg a.s./L
Percent Hatch:	NOEC	9.72 mg a.s./L*	LOEC	> 9.72 mg a.s./L
Time to Hatch:	NOEC	9.72 mg a.s./L*	LOEC	> 9.72 mg a.s./L
Growth (Length):	NOEC	9.72 mg a.s./L*	LOEC	> 9.72 mg a.s./L
Growth (Dry Weight):	NOEC	9.72 mg a.s./L*	LOEC	> 9.72 mg a.s./L
Morphological & Behavioral Effects:	NOEC	9.72 mg a.s./L*	LOEC	> 9.72 mg a.s./L

* highest concentration tested

Since at the NOEC of 9.72 mg/L less than 10% effects were observed, the EC_{10} will be > 9.72 mg/L and thus not influence the risk assessment. For this reason a calculation of an EC_{10} and EC_{20} is considered not necessary.

Conclusion:
The 35-day exposure to Amidosulfuron Technical resulted in a NOEC of 9.72 mg a.s./L (highest concentration tested) and a LOEC of > 9.72 mg a.s./L.

CA 8.2.2.2 Fish full life cycle test

A fish full life cycle test with amidosulfuron is not triggered as the compound has no potential for bioconcentration and is not persistent in water-sediment systems.

CA 8.2.2.3 Bioconcentration in fish

Due to the low P_{OW} amidosulfuron has no potential for bioconcentration (see Sec. 2, CA 2.7).

CA 8.2.3 Endocrine disrupting properties

Fish
Population relevant effects of Amidosulfuron on fish were studied in an early life-stage test (ELS). No effects on embryo survival at hatch or on survival and growth (wet weight, dry weight, and total length) or behaviour were seen at the highest tested concentration of 9.72 mg/L(mean measured, 10 mg/L nominal).

The fish NOEC is orders of magnitude above regulatory acceptable concentration, driven by aquatic plants.

Based on the absence of relevant effects it can be concluded that Amidosulfuron is not a (potential) endocrine disrupter in fish.

No further testing is indicated to evaluate the endocrine disrupter potential of Amidosulfuron to fish.

Amphibians and Reptiles
Currently no test methods are established to assess the population relevant effects of chemicals to amphibians or reptiles. While an amphibian metamorphosis test method exists, this test was developed to evaluate to potential effect on the thyroid system, and not to measure population relevant effects. Therefore no further studies can be suggested at this time for this group of organisms.

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to Daphnia magna

Some metabolites relevant for risk assessment have been tested for their acute toxicity on *Daphnia magna*, these are the primary metabolites A-desmethyl and ADMP. In addition a secondary metabolite (ADHP) was also tested.
Despite the presence of the structural group responsible for sulfonylureas herbicidal activity (Sinclair, 2009[5]), tests demonstrated a lack of herbicidal activity for both A-desmethyl (KCA 8.6.1 /02) and A-

[5] CJ Sinclair PhD Thesis University of York Predicting the environmental fate and ecotoxicological and toxicological effects of pesticide transformation products
https://www.researchgate.net/publication/235934684_Predicting_the_environmental_fate_and_ecotoxicological_and_toxicological_effects_of_pesticide_transformation_products

desmethyl-chloropyrimidine (KCA 8.6.1 /06). Moreover, the same results were obtained with ADMP (KCA 8.6.1 /08), ADHP (KCA 8.6.1 /03), A-guanidine (KCA 8.6.1 /06) and A-biuret (KCA 8.6.1 /07). Therefore all metabolites lost the toxophore responsible for the biological target activity (i.e. herbicidal activity). According to the risk assessment scheme for metabolites (pp 143-144) of the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013[6]), risk assessment for metabolites without toxophore can be based on active substance data. Consequently further tests on *Daphnia magna* with A-desmethyl chloropyrimidine, A-guanidine and A-biuret were not deemed necessary.

Guanidinocarbonyl sulfamic acid is also a major metabolite for the aquatic environment. No tests were performed to characterize neither its toxicity nor its herbicidal activity. It is a tertiary metabolite resulting from the degradation of amidosulfuron-guanidine which does not show any herbicidal activity. Consequently, it is assumed that the toxophore is no longer present in this metabolite and the risk assessment can be addressed using information from the parent substance.

Table CA 8.2.4- 4: Acute toxicity data of amidosulfuron and metabolites to *Daphnia magna* presented in this chapter

Test species	Test system	Test duration	Endpoint		Reference
Amidosulfuron					
Daphnia magna (water flea)	static acute	48 h	EC_{50}	55 (mm)	[redacted]; 1988; M-120328-01-1 KCA 8.2.4.1 /01
			EC_{50}	**36** (nom)	[redacted]; 1987; M-119379-01-1 KCA 8.2.4.1 /02
Amidosulfuron-desmethyl					
Daphnia magna (water flea)	static acute	48 h	EC_{50}	> **55** (mm)	[redacted]; [redacted]; 2003; M-211220-01-1 KCA 8.2.4.1 /03
			EC_{50}	3.6 (nom)	[redacted]; 1993; M-131833-01-1 KCA 8.2.4.1 /04
Amidosulfuron-ADMP					
Daphnia magna (water flea)	static acute	48 h	EC_{50}	**223** (nom)	[redacted]; 1993; M-131382-01-1 KCA 8.2.4.1 /05
Amidosulfuron-ADHP					
Daphnia magna (water flea)	static acute	48 h	EC_{50}	>100 (nom)	[redacted]; 1993; M-131835-01-1 KCA 8.2.4.1 /06

[6] 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

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
Amidosulfuron-Lysimeter leachate				
Daphnia magna (water flea)	static acute	48 h	EC_{50} **no effects**	[redacted]; 1993; M-131134-01-1 KCA 8.2.4.1 /07
			EC_{50} **no effects**	[redacted]; 1993; M-131133-01-1 KCA 8.2.4.1 /08
			EC_{50} no effects [untreated control]	[redacted]; 1993; M-131854-01-1 KCA 8.2.4.1 /09

Bold letters: Values considered relevant for risk assessment in the MCP document
(nom) nominal concentration; (mm) mean measured concentration

Studies on amidosulfuron:

Report: KCA 8.2.4.1/01; [redacted]; 1988; M-120328-01-1
Title: The Effect of Hoe 075032 - substance, technical (Identification code : Hoe 075032 OH ZC96 0001) to Daphnia magna (Waterflea) in a Static-Acute Toxicity Test (method OECD)
Report No.: A38705
Document No.: M-120328-01-1
Guideline(s): **OECD: 202 (1984)**
Guideline deviation(s): **Deviation not specified**
GLP/GEP: **yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a static acute toxicity test on *Daphnia magna* with amidosulfuron technical a.s. No mortality was observed in the controls and up to a concentration of 18 mg a.s./L. 10 % and 40 % of animals died at test concentrations of 32 and 56 mg a.s./L. After 48 hours all animals were found dead at concentrations of 100 mg a.s./L. A 48 h EC_{50} = 55 mg a.s./L and a 48 h NOEC = 18 mg a.s./L were reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment. However, no EU agreed endpoint was derived from this test, since a lower endpoint resulted from the corresponding study KCA 8.2.4.1/02.

Report: KCA 8.2.4.1/02; [redacted]; 1987; M-119379-01-1
Title: The Effect of Hoe 075032 - substance, technical (Identification code : Hoe 075032 OH ZC96 0001) to Daphnia magna (Waterflea) in a Static-Acute Toxicity Test (method EPA)
Report No.: A37699
Document No.: M-119379-01-1
Guideline(s): **USEPA (=EPA): EPA-660/3-75-009**
Guideline deviation(s): **Deviation not specified**
GLP/GEP: **yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a static acute toxicity test on *Daphnia magna* with amidosulfuron technical a.s. No mortality was observed in the controls and up to a concentration of 10 mg a.s./L. 25 % and 30 % of animals died at test concentrations of 18 and 32 mg a.s./L. After 48 hours all animals were found dead at concentrations of 56 and 100 mg a.s./L. A 48 h EC_{50} = 36 mg a.s./L and a 48 h NOEC = 10 mg a.s./L were reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment. An EU agreed endpoint of EC_{50} = 36 mg/L was derived from this test.

Report:	KCA 8.2.4.1/10; [redacted]; [redacted] 2003; M-249534-01-1
Title:	Statement of Bayer CropScience on Questions from the Austrian UBA regarding the submission of the Dossier for Amidosulfuron (AE F075032) Aquatic organisms
Report No.:	C048106
Document No.:	M-249534-01-1
Guideline(s):	**SANCO: Sanco/3268/2001 rev.4**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**no**

This expert statement responds to a question by the former RMS why no analytical measurements were made in the above two Daphnia toxicity studies (KCA 8.2.4.1/01 and KCA 8.2.4.1/02), and in green algae study KCA 8.2.6.1/01. It was argued that

- the procedure is in-line with recommendation given in the Guidance Document on Aquatic Ecotoxicology for handling older studies lacking analysis, since neither daphnia nor algae are driving aquatic risk assessment.
- hydrolysis and photolysis data let expect that amidosulfuron would have been stable under the conditions and over the in-life phase periods of these tests

The case is discussed in the previous DAR (2006), it was concluded in a general comment on the studies with crustaceans: "Since daphnids were not the most sensitive group of organisms and do not represent the most critical endpoint for the aquaitc risk assessment no new studies with daphnids are considered to be necessary."

<u>Studies on the metabolites of amidosulfuron:</u>

Amidosulfuron-desmethyl:
Two studies were submitted and evaluated for the first inclusion of amidosulfuron on Annex I. The lowest endpoint EC_{50} = 3.6 mg/L was selected for this metabolite, while in the other study, no immobilization was observed up to 100 mg/L, the highest concentration tested.
However, the grades of the metabolite tested in both studies are rather different. The highest toxicity is observed for the study with the grade of lower purity (93.0% vs 97.2%) and most importantly, the highest amount of unknown material (5.5% vs 0.9%).
The impurities present in the tested material result from the synthesis of the metabolite and are not relevant for the risk assessment of this metabolite in the environment, as this compound is produced during the degradation of the active ingredient, and not from chemical synthesis. It is therefore proposed to consider only the study performed with the best grade.

Report:	KCA 8.2.4.1/03; [redacted]; [redacted]; 2003; M-211220-01-1
Title:	Acute toxicity to Daphnia magna (waterflea) under static testing conditions AE F101630 substance, technical metabolite of amidosulfuron Code: AE F101630 00 1C97 0001
Report No.:	C026338
Document No.:	M-211220-01-1
Guideline(s):	**EEC directive 92/69/EWG Annex Part C: C.2; OECD: 202**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a static acute toxicity test on *Daphnia magna* with the metabolite amidosulfuron-desmethyl. No immobilization occurred in the controls. One individual died at the lowest test concentration of 1 mg/L. This mortality event was considered as being not related to the test substance since no immobile animals were observed at all other test concentrations. A 48 h EC_{50} > 100 mg/L and a 48 h NOEC = 100 mg/L were reported. Measured concentrations at t0 for the 2 highest concentrations were below 80%, due to solubility issue. It is then proposed to base the results on mean measured values (geomean): 48 h EC_{50} > 55 mg/L and a 48 h NOEC = 55 mg/L.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment. However, no EU agreed endpoint was derived from this test, since a lower endpoint resulted from the corresponding study KCA 8.2.4.1/04.

Report:	KCA 8.2.4.1/04; [redacted]; 1993; M-131833-01-1
Title:	Hoe 101630 substance, technical (Hoe 101630 00 ZC93 0001) Effect to Daphnia magna (waterflea) in a Static-Acute Toxicity Test (method OECD)
Report No.:	A50827
Document No.:	M-131833-01-1
Guideline(s):	**OECD: 202 (1984)**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a static acute toxicity test on *Daphnia magna* with the metabolite amidosulfuron-desmethyl. No immobile daphnids were observed in the control, solvent control and in the test substance up to a concentration of 0.56 mg/L. The percentage of dead individuals rose from 10 to 85 % at the tested concentrations of 1 – 10 mg metabolite/L. The highest mortality of 90 % of the tested animals was observed at a concentration of 5.6 mg metabolite/L. A 48 h EC_{50} = 3.6 mg metabolite/L and a 48 h NOEC = 0.56 mg metabolite/L were reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment. An EU agreed endpoint of EC_{50} = 3.6 mg/L was derived from this test.

It is proposed to change this endpoint to EC_{50} > 55 mg /L based on the further study KCA 8.2.4.1 /03, for the arguments on test item purity discussed above.

Amidosulfuron-ADMP:

Report:	KCA 8.2.4.1/05; [redacted]; 1993; M-131382-01-1
Title:	Hoe 092944 - substance, technical (Hoe 092944 00 ZD99 0001) Effect to Daphnia magna (waterflea) in a Static -Acute Toxicity Test (method OECD)
Report No.:	A50353
Document No.:	M-131382-01-1
Guideline(s):	**OECD: 202 (1984)**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a static acute toxicity test on *Daphnia magna* with the metabolite amidosulfuron-ADMP. No immobile daphnids were observed in the controls or in the tested concentrations up to 32 mg metabolite/L. The percentages of dead individuals were 20 %, 15 %, 20 %, 95 % and 100 % at the tested concentrations of 56, 100, 180, 320 and 560 mg metabolite/L. A 48 h EC_{50} = 223 mg metabolite/L and a 48 h NOEC = 32 mg metabolite/L were reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment. An EU agreed endpoint of EC_{50} = 223 mg/L was derived from this test.

Amidosulfuron-ADHP:

Report:	KCA 8.2.4.1/06; [redacted]; 1993; M-131835-01-1
Title:	Hoe 094206 - Substance, technical (Hoe 094206 00 ZC99 0002) - Effect to Daphnia magna (waterflea) in a static-acute toxicity test (Method OECD)
Report No.:	A50833
Document No.:	M-131835-01-1
Guideline(s):	**OECD: 202 (1984)**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a static acute toxicity test on *Daphnia magna* with the metabolite amidosulfuron-ADHP. No immobile animals were observed in the controls or in the tested concentrations of the metabolite. A 48 h EC_{50} > 100 mg metabolite/L and a 48 h NOEC = 100 mg metabolite/L were reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

Although the concentration of the test substance was not measured in the test vessels, the study was considered to be acceptable and was used for the risk assessment. An EU agreed endpoint of EC_{50} > 100 mg/L was derived from this test.

Amidosulfuron-Lysimeter leachates

Report:	KCA 8.2.4.1/07; [redacted]; 1993; M-131134-01-1
Title:	Hoe 075032: The Effect of lysimeter percolates (Lysimeter no. IX) to Daphnia magna (waterflea) in a static-acute toxicity test (method DIN)
Report No.:	A50100
Document No.:	M-131134-01-1
Guideline(s):	**Guidelines of Deutsche Normung DIN 38412 – L30 (1989)**
Guideline deviation(s):	**Yes, see report**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The static acute study reports on the effect of lysimeter percolates treated with amidosulfuron technical a.s. to *Daphnia magna*. No immobile animals were observed in the tested percolates and in the control until the end of the test after 48 hours. The percolates from a lysimeter treated with 49 g a.s./ha were not toxic to *Daphnia magna*.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment. An EU agreed endpoint "no effects" was derived from this test.

Report:	KCA 8.2.4.1/08; [redacted]; 1993; M-131133-01-1
Title:	Hoe 075032: The Effect of lysimeter percolates (Lysimeter no. X) to Daphnia magna (waterflea) in a static-acute toxicity test (method DIN)
Report No.:	A50099
Document No.:	M-131133-01-1
Guideline(s):	**Guidelines of Deutsche Normung DIN 38412 – L30 (1989)**
Guideline deviation(s):	**Minor deviation see Point 4.1**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The static acute study reports on the effect of lysimeter percolates treated with amidosulfuron technical a.s. to *Daphnia magna*. No immobile animals were observed in the tested percolates and in the control until the end of the test after 48 hours. The percolates from a lysimeter treated with 54 g a.s./ha were not toxic to *Daphnia magna*.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment An EU agreed endpoint "no effects" was derived from this test.

Report:	KCA 8.2.4.1/09; [redacted]; 1993; M-131854-01-1
Title:	The Effect of lysimeter percolates (untreated control lysimeter no. IV) to Daphnia magna (waterflea) in a static-acute toxicity test (method DIN)
Report No.:	A50855
Document No.:	M-131854-01-1
Guideline(s):	**Guidelines of Deutsche Normung DIN 38412 – L30 (1989)**
Guideline deviation(s):	**For deviation see Point 4.1**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The static acute study reports on the effect of untreated control lysimeter percolates to *Daphnia magna*. No immobile animals were observed in the tested percolates and in the control until the end of the test after 48 hours. The percolates from the untreated lysimeter was not toxic to *Daphnia magna*.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The results of the study were rated acceptable, however the study was considered not to be relevant for risk assessment. No EU agreed endpoint was derived from this test.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

One acute study on *Mysidopsis bahia* was performed. Details of the study are provided in the following table.

Table CA 8.2.4.2- 1: Acute toxicity data of amidosulfuron to *Mysidopsis bahia* presented in this chapter

Test organism	Test system	Test duration	Endpoint [mg/L]	Reference
Mysidopsis bahia (mysid shrimp)	static acute	96 h	LC_{50} (nom) > 94 NOEC 56	[redacted]; 1989; M-123930-01-1 KCA 8.2.4.2 /01

(nom) nominal concentration

Report:	KCA 8.2.4.2/01; [redacted]; 1989; M-123930-01-1
Title:	Static acute toxicity of sample number Hoe 075032 to the mysid, Mysidopsis bahia
Report No.:	A40985
Document No.:	M-123930-01-1
Guideline(s):	**USEPA=EPA 72-3**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a static acute toxicity test on *Mysidopsis bahia* with amidosulfuron technical a.s. One individual died in the solvent control and one was found dead at a concentration of 38 mg a.s./L after 24 hours. No further animals died at the tested concentration of 38 mg a.s./L and at concentrations up to 56 mg a.s./L until the end of test after 96 hours. Therefore, the mortality observed at the concentration of 38 mg a.s./L was considered not being related to the test substance. 95 % of the mysids died at the highest test concentration of 94 mg a.s./L within 96 hours. A 96 h LC_{50} = 75 mg a.s./L and a 96 h NOEC = 56 mg a.s./L were reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The results of the study were used as confirmatory data only, but were not used for the risk assessment. No EU agreed endpoint was derived from this test.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

One reproductive study on *Daphnia magna* was performed. Details of the study are provided in the following table.

Table CA 8.2.5.1- 1: Reproductive toxicity data of amidosulfuron to *Daphnia magna* presented in this chapter

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
Daphnia magna (water flea)	chronic	21 d	NOEC reproduction: 1 (nom) EC_{10} reproduction: 0.8 EC_{20} reproduction: 1.4	[redacted]; 1991; M-130193-01-1 KCA 8.2.5.1 /01 [redacted]; 2016; M-551834-01-1 KCA 8.2.5.1 /02

Bold letters: Values considered relevant for risk assessment in the MCP document
(nom) nominal concentration

Report: KCA 8.2.5.1/01; [redacted]; 1991; M-130193-01-1
Title: Hoe 075032 - substance, technical (Hoe 075032 00 ZC94 0001) Effect to Daphnia magna (Waterflea) in a 21-day Reproduction Test (method OECD)
Report No.: A46135
Document No.: M-130193-01-1
Guideline(s): **OECD: 202 (1984)**
Guideline deviation(s): **Deviation not specified**
GLP/GEP: **yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

Report: KCA 8.2.5.1/02; [redacted]; 2016; M-551834-01-1
Title: EC10/EC20 calculation for Daphnia reproduction study with amidosulfuron (M-130193-01-1, [redacted], 1991)
Report No.: M-551834-01-1
Document No.: M-551834-01-1
Guideline(s): **Not relevant**
Guideline deviation(s): none
GLP/GEP: **no**

The study reports on a semi static 21-day reproduction test on *Daphnia magna* with amidosulfuron technical a.s. All animals died at the highest test concentration of 10 mg a.s./L within 7 days of exposure. Immobilization of adult individuals and delayed brood was observed at a concentration of 3.2 mg a.s./L. Release of brood started at day 9 at concentrations below 3.2 mg a.s./L and in controls. The number of living juveniles was not significantly different from the controls up to a concentration of 1 mg/L. A 21 d NOEC = 1 mg a.s./L and a 21 d EC_{50} = 3.2 mg a.s./L were reported.
EC_{10} and EC_{20} are 0.8 (0.3 – 1.1) and 1.4 (0.9 – 1.8) mg/L, respectively (M-551834-01-1).

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment. An EU agreed endpoint of $NOEC_{reproduction}$ = 1 mg/L was derived from this test.

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

Amidosulfuron has no insecticidal activity and no relevant chronic effects on *Daphnia magna* have been observed. No additional chronic testing with aquatic invertebrate species is deemed necessary.

CA 8.2.5.3 Development and emergence in *Chironomus riparius*

Amidosulfuron has no insecticidal activity, is not a growth regulator, and no relevant chronic effects on *Daphnia magna* have been observed. No additional chronic testing with aquatic invertebrate species is deemed necessary.

CA 8.2.5.4 Sediment dwelling organisms

Amidosulfuron is highly water soluble and does not accumulate in the sediment. No testing with sediment dwelling organisms is triggered.

CA 8.2.6 Effects on algal growth

Some metabolites relevant for risk assessment have been tested for their toxicity on algal growth, these are the primary metabolites A-desmethyl and ADMP. In addition a secondary metabolite (ADHP) was also tested.

Despite the presence of the structural group responsible for sulfonylureas herbicidal activity (Sinclair, 2009[7]), tests demonstrated a lack of herbicidal activity for both A-desmethyl (KCA 8.6.1 /02) and A-desmethyl-chloropyrimidine (KCA 8.6.1 /06). Moreover, the same results were obtained with ADMP (KCA 8.6.1 /08), ADHP (KCA 8.6.1 /03), A-guanidine (KCA 8.6.1 /06) and A-biuret (KCA 8.6.1 /07). Therefore all metabolites lost the toxophore responsible for the biological target activity (i.e. herbicidal activity). According to the risk assessment scheme for metabolites (pp 143-144) of the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013[8]), risk assessment for metabolites without toxophore can be based on active substance data. Consequently further tests on algae with A-desmethyl chloropyrimidine, A-guanidine and A-biuret were not deemed necessary.

Guanidinocarbonyl sulfamic acid is also a major metabolite for the aquatic environment. No tests were performed to characterize neither its toxicity nor its herbicidal activity. It is a tertiary metabolite

[7] CJ Sinclair PhD Thesis University of York Predicting the environmental fate and ecotoxicological and toxicological effects of pesticide transformation products
https://www.researchgate.net/publication/235934684_Predicting_the_environmental_fate_and_ecotoxicological_and_toxicological_effects_of_pesticide_transformation_products

[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

resulting from the degradation of amidosulfuron-guanidine which does not show any herbicidal activity. Consequently, it is assumed that the toxophore is no longer present in this metabolite and the risk assessment can be addressed using information from the parent substance.

Table CA 8.2.6- 1: Growth effect data of amidosulfuron and its metabolites to algae presented in this chapter

Test species	Test system	Test duration	Endpoint [mg/L]	Reference
Amidosulfuron				
Scenedesmus subspicatus (green alga)	growth inhibition	72 h	E_rC_{50} [1)] **145**(nom) E_rC_{10} 13 E_rC_{20} 29	[redacted]; 1988; M-120327-01-1 KCA 8.2.6.1 /01 [redacted]; 2016; M-549424-01-1 KCA 8.2.6.1/08
		72 h[9]	NOEC 3.2	
Navicula pelliculosa (diatom)	growth inhibition	72 h /96 h	E_rC_{50} [1)] > 84.2(nom) E_rC_{10} > 84.2 E_rC_{20} > 84.2 NOEC 84.2	[redacted]; [redacted]; [redacted]; 1999; M-181803-01-1 KCA 8.2.6.2 /01
Amidosulfuron-desmethyl				
Scenedesmus subspicatus (green alga)	growth inhibition	72 h	E_rC_{50} [1)] >1000(nom) E_rC_{10} 978 E_rC_{20} > 1000 NOEC 100	[redacted]; 1993; M-132028-01-1 KCA 8.2.6.1 /02 [redacted]; 2016; M-549438-02-1 KCA 8.2.6.1 /09
Amidosulfuron-ADMP				
Scenedesmus subspicatus (green alga)	growth inhibition	72 h	E_rC_{50} [1)] > **560**(nom) E_rC_{10} 283 E_rC_{20} 463 NOEC 56	[redacted]; 1993; M-131421-01-1 KCA 8.2.6.1 /03 [redacted]; 2016; M-549790-01-1 KCA 8.2.6.1/11
Amidosulfuron-ADHP				
Scenedesmus subspicatus (green alga)	growth inhibition	72 h	E_rC_{50} [1)] > 100(nom) E_rC_{10} 51 E_rC_{20} 72 NOEC 32	[redacted]; 1993; M-138952-01-1 KCA 8.2.6.1 /07 [redacted]; 2016; M-549441-02-1 KCA 8.2.6.1/12

[9] The study duration is 72 h but the endpoints were calculated at 48h for validity reasons (see: KCA 8.2.6.1/08 for further explanations)

Test species	Test system	Test duration	Endpoint [mg/L]		Reference
Amidosulfuron-Lysimeter leachate					
Scenedesmus subspicatus (green alga)	growth inhibition	72 h	E_rC_{50} [1)]	**no effects**	[redacted]; 1992; M-138143-01-1 KCA 8.2.6.1 /04
				no effects	[redacted]; 1993; M-138605-01-1 KCA 8.2.6.1 /05
				no effects [untreated control]	[redacted]; 1993; M-131492-01-1 KCA 8.2.6.1 /06

Bold letters: Values considered relevant for risk assessment in the MCP document
[1)] Since the new aquatic GD10 focusses on endpoints based on growth rates the EbC50 figures were omitted from the table above.
(nom) nominal concentration

CA 8.2.6.1 Effects on growth of green algae

Studies on amidosulfuron:

Report: KCA 8.2.6.1/01; [redacted]; 1988; M-120327-01-1
Title: The Effect of Hoe 075032 OH substance, technical (Identification code : Hoe 075032 OH ZC96 0001 00) on Scenedesmus subspicatus CHODAT (Green alga) in a Growth Inhibition Test (method OECD)
Report No.: A 38704
Document No.: M-120327-01-1
Guideline(s): **OECD: 201 (1984)**
Guideline deviation(s): **Deviation not specified**
GLP/GEP: **yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

Report: KCA 8.2.6.1/08; [redacted]; 2016; M-549424-01-1
Title: Validity check and calculation of ErC50 for amidosulfuron study on Desmodesmus subspicatus (M-120327-01-1, [redacted] 1988)
Report No.: M-549424-01-1
Document No.: M-549424-01-1
Guideline(s): **OECD: 201 (2011)**
Guideline deviation(s): **Deviation none**
GLP/GEP: **no**

The study reports on a static 72-hour growth inhibition test on *Scenedesmus subspicatus* with amidosulfuron technical a.s. The test was performed in two dilution series of amidosulfuron. In the first dilution series low growth inhibition at the highest concentration and an equivocal concentration-effect relationship were observed. Therefore, the results of the second dilution series were used for the

[10] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290

calculation of the E_bC_{50} value. In the second dilution series growth inhibition was observed at concentrations at and above 10 mg a.s./L. No significant effects on growth were found up to a concentration of 3.2 mg a.s./L. A 72 h E_bC_{50} = 47 mg a.s./L and a 72 h NOEC = 3.2 mg a.s./L were reported.

The validity criteria of the new version of the OECD guideline 201 (July 2011) have been checked for this study:

- The biomass in the control increased by a factor 49 over the 72 h test period (guideline criteria = 16);
- The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%, for this specific study, the observed value is 52.1;
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%, it was 3.3 in this study.

The second criterium is not met, this is explained by the fact that the growth was no longer in exponential phase after 48 h, there was an obvious decrease of the growth rate.

The OECD guideline 201 (July 2011) states that:
The test period may be shortened to at least 48 hours to maintain unlimited, exponential growth during the test as long as the minimum multiplication factor of 16 is reached.(§ 11, page 2). The validity of the study at 48h was also checked and all criteria were met: the biomass increased by a factor of 28, the coefficient of variation of average specific growth rates is 17,6 and the coefficient of variation of average specific growth rates is 6,6.

Consequently the 48h E_rC_{50} is considered to be the relevant endpoint: 48h E_rC_{50} = 145 mg a.s./L (95% confidence interval: 106-198 mg a.s./L). The corresponding E_rC_{10} and E_rC_{20} are 13 (5.5-21.3) and 29 (16-43) mg a.s./L, respectively (M-549424-01-1).

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. An EU agreed endpoint of E_bC_{50} = 47 mg/L for *Scenedesmus subspicatus* was derived based on this test. It is proposed to change this endpoint to the E_rC_{50} = 145 mg a.s./L according to EFSA Aquatic Guidance document.

Studies on the metabolites of amidosulfuron:

Amidosulfuron-desmethyl:

Report:	KCA 8.2.6.1/02; [redacted]; 1993; M-132028-01-1
Title:	Hoe 101630 - substance, technical (Hoe 101630 00 ZC93 0001) Effect to Scenedesmus subspicatus (Green alga) in a Growth Inhibition Test (method OECD)
Report No.:	A51046
Document No.:	M-132028-01-1
Guideline(s):	**OECD: 201 (1984)**
Guideline deviation(s):	**not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

Report:	KCA 8.2.6.1/09; [redacted]; 2016; M-549438-02-1
Title:	Validity check and EC10/EC20 calculation for amidosulfuron-desmethyl study on Desmodesmus subspicatus (M-132028-01-1, [redacted], 1993)
Report No.:	M-549438-02-1
Document No.:	M-549438-02-1
Guideline(s):	**OECD: 201 (2011)**
Guideline deviation(s):	**Deviation none**
GLP/GEP:	**no**

The study reports on a static 72-hour growth inhibition test on *Scenedesmus subspicatus* with the metabolite amidosulfuron-desmethyl. A dose dependant biomass growth inhibition ranging from -1.2 % to 37.7 % was observed at the tested concentrations from 56 mg/L to 1000 mg/L. Significant growth inhibition was found at a concentration of 180 mg metabolite/L. No calculation of the E_bC_{50}/E_rC_{50} values was conducted because 50 % growth inhibition was not exceeded at the tested concentrations. A 72 h E_bC_{50}/E_rC_{50} > 1000 mg metabolite/L and a 72 h NOEC = 100 mg metabolite/L were reported.

72h E_rC_{10} and E_rC_{20} are 978 (910-1063) and > 1000 mg metabolite/L, respectively.

The validity criteria of the new version of the OECD guideline 201 (July 2011) have been checked for this study:

- -The biomass in the control increased by a factor 140 over the 72 h test period (guideline criteria = 16);
- The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%, for this specific study, the observed value is 11.5;
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%, it was 1.1 in this study.
-

All 3 criteria were met, the study is considered to be valid (M-549438-02-1).

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. An EU agreed endpoint of 72h E_bC_{50} = E_rC_{50} >1000 mg/L for *Scenedesmus subspicatus* was derived based on this test.

Amidosulfuron-ADMP:

Report:	KCA 8.2.6.1/03; [redacted]; 1993; M-131421-01-1
Title:	Hoe 092944 - Substance Technical (Hoe 092944 00 ZD99 0001) Effect to Scenedesmus subspicatus (Green alga) in a Growth Inhibition Test (method OECD)
Report No.:	A50395
Document No.:	M-131421-01-1
Guideline(s):	**OECD: 201 (1984)**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

Report:	KCA 8.2.6.1/10; [redacted]; 2016; M-549437-01-1
Title:	Validity check for amidosulfuron-ADMP study on Desmodesmus subspicatus (M-131421-01-1, [redacted] 1993)
Report No.:	M-549437-01-1
Document No.:	M-549437-01-1
Guideline(s):	**OECD: 201 (2011)**
Guideline deviation(s):	**Deviation none**
GLP/GEP:	**no**

Report:	KCA 8.2.6.1/11; [redacted]; 2016; M-549790-01-1
Title:	Calculation of EC10 and EC20 for algae study (M-131421-01-1) in context of mesosulfuron approval renewal (EFSA request, Point 42)
Report No.:	M-549790-01-1
Document No.:	M-549790-01-1
Guideline(s):	**Not relevant**
Guideline deviation(s):	**none**
GLP/GEP:	**no**

The study reports on a static 72-hour growth inhibition test on *Scenedesmus subspicatus* with the metabolite amidosulfuron-ADMP. No growth inhibition was observed up to a concentration of 100 mg metabolite/L. A dose dependant biomass growth inhibition ranging from 22.4 % to 67.6 % was observed at the tested concentrations from 180 mg/L to 560 mg/L. A 72 h E_bC_{50} = 403 mg metabolite/L and a 72 h NOEC = 56 mg metabolite/L were reported.

72h E_rC_{10} and E_rC_{20} are 283 (235-321) and 403 (421-511) mg metabolite/L respectively (M-549790-01-1).

The validity criteria of the new version of the OECD guideline 201 (July 2011) have been checked for this study:

- -The biomass in the control increased by a factor 107 over the 72 h test period (guideline criteria = 16);
- The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%; for this specific study, the observed value is 22.6;
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%, it was 6.3 in this study.

All 3 criteria were met, the study is considered to be valid (M-549437-01-1).

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. An EU agreed endpoint of 72h E_bC_{50} = 403 mg/L for *Scenedesmus subspicatus* was derived based on this test.

The new aquatic guidance document (EFSA 2013) only regards endpoints based on growth rates as relevant. Accordingly, the listed endpoint should be revised to:
E_rC_{50} > 560 mg/L.

Amidosulfuron-ADHP:

Report:	KCA 8.2.6.1/07; [redacted]; 1993; M-138952-01-1
Title:	Hoe 094206 - Substance, technical (Hoe 094206 00 ZC99 0002) - Effect to Scenedesmus subspicatus (geen alga) in a growth inhibition test (method OECD)
Report No.:	A49948
Document No.:	M-138952-01-1
Guideline(s):	**OECD: 201 (1984)**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

Report:	KCA 8.2.6.1/12; [redacted]; 2016; M-549441-02-1
Title:	Validity check and EC10/EC20 calculation for amidosulfuron-ADHP study on Desmodesmus subspicatus (M-138952-01-1; [redacted], 1993)
Report No.:	M-549441-02-1
Document No.:	M-549441-02-1
Guideline(s):	**OECD: 201 (2011)**
Guideline deviation(s):	**Deviation none**
GLP/GEP:	**no**

Executive Summary:
The aim of this study was to determine the effect of the metabolite amidosulfuron-ADHP to *Scenedesmus subspicatus* in a growth inhibition test. The test was performed in accordance with OECD guideline 201 (1984). Algae were exposed for 72 hours under static exposure conditions to nominal concentrations of 0.032, 0.10, 0.32, 1.0, 3.2, 10, 18, 32, 56 and 100 mg metabolite/L in comparison to untreated control. Three replicates for each treated group and six replicates for the control were used. 24, 48 and 72 hours after test initiation cell counts were determined for each test flask. The concentrations of test substance inhibiting the growth and the resulting E_bC_{50} and E_rC_{50} were determined by using computerized programs. The concentration of no observed effects (NOEC) was verified by a multiple t-test. Based on analytical findings, the biological endpoints are reported as nominal figures. After 72 hours the E_bC_{50} was determined as 70 mg/L (95 % CL: 56 – 100 mg/L). The E_rC_{50} could not be calculated because the inhibition of the specific growth rate did not exceed 34% in the highest tested concentration. Therefore the E_rC_{50} is > 100 mg/L. The no observed effect concentration (NOEC) was found after 72 hours at 32 mg/L. The lowest observed effect concentration was found at 56 mg/L.

Material and methods:
Test item: Amidosulfuron-ADHP; Material: Hoe 094206, Code: Hoe 094206 00 ZC99 0002, Sample-No.: Roe/GY 751, YP 129, Purity: 99.9% w/w.

Scenedesmus subspicatus CHODAT (unicellular planktonic green algae) were exposed in a growth inhibition test for 72 hours to nominal concentrations of 0.032, 0.10, 0.32, 1.0, 3.2, 10, 18, 32, 56 and 100 mg metabolite/L in comparison to untreated control. The test volume was 100 mL test medium per replicate. The study was carried out with 3 replicates in each of the treated groups and 6 replicates in the control group. 24, 48 and 72 hours after test initiation cell counts were determined for each test flask. At the same time the pH values at each test flask and temperature at each concentration step were assessed. The concentrations of test substance inhibiting the growth and the resulting E_bC_{50} and E_rC_{50} were determined by using computerized programs. The concentration of no observed effects (NOEC) was verified by a multiple t-test. At the end of testing the growth curves and the concentration effect relationship were plotted using a computer program.

The pH values ranged from 7.5 to 10.0 in all replicates and the incubation temperature ranged from 24.5 °C to 28.8 °C over the whole period of testing at a continuous illumination using white spectrum fluorescent lamps and a quantum flux density of 180 ± 12 $\mu E.m^{-2}.s^{-1}$.

Chemical analyses of the test substance concentration in the test water was made in a stability test after 0, and 72 hours test duration from the nominal concentration of 18.0 mg/L by HPLC-analysis.

Dates of experimental work: July 28, 1992 to September 11, 1992

Results:

Validity:
No unforeseen circumstances were observed which may have affected the quality or integrity of this study.

Moreover, the criteria of the latest version of the OECD 201 guideline (July 2011) are all met. The biomass in the control increased by a factor of 150 over the 72h test period. The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%, for this specific study, the observed value is 21.3. And the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%, it was 5.3 in this study. (M-549441-02-1).

In a Growth Inhibition Test (report CE92/006) carried out January 27-30, 1992 to determine the effects of the reference substance potassium bichromate to *Seenedesmus subspicatus* (Green alga) the E_bC_{50} after 72 hours test duration was calculated with 95% confidence limits at 0.19 (0.10 - 0.32) mg/L when compared with the untreated control group. The no observed effect concentration was found at 0.1 mg/L.

Analytical findings:
In the stability test it was shown that the test substance is stable under test conditions with analysed values >80% of the nominal concentrations. Therefore nominal concentrations are used for reporting. In the concentrations higher than 18 mg/L the test substance was not completely solubilized and flocculated.

Table CA 8.2.6.1- 1: Concentrations of amidosulfuron-ADHP in the test solutions at day 0 and 3

		Nominal concentration (mg amidosulfuron-ADHP/L)	
		Control	**18.0**
Theoretical content of the test item in the test medium in mg metabolite/L		0.0	18.0
Theoretical content of the metabolite calculated from amount in test item (purity: 99.9%) in mg metabolite/L		0.0	17.982
Actual concentration in mg metabolite/L	Day 0	0.508	16.304
	Day 3	0.588	14.743
	Mean	0.55	15.52

Table CA 8.2.6.1- 2: Concentrations of amidosulfuron-ADHP in the test solutions at day 0 and 3 (continued)

		Nominal concentration (mg amidosulfuron-ADHP/L)	
		Rec.[1]	**18.0**
Actual concentration in % metabolite/L	Day 0	87.2[2]	90.7
	Day 3	87.2	82.0
	Mean	87.2	86.3

[1] Concurrent recovery rate of laboratory fortifications prepared on the corresponding test day.
Reference substance: w (Hoe 094206 00 ZC99 0001) = 99.6 %

[2] Recovery from Day 3.

Biological findings:
Observations are listed as follows:

Table CA 8.2.6.1- 3: Effects after 72 hour on algae growth inhibition test

Nominal concentration [mg metabolite/L]	Mean area under the growth curves in 10^4 cells/mL*h	Mean growth rate in h^{-1}	Percent inhibition cell growth (area)	Percent inhibition growth rate
control	2873.2	0.069	0.0	0.0
1.0	2996.8	0.070	-4.302	-0.4792
3.2	2985.2	0.070	-3.898	-1.2165
10.0	3050.4	0.070	-6.167	-1.2496
18.0	3452.8	0.072	-20.173	-4.3855
32.0	2618.4	0.068	8.868	2.0839
56.0	1798.4	0.061	37.408	12.4357
100.0	857.6	0.046	70.152	33.7216

After 72 hours test duration the E_rC_{50} was calculated at 70 mg/L (95% confidence limits 56 - 100 mg/L) in comparison with the untreated control. The E_rC_{50} could not be calculated because the inhibition of the specific growth rate did not exceed 34% in the highest tested concentration. It is therefore > 100 mg/L.

Significant inhibition of growth in comparison with the control was observed after 72 hours at concentrations to and higher than 56 mg/L.

In the concentrations of 56 and 100 mg/L cell nuclei were enlarged and several cells formed lumps.
The no observed effect concentration (NOEC) as defined in the OECD-test guidelines (no significant growth inhibition and no cell deformation) was found after 72 hours at 32 mg/L. The lowest observed effect concentration was found at 56 mg/L. 72h E_rC_{10} and E_rC_{20} are 51 (46-55) and 72 (68-75) mg metabolite/L, respectively.

Conclusions:
After 72 hours test duration the E_bC_{50} was calculated at 70 (95% confidence limits 56 - 100) mg/L in comparison with the untreated control group. The E_rC_{50} is > 100 mg/L. The no observed effect concentration (NOEC) as defined in the OECD-test guidelines (no significant growth inhibition and no cell deformation) was found after 72 hours at 32 mg/L. The 72h E_rC_{10} and E_rC_{20} are 51 (46-55) and 72 (68-75) mg metabolite/L, respectively.

Amidosulfuron-Lysimeter leachate:

Report:	KCA 8.2.6.1/04; [redacted]; 1992; M-138143-01-1
Title:	Hoe 075032: The Effect of lysimeter percolate (Lysimeter no. IX) to Scenedesmus subspicatus Chodat (geen alga) in a growth inhibition test (method DIN/ISO)
Report No.:	A49062
Document No.:	M-138143-01-1
Guideline(s):	**Guidelines of Deutsche Normung DIN 38412 - L9 (adopted May 1989); International Standard ISO 8692 (adopted November 1989)**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

Report:	KCA 8.2.6.1/13; [redacted]; 2016; M-549422-01-1
Title:	Validity check for amidosulfuron lysimeter-leachate study on Desmodesmus subspicatus (M-138143-01-1, [redacted] 1992)
Report No.:	M-549422-01-1
Document No.:	M-549422-01-1
Guideline(s):	**OECD: 201 (2011)**
Guideline deviation(s):	**Deviation none**
GLP/GEP:	**no**

The static 72-hour growth inhibition study reports on the effect of lysimeter percolates treated with amidosulfuron technical a.s. to *Scenedesmus subspicatus*. No significant growth inhibition was observed at all tested dilutions and undiluted percolates. The percolate from a lysimeter treated with 49 mg a.s./ha did not inhibit the growth of *Scenedesmus subspicatus*.

The validity criteria of the new version of the OECD guideline 201 (July 2011) have been checked for this study:

- The biomass in the control increased by a factor 83 over the 72 h test period (guideline criteria = 16);
- The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%, for this specific study, the observed value is 27.8;
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%, it was 3.2 in this study.

All 3 criteria were met, the study is considered to be valid (M-549422-01-1).

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment. An EU agreed endpoint "no effects" was derived from this test.

Report:	KCA 8.2.6.1/05; [redacted]; 1993; M-138605-01-1
Title:	Hoe 075032: The Effect of lysimeter percolate (Lysimeter no. X) to Scenedesmus subspicatus Chodat (geen alga) in a growth inhibition test (method DIN/ISO)
Report No.:	A49569
Document No.:	M-138605-01-1
Guideline(s):	**Guidelines of Deutsche Normung DIN 38412 - L9 (adopted May 1989); International Standard ISO 8692 (adopted November 1989)**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

Report:	KCA 8.2.6.1/14; [redacted]; 2016; M-549423-01-1
Title:	Validity check for amidosulfuron lysimeter leachate study on Desmodesmus subspicatus (M-138605-01-1, [redacted], 1993)
Report No.:	M-549423-01-1
Document No.:	M-549423-01-1
Guideline(s):	**OECD: 201 (2011)**
Guideline deviation(s):	**Deviation none**
GLP/GEP:	**no**

The static 72-hour growth inhibition study reports on the effect of lysimeter percolates treated with amidosulfuron technical a.s. to *Scenedesmus subspicatus*. No significant growth inhibition was observed at all tested dilutions of the percolates. The percolate from a lysimeter treated with 54 mg a.s./ha did not inhibit the growth of *Scenedesmus subspicatus*.

The validity criteria of the new version of the OECD guideline 201 (July 2011) have been checked for this study:

- -The biomass in the control increased by a factor 135 over the 72 h test period (guideline criteria = 16);
- The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%, for this specific study, the observed value is 9.8;
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%, it was 4.1in this study.

All 3 criteria were met, the study is considered to be valid (M-549423-01-1).

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment. An EU agreed endpoint “no effects” was derived from this test.

Report:	KCA 8.2.6.1/06; [redacted]; 1993; M-131492-01-1
Title:	The Effect of Lysimeter Percolate (Untreated Control Lysimeter no. IV) to Scenedesmus subspicatus CHODAT (Green alga) in a Growth Inhibition Test (method DIN/ISO)
Report No.:	A90475
Document No.:	M-131492-01-1
Guideline(s):	**Guidelines of Deutsche Normung DIN 38412 - L9 (adopted May 1989); International Standard ISO 8692 (adopted November 1989)**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

Report:	KCA 8.2.6.1/15; [redacted]; 2016; M-549418-01-1
Title:	Validity check for amidosulfuron lysimeter leachate study on Desmodesmus subspicatus (M-131492-01-1, [redacted], 1993)
Report No.:	M-549418-01-1
Document No.:	M-549418-01-1
Guideline(s):	**OECD: 201 (2011)**
Guideline deviation(s):	**Deviation none**
GLP/GEP:	**no**

The static 72-hour growth inhibition study reports on the effect of untreated control lysimeter percolates to *Scenedesmus subspicatus*. A statistically significant effect in growth rates was observed at a dilution of 2. However, growth inhibition was very low and the significance of the value is

probably linked to the very low variation in the control. No statistically significant effect was observed at the dilution factor of 4.

The validity criteria of the new version of the OECD guideline 201 (July 2011) have been checked for this study:

- The biomass in the control increased by a factor 149 over the 72 h test period (guideline criteria = 16);
- The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%, for this specific study, the observed value is 33.4;
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%, it was 1.0 in this study.

All 3 criteria were met, the study is considered to be valid (M-549418-01-1).

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The results of the study were rated acceptable, however the study was considered to not be relevant for risk assessment. No EU agreed endpoint was derived from this test.

CA 8.2.6.2 Effects on growth of an additional algal species

For amidosulfuron, an aquatic toxicity study on an additional algal species, *Navicula pelliculosa,* was performed.

Report:	KCA 8.2.6.2/01; [redacted]; [redacted]; [redacted]; 1999; M-181803-01-1
Title:	Amidosulfuron (prov. approved ISO) substance, technical Code: AE F075032 00 1 D990004 Algal growth inhibition - Navicula pelliculosa
Report No.:	C001109
Document No.:	M-181803-01-1
Guideline(s):	**EU directive 93/69/EWG Annex Part C, 8.3; OECD: 201; USEPA (=EPA) Subdivision J, § 123-2**
Guideline deviation(s):	**for deviation see Point 2.5**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

Report:	KCA 8.2.6.2/02; [redacted], 2016; M-549436-01-1
Title:	Validity check for amidosulfuron study on Navicula pelliculosa (M-181803-01-1, [redacted], 1999)
Report No.:	M-549436-01-1
Document No.:	M-549436-01-1
Guideline(s):	**OECD: 201 (2011)**
Guideline deviation(s):	**Deviation none**
GLP/GEP:	**no**

The study reports on a static 96-hour growth inhibition test on *Navicula pelliculosa* with amidosulfuron technical a.s. Growth inhibition was more pronounced after 72 hours than after 96 hours of exposure. Statistically significant inhibition of growth was not observed at a significance level of p = 0.05. A 96 h E_bC_{50}/E_rC_{50} > 84.2 mg a.s./L and a 96 h NOEC = 84.2 mg a.s./L were reported.

No growth inhibition was observed at the highest concentration so EC_{10} and EC_{20} values can not be calculated, they are both > 84.2 mg a.s./L.

The validity criteria of the new version of the OECD guideline 201 (July 2011) have been checked for this study:

- -The biomass in the control increased by a factor 16 over the 96 h test period (guideline criteria = 16);
- The mean coefficient of variation for section-by-section specific growth rates in the control cultures must not exceed 35%, for this specific study, the observed value is 52.8;
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 10%, it was 7.5 in this study.

One of the validity criteria is not met. Nevertheless the study was not repeated for several reasons:

- The section-by section high variability is due to the initial lag phase, whereas no stationary phase is reached over the study duration, which is an important criteria to assess the validity of the study,
- No effects were observed in this study up to the highest concentration,
- Diatoms are less sensitive than green algae which are far less sensitive than macrophytes (4 orders of magnitude). Therefore Diatoms are not critical for the risk assessment.
- Technical reasons such as the counting method and timing, were not optimum in old studies, not designed to minimize the growth variability in the controls (M-549436-01-1)

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. An EU agreed endpoint of (96h) E_bC_{50} = E_rC_{50} >84.2 mg/L for *Navicula pelliculosa* was derived based on this test.

CA 8.2.7 Effects on aquatic macrophytes

For amidosulfuron, toxicity studies on aquatic macrophytes clearly indicated vascular plant to represent the overall most sensitive group of aquatic organisms. To provide a thorough description of susceptibility, the following tests on active substance are summarised:

Standard test:

KCA 8.2.7.01: Lemna growth inhibition test with 14 days exposure time

Standard test including investigations on recovery potential:

KCA 8.2.7.02: Lemna laboratory growth inhibition & recovery test with
7 days exposure phase followed by 7 days recovery phase

Supportive information for effect on a further aquatic macrophyte species:

KCA 8.2.7.10 & 11: public literature data on sensitivity of *Myriophyllum*.

Moreover, all primary and secondary metabolites relevant for risk assessment, and the hydolysis product amidosulfuron-sulfamic acid, have been tested for growth inhibition on *Lemna*. Even though this species represents the most sensitive aquatic organism for parent substance amidosulfuron, effect of all metabolites was found orders of magnitude lower than of parent substance, or fully absent.

Guanidinocarbonyl sulfamic acid is a tertiary degradate observed in water/sediment. No tests were performed to characterize its effect on aquatic macrophytes, however the component results from the

degradation of amidosulfuron-guanidine which does not show relevant effect on aquatic macrophytes, nor herbicidal activity (cf. KCA 8.6.1 /06 and KCA 8.6.1 /07). Consequently, it is assumed that the toxophore is no longer present in this metabolite. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA, 2013[11]) proposes that in a non-testing approach risk assessment for metabolites without toxophore can be conservatively based on active substance data.

An overview summary of endpoint information on aquatic macrophytes is provided in Table CA 8.2.7-1 below

Table CA 8.2.7- 1: Effect data of amidosulfuron and metabolites to aquatic macrophytes presented in this chapter

Test species	Test system	Test duration	Endpoint	[mg as/L]	Reference
Amidosulfuron					
Lemna gibba (duck weed)	growth inhibition, static	14 d	E_rC_{50} NOEC	0.0176 0.0074	[redacted]; 1993; M-138621-01-1 KCA 8.2.7 /01
Lemna gibba (duck weed)	growth inhibition + recovery	7 d + 7 d	exposure phase: 7 d E_rC_{50} 7 d NOEC recovery phase: 7 d E_rC_{50} 7 d NOEC	**~0.0092 (mm)** [2] ~ 0.0046 (mm) 0.0799 (mm) 0.0092 (mm)	[redacted]; 2002; M-208657-01-1 KCA 8.2.7 /02
Myriophyllum aquaticum	growth inhibition	14 d	EC_{50} for Chlorophyll a and b and Carotinoids: 0.325 EC_{50} for growth endpoints: 0.970 to 0.974		[redacted]; [redacted]; 2002; M-255960-02-1 KCA 8.2.7 /10
Amidosulfuron-desmethyl					
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1] NOE$_r$C	**0.92** 0.16	[redacted]; 2003; M-213899-01-1 KCA 8.2.7 /03
Amidosulfuron-desmethyl-chloropyrimidine					
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1] NOE$_r$C	**>100** 100	[redacted]; 2010; M-365833-01-1 KCA 8.2.7 /06
Amidosulfuron-guanidine					
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1] NOE$_r$C	**>100** 6.25	[redacted]; 2010; M-365913-02-1 KCA 8.2.7 /07
Amidosulfuron-biuret					
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1] NOE$_r$C	**> 10** ≥ 10	[redacted]; 2015; M-510513-01-1 KCA 8.2.7 /08
Amidosulfuron-ADMP					
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} [1] NOEC	**> 100** 100	[redacted]; [redacted]; 2000; M-

[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

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
				186916-01-1 KCA 8.2.7 /04
Amidosulfuron-ADHP				
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} 1) > 100 NOEC 18	[redacted]; 2003; M-213897-01-1 KCA 8.2.7 /05
Amidosulfuron-sulfamic acid				
Lemna gibba (duck weed)	growth inhibition	7 d	E_rC_{50} 1) **> 10** NOE_rC ≥ 10	[redacted]; 2013; M-464386-01-1 KCA 8.2.7 /09

Bold letters: Values considered relevant for risk assessment in the MCP document
$_{mm}$ = mean measured; $_{nom}$ = nominal

1) Since the new aquatic GD focusses on endpoints based on growth rates the old E_bC_{50} figures were omitted from the table above.

2) EU-agreed endpoint; at 9.2 µg/L the % inhibition was 53.01%

Studies on amidosulfuron:

Report: KCA 8.2.7/01; [redacted]; [redacted]; 1993; M-138621-01-1
Title: Technical Hoe 075032 (Hoe 075032 00 ZD99 0001) Acute toxicity to duckweed (Lemna gibba) G3) under Static test conditions
Report No.: A49587
Document No.: M-138621-01-1
Guideline(s): **USEPA (=EPA) Subdivision E: § 123-2**
Guideline deviation(s): **for deviation see Point 4.0**
GLP/GEP: **yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a growth inhibition test on *Lemna gibba* with amidosulfuron technical a.s. A dose dependant reduction of growth was observed with increasing concentration of amidosulfuron from a mean measured concentration of 8.74 up to 65.5 µg a.s./L. A 14-d E_rC_{50} = 17.6 µg a.s./L and a 14-d NOEC = 8.74 µg a.s./L were reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment. However, no EU agreed endpoint was derived from this test, since a lower endpoint resulted from the corresponding study KCA 8.2.7/02.

Report: KCA 8.2.7/02; [redacted]; 2002; M-208657-01-1
Title: Duckweed (Lemna gibba G3) growth inhibition test with recovery phase AE F075032 substance, technical Code: AE F075032 00 1D99 0013
Report No.: C025093
Document No.: M-208657-01-1
Guideline(s): **ASTM: E 1415-91; OECD: draft June 1998; USEPA (=EPA): Subdivision J, § 123-2**
Guideline deviation(s): **Deviation not specified**
GLP/GEP: **yes**

Objective:

The aim of the study was to determine the effects of Amidosulfuron (AE F075032 00 1D99 0013 purity 99.4% w/w) to duckweed, *Lemna gibba* under semistatic conditions and the potential for recovery after the treatment. The study was designed to meet OECD criteria.

Material and methods:
Lemna cultures with an initial frond number of 12 fronds per replicate were exposed to the test item in 20X-AAP medium at five nominal treatment levels (i.e. 10, 18, 32, 56 and 100 µg/L). During the treatment phase six replicates were involved in which growth and abnormal appearance of fronds were determined on test days 3, 5 and 7. At day 7 three replicates were sacrificed in order to obtain dry weight data. The test continued with the remaining three replicates but with untreated nutrient solutions (recovery phase). Again, growth and abnormal appearance of fronds were determined at days 10, 12 and 14.

Findings:
Time-weighted average concentrations during the seven-day treatment period were between 72.5 and 91.96 % of nominal. Therefore, the following time-weighted average treatment levels were used to calculate the biological endpoints: 9.20, 13.05, 25.63, 45.97 and 79.17µg/L.
Aged water from day 10 (day 3 of the recovery phase) revealed that the test item concentrations in the recovery phase were below the limit of detection (LOD).

Inhibition during the treatment phase was quantified as follows:

Table CA 8.2.7- 2: Inhibition during the treatment phase

treatment level (µg/L)	mean growth rate (d^{-1})	percentual inhibition of growth rate	mean increase in biomass (mg)	percentual inhibition of biomass increase
untreated control	0.387	0.00	19.9	0.0
10	0.174	55.03	9.4	53.01
18	0.148	61.82	8.2	59.03
32	0.098	74.78	5.7	71.24
56	0.091	76.59	6.0	69.73
100	0.079	79.55	6.5	67.39

At day 7 vaulted fronds were observed at 10 and 18 µg/L. Since no observations regarding plant appearance were made in higher treatment levels, vaulted fronds are not regarded as a test item related intoxication symptom.

Inhibition during the recovery phase was quantified as follows:

Table CA 8.2.7- 3: Inhibition during the recovery phase

treatment level (µg/L)	mean growth rate (d^{-1})	percentual inhibition of growth rate	mean increase in biomass (mg)	percentual inhibition of biomass increase
untreated control	0.396	0.00	24.4	0.00
10	0.392	0.98	22.8	6.82
18	0.364	8.02	22.1	9.55
32	0.367	7.33	20.7	15.28
56	0.326	17.58	17.7	27.69
100	0.311	21.38	18.1	26.06

The occurence of symptoms changed between the different days of the assessment during the recovery phase. This indicates that these symptoms were transient. The fact, that at day 14 only some (but not

all!) fronds were smaller also confirms the transient character of the morphological appearance. Therefore, these observations are not regarded to be severe intoxication symptoms.

Conclusion:
The inhibitory effect of Amidosulfuron (AE F075032 00 1D99 0013) to duckweed, *Lemna gibba* was determined as follows:

7 d E_rC_{50}	**<**	**9.20 µg/L**	
7 d E_bC_{50}	**<**	**9.20 µg/L**	
7 d NOEC	**<**	**9.20 µg/L**	**regarding growth rate (frond number)**
7 d NOEC	**<**	**9.20 µg/L**	**regarding growth rate (biomass)**

Time-weighted average figures during the seven-day static renewal **recovery phase**:

7 d E_rC_{50}	**>**	**79.17 µg/L**	
7 d E_bC_{50}	**>**	**79.17 µg/L**	
7 d NOEC	**=**	**9.20 µg/L**	**regarding growth rate (frond number)**
7 d NOEC	**=**	**13.05 µg/L**	**regarding growth rate (biomass)**

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006). The study was considered to be acceptable and was used for the risk assessment. Based on the fact that the percentages of inhibition at the actual test concentration of 9.2 µg/L were only very slightly exceeding 50% level (55.03 % for growth rate and 53.01% for biomass increase), it was concluded that for risk assessment purposes 7d ErC50 / EbC50 can be assumed as the discrete value of 9.2 µg/L.

An EU agreed endpoint of 7d E_rC_{50} = E_bC_{50} = 9.2 µg/L for *Lemna gibba* was derived from this test.

***
To provide information on the sensitivity of further aquatic macrophyte species to the active substance amidosulfuron, two public literature articles reporting on endpoints for *Myriophyllum aquaticum* are submitted. Even though the key methodology applied by the authors of these publications did not exactly follow current OECD 239 guidance, and the experiments were non-GLP tests, the data generated allows for a clear conclusion of a significantly lower sensitivitiy of *Myriophyllum* than was previously observed for *Lemna*. Such finding is also in-line with general trends in the class of sulfonylurea herbicide compounds.

For the above reasons, these public literature articles are classified as supplemental information. The endpoints reported are considered suitable to characterise sensitivity of *Myriophyllum* as notably lower than *Lemna*, but will not be used for numeric risk assessments of products.

Report: KCA 8.2.7/10; [redacted]; [redacted]; 2002; M-255960-02-1
Title: Sensitivity of the rooted macrophyte Myriophyllum aquaticum (Vell.) verdcourt to seventeen pesticides determined on the basis of EC50
Source.: Bull. Environ. Contam. Toxicol., Vol. 69, p. 601-608
Document No.: M-255960-02-1
Guideline(s): none
Guideline deviation(s): none
GLP/GEP: **No (published paper)**

EXECUTIVE SUMMARY
The aim of the study was to determine the sensitivity of *Myriophyllum aquaticum* to amidosulfuron and 16 other pesticides (2,4-D (acid), dichlorprop, dicamba, pyridate, propiquizafop, terbutryn, triflusulfuron-methyl, rimsulfuron, metsulfuron-methyl, thifensulfuron-methyl, glyphosate, trifluralin, pendimethalin, chlorothalonil, propiconazole and parathion) using several endpoints (contents of

chlorophyll a, chlorophyll b, carotenoid, and increases in shoot length, root number, total root length, fresh weight, side shoot number, and side shoot length). For the purpose of the present document, the summary presented will focus on amidosulfuron only.

Plants were exposed for 14 days to 7-8 concentration levels of amidosulfuron. Each concentration was replicated five times. During the test, plant length was measured regularly on alternate days. After the fourteen days of exposure, the plants were removed from the test tubes and shoot length, total root length, root number, side shoot number and length were measured and recorded. Additionally, 50 mg apical segment were weighed and the contents of chlorophyll a, chlorophyll b and carotenoid contents were measured. EC_{50} values were calculated by a non-linear regression model.

The results showed that the pigment content were mostly more sensitive than the remaining endpoints. The EC_{50} values for *M. aquaticum* exposed to amidosulfuron were 0.325 mg/L for change in chlorophyll a, chlorophyll b and carotenoid contents 14 days after exposure. The respective 14-d EC_{50} values for area under growth curve, increase in fresh weight and increase in shoot length were 0.974, 0.970 and 0.974 mg/L.

MATERIAL AND METHODS

A. Material

1. Test material

Test item:	Amidosulfuron 75% SC
Active substance(s):	Amidosulfuron
Chemical state and description:	Not reported
Source of test item:	Germany
Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported
Water solubility:	Not reported

2. Test solutions

Vehicle/solvent:	-water
Source of vehicle/solvent:	Not reported
Concentration of vehicle/solvent:	Not reported
Method of preparation:	Not reported
Evidence of unsolved material:	Not reported

3. Test organism(s)

Species:	*Myriophyllum aquaticum* (Vell.) Verdcourt
Common name:	Not reported
Source of test species:	Not reported

4. Culture conditions of test organism(s)

Culture medium:	50 mL of sterile liquid growth medium supplemented with 3% sucrose and filled with 5 g Turface®. All experiments were conducted using 50 mL of sterile Hoagland nutrient medium with 30 g/L sucrose added.
Temperature:	25°C (light),18-20°C (dark)
Photoperiod:	16 hours light, 8 hours dark
Light intensity:	120-180 µmol/m²/s
pH:	Not reported
Oxygen saturation:	Not reported
Food and feeding regime:	Does not apply
Acclimatisation prior to testing:	Incubation started 14 days before testing
Observations during acclimatisation:	Not reported

B. Study design and methods

1. Test procedure

Test system:	Tubes covered with sterile plain closures
Test concentration(s):	7 or 8 test concentrations
Control(s):	Not reported
Number of replicates:	5 replicates
Test conditions:	Not reported (most likely same conditions as during incubation period)
Feeding:	Does not apply
Medium renewal:	None
Frequency of test item application:	Once
Test duration:	14 days
Endpoints:	Content of chlorophyll a, chlorophyll b and carotenoid as well as shoot length, root number, total root length, fresh weight, side shoot number and side shoot length
Statistics:	EC_{50} values were calculated using non-linear regression analysis using transformed concentrations of the active component (Sigma Plot, version 4.0)

2. Measurements during the test

Water/medium parameters:	Not reported

3. Sampling

Sampling frequency:	Not reported
Transport/storage of samples:	Not reported

4. Chemical analysis

Guideline/protocol:	Not reported
Method:	Not reported
Pre-treatment of samples:	Not reported
Conduction:	Not reported
Reference item:	Not reported
Recovery:	Not reported
Limit of detection:	Not reported
Limit of quantification:	Not reported

RESULTS AND DISCUSSION

1. Validity criteria:

No validity criteria were mentioned.

2. Biological findings:

Compared to the other sixteen herbicides tested Amidosulfuron caused medium effects to *Myriophyllum aquaticum* after 14 days of exposure. EC_{50} values are presented in the following table.

Table CA 8.2.7- 4: Effective concentrations (EC_{50}) for *M. aquaticum* tested with amidosulfuron. All units are mg/L. Endpoints were determined at 14 d.

Endpoint	EC_{50} (mg/L)
Chlorophyll a	**0.325**
Chlorophyll b	**0.325**
Carotenoid	**0.325**
Area under growth curve	**0.974**
Increase in fresh weight	**0.970**
Increase in shoot length	**0.974**
Total root length	**nd**
Root number	**nd**

nd: not determinable

RESULTS SUMMARY

The 14d-EC_{50} values for *Myriophyllum aquaticum* exposed to amidosulfurion ranged from 0.325 to 0.974 mg/L for the various endpoint parameters assayed.

Report: KCA 8.2.7.1; [redacted]; 2014; M-488799-01-1
Title: The impact of pesticides toward parrotfeather when applied at the predicted environmental concentration
Source: Chemosphere 66 (2007) 469–473
Document No.: M-488799-01-1
Guideline(s): not applicable
Guideline deviation(s): not applicable
GLP/GEP: **No. Published study (peer-reviewed article).**

EXECUTIVE SUMMARY

The effects of 18 pesticides to parrotfeather (*Myriophyllum aquaticum*) were evaluated in a water-sediment system over a period of 14 days. A single test concentration was assayed, considered to represent a worst case estimated environmental concentration by the authors. Material and method as well as results will be summarized in the following for amidosulfuron only.

MATERIAL AND METHODS

A. Material

1. Test material

Test items:	Amidosulcuron 75% (SC)
Active substance(s):	Amidosulfuron
Chemical state and description:	Not reported
Source of test item:	Not reported
Batch number:	Not reported
Purity:	Not reported
Storage conditions:	Not reported
Solubility at room temperature:	Not reported

2. Test organism(s)

Species:	*Myriophyllum aquaticum* (Vell.) Verdcourt
Common name:	Parrotfeather
Source(s):	Not reported
Culturing conditions:	An axenic culture of was established aseptically, stock cultures were maintained by cutting 1 cm long stems into vessels and transferring them into culture vessels as described by Turgut and Fomin (2001)[12]

B. Study design and methods

1. Test procedure

Test principle:	Laboratory toxicity test
Test conduct:	After 4–6 weeks in stock culture, 3 cm long axillary buds from stock plants were transferred into the culture tubes (200 × 25 mm) containing 50 ml of sterile liquid growth medium supplemented with the addition of 30 g/l sucrose and filled with 5 g Turface®.All experiments were conducted using 50 ml of sterile Hoagland nutrient medium[13] with adding 30 g/l sucrose. Plants were incubated for 14 days
Test concentrations:	**single dose level; applied concentration for amidosulfuron = 0.0075 mg/L**
Solvent:	Not reported
Control:	untreated
Replicates:	6 replicates
Test conditions:	25°C for 16 h with a light influence rate 120–180 µmol m^{-2} s^{-1} and 18–20°C during an 8 h dark period[14]
Test duration:	14 days
Measurements:	Shoots, total root length, root number, side shoot number and length were measured and recorded after 14-days exposure. 50 mg apical segments were weighed, placed into 10 ml of 96% ethanol, stored in the dark at 4°C for 24 h and measured with a spectrophotometer at 470, 649 and 665 nm for chlorophyll-a, -b and carotenoids, respectively[15].
Statistics:	Pairwise comparison were made between pesticide treatments and controls via Tukey´s test in SigmaStat 3.1 (SPPS Science, Chicago, IL, USA).
Risk assessment:	Calculation of EEC (predicted environmental concentration in a 30cm deep water body for a worst case exposure scenario) and EC_{50} (mean lethal concentration). EC_{50} values were obtained from Turgut and Fomin (2002a). The following formula of risk quotient (RQ) was used to calculate the risk (Goktepe et al., 2004) RQ = EEC / EC_{50}.

[12] Turgut, C., Fomin, A., 2001. J. Appl. Bot. 75, 80–84.
[13] Selim, S.A., O'Neal, S.W., Ross, M.A., Lembi, C.A., 1989. Weed Sci. 37, 810–814.
[14] Roshon, R.D., Stephenson, G.R., Horton, R.F., 1996. Hydrobiologia 340, 17–22.
[15] Lichtenthaler, H.K., Wellburn, A.R., 1983. Biochem. Soc. Trans. 11, 1982–1983

RESULTS

1. Validity criteria:

No validity criteria are mentioned.

1. Biological findings:

In case of the test substance amidosulfuron, for none of the assessed parameters any statistically significant difference to the controls was observed. Numeric results for inhibition of pigment content and shoot length are presented in Table CA 8.2.7- 5; inhibition of root length, root number and fresh weight are shown in Table CA 8.2.7- 7; negative values indicate increase over controls.

Table CA 8.2.7- 6: Inhibition of pesticide used on pigment content and increase in shoot length (%) at the test concentration of 0.0075 mg amidosulfuron/L

Pesticide	Chlorophyll-a	Chlorphyll-b	Carotenoids	Increase in shoot length
Amidosulfuron	-20.3 ± 1.7	-11.9 ± 4.1	-8.9 ± 2.0	-10.0 ± 3.4

* = Statistically different ($p < 0.05$) from controls using Tukey test.

Table CA 8.2.7- 7: Inhibition of root length, root number and fresh weight at the test concentration of 0.0075 mg amidosulfuron/L

Pesticide	Root number	Root length	Fresh weight
Amidosulfuron	0.00 ± 0.00	1.67 ± 1.5	-20.38 ± 5.2

Risk assessment was done via risk quotient (RQ) comparison of EEC vs. EC_{50}. EC_{50} values were obtained from Turgut and Fomin (2002)[16] [summarised above, KCA 8.2.7 /10]. The resulting RQ values are presented in Table CA 8.2.7- 8. Amidosulfuron was considered of no concern for *Myriophyllum* since the RQ was below 0.05.

Table CA 8.2.7- 8: RQ values for risk quotient

Pesticide	Chlorophyll-a	Chlorphyll-b	Carotenoid
Amidosulfuron	0.023	0.023	0.023

RESULTS SUMMARY

Amidosulfuron, when tested at a concentration of 0.0075 mg/L, was found to not cause any statistically significant difference to controls for the evaluated parameters pigment content, shoot length increase and fresh weight of *Myriophyllum aquaticum* (Vell.) Verdcourt.

Based on worst case expected environmental concentration as calculated by the authors of the present publication, and EC_{50} data reported in a publication by Turgut and Fomin (2002) [KCA 8.2.7 /10], a risk quotient RQ < 0.05 was derived and considered to indicate no concern.

[16] Turgut, C., Fomin, A., 2002. J. Appl. Bot. 76, 62–65.

Studies on metabolites of amidosulfuron:

Amidosulfuron-desmethyl:

Report:	KCA 8.2.7/03; [redacted]; 2003; M-213899-01-1
Title:	Duckweed (Lemna gibba G3) growth inhibition test AE F101630; substance, technical Metabolite of amidosulfuron Code: AE F101630 00 1C97 0002
Report No.:	C027728
Document No.:	M-213899-01-1
Guideline(s):	**ASTM: E 1415-91; OECD: Draft June 1998; USEPA (=EPA): OPPTS 850.4450**
Guideline deviation(s):	**for deviation see Point 3.9**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a semi-static growth inhibition test on *Lemna gibba* with the metabolite amidosulfuron-desmethyl. A significant reduction of growth in terms of frond number was observed in a concentration of 0.27 mg/L and above. A significant enhanced growth (in terms of biomass) was observed at a concentration of 2.7 mg/L and above. This enhanced growth was not regarded as an adverse effect. No significant growth inhibition and no changes in plant appearance were observed at a time weighted average concentration of 0.16 mg/L. A 7-d E_rC_{50} = 0.92 mg/L, a 7-d E_bC_{50} = 0.75 mg/L and a 7-d NOEC = 0.16 mg/L were reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. EU agreed endpoints of E_rC_{50} = 0.92 mg/L and E_bC_{50} = 0.75 mg/L for *Lemna gibba* were derived based on this test.

Amidosulfuron-desmethyl-chloropyrimidine:

Report:	KCA 8.2.7/06; [redacted]; 2010; M-365833-01-1
Title:	Lemna gibba G3 growth inhibition test with Amidosulfuron-desmethylchloropyrimidine (BCS-CO78570) under static conditions
Report No.:	EBBEL008
Document No.:	M-365833-01-1
Guideline(s):	**OECD Guideline 221 (March 23, 2006)**
Guideline deviation(s):	**Deviation none**
GLP/GEP:	**yes**

Note: *This study has been previously submitted to former RMS (Austria) to support the post Annex I process of amidosulfuron. It was evaluated by Austria and is part of the DAR Addendum (Feb. 2011 – Addendum to monograph prepared in the context of post Annex I procedure (new Annex II data).Upon request of the new RMS Finland, the study has nevertheless been included in the supplemental dossier.*

Executive Summary:
The objective of this growth inhibition test was to verify the assumption that the metabolite amidosulfuron-desmethyl-chloropyrimidine (BCS-CO41838), tested in form of its sodium salt (BCS-CO78570) will cause no adverse effects on the growth of *Lemna gibba* G3 at the test item concentration of 100 mg pure metabolite (p.m.)/L. The test was performed according to the OECD Guideline 221.

6 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multi-generation test for 7 days under static exposure conditions to the nominal concentrations of 100 mg/L in comparison to control. Visual observations were made on study days 3, 5, and 7. Quantitative amounts

of amidosulfuron-desmethyl-chloropyrimidine were measured in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period. Based on analytical findings the biological endpoints are reported as nominal figures. The 7-day-E_rC_{50} was > 100 mg/L, the 7-day-NOEC was determined to be 100 mg/L.

Material and methods:
Test item: Amidosulfuron-desmethyl-chloropyrimidine, sodium salt (BCS-CO78570); Origin batch No.: BCOO5766-3-3; Batch code: BCS-CO78570-01-01; LIMS No.: 0922452; Certificate No.: AZ 16057; TOX-No.: 08625-00; Analysed content: 88.7 % w/w.

Cultures of *Lemna gibba* G3 with an initial frond number of 12 fronds per replicate (6 replicate vessels per test level and 6 replicate vessels per control) were exposed for 7 days under static exposure conditions to the test item in 20X-AAP medium at the nominal concentration of 100 mg/L in comparison to control. The pH values ranged from 7.5 to 8.7 and the incubation temperature ranged from 23°C to 26°C measured over the whole period of testing at a continuous illumination of 8260 lux (mean). Visual observations were made on study days 3, 5, and 7 to determine the growth rate. Quantitative amounts of amidosulfuron-desmethyl-chloropyrimidine were measured in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period.

Dates of experimental work: November 04, 2009 – November 23, 2009

Results:
Validity criteria:
The frond number increased in the control by a factor of 14.5 within 7 days corresponding to a doubling time (T_d) of 1.8 days. Test conditions met all validity criteria given by the mentioned guideline.

Analytical findings:
The chemical analysis of amidosulfuron-desmethyl-chloropyrimidine revealed recoveries of 100% of the nominal concentration on day 0 and day 7. Based on the analytical findings all results are given as initial nominal concentrations of the test item in the test medium.

Biological findings:
The static 7-day growth inhibition test provided the following tabulated effects:

Table CA 8.2.7- 9: Effects of amidosulfuron-desmethyl-chloropyrimidine on *Lemna gibba*

Nominal test level [mg/L]	Measured concentration of amidosulfuron-desmethyl-chloropyrimidine at day 7 [mg/L]	Mean final frond number on day 7	Mean final total frond area of plants [mm²]	% inhibition* of average growth rate of	
				frond numbers	total frond area of plants
control	< 0.102	174	733	--	--
100	100	202	866	- 5.29	- 2.00

* Negative values mean growth stimulation

No morphological effects and effects on biomass and growth rate were observed.

Conclusions:
The E_rC_{50} (7 d) is >100 mg/L and the NOEC (7 d) is 100 mg/L (based on nominal initial concentrations).

Amidosulfuron-guanidine:

Report:	KCA 8.2.7/07; [redacted]; 2010; M-365913-02-1
Title:	Lemna gibba G3 growth inhibition test with Amidosulfuron-guanidine under static conditions [amended 2016-05-03 for typo correction]
Report No.:	EBBEL009
Document No.:	M-365913-02-1
Guideline(s):	**OECD Guideline 221 (March 23, 2006)**
Guideline deviation(s):	**Deviation none**
GLP/GEP:	**yes**

Note: *This study has been previously submitted to former RMS (Austria) to support the post Annex I process of amidosulfuron. It was evaluated by Austria and is part of the DAR Addendum (Feb. 2011 – Addendum to monograph prepared in the context of post Annex I procedure (new Annex II data)).Upon request of the new RMS Finland, the study has nevertheless been included in the supplemental dossier.*

Executive Summary:
Aim of the study was to determine the influence of the metabolite amidosulfuron-guanidine (BCS-CO41839) on exponentially growing Lemna gibba G3 expressed as NOEC, LOEC and ECx for growth rate of the response variables, frond number and total frond area of plants. The test was performed according to the OECD Guideline 221. 3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multi-generation test for 7 days under static exposure conditions to the nominal concentrations of 1.56, 3.13, 6.25, 12.5, 25.0, 50.0 and 100 mg test item/L in comparison to control. Visual observations were made on study days 2, 5, and 7 to determine the growth rate. Quantitative amounts of amidosulfuron-guanidine were measured in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period. Based on analytical findings the biological endpoints are reported as nominal figures. The 7-day-E_rC_{50} was > 100 mg/L, the 7-day-NOEC was determined to be 6.25 mg/L.

Material and methods:
Test item: Amidosulfuron-guanidine (BCS-CO41839); Origin batch No.: RDL 603-16-20; Batch code: BCS-CO41839-01-01; LIMS No.: 0920454; Certificate No.: AZ 16021; TOX-No.: 08626-00; Analysed content: 98.3 % w/w.

Cultures of *Lemna gibba* G3 with an initial frond number of 12 fronds per replicate (3 replicate vessels per test level and 3 replicate vessels per control) were exposed for 7 days under static exposure conditions to the test item in 20X-AAP medium at the nominal concentrations of 1.56, 3.13, 6.25, 12.5, 25.0, 50.0 and 100 mg/L in comparison to control The pH values ranged from 7.4 to 8.8 and the incubation temperature ranged from 22°C to 24°C measured over the whole period of testing at a continuous illumination of 7840 lux (mean). Visual observations were made on study days 2, 5, and 7 to determine the growth rate. Quantitative amounts of amidosulfuron-guanidine were measured in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period.

Dates of experimental work: September 09, 2009 – October 20, 2009

Results:
Validity criteria:
The frond number increased in the control by a factor of 17.7 within 7 days corresponding to a doubling time (T_d) of 1.7 days. Test conditions met all validity criteria, given by the mentioned guideline.

Analytical findings:
The analytical findings of amidosulfuron-guanidine determined in all test levels on day 0 ranged between 89 and 104 % (average 94 %), on day 7 the analysed concentrations ranged between 82 and 115 % (average 96 %) of nominal concentrations. Based on the analytical findings all results are given as initial nominal concentrations of the test item in the test medium.

Biological findings:
The static 7-day growth inhibition test provided the following tabulated effects:

Table CA 8.2.7- 10: Effects of amidosulfuron-guanidine on *Lemna gibba*

Nominal test level [mg/L]	Measured concentration of amidosulfuron-guanidine at day 7 [mg/L]	Mean final frond number on day 7	Mean final total frond area of plants [mm²]	% inhibition of average growth rate of	
				frond numbers	total frond area of plants
control	< 0.0995	212	694	--	--
1.56	1.54	190	638	3.68	2.27
3.13	2.88	167	516	8.47	4.90
6.25	5.13	178	614	5.99	6.11
12.5	14.4	158	521	10.6	6.74*
25.0	25.1	148	473	12.7	10.3*
50.0	46.8	166	507	8.44	9.74*
100	91.7	76	216	36.5	41.9*

* significant difference to the control ($p = 0.05$)

No morphological effects were observed.
The most sensitive response variable was total frond area of plants resulting in (0 – 7-day) E_rC_{50} of > 100 mg/L (131 mg/L) and a lowest (0 – 7-day) NOE_rC of 6.25 mg/L.

Conclusions:
The E_rC_{50} (7 d) is > 100 mg/L and the NOEC (7 d) is 6.25 mg/L (based on nominal initial concentrations).

Amidosulfuron-biuret:

Report: KCA 8.2.7/08, [redacted], 2015; M-510513-01-1
Title: Lemna gibba G3 - Growth inhibition test with BCS-CQ51287 (amidosulfuron-biuret) under static conditions
Report No.: EBBEN033
Document No.: M-510513-01-1
Guideline(s): **EU Directive 91/414/EEC; Regulation (EC) Number 1107/2009; US EPA OCSPP 850.4400**
Guideline deviation(s): **for deviation see Point 4**
GLP/GEP: **yes**

Executive Summary:
The objective of this growth inhibition test was to verify the assumption that the metabolite amidosulfuron-biuret (BCS-CQ51287) will cause no adverse effects on the growth of *Lemna gibba* G3 at the only test item concentration of 10 mg pure metabolite (p.m.)/L. This test was conducted according to the OECD Guideline 221.
At test start 8 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditions to a nominal concentration of 10.0 mg p.m./L in comparison to a control.

Plant frond numbers and total frond area of plants were recorded at the beginning of the test, at test termination, and at two occasions during the 7 day period. Growth and growth inhibition were calculated. The E_rC_{50} was > 10.0 mg p.m./L and the NOE_rC was ≥ 10.0 mg p.m./L.

Material and methods:
Test item: Amidosulfuron-biuret (BCS-CQ51287); Batch Code: BCS-CQ51287-01-02; Origin batch No.: GSE61653-3-3; TOX no.: 10517-00; Analysed purity: 93.6 % w/w.

At test start 8 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditions to a nominal concentration of 10.0 mg p.m./L in comparison to a control. The pH values ranged from 7.4 to 8.7 in the control and the incubation temperature ranged from 24.1°C to 24.6°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6.69 klux (average of nine measurements).
Quantitative amounts of the test item were measured in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period.

Dates of experimental work: November 07, 2014 – November 27, 2014

Results:
Validity Criteria:
The doubling time of frond number in the control was 1.8, corresponding to a 13.8 fold increase in 7 days. The control coefficient of variation (CV) for yield and growth is < 20 % at test termination. Therefore the study met all validity criteria, requested by the mentioned guidelines.

Analytical findings:
The analytical finding of amidosulfuron-biuret found on day 0 was 118 % of nominal. In the aged test media the concentration of amidosulfuron-biuret increased in the treatment and the nominal range of 120 % was exceeded (135 %). The high recoveries at the test end can be explained by loss of media in the replicates due to evaporation.
Since a correct dosing of the test item is proven by the analytical measurement all reported results are based on nominal values of the test item.

Table CA 8.2.7- 11: Concentrations of amidosulfuron-biuret in the test solutions at day 0

Day 0				
Nominal Concentration in mg p.m./L	**Actual Concentration (µg amidosulfuron-biuret/L)**			
	1. Determination	**2. Determination**	**Average**	**%**
Control	<0.493	<0.493	<0.493	--
10.0	11800	11800	11800	118

Table CA 8.2.7- 12: Concentrations of amidosulfuron-biuret in the test solutions at day 7

Day 7				
Nominal Concentration in mg p.m./L	**Actual Concentration (µg amidosulfuron-biuret /L)**			
	1. Determination	**2. Determination**	**Average**	**%**
Control	<0.493	<0.493	<0.493	--
10.0	13400	13500	13450	135

Biological findings:
Effects are summarized in the following table.

Table CA 8.2.7- 13: Effects of amidosulfuron-biuret on *Lemna gibba* in a static 7-day test

Nominal test concentration [mg p.m./L]	Frond no. (day 7), mean values from 8 replicates	Total frond area of plants (day 7), mean values from 8 replicates [mm^2]	% inhibition	
			Mean growth rate for frond no.	Mean growth rate for total frond area of plants
Control	166	1474	--	--
10.0	179	1566	-2.8	-0.3

No sublethal effects on *Lemna gibba* were observed. No remarkable observations of the test item in the test medium were recorded. Over the whole test period, the media were clear and colourless.

Table CA 8.2.7- 14: Results based on nominal concentrations of amidosulfuron-biuret

Endpoint (0-7 day)	Effect on mean growth rate of frond no. [mg p.m./L]	Effect on mean growth rate of total frond area of plants [mg p.m.L]
E_rC_{50} (Cl 95 %)	**>10.0**	**>10.0**
LOE_rC	>10.0	>10.0
NOE_rC	≥10.0	≥10.0

The E_rC_{50}, LOE_rC and NOE_rC determination is based on statistical data analysis.

Conclusions:
Amidosulfuron-biuret caused no adverse effects on the growth of *Lemna gibba* G3 up to the limit test item concentration of 10.0 mg pure metabolite/L.

Amidosulfuron-ADMP:

Report: KCA 8.2.7/04; [redacted]; 2000; M-186916-01-1
Title: Duckweed (Lemna gibba G3) growth inhibition test - AE F092944, substance technical (metabolite of ethoxysulfuron AE F095404 and amidosulfuron AE F075032) Code: AE F092944 00 1C99 000b
Report No.: C003865
Document No.: M-186916-01-1
Guideline(s): ASTM: E 1415-91; OECD: Draft June 1998; USEPA (=EPA): Subdivision J § 123-2
Guideline deviation(s): Deviation not specified
GLP/GEP: yes

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a semi-static growth inhibition test on *Lemna gibba* with the metabolite amidosulfuron-ADMP. Exponential growth was observed at all treatment levels and control. A significant inhibition of growth was not observed up to the highest test concentration of 100 mg/L. A 7-d E_rC_{50} > 100 mg/L, a 7-d E_bC_{50} > 100 mg/L and a 7-d NOEC = 100 mg/L were reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment. An EU agreed endpoint of 7d E_rC_{50} = E_bC_{50} >100 mg/L for *Lemna gibba* was derived from this test.

Amidosulfuron-ADHP:

Report:	KCA 8.2.7/05; [redacted]; 2003; M-213897-01-1
Title:	Duckweed (Lemna gibba G3) growth inhibition test AE F094206; substance, technical (metabolite of amidosulfuron) Code: AE F094206 00 1C99 0001
Report No.:	C027727
Document No.:	M-213897-01-1
Guideline(s):	**ASTM: E 1415-91; OECD: Draft June 1998; USEPA (=EPA): OPPTS 850.4400**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a semi-static growth inhibition test on *Lemna gibba* with the metabolite amidosulfuron-ADHP. A significant inhibition of growth in terms of frond number was observed at treatment levels of 32 mg/L and higher. Enhanced growth in terms of biomass was observed at all treatment levels of 18 mg/L and above. However, the effect was not significant and was not regarded as an adverse effect. A 7-d E_rC_{50} > 100 mg/L, a 7-d E_bC_{50} > 100 mg/L and a 7-d NOEC (growth rate) = 18 mg/L were reported.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered to be acceptable and was used for the risk assessment. An EU agreed endpoint of 7d E_rC_{50} = E_bC_{50} >100 mg/L for *Lemna gibba* was derived from this test.

Amidosulfuron-sulfamic acid:

Report:	KCA 8.2.7/09; [redacted]; 2013; M-464386-01-1
Title:	Lemna gibba G3 - Growth inhibition test with BCS-AW41401 under static conditions
Report No.:	EBFSN012
Document No.:	M-464386-01-1
Guideline(s):	**EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4400; OECD Guideline 221 (March 23, 2006)**
Guideline deviation(s):	**Deviation none**
GLP/GEP:	**yes**

Executive Summary:
The objective of this growth inhibition test was to verify the assumption that the metabolite amidosulfuron-sulfamic acid (BCS-AW41401) will cause no adverse effects on the growth of *Lemna gibba* G3 at the only test item concentration of 10 mg pure metabolite/L. Fronds of *Lemna gibba* G3 were exposed in a chronic multigeneration test for 7 days under static exposure conditions to a nominal concentration of 10 mg pure metabolite in comparison to a water control. Plant frond numbers and total frond area of plants were recorded at the beginning of the test, at test termination, and at two occasions during the 7 day period. Growth and growth inhibition were calculated. The concentration which inhibited the growth of this species by 50 percent (EC_{50}) was determined where possible.
Amidosulfuron-sulfamic acid (BCS-AW41401) caused no adverse effects on the growth of *Lemna gibba* G3 up to the limit test item concentration of 10 mg pure metabolite/L.

Material and methods:
Test item: BCS-AW41401; Origin Batch No.: GSE 61222-2-3; Batch ID: BCS-AW41401-01-01; Customer Order No.: TOX09976-00; analysed content: 89.7 %; Certificate No.: AZ 18815; LIMS No.: 1320720.

6 x 12 fronds of *Lemna* gibba G3 per test concentration were exposed in a chronic multigeneration test for 7 days under static exposure conditions to a nominal concentration of 10.0 mg pure metabolite/L in comparison to a water control. The pH values ranged from 7.6 to 8.8 in the control and the incubation temperature ranged from 24.4°C to 24.9°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6.70 klux (average of nine measurements).

Amidosulfuron-sulfamic acid (BCS-AW41401) was quantitatively determined in all freshly prepared test levels on day 0 and additionally in all aged test levels on day 7 of the exposure period.

Dates of experimental work: July 10, 2013 – August 20, 2013

Results:
Validity Criteria:
The study met all validity criteria, requested by the mentioned guideline.

Analytical findings:
The analytical finding of BCS-AW41401 found on day 0 was 113 % of nominal and 115 % of nominal on day 7. All reported results are based on nominal values of the test item amidosulfuron-sulfamic acid (BCS-AW41401).

The static 7 day growth inhibition test provided the following tabulated effects:

Table CA 8.2.7- 15: Survey of biological results and derived inhibition percentages based on growth rates

nominal test concentration [mg p.m./L]	final frond no. (replicate means, day 7)	final total frond area of plants (replicate means) [mm^2]	% inhibition	
			mean growth rate for frond no.	mean growth rate for total frond area of plants
control	1940	1400.8	--	--
10.0	2078	1469.1	-2.5	-2.1

Observed visual effects:
There were no visual effects observed in any of the test concentrations.
Observed visual effects on the test item: none

The results based on nominal concentrations of the test item amidosulfuron-sulfamic acid (BCS-AW41401) are shown in the table below.

Table CA 8.2.7- 16: Survey of 7-day endpoints for amidosulfuron-sulfamic acid

end point (0-7 day)	effect on mean growth rate of frond no. [mg p.m./L]	effect on mean growth rate of total frond area of plants [mg p.m./L]
E_rC_{50}	**>10.0**	**>10.0**
LOE_rC	>10.0	> 10.0
NOE_rC	≥10.0	≥ 10.0

Conclusions:
Amidosulfuron-sulfamic acid (BCS-AW41401) caused no adverse effects on the growth of *Lemna gibba* G3 up to the limit test item concentration of 10 mg pure metabolite/L.

CA 8.2.8 Further testing on aquatic organisms

No further aquatic organisms were tested. Further studies are not required under Commission Regulation (EU) No 283/2013 in accordance with Regulation (EC) No 1107/2009.

CA.8.3 Effect on arthropods

CA 8.3.1 Effects on bees

Acute laboratory studies with technical amidosulfuron (oral test: [redacted]; 1989; M-124106-01-1, KCA 8.3.1.1.1 /01; and contact test: [redacted]; 1989; M-124103-01-1, KCA 8.3.1.1.2 /01) had been available but were described and rated as invalid due to deficiencies in the test design (missing toxic standard) in the EU review for the first inclusion of amidosulfuron on Annex I. Therefore, for approval renewal, these studies are superseded by a new guideline-conform acute oral and contact toxicity test on honey bees ([redacted]; 2014, M-503119-01-1, KCA 8.3.1.1.1 /02) performed with amidosulfuron tech.

Moreover, an acute oral (KCA 8.3.1.1.1 /03) and an acute contact toxicity study (KCA 8.3.1.1.2 /03) with amidosulfuron techn. in bumble bees have been conducted in order to benchmark potential amidosulfuron-inherent sensitivity differences to honey bees.

In addition, a chronic 10 day adult feeding limit test was conducted with Amidosulfuron WG 75 ([redacted]; 2016; M-549770-01-1, KCA 8.3.1.2 /01).

Potential effects on bee brood were investigated in a bee brood feeding study ([redacted]; 2014; M-482118-01-1, KCA 8.3.1.3 /01) where entire honey bee colonies were exposed to Amidosulfuron WG 75 via feeding of treated sugar solution and additionally in a semi-field honey bee brood study performed under forced exposure conditions in flowering *Phacelia* sprayed with Amidosulfuron WG 75 at full bloom ([redacted]; 2016; M-545720-01-1, KCA 8.3.1.3 /02).

The information derived from these studies is summarised in the following table.

Table CA 8.3.1- 1: Toxicity endpoints of/for amidosulfuron (tech. and representative formulation Amidosulfuron WG 75)

Test substance	Ecotoxicological endpoint		Reference
Acute oral and contact toxicity to honey bees			
Amidosulfuron, tech.	LD_{50}-oral, 48 h	LD_{50} > 916.4 µg a.s./bee [1)]	[redacted]; 1989; M-124106-01-1 KCA 8.3.1.1.1 /01
Amidosulfuron, tech.	LD_{50}-oral, 48 h LD_{50}-contact, 48 h	LD_{50} > 109.2 µg a.s./bee LD_{50} > 100.0 µg a.s./bee	[redacted]; 2014; M-503119-01-1 KCA 8.3.1.1.1 /02 KCA 8.3.1.1.2 /02
Amidosulfuron, tech.	LD_{50}-contact, 48 h	LD_{50} > 100 µg a.s./bee [1)]	[redacted]; 1989; M-124103-01-1 KCA 8.3.1.1.2 /01
Acute oral and contact toxicity to bumble bees			
Amidosulfuron, tech.	LD_{50}-oral, 48 h	LD_{50} > 203 µg a.s./bumble bee	[redacted]; 2016; M-545712-01-1 KCA 8.3.1.1.1 /03
Amidosulfuron, tech.	LD_{50}-contact, 48 h	LD_{50} > 100 µg a.s./bumble bee	[redacted]; 2015; M-525139-01-1 KCA 8.3.1.1.2 /03
Chronic toxicity to adult honey bees			
Amidosulfuron WG 75	10 d adult feeding study	LC_{50} > 3333 mg a.s./kg LDD_{50} > 78.4 µg a.s./bee/d	[redacted]; 2016; M-549770-01-1 KCA 8.3.1.2 /01
Honey bee brood feeding study			
Amidosulfuron WG 75	Honey bee brood feeding (Qomen *et al.*, 1992)	No adverse effects on bee mortality (adult, pupae and larvae), bee brood development (eggs, young larvae, old larvae), and behaviour, by feeding honey bee colonies sugar syrup at a concentration typically present in the spray tank (0.114 g a.s./L)	[redacted]; [redacted]; 2014; M-482118-01-1 KCA 8.3.1.3 /01
Semi field honey bee brood study			
Amidosulfuron WG 75	Semi-field honey bee brood study (OECD No. 75; forced exposure conditions) in *Phacelia*; application during full-bloom and bees actively foraging	No adverse effects on mortality (adults, pupae and larvae), foraging activity, behaviour, colony condition, colony strength and bee brood development at 45 g a.s./ha	[redacted]; 2016; M-545720-01-1 KCA 8.3.1.3 /02

[1)] Study not considered valid

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

Report:	KCA 8.3.1.1.1/01; [redacted]; 1989; M-124106-01-1
Title:	Report on laboratory investigations into the oral toxicity of HOE 075032 OH ZC94 0001 to the honey bee Apis mellifera L.
Report No.:	A41103
Document No.:	M-124106-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on an acute oral toxicity test on honey bees for the technical active substance. The acute oral LD_{50} of amidosulfuron was reported to be > 916.4 µg a.s./bee (measured substance uptake, 48 h).

The study was rated invalid for reason of a lack in the test design (missing toxic standard) in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

In consequence, no EU agreed endpoint was derived from this test.

For approval renewal, the study is superseded by a new guideline-conform oral acute toxicity test on honey bees, see reported below under KCA 8.3.1.1.1 /02.

Report:	KCA 8.3.1.1.1/02; [redacted]; 2014; M-503119-01-1
Title:	Effects of amidosulfuron tech. (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	91051035
Document No.:	M-503119-01-1
Guideline(s):	**OECD 213 and 214 (1998)**
Guideline deviation(s):	**none**
GLP/GEP:	**yes**

Executive Summary:
The purpose of this study was to determine the acute contact and oral toxicity of the active substance amidosulfuron to the honey bee (*A. mellifera* L.) under laboratory conditions following the current valid test guideline (OECD 213 and 214). For this purpose 50 female worker bees were exposed for 48 hours to a single dose of 100.0 µg a.s. per bee by topical application (contact limit test) and to a single dose of 109.2 µg a.s. per bee by feeding (oral limit test, value based on the actual intake of the test item). Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

The contact LD_{50} (48 h) was > 100.0 µg a.s./bee. The oral LD_{50} (48 h) was > 109.2 µg a.s./bee.

Material and methods:
Test item: Amidosulfuron, technical (AE F075032); Specification No.: 102000000551-03, Batch code: AE F075032-01-05; Origin batch no.: 2014-000761; TOX-No: 10494-00; Analysed content: 98.1 % w/w; Certificate of Analysis No.: AZ 19335.

Test units were stainless steel cages of 10 cm x 8.5 cm x 5.5 cm (length x height x width). 10 bees were used per test unit. 5 test units were used per test item dose level, control and reference item dose level, respectively. 50 worker bees were exposed for 48 hours to a single dose of 100.0 µg a.s. per bee by topical application (contact limit test) and 50 worker bees (*Apis mellifera*) were exposed for 48 hours to a single dose of 109.2 µg a.s. per bee by feeding (oral limit test, value based on the actual intake of the test item).

For the contact test one 5 µL droplet of amidosulfuron tech. dissolved in acetone was applied tow times on the dorsal bee thorax. The reference item was applied as one 5 µL droplet of dimethoate dissolved in tap water containing 0.5 % Adhäsit. For the control, one 5 µL droplet of tap water containing 0.5 % Adhäsit was used. For the solvent control, one 5 µL droplet of pure acetone was used.

For the oral test the test item was diluted in acetone and then applied in 50 % w/v sucrose solution, which was used as carrier (food) in the oral test. The reference item was diluted in tap water and applied in 50 % w/v sucrose solution. For the control pure 50 % w/v sucrose solution was offered to the bees and for the solvent control 50% w/v sucrose solution with 5 % acetone was offered to the bees. The treated food was offered in syringes, which were weighed before and after introduction into the cages. After a maximum of 2 hours 20 minutes, the uptake was complete and the syringes were removed, weighed and replaced by ones containing fresh, untreated food.

The number of dead bees was recorded after 4 (± 0.5 h) hours (first day); 24 and 48 (± 2 h) hours. Behavioural abnormalities (e.g. vomiting, apathy, intensive cleaning) were assessed after 4 (± 0.5 h) hours (first day); 24 and 48 (± 2 h) hours. Temperature during the test was 24 – 25 °C; relative humidity was 28 – 72 %. Bees were kept in darkness (except during observation).

Dates of work: April 14, 2014 – April 17, 2014

Results:

Table CA 8.3.1.1.1- 1: Validity criteria

Validity Criteria		Recommended	Obtained
Control mortality	Contact Test		
	Water control	< 10%	0.0%
	Acetone control:	< 10%	0.0%
	Oral Test		
	Sugar control	< 10%	0.0%
	Acetone/sugar control:	< 10%	0.0%
LD_{50} of reference item (24 h)	Contact Test		
		0.10 - 0.30 µg a.s./bee	0.24 µg a.s./bee
	Oral Test		
		0.10 - 0.35 µg a.s./bee	0.12 µg a.s./bee

The contact and oral test is considered valid as the control mortality in each case was < 10% and the LD_{50} values obtained with the reference item (dimethoate) were within the required ranges.

Biological results:

Contact Test:

At the end of the contact toxicity test (48 hours after application), there was no mortality at 100.0 µg a.s./bee. Also no mortality occurred in the water control group (water + 0.5 % Adhäsit) and in the solvent control group (acetone), respectively.

No test item induced behavioural effects were observed at any time in the contact toxicity test.

Oral Test:
The maximum nominal test level of amidosulfuron tech. (*i.e.* 100 µg a.s./bee) corresponded to an actual intake of 109.2 µg a.s./bee. This dose level led to no mortality after 48 hours. No mortality occurred in the water control group (sucrose 50 % w/v solution) and in the solvent control group at the end of the oral toxicity test (after 48 hours).

No test item induced behavioural effects were observed at any time in the oral toxicity test.

Table CA 8.3.1.1.1- 2: Toxicity of amidosulfuron to honey bees; acute contact and oral laboratory test

Test Item	Amidosulfuron tech.	
Test Object	*Apis mellifera*	
Exposure	**contact (solution in acetone)**	**oral (sugar/acetone/water solution)**
Application rate µg a.s./bee	100.0	109.2
LD_{50} µg a.s./bee	> 100.0	> 109.2
LD_{20} µg a.s./bee	> 100.0	> 109.2
LD_{10} µg a.s./bee	> 100.0	> 109.2
NOED µg a.s./bee*	≥ 100.0	≥ 109.2

* The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).

The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.24 and 0.12 µg a.s./bee, respectively.

Conclusions:
The toxicity of amidosulfuron tech. was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact LD_{50} (48 h) was > 100.0 µg a.s./bee. The oral LD_{50} (48 h) was > 109.2 µg a.s./bee.

Report: KCA 8.3.1.1.1-03; [redacted]; 2016; M-545712-01-1
Title: Amidosulfuron technical: Acute oral toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions
Report No.: SLS-00342
Document No.: M-545712-01-1
Guideline(s): no specific guidelines available, based on OECD Guidelines No. 213 (1998), OEPP/EPPO 170 (4) (2010), review article of VAN DER STEEN (2001) and the recent recommendations of the ICPPR bumble bee ring test group (2015)
Guideline deviation(s): none
GLP/GEP: yes

Executive summary:
The aim of this study was to determine the oral toxicity of amidosulfuron tech. to the bumble bee (*Bombus terrestris* L.) under laboratory conditions. For this purpose bumble bees were exposed to a single dose of amidosulfuron, one dose of the reference item, as well as a control (50 % (w/v) aqueous sucrose solution) and a solvent control (50 % (w/v) aqueous sucrose solution containing 5 % acetone and 0.1 % Xanthan) for the same period of time under identical exposure conditions. Mortality and sub-lethal effects were assessed 24 and 48 hours after test start. In the test item treatment group, 2.0 % mortality was observed at the dose level corresponding to 203 µg a.s./bumble bee (based on the actual uptake) at the final assessment after 48 hours. Only two affected bees were observed during the 48 hour test period in the test item group. The oral LD_{50} (48 h) was determined to be > 203 µg a.s./bumble bee. The oral NOED (48 h) was determined to be ≥ 203 µg a.s./bumble bee.

Material and methods:
Test item: Amidosulfuron, technical (AE F075032); Specification No.: 102000000551-03; Batch code: AE F075032-01-05; Origin batch No.: 2014-000761; TOX-No: 10494-00; Analysed content: 98.1 % w/w; Certificate of Analysis No.: AZ 19335.

In an acute oral limit test, adult worker bumble bees (*Bombus terrestris L.*) were exposed for 48 hours under laboratory conditions to one dose of the test item, a dose of the reference item, a control and a solvent control.

In the test item group (50 replicates of 1 bee each), the bumble bees were exposed to a single dose of 203 µg a.s./bumble bee by feeding (value based on actual uptake). The target dose was 250 µg a.s./bumble bee.

In the toxic reference item group (50 replicates of 1 bee each), bumble bees were exposed to a single dose of 1.4 µg dimethoate/bumble bee by feeding (value based on actual uptake). The target dose was 1.5 µg dimethoate/bumble bee. As a toxic reference item Perfekthion was used, containing 420.3 g dimethoate/L (analysed content).

In addition, two untreated controls (50 replicates of 1 bee each) were tested, one being a control with 50% (w/v) aqueous sucrose solution and the other being a solvent control with 50% (w/v) aqueous sucrose solution containing 5% acetone and 0.1% xanthan.

Bumble bees were randomly collected from the hive and introduced into the test units one day before test start. The collected bumble bees were kept under test conditions until test start. During the acclimatisation period they were fed *ad libitum* with untreated 50% (w/v) aqueous sucrose solution.

The bumble bees were exposed to test item by individual feeding. Temperature during the test was between 24.3 and 25.0°C; relative humidity was 55.9 – 63.8%. Bees were kept in darkness (except during application and observation).

Assessments of mortality were made 24 and 48 hours after test start. Behavioural abnormalities such as symptoms of poisoning in comparison to the controls were recorded at each observation interval.

Dates of work: September 29, 2015 – October 01, 2015

Results:

Validity criteria:
The study is considered valid since the validity criteria were met:
- the mean control mortality was ≤ 10 % at the end of the test
- the mean reference item mortality was ≥ 50 % at the end of the test

Statistics:
Fisher's Exact Binominal Test (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 at the end of the test.

Biological results:
In the control group, treated with untreated 50 % (w/v) aqueous sucrose solution, 2.0 % mortality was observed during the 48 h test period. In the solvent control group, treated with 50 % (w/v) aqueous sucrose solution containing 5 % acetone and 0.1 % xanthan, no mortality was observed during the 48 h test period.

The reference item mortality of 90.0 % (corrected mortality: 89.8 %) at the end of the test (48 hours after application) was within the required range.

In the test item treatment group, 2.0 % mortality was observed at the dose level corresponding to 203 µg a.s./bumble bee (based on the actual uptake) at the final assessment after 48 hours.

The results described above are presented in the following table:

Table CA 8.3.1.1.1- 3: Mortality and actual uptake in the acute oral toxicity test on bumble bees with amidosulfuron (tech.) in the control, test item and reference item groups

Treatment group (Target dose)	Actual uptake	Mortality [%]		Corrected mortality [%]	
		24 h	48 h	24 h	48 h
Control	--	0.0	2.0	-	-
Solvent control	--	0.0	0.0	-	-
Test item: Amidosulfuron [µg a.s./bumble bee]					
250	203	0.0	2.0		
Reference item: Perfekthion [µg a.s./bumble bee]					
1.5	1.44	90.0	90.0	90.0	89.8

In the test item group only two affected bees were observed during the 48 hour test period.

Table CA 8.3.1.1.1- 4: Acute toxicity of amidosulfuron to bumble bees; oral laboratory test

Test Item	Amidosulfuron tech.
Test Object	*Bombus terrestris* L.
Exposure	**Oral toxicity test [µg amidosulfuron/bumble bee]**
LD_{50} (24 h)	> 203
LD_{50} (48 h)	> 203
NOED* (48 h)	≥ 203

* NOED = No Observed Effect Dose, Fisher's Exact Test (one-sided, $p \leq 0.05$)

Conclusion:
The 48 hour oral LD_{50} value for amidosulfuron technical was determined to be >203 µg a.s./bumble bee. The oral NOED was determined to be ≥ 203 µg a.s./bumble bee.

CA 8.3.1.1.2 Acute contact toxicity

Report: KCA 8.3.1.1.2/01; [redacted]; 1989; M-124103-01-1
Title: Report on laboratory investigations into the contact toxicity of HOE 075032 OH ZC94 2001 to the honey bee Apis mellifera L.
Report No.: A41182
Document No: M-124103-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: yes

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on an acute contact toxicity test on honey bees for the technical active substance. Acute contact LD_{50} of amidosulfuron was reported to be > 100 µg a.s./bee (48 h).

The study was rated invalid for reason of a lack in the test design (missing toxic standard) in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

Nevertheless, an EU agreed endpoint of acute contact LD_{50} > 100 µg a.s./bee was derived from this test, footnoting "study of limited validity".

For approval renewal, the study is superseded by a new guideline-conform acute contact toxicity test on honey bees, reported here below as KCA 8.3.1.1.2 /02. This new study confirmed the above endpoint for acute contact toxicity. It is hence proposed to change study reference to this EU endpoint, whilst no update is triggered for the numeric parameter itself.

Report:	KCA 8.3.1.1.2/02; [redacted]; 2014; M-503119-01-1
Title:	Effects of amidosulfuron tech. (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No.:	91051035
Document No.:	M-503119-01-1
Guideline(s):	**OECD 213 and 214 (1998)**
Guideline deviation(s):	**none**
GLP/GEP:	**yes**

The study reports on a combined test covering aspects of both data points acute oral (CA 8.3.1.1.1) and acute contact (CA 8.3.1.1.2) toxicity to honey bees.

A study summary has been provided before under point KCA 8.3.1.1.1 /01.

Study endpoint for acute contact toxicity for honey bee: LD_{50} >100 µg a.s./bee.

Report:	KCA 8.3.1.1.2/03; [redacted]; 2015; M-525139-01-1
Title:	Amidosulfuron technical: Acute contact toxicity to the bumble bee, Bombus terrestris L. under laboratory conditions
Report No.:	S14-00620
Document No.:	M-525139-01-1
Guideline(s):	**No specific guidelines are available. The test design is based on OEPP/EPPO 170 (4) (2010), OECD Guideline 214 (1998), and on the review article of VAN DER STEEN (2001)**
Guideline deviation(s):	**none**
GLP/GEP:	**yes**

Executive Summary:
The aim of this study was to determine the acute contact toxicity of amidosulfuron tech. to the bumble bee (*Bombus terrestris* L.) under laboratory conditions. For this purpose bumble bees were exposed for 48 hours to a single dose of 100.0 µg a.s. per bumble bee by topical application (contact limit test). One control group was exposed for the same period of time under identical exposure conditions to DMSO the other control group was exposed to tap water. Mortality and sub-lethal effects were assessed 24 and 48 hours after application. Only one affected bumble bee was observed during the 48 hour test period in the test item group. The contact LD_{50} (48 h) was > 100.0 µg a.s./bumble bee. The contact NOED (48 h) was determined to be ≥100 µg a.s./bumble bee.

Material and methods:
Test item: Amidosulfuron, technical (AE F075032); Specification No.: 102000000551-03; Batch code: AE F075032-01-05; Origin batch No.: 2014-000761; TOX-No.: 10494-00; Analysed content: 98.1% w/w; Certificate of Analysis No.: AZ 19335.

In the laboratory, 50 bumble bees (*Bombus terrestris* L.), were exposed for 48 hours to a single dose of 100.0 µg a.s. per bumble bee by topical application (contact limit test). Test units were plastic boxes

(base: 13 cm x 17 cm, length: 6 cm). 10 bumble bees were used per test unit. Five replicates were used per test item dose and three replicates were used for controls and reference item treatments. One control group was exposed to DMSO (solvent control) the other control group was exposed to tap water. The reference item group was exposed to the reference item Perfekthion (a.s. dimethoate). The tested dose was 13 µg dimethoate/bumble bee.

For the contact toxicity test, amidosulfuron was applied using DMSO as carrier. A single 2 µL droplet of the preparation of the test item was placed on the dorsal bumble bee thorax, likewise for the toxic reference solution (dimethoate, with water as carrier), the control (tap water) and solvent control (DMSO). After the application, the bumble bees were returned to the test cages and fed with a 50 % aqueous sucrose solution *ad libitum*. Temperature during the test was between 23.8 and 26.2°C; relative humidity was 47.0 – 61.8%. Bees were kept in darkness (except during application and observation).

The number of dead bumble bees was recorded 24 and 48 hours after dosing. Sub-lethal effects as symptoms of poisoning or any abnormal behaviour in comparison to the control were recorded at each observation interval.

Dates of experimental work: November 11, 2014 – November 13, 2014

Results:
Validity criteria:

Table CA 8.3.1.1- 1: Validity criteria

Validity Criteria		Recommended	Obtained
Mean mortality in the control groups at the end of the test	water control	≤ 10%	0%
	DMSO control	≤ 10%	0%
Mean mortality in the reference item treatment at the end of the test		≥ 50 %	86.7%

All validity criteria for the study were met.

Statistics:
Fisher's Exact Binominal Test (one-sided greater, $\alpha = 0.05$) was used to evaluate whether there are significant differences between the mortality data of the control and the test item treatment group at the end of the test.

Biological results:
In the control groups, treated with DMSO or tap water, no mortality was observed during the 48 h test period.

The reference item mortality of 86.7 % at the end of the test (48 hours after application) was within the required range.

In the test item treatment group, a mortality of 2.0 % was observed at the dose level corresponding to 100 µg amidosulfuron /bumble bee at the final assessment after 48 hours.

In the test item treatment group only one single affected bee was observed during the entire observation period. In the final assessment (48 hours after test start) no sub-lethal effects were noticed.

Table CA 8.3.1.1- 2: Acute toxicity of amidosulfuron to bumble bees; contact laboratory test

Test Item	Amidosulfuron tech.
Test Object	*Bombus terrestris* L.
Exposure	**Contact toxicity test [µg amidosulfuron/bumble bee]**
LD_{50} (24 h)	> 100
LD_{50} (48 h)	> 100
NOED* (48 h)	≥ 100

* NOED = No Observed Effect Dose, Fisher's Exact Test (one-sided, $p \leq 0.05$)

Conclusions:
The 48 hour contact LD_{50} value for amidosulfuron technical was determined to be >100 µg a.s./bumble bee. The contact NOED (48 h) was determined as ≥ 100 µg a.s./bumble bee.

CA 8.3.1.2 Chronic toxicity to bees

Note: The following study has been conducted using the formulation Amidosulfuron WG75, to serve as a vehicle for delivery of the active substance amidosulfuron to the test system. Since primary intent of the study is the generation of active substance information, it is summarised and evaluated on document MCA level.

Report: KCA 8.3.1.2/01 [redacted] 2016; M-549770-01-1
Title: Amidosulfuron WG 75A W: 10-day chronic feeding test on the honey bee (Apis mellifera L.) in the laboratory
Report No.: M-549770-01-1
Document No.: M-549770-01-1
Guideline(s): **Regulation (EC) No. 1107/2009**
OECD 213 (1998) and CEB No. 230 with current recommendations of the ring test group by Kling and Schmitzer (2015)
Directive 2003-01 (Canada/PMRA)
US EPA OCSPP Not Applicable
Guideline deviation(s): **none**
GLP/GEP: **yes**

Executive Summary:
The purpose of this study was to determine the chronic oral toxicity of Amidosulfuron WG 75 (750 g/kg) to the honey bee (*Apis mellifera* L.) for a period of ten days in the laboratory.

The test item was daily administered to young honey bees in a sugar solution at the concentration of 3333 mg a.s./kg feeding solution. An untreated control and a reference item were included in this study. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

The LC_{50} (10 days) was >3333 mg a.s./kg feeding solution, corresponding to the LDD_{50} of >78.4 µg a.s./bee/day. The NOEC (10 days) was ≥ 3333 mg a.s./kg feeding solution, corresponding to the NOEDD of ≥ 78.4 µg a.s./bee/day.

Material and methods:
Test item: Amidosulfuron WG 75A W; Specification No.: 102000000550, Batch ID: EFKE002780, Analysed content of active substance (Amidosulfuron, AE F075032): 74.9 % w/w; Sample Description: FAR01779-00.

The chronic toxicity of the test item Amidosulfuron WG 75 on the honey bee, *Apis mellifera* L. was assessed in a 10 days continuous oral feeding test in the laboratory (limit test). Over a period of

10 days, young honey bees (2 days old worker bees) were exposed by continuous and *ad libitum* feeding via 50 % (w/v) aqueous sucrose solution containing 3333 mg a.s./kg. An untreated control (50 % w/v sucrose solution) and a reference item (Perfekthion EC; 400 g/L dimethoate) at a concentration of 1 mg dimethoate/kg feeding solution were included in this study. The test was conducted with 5 replicates per treatment, each consisting of 10 bees per test cage. The temperature in the test environment (incubator) ranged from 31.0 to 34.0 °C, the relative humidity ranged from 57 to 90% (mean relative humidity: 84 %) at 24 h darkness (except during observation).

Mortality and behavioural abnormalities were assessed every day throughout the 10 days exposure period. Furthermore, the daily food uptake was determined.

Samples of the feeding solutions prepared freshly every day throughout the 10 days continuous feeding period were taken daily for subsequent chemical analysis in order to reveal the actual concentration of the test item.

Dates of experimental work: June 09, 2015 – July 17, 2015

Results:

Validity criteria:
The chronic oral test is considered valid as the control mortality was <15 % and the mortality of the reference item (dimethoate) was within the required range.

Analytical findings:
The actual concentration of Amidosulfuron in the feeding solutions ranged between 81 and 103%.

Biological findings:
The test item was daily administered to the bees in a sugar solution at the following concentration: 3333 mg a.s./kg sugar solution. This concentration led to an actual daily mean dose of 78.4 µg a.s./bee/day after 10 days.

At test end, 10 days following start of exposure, 6.0 % mortality occurred in the untreated water control (50 % w/v sucrose solution). At 3333 mg a.s./kg (corresponding to 78.4 µg a.s./bee/day) 2.0 % mortality occurred. This was statistically not significantly different from the control (Fisher`s Exact Test, $\alpha = 0.05$).

One moribund bee was observed at day 5 in the test item treatment group.

The reference item (dimethoate) at a concentration of 1 mg dimethoate/kg feeding solution corresponding to actually 0.018 µg a.s./bee/day caused 100 % mortality at day 6.

Table CA 8.3.1.2- 1: Ten-Day Chronic Feeding of Amidosulfuron WG 75 to young honey bees (laboratory test)

Test Object		*Apis mellifera carnica*	
Treatment Group	Concentration [mg a.s./kg]	Dose Level [1] [µg a.s./bee/day]	Mortality at day 10 [2], [% Mean]
Amidosulfuron WG 75	3333	78.4	2.0 (n.s.)
Water control	0.0	0.0	6.0
Reference Item	1.0	0.018	100.0
Endpoint at test termination (day 10)			
LC_{50}	**LDD_{50}**	**NOEC**	**NOEDD**
> 3333 mg a.s./kg	**> 78.4 µg a.s./bee/day**	**≥ 3333 mg a.s./kg**	**≥ 78.4 µg a.s./bee/day**

[1]mean dose per bee per day; dose measured based on consumed feeding solution
[2]Mortality at study termination 10 days after start of first feeding
Statistics:
Mortality: Fisher`s Exact Test, pairwise comparison, one-sided greater, $\alpha = 0.05$
NOEC/NOEDD: was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).
n.s. = no statistical significant difference compared to the control, * = statistically significant different compared to the control ($\alpha = 0.05$)

Conclusion:
The LC_{50} (10 days) was > 3333 mg a.s./kg feeding solution, corresponding to the LDD_{50} of > 78.4 µg a.s./bee/day.
The NOEC (10 days) was ≥ 3333 mg a.s./kg feeding solution, corresponding to the NOEDD of ≥ 78.4 µg a.s./bee/day.

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

Note: The following two studies have been conducted using the formulation Amidosulfuron WG75 as a vehicle for delivery of the active substance amidosulfuron to the test system. Since primary intent of the study is the generation of active substance information, it is summarised and evaluated on document MCA level.

Report: KCA 8.3.1.3/01; [redacted]; 2014; M-482118-01-1
Title: Amidosulfuron WG 75A W (750 g/kg): Effects on honey bee brood (Apis mellifera L.) - Brood feeding test
Report No.: M-482118-01-1
Document No.: M-482118-01-1
Guideline(s): [redacted]**. (1992). Method for honeybee brood feeding tests with insect growth-regulating insecticides. EPPO Bulletin, 22, 613-616 (1992)**
Guideline deviation(s): none
GLP/GEP: yes

Executive summary:
The purpose of the honey bee brood feeding study was to evaluate potential effects of Amidosulfuron WG 75 W on brood development and mortality of adult worker honey bees and pupae, *Apis mellifera*. 0.15 g test item in 1 L commercial ready-to-use sugar syrup (Apiinvert; 30 % sucrose, 31 % glucose, 39 % fructose) per colony was used (= 0.114 g amidosulfuron/L). As toxic reference 3.0 g reference item (Insegar; 25 % fenoxycarb) in 1 L commercial ready-to-use sugar syrup (Apiinvert) per colony was used (= 0.75 g fenoxycarb/L). As untreated control 1 L untreated commercial ready-to-use sugar syrup (Apiinvert) per colony was used. The ready prepared sugar solutions were offered per colony in a feeding trough. Three bee colonies were used per treatment group. Ontogenesis of honey bee eggs, young and old larvae was observed for a period of 21 days after application. Mortality of adult bees and pupae and sublethal effects such as changes in behaviour were also monitored. No adverse effects on any endpoints assessed were observed (i.e. on the survival of adult bees, larvae and pupae, brood

development). The method of investigating the development of the honey bee brood is based on the method of Oomen *et al.* (1992)[17].

Overall, it can be concluded that the administration of Amidosulfuron WG 75 fortified sugar syrup (0.114 g amidosulfuron/L) to honey bee colonies does neither adversely affect honey bee colonies nor bee brood development.

Material and methods:
Test item: Amidosulfuron WG 75A W; Specification No.: 10200000550-03; Batch-ID: EFKE002307; Sample description: TOX10124-00; Analysed content of a.s: 75.9 % w/w amidosulfuron (AE F075032).

Test species: Honey bees (*Apis mellifera* L.); honey bee colonies were maintained according to normal beekeeping practice, containing two magazines with 10 combs, each. The preliminary brood check indicated healthy colonies with all brood stages present and a sufficient supply of nectar and pollen. The mean strength of the colonies per treatment group, two days before application, ranged between 10035 and 17100 adult bees. Colonies were free flying, with access to natural food sources, but due to the season, there were no main flowering, bee-attractive crops or flowering weeds in the surrounding area.

Test design: A bee brood test was conducted, in order to assess the effect of Amidosulfuron WG 75A W to the honey bee brood. An untreated control and a toxic reference were included in the study. Three bee colonies were used per treatment group. The test item and reference item solutions were mixed with ready-to-use sugar syrup (Apiinvert) and applied to the bee colonies via a feeding trough, which was put directly into the colony on top of the second magazine. Pure sugar syrup (Apiinvert) was used for the controls. Ontogenesis of a defined number of honey bee eggs, young- and old larvae was observed for a period of 21 days following the application for each treatment group and colony. This was assessed one day before the application, by selecting one (or several) brood comb(s) of each colony and by taking a digital photo of this (these) brood comb(s). After saving the photo-file on a computer, eggs, young- and old larvae were marked at this first Brood area Fixing Day (BFD0). For each subsequent brood assessment (BFDn), again the same comb(s) was (were) selected from the respective colony and another digital photo was taken, in order to investigate the progress of brood development. Ontogenesis of the bee brood was observed for a period of 21 days after application (*i.e.* 22 days following BFD 0). Mortality of adult bees and pupae was also assessed.

Endpoints:

- Mortality of adult bees as well as pupae or larvae: between 3 days before to 21 days after application (= end of the trial);
- Bee brood development (eggs, young- and old larvae): one day before (= BFD0) and 4 (= BFD 5), 8 (= BFD 9), 15 (= BFD 16), 21 (= BFD 22) days after the application.

Test concentrations:
Control: 1 L untreated commercial ready-to-use sugar syrup (Apiinvert; 30 % sucrose, 31 % glucose, 39 % fructose) per colony.
Test Item: 0.15 g test item (Amidosulfuron WG 75A W) in 1 L commercial ready-to-use sugar syrup per colony, equivalent to an active substance concentration of 0.114 g amidosulfuron/L.
Reference Item: 3.0 g reference item (Insegar; 25 % fenoxycarb) in 1 L commercial ready-to-use sugar syrup per colony, equivalent to a nominal active substance concentration of 0.75 g fenoxycarb/L.

[17] [redacted]. (1992). Method for honeybee brood feeding tests with insect growth-regulating insecticides. EPPO Bulletin, 22, 613-616 (1992)

Test conditions: Natural field conditions. Temperature, relative humidity and rain were recorded during the experimental time

Statistics: Statistical evaluation was done for mortality and the brood termination rates using Shapiro-Wilk`s test (check for normal distribution), Levene`s test (check for homogeneity of variance), Student`s t-test (pairwise).

Dates of work: July 15, 2013 to August 08, 2013

Results:

Validity criteria:
For the validity of the test, the mean control mortality should not be considerable and in the reference item group there should be a high number of impacted brood, which either died in the larval, pupae or adult stage.

The overall mean mortality in the control group was low (21.2 dead bees/colony/day), and is within the range of normal mortality levels of colonies of the employed size under field conditions. In addition, a mean of 0.9 dead pupae/colony/day were found during the 21 days post-application period and represents a biologically typical number of dead pupae over the period of 21 days.

In the reference group, there was a high number of impacted bee brood, which resulted in 81.1% mean loss of the initial observed cells (100% eggs, 99.6% young larvae and 43.8 old larvae stages). The termination rate of the eggs was 100 % and the values of the young and old larvae termination rates were statistically significantly higher compared to the control. Thus, the reference item values were on an absolute scale sufficiently high to demonstrate the sensitivity of the test system and the validity of the test conditions.

Biological results:

Table CA 8.3.1.3- 1: Effects of Amidosulfuron WG 75 on honey bee brood

Test item	**Amidosulfuron WG 75A W**		
Test species	**Honey bees (*Apis mellifera* L) (complete colonies)**		
Exposure	***via* treated sugar solution**		
Treatment	Untreated control	Amidosulfuron WG 75A W	Reference item (Insegar, a.s. = fenoxycarb)
Rate per L sugar solution [product][1)]	-	0.15 g/L	3.0 g/L
Rate per L sugar solution [a.s.][1)]	-	0.114 g/L	0.75 g/L
Termination rate of the eggs [%][2)]	17.8 %	21.6 % (n.s.)	100 % (n.d.)
Termination rate of the young larvae [%][2)]	10.2 %	11.1 % (n.s.)	99.6 % (*)
Termination rate of the old larvae [%][2)]	6.4 %	6.2 % (n.s.)	43.8 % (*)
Mean brood termination rate over all stages	11.5 %	13.0 % (n.s.)	81.1 % (*)
Mean mortality of worker bees/colony/day during pre-application phase[3)] during the entire post-application phase[3)]	 20.3 21.2	 19.2 (n.s.) 18.0 (n.s.)	 5.8 (n.s.) 25.4 (n.s.)
Mean mortality of pupae+larvae/colony/day during pre-application phase[4)] during the entire post-application phase[4)]	 0.9 0.9	 0.1 (n.s.) 0.0 (n.s.)	 2.0 (n.s.) 1.3 (n.s.)
Mean number of bees before application[5)]	15030	17100	10035

[1)] test and reference item were mixed with sugar solution
[2)] mean termination rate of 3 colonies per treatment group
[3)] mean number of dead honeybees per day and colony found in dead bee traps
[4)] mean number of dead pupae/larvae per day and colony found in dead bee traps
[5)] mean number of bees per colony
Statistics: n.s. = not statistically significant compared to the control; * = statistically significant compared to the control; n.d. = not determined; Student t-test, α = 0.05, pairwise comparison, two-sided (before application), one-sided greater (after application)

Although the mean termination rate of eggs was slightly higher in the test item treatment group (21.6 %) when compared to the values of the control group (17.8 %), there was no statistically significant difference. Thus, there was no effect on the development of eggs following the consumption of the test item via treated sugar solution.

The development success of the young larvae in the test item treatment group was slightly lower and resulted in a mean termination rate of 11.1 % compared to 10.2 % in the control group. This difference was not statistically significant compared to the control group. Thus, there was also no effect on the development of young larvae after consumption of the test item via treated sugar solution.

No effect on the development of old larvae was observed after consumption of the test item treated sugar solution. The mean termination rate of old larvae in the test item treatment group was lower with a mean of 6.2 % compared to 6.4 % in the control group. Accordingly, this was not statistically significant compared to the control group.

Adult bee mortality in the test item treatment group was lower (mean of 18.0 dead bees per day) when compared to the control group (21.2 dead bees per day) and not statistically significantly different.

Nearly no dead larvae and pupae were found in the dead bee traps after treatment with Amidosulfuron WG 75. Thus, there was no effect of the test item on honey bee pupae and larvae.

No behavioural impairments were noted at any time in any of the test or reference item treatment groups until test end. Also no behavioural abnormalities were observed in the control group.

The reference item treatment (Insegar, a.s. = fenoxycarb) resulted in an egg termination rate of 100 % and a statistically significantly increase of unsuccessful young- and old larvae development and thus confirmed the sensitivity of the test system and the validity of the test conditions.

Conclusion:
Overall, it can be concluded according to the results of this study that the administration of Amidosulfuron WG 75 fortified sugar syrup (0.114 g amidosulfuron/L) to honey bee colonies does neither adversely affect honey bee colonies nor bee brood development.

Report:	KCA 8.3.1.3/02; [redacted]; 2016; M-545720-01-1
Title:	Amidosulfuron WG 75A W (750 g/kg): Effects on honey bee brood (Apis mellifera L.) under semi-field conditions - Tunnel Test - Final report
Report No.:	EBBEN041
Document No.:	M-545720-01-1
Guideline(s):	**OEPP/EPPO guideline No. 170 (4) (OEPP/EPPO, 2010), OECD Number 75 (2007) and recommendations of the AG Bienenschutz (2011)**
Guideline deviation(s):	**Yes, see report**
GLP/GEP:	**yes**

Executive summary:
A higher tier semi-field honey bee brood study (following OECD Guidance Document 75 (2007) and the Guideline OEPP/EPPO No. 170 (4) (2010)) was conducted under forced confined exposure conditions. As deviations to the guidelines the post-application exposure phase in the tunnel was reduced to 3 days due to the herbicide mode of action of the test item against the *Phacelia*-crop.

Amidosulfuron WG 75A W (750 g/kg) was applied at a rate of 60.1 g product in 400 L tap water/ha (corresponding to 45 g amidosulfuron/ha) to full flowering *Phacelia tanacetifolia*, a highly bee attractive surrogate crop.

The test was designed as a replicated tunnel study to assess potential effects of amidosulfuron to honey bee colonies, including a very detailed assessment of brood development. Tunnels (20 m length x 5.5 m width x 2.5 m height) were set up on a ca. 75 m² plot of *Phacelia tanacetifolia* (2 x 36 m²). Small bee colonies (11 frames, ~6000 bees per colony) were introduced to the tunnels 4 days before the application. One honey bee colony was used per tunnel.

The test item, water and a reference item were applied on the whole plot of plants in two operations, with foraging bees present. The trial was carried out using four tunnels (i.e. replicates) for the test item treatment, the control and the reference item treatment (Insegar, 250 g/kg fenoxycarb), respectively. The confined exposure phase of the honey bees inside the treated crop was 3 days following the test item application. At the end of the 3rd day after application, due to the herbicide mode of action of the test item, the *Phacelia*-crop was no longer attractive to bees (faded crop) and did not longer support the confined colonies. Thus, all bee colonies (i.e. the colonies from the test item, the water and the reference item group, respectively) were relocated after 3 complete days of confined exposure from their respective tunnels and placed in an area with no main flowering, bee attractive crops.

Applications were conducted during daytime and during full flowering of the *Phacelia*-crop (BBCH 65), with confined honey bees actively foraging on the crop during application. After foliar (spray) application of the water (control), test item (Amidosulfuron WG 75A W (750 g/kg)) and the reference item (fenoxycarb), ontogenesis of a defined number of honey bee eggs was observed for each group and colony. Mortality of adult bees and pupae/larvae as well as foraging activity of the adult bees was also assessed. The condition of the colonies was assessed in regular intervals until the end of the trial.

Ontogenesis of the bees from egg to adult workers was observed for a period of 22 days (i.e. one complete honey bee brood cycle). This was done one day before the application by taking out one or more brood combs and taking a digital picture of the brood combs. After saving the file on a computer,

250 eggs per colony were marked at this first brood area fixing day BFD0 (BFD = Brood Area Fixing Day). For each subsequent brood assessment (BFDn), again, the respective comb(s) was taken out of the hive and another digital photo was taken in order to investigate the progress of the brood development until day 21 following the application (BFD22 following BFD0).

Statistical evaluation was done for mortality, foraging activity, colony strength, brood termination rate and brood indices using Shapiro-Wilk`s test (check for normal distribution), Levene`s test (check for homogeneity of variance), Student or Welch t-test (pairwise comparison).

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 was 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 Amidosulfuron WG 75A W (750 g/kg) does not adversely affect honey bees and honey bee brood when applied at a rate of 60.1 g product in 400 L tap water/ha (corresponding to 45 g amidosulfuron/ha), during honey bees actively foraging on a bee-attractive, flowering crop. The observed, characteristic brood effects of the reference item Insegar (a.s. fenoxycarb) in terms of typicality, time of occurrence and extent, showed that the prevailing test conditions allowed for a profound detection of effects on immature honey bee life stages.

Material and methods:

Test Item: Amidosulfuron WG 75A W (750 g/kg); Specification No.: 102000000550; Batch ID: EFKE002780; Sample Description: FAR01779-00; analysed content of a.s.: 74.9 % w/w amidosulfuron (AE F075032).

Test Species: Honey bees (*Apis mellifera carnica* L.); small bee colonies, maintained according to normal beekeeping practice, containing 11 combs with honey, pollen and brood. The preliminary brood check indicated healthy colonies with all brood stages present and a minimum reserve of food (uncontaminated nectar and pollen) to guarantee colony viability and brood status but also to ensure that enough space is available for exposure of the brood to new food sources. The mean strength of the colonies per treatment group, one day before the application, was similar and ranged between 6030 and 6739 adult bees per colony.

Test Design: The test was conducted under forced/confined exposure conditions (tunnel), in order to assess potential effects of Amidosulfuron WG 75A W (750 g/kg) to honey bee colonies including brood development under semi-field conditions. Tunnels (20 m length x 5.5 m width x 2.5 m height) were set up on a ca. 75 m² plot of *Phacelia tanacetifolia* (2 x 36 m²). Small bee colonies were introduced to the tunnels 4 days before the application. One honey bee colony was used per tunnel.
The test item, water and a reference item were applied on the whole plot of plants in two operations, with foraging bees present. The trial was carried out using four tunnels (i.e. replicates) for the test item treatment, the control and the reference item treatment (Insegar, 250 g/kg fenoxycarb), respectively. The confined exposure phase of the honey bees inside the treated crop was 3 days following the test item application. At the end of the 3rd day after application, due to the herbicide mode of action of the test item, the *Phacelia*-crop was no longer attractive to bees (faded crop) and no longer supported the confined colonies. Thus, all bee colonies (i.e. the colonies from the test item, the control and the reference item group, respectively) were relocated after 3 complete days of confined exposure from their respective tunnels and placed in an area with no main flowering, bee attractive crops.

After foliar (spray) application of the water (control), test item and the reference item, ontogenesis of a defined number of honey bee eggs was observed for each group and colony. Mortality of adult bees and pupae/larvae as well as foraging activity of the adult bees was also assessed. The condition of the colonies was assessed in regular intervals until the end of the trial.

Ontogenesis of the bees from egg to adult workers was observed for a period of 22 days (i.e. one complete honey bee brood cycle). This was done one day before the application by taking out one or

more brood combs and taking a digital picture of the brood comb(s). After saving the file on a computer, 250 eggs per colony were marked at this first brood area fixing day BFD0 (BFD = Brood Area Fixing Day). For each subsequent brood assessment (BFDn), again, the respective comb(s) was taken out of the hive and another digital photo was taken in order to investigate the progress of the brood development until day 21 following the application (BFD22 following BFD0).

Test Parameters:

- Mortality of adult bees and pupae: 3 days before to 27 days after application (= end of the trial);
- Behavioural abnormalities: 3 days before to 27 days after application (= end of the trial);
- Foraging activity of the bees: 3 days before to 3 days after application;
- Condition of the colonies (food stores, brood status and colony strength): 1 day before and 4, 8, 15, 21, 27 and 41 days after application;
- Bee brood development (eggs): 1 day before (= BFD0) and 4 (= BFD 5), 8 (= BFD 9), 15 (= BFD 16), 21 (= BFD 22) days after the application.

Application Rates:

Control:	400 L tap water/ha,
Test Item:	45 g amidosulfuron/ha via 400 L spray solution/ha; according to Certificate of Analysis: 60.1 g Amidosulfuron WG 75A W (750 g/kg) in 400 L tap water/ha (corresponding to 0.15 g Amidosulfuron WG 75A W (750 g/kg)/L tap water),
Reference Item:	300 g fenoxycarb (1200 g product)/ha in 400 L spray solution/ha (corresponding to nominally 3.00 g product/L);

All applied during full flowering of the crop when honey bees were actively foraging on the *Phacelia*-crop

Test Conditions: Natural field conditions. On the application day, it was very warm and sunny and accordingly there was a high honeybee foraging activity on the crop within the tunnels. Mean temperature during the whole experiment (until day 27 following application) was between 16.4 and 30.9°C. No rain occurred during the exposure phase of the bees to the treated crop in the tunnels. First precipitation (4 mm) occurred between night of day 5 and morning of day 6. Thereafter rain occurred on 10 occasions during the next 21 days, predominantly in the second half of the experiment.

Statistics: Statistical evaluation was done for mortality, foraging activity, colony strength, brood termination rate and brood indices using Shapiro-Wilk`s test (check for normal distribution), Levene`s test (check for homogeneity of variance), Student or Welch t-test (pairwise comparison)

Dates of work: June 29, 2015 – August 12, 2015

Results:

Mortality of the adult bees (worker bees)

Pre-application phase (day- 3 to day 0 before application):

Mortality of the pre-application phase in the control, test item and reference item group was 77.2, 104.9 and 96.5 dead bees/colony/day, respectively. This was not statistically significantly different compared to the water control (Student t-test, pairwise comparison to the control, two-sided, $\alpha = 0.05$).

Exposure phase in the tunnels (day 0 after application to day 3):

Mortality of adult bees in the test item treatment was slightly higher compared to the control group. The comparison of the daily mortality values between the test item treatment and the control group showed a statistical significant difference to the control on the day of application (day 0). Thereafter from day 1 to day 3 following application, no statistical significant difference to the control group was detected. A statistical evaluation of the mean mortality levels from the period of day 0 after application to day 3 resulted in no statistical significant difference when compared to the control group

(Student t-test, pairwise comparison, one-sided greater, α = 0.05). Average control mortality of adult bees during the exposure phase (day 0 to day 3 following the application) was 95.8 dead bees/colony/day. The average mortality in the test item group was higher with 195.4 dead bees/colony/day. Reference Item mortality was 147.7 dead bees/colony/day.

Phase outside the tunnels (day 4 after application to day 27):
The number of dead bees in the test item treatment was low with a mean of 2.6 dead bees per day and colony during the period from day 4 to day 27 after treatment. This was lower and accordingly not statistical significant different to the control (2.8 dead bees/day/colony) (Student t-test, pairwise comparison, one-sided greater, α = 0.05). The overall comparison from day 0 to day 27 showed that the number of dead bees found in the test item treatment (30.1 dead bees /day/tunnel) was not statistical significant compared to the number of dead bees found in the control group (16.0 dead bees/day/colony).

After treatment with the reference item to the adult bees, mortality was slightly increased (4.9 dead bees/day/colony) but this was not statistically significant different to the control value (2.8 dead bees/day/colony. The day wise comparison indicated a statistical significant difference to the control on days 4, 5, 15 and 20 (Student t-test, pairwise comparison, one-sided greater, α = 0.05).

Mortality of pupae
Pre-application phase (day -3 to day 0 before application):
No dead pupae were found in the control and reference item treatment before application. In the test item treatment 4 dead pupae were found over the pre-application period of 3.5 days in all 4 colonies (0.25 dead pupae/day/colony). These numbers were not statistically significant (Welch t-test, pairwise comparison to the control, two-sided, α = 0.05).

Exposure phase in the tunnels (day 0 after application to day 3):
Mean pupae mortality during exposure phase in the test item treated group was 0.38 dead pupae/day/colony and not statistically significantly different to the control group (0.25 dead pupae/day/colony) (Welch t-test, pairwise comparison one-sided greater, α = 0.05).
No dead pupae were found after the application of the reference item following the first 3 days after treatment.

Phase outside the tunnels (day 4 after application to day 27):
In the test item treatment group only 2 dead pupae were found during the period from day 4 to 27 in all 4 colonies (0.02 dead pupa/day/colony). No dead pupa was found in the control group for this period. The mean number of dead pupae found in the test item treatment for the period from day 4 to 27 and 0 to 27 was not statistically significantly different to the control group.
Pupae mortality in the reference item group was distinctly increased with means of 4.85 and 4.16 dead pupae/day/colony for both post-application periods from day 4 to 27 and 0 to 27 and both were statistically significant different to the control group (Welch t-test, pairwise comparison one-sided greater, α = 0.05).

Foraging Activity
Pre-application phase (day -3 to day 0 before application):
The mean foraging activity in the intended test item and reference item groups was comparable to the control group, resulting in overall daily mean values of 23.3, 24.3 and 21.7 bees/m²/day in the control, test item group and reference item groups, respectively. No statistically significant differences were found between the control, the test and reference item treatment groups at the overall daily mean comparison of this period (Student`s t-test, α = 0.05, two-sided).

Exposure phase in the tunnels (day 0 after application to day 3):
Over the first two days following application (day 0 and day 1), foraging activity in the test item group was not reduced when compared to the control group or the situation before application. From day 2

onwards foraging activity was decreasing due to the fading attractiveness of the crop as the result of the herbicide action of the test item. On day 3 foraging activity was distinctly decreased to the control group and the bees were removed from the tunnels in the evening of day 3. Accordingly, the overall daily mean foraging activity from day 0 to day 3 in the test item group was little lower with 18.3 bees/m²/day compared to 24.7 bees/m²/day in the control group. As foraging activity for the first 2 days was comparable to the control group, the mean value over the post application period was not statistically significant different (Student t-test, pair-wise comparison to the control, one-sided smaller, $\alpha = 0.05$).

The reference item (Insegar) resulted in no reduction of the foraging activity.

Behavioural abnormalities
No test item related behavioural abnormalities occurred at any time during the whole assessment period (up to day 27). No behavioural abnormalities were observed in the control group and in the reference item group.

Condition of the Colonies
At the beginning of the trial, all queens (or eggs) and all brood stages (eggs, larvae and closed brood) were found in all colonies as an indication of healthy colonies. 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.

An additional brood and colony check was assessed on day 41 (42 days following BFD0) in order to cover 2 whole brood cycles of the honey bees (2 x 21 days).

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

After application, no indication of a test item related effect on the condition of the colonies was observed. Compared to the control, a similar amount of all single brood stages (*i.e.* eggs, larvae or closed brood (pupae) could be found during the assessments with no indication of a test item related effect. On all colony assessment days (*i.e.* 1 day before and on days 4, 8, 15, 21, 27 and 41 after the applications the total number of brood in the test item treated colonies followed the same pattern as the control colonies. All test item treated colonies remained vital with increasing bee numbers and healthy brood. There was no indication of any hazard of the test item on the condition of the bee colonies.

Colony Strength
The mean number of honey bees per colony in all treatment groups was similar one day before application and did not differ statistically significantly (mean of 6030 to 6739 per colony). The subsequent development of the colony strength among the colonies in the control and test item treatment groups followed the same pattern. Following re-movement of the colonies from the tunnels, there was a continuous increase of colony strength observable, which was very similar or even higher in the test item group compared to the control group. No statistically significant difference in the colony strength between the test item treated colonies and the control colonies occurred at any assessment date (Student t-test, pair-wise comparison to the control, one-sided smaller, $\alpha = 0.05$). Overall, no adverse effects of the test item on colony strength and population development have been observed throughout the study. Development in the reference item group was decreased.

Considering the initial mean number of bees per treatment group before the application as 100 %, the following relative mean numbers of bees were determined:

Treatment Group	Day[1] -1	Day +4	Day +8	Day +15	Day +21	Day 27	Day 41
Control	100%	114%	129%	150%	133%	127%	163%
Test Item	100%	113% (n.s.)	143% (n.s.)	154% (n.s.)	132% (n.s.)	133% (n.s.)	163% (n.s.)
Reference Item	100%	110% (n.s.)	130% (n.s.)	118% (n.s.)	121% (n.s.)	99% (n.s.)	113% (*)

[1] in relation to the application
n.s. = not statistically significant to the control, *. = statistically significant to the control; Student t-test, α=0.05, pairwise; one-sided smaller.

Development of Bee 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 BFD (Brood Fixing Day) 22 in the test item group was with a mean of 47.4 % only higher compared to the control group (29.7 %). This higher 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).

Brood Compensation Index:

The Brood Compensation Index is an indication for recovery and shows the development of the brood at each assessment. A continuous brood development was observed in the test item as well as in the control group. The Brood Compensation Indices following the labelling of the egg stage up to day 21 after application (BFD+22) were only slightly lower in the test item group compared to the control. Differences in the Brood Compensation Index between test item and control were not statistically significant. The high brood 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.

Treatment Group	BFD +5	BFD +9	BFD +16	BFD +22
Control	2.5	3.0	3.0	3.9
Test Item	1.8 (n.s.)	2.6 (n.s.)	2.8 (n.s.)	3.8 (n.s.)
Reference Item	0.7 (*)	0.8 (*)	1.0 (*)	2.3 (*)

n.s. = not statistically significant to the control, * = statistically significant to the control, Student t-test, α=0.05, pairwise; one-sided smaller.

Brood Index:

The Brood Index as an additional indicator for the bee brood development facilitates a comparison between the different treatments. Following the labelling of the egg stage, the Brood Indices of the test item group were as well only slightly lower compared to the control values. Differences in the Brood Index between test item and control were not statistically significant. After treatment with the reference item Insegar (a.s.: fenoxycarb), following the labelling of the eggs, the mean Brood Indices were statistically significant lower compared to the control indices.

Treatment Group	BFD +5	BFD +9	BFD +16	BFD +22
Control	2.4	2.8	2.8	3.5
Test Item	1.8 (n.s.)	2.2 (n.s.)	2.1 (n.s.)	2.6 (n.s.)
Reference Item	0.5 (*)	0.6 (*)	0.5 (*)	0.6 (*)

n.s. = not statistically significant to the control, * = statistically significant to the control, Student t-test, α=0.05, pairwise; one-sided smaller.

Accordingly, 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).

Table CA 8.3.1.3- 2: Effects of Amidosulfuron WG 75A W (750 g/kg) on honey bee brood under semi-field conditions (Tunnel Test)

Parameter	Treatment group[1]		
	Control	**Amidosulfuron WG 75A W (750 g/kg)**	**Reference Item Insegar [0.3 kg a.s./ha]**
Mean mortality of worker bees / colony / day [n] during			
pre-application phase [2]	77.2 ± 42.9	104.9 ± 60.6 (n.s.)	96.5 ± 50.9 (n.s.)
exposure phase in the tunnels [2]	95.8 ± 43.4	195.4 ± 104.5 (n.s.)	147.7 ± 73.9 (n.s.)
phase outside the tunnels [3]	2.8 ± 6.4	2.6 ± 4.8 (n.s.)	4.9 ± 7.6 (n.s.)
overall after application	16.0 ± 36.6	30.1 ± 77.2 (n.s.)	25.3 ± 57.0 (n.s.)
Mean mortality of larvae and pupae [n] during			
pre-application phase [4]	0.00 ± 0.00	0.25 ± 0.35 (n.s.)	0.00 ± 0.00 (n.d.)
exposure phase in the tunnels [4]	0.25 ± 0.20	0.38 ± 0.48 (n.s.)	0.00 ± 0.00 (n.d.)
phase outside the tunnels [5]	0.00 ± 0.00	0.02 ± 0.07 (n.s.)	4.85 ± 8.34 (*)
overall after application	0.04 ± 0.11	0.07 ± 0.21 (n.s.)	4.16 ± 7.89 (*)
Mean foraging activity / m² / colony / day [n] during			
pre-application phase	23.3 ± 5.7	24.3 ± 5.4 (n.s.)	21.7 ± 4.7 (n.s.)
exposure phase in the tunnels	24.7 ± 4.5	18.3 ± 8.5 (n.s.)	22.2 ± 5.5 (n.s.)
Mean brood termination rate [%] [6]	29.7	47.4 (n.s.)	88.3 (*)

[1] each with four tunnels (replicate)
[2] mean number of dead honey bees per day and colony found in dead bee traps and on gauze strips in the tunnels
[3] mean number of dead honey bees per day and colony found in dead bee traps, only
[4] mean number of dead pupae/larvae per day and colony found in dead bee traps and on gauze strips in the tunnels
[5] mean number of dead pupae/larvae per day and colony found in dead bee traps, only
[6] at BFD 22
Statistic: Student or Welch t-test, α=0.05, pairwise; before application: two-sided; after application one-sided greater (mortality and termination rate), one-sided smaller (foraging activity).
n.s. = not statistically significant compared to the control; * = statistically significant compared to the control
"n.d." = not determined due to "0" response

Conclusions:
To assess the potential effects of Amidosulfuron WG 75A W (750 g/kg) on honey bee colonies including brood development, 60.1 g product in 400 L tap water/ha (corresponding to 45 g amidosulfuron/ha), tap water for the control and a reference item were applied to a full-flowering and highly bee-attractive crop (i.e. *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 was 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 Amidosulfuron WG 75A W (750 g/kg) does not adversely affect honey bees and honey bee brood when applied at a rate 60.1 g product in 400 L tap water/ha (corresponding to 45 g amidosulfuron/ha), during honey bees actively foraging on a bee-attractive, flowering crop.

The observed, characteristic brood effects of the reference item Insegar (a.s.: fenoxycarb) in terms of typicality, time of occurrence and extent, showed that the prevailing test conditions allowed for a profound detection of effects on immature honey bee life stages.

CA 8.3.1.4 Sub-lethal effects

There is no particular study design / test guideline to assess "sub-lethal effects" in honey bees. However, in each laboratory study as well as in any higher-tier study, sub-lethal effects, if occurring, are described and reported.

CA 8.3.2 Effects on non-target arthropods other than bees

No studies on the pure active substance were generated. Tests on non-target arthropods other than bees are product related information and as such found reported in document MCP for the representative formulation.

CA 8.3.2.1 Effects on Aphidius rhopalosiphi

(see comment under CA 8.3.2)

CA 8.3.2.2 Effects on Typhlodromus pyri

(see comment under CA 8.3.2)

CA 8.4 Effects on non-target soil meso and macrofauna

In the new European dossier format data requirements there is no data point that corresponds to acute toxicity to earthworms, and acute risk assessment for earthworms is not required anymore. Nevertheless, one acute study (on the active substance) and two statements (on metabolites) are mentioned here as supportive information, since they are contained in the baseline dossier and in the List of Endpoints from the first EU review.

Studies submitted and evaluated for the first inclusion of amidosulfuron on Annex I:

Report:	KCA 8.4/01; [redacted]; 1987; M-119372-01-1
Title:	The Effect of Hoe 075032 - substance, technical (Identification code : Hoe 075032 OH ZC96 0001) to Eisenia fetida (earthworm) in a 14 day Artificial Soil Test (method OECD)
Report No.:	A37692
Document No.:	M-119372-01-1
Guideline(s):	**OECD: 207 (1984)**
Guideline deviation(s):	**Deviation not specified**
GLP/GEP:	**yes**

The study reports on an acute earthworm toxicity test for the technical active substance. The study was rated valid in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

An EU agreed endpoint of LC_{50} > 1000 mg a.s./kg d.w.soil was derived from this test.

Note: In context of application for EU approval renewal of amidosulfuron, this endpoint is ranked as supportive information, since acute earthworm testing is no longer a data requirement under Regulation 1107/2009. The updated List of Endpoints will include only data from a corresponding chronic earthworm test.

Report:	KCA 8.4/02; [redacted]; 1999; M-188950-01-1
Title:	Assessment of the ecological risk of soil metabolites of amidosulfuron to earthworms Code: AE F075032
Report No.:	C004996
Document No.:	M-188950-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	no

Report:	KCA 8.4/03; [redacted]; 2004; M-236891-01-1
Title:	Amidosulfuron AE F075032: Effects of soil metabolites on earthworms Additional remarks to document C004996
Report No.:	C045228
Document No.:	M-236891-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	no

The two statements predict earthworm toxicity to be low for soil metabolites of amidosulfuron, founded on structural considerations. Both documents were rated valid in the EU review for the first inclusion of amidosulfuron on Annex I, a review is found in the previous DAR (2006).

No EU agreed endpoints were derived from these studies.

Note: In context of application for EU approval renewal of amidosulfuron, these statements are superseded since acute earthworm risk assessment is no longer a data requirement under Regulation 1107/2009. Chronic earthworm tests are now available for all soil metabolites considered relevant for risk assessment.

CA 8.4.1 Earthworm, sub-lethal effects

For amidosulfuron and its metabolites amidosulfuron-desmethyl, amidosulfuron-desmethyl-chloropyrimidine, amidosulfuron-guanidine, amidosulfuron-ADMP, and amidosulfuron-ADHP, reproductive toxicity studies on *Eisenia fetida* were performed. In these studies, no mortality occurred. No-Observable-Effect levels ranged from 9.98 mg/kg dws for the metabolite amidosulfuron-ADMP to ≥ 983 mg/kg dws for the metabolite amidosulfuron-guanidine. An overview of all studies is provided in the following table.

Table CA 8.4.1- 1: Reproductive toxicity data of amidosulfuron and metabolites to *Eisenia fetida* presented in this chapter

Test species	Test system	Test duration	Endpoint	Reference
Amidosulfuron				
Eisenia fetida (earthworm)	reproduction test[1)]	56 d	NOEC 56 mg prod./kg dws 42.5 mg a.s./kg dws	[redacted]; 2015; M-524933-01-1 KCA 8.4.1/03
Amidosulfuron-desmethyl				
Eisenia fetida (earthworm)	reproduction test	56 d	NOEC ≥ 95.8 mg p.m./kg dws[2)]	[redacted]; 2015; M-529709-01-1 KCA 8.4.1/04
Amidosulfuron-desmethyl-chloropyrimidine				
Eisenia fetida (earthworm)	reproduction test	56 d	NOEC ≥ 887 mg p.m./kg dws[3)]	[redacted]; 2009; M-359724-01-1 KCA 8.4.1/01
Amidosulfuron-guanidine				
Eisenia fetida (earthworm)	reproduction test	56 d	NOEC ≥ 983 mg p.m./kg dws[4)]	[redacted]; 2009; M-358183-01-1 KCA 8.4.1/02
Amidosulfuron-ADMP				
Eisenia fetida (earthworm)	reproduction test	56 d	NOEC 9.98 mg p.m./kg dws[5)]	[redacted]; 2013; M-461051-01-1 KCA 8.4.1/05
Amidosulfuron-ADHP				
Eisenia fetida (earthworm)	reproduction test	56 d	NOEC ≥ 99.5 mg p.m./kg dws[6)]	[redacted]; 2015; M-533011-01-1 KCA 8.4.1/06

dws = dry weight soil; prod. = product, a.s. = active substance,; p.m. = pure metabolite
1) conducted with WG 75 formulation
2) corrected to an anlysed purity of 95.8 %
3) corrected to an anlysed purity of 88.7 %
4) corrected to an anlysed purity of 98.3 %
5) corrected to an anlysed purity of 99.8 %
6) corrected to an anlysed purity of 99.5 %

The metabolite amidosulfuron-biuret was detected as a minor and transient soil metabolite. Maximum occurrence detected in soil was 6.3 %. No potential for persistence of amidosulfuron-biuret is indicated based on the soil half-life calculated to range from 18.6 to 65.7 days.
The chemical structure of amidosulfuron-biuret is very close to the structure of the metabolite amidosulfurone-guanidine, so that similar ecotoxicological properties of both substances may be expected. The latter component, being formed in soil in more relevant quantity and being characterized by longer degradation half-life, has been tested in reproductive toxicity studies on *Eisenia fetida*.
Therefore, for amidosulfuron-biuret no reproductive toxicity testing on *Eisenia fetida* was deemed required.

Studies on amidosulfuron:

Note: The following study has been conducted using formulation Amidosulfuron WG75 as a vehicle for delivery of the active substance amidosulfuron to the test system. Since primary intent of the study is the generation of active substance information, it is summarised and evaluated on document MCA level.

Report:	KCA 8.4.1/03;[redacted]; 2015; M-524933-01-1
Title:	Amidosulfuron WG 75 W: Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil
Report No.:	kra/Rg-R-164/14
Document No.:	M-524933-01-1
Guideline(s):	**The OECD Guideline No.: 222 for the Testing of Chemicals "Earthworm Reproduction Test (Eisenia fetida / Eisenia andrei)" adopted April 13, 2004; ISO 11268-2 "Soil quality - Effects of pollutants on earthworms (Eisenia fetida) Part 2: Determination of effects on reproduction" adopted July 1998**
Guideline deviation(s):	**for deviation see Point 2.3**
GLP/GEP:	**yes**

Executive summary:

The purpose of this study was to assess the effect of Amidosulfuron WG 75 on survival, growth, and reproduction of the earthworm *Eisenia fetida* during an exposure in an artificial soil with 8 different test concentrations. The test was performed according to the recommendations of the OECD Guideline 222 (2004) and the International Standard ISO 11268-2 (1998). As minor deviation from the guideline the age of the worms from the synchronised culture differed not more than 8 weeks (instead of 4 weeks).

Ten adult earthworms (*Eisenia fetida*, approximately 8 months old) per replicate (4 replicates per test concentration of the treatment group, 8 replicates for the control group) were exposed in artificial soil (containing 70% industrial quartz sand, 20% kaolinite clay and 10% sphagnum peat) to an untreated control (quartz sand only) and to nominal concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration were determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for this study is 56 mg test item/kg dry weight artificial soil (equals 42.5 mg a.s./kg dws). Thus, the overall LOEC is determined to be 100 mg test item/kg dry weight artificial soil (equals 75.9 mg a.s./kg dws).

Material and methods:

Test item: Amidosulfuron WG 75 W; Batch ID: EFKE002307; Specification No.: 102000000550-03, Material No.: 05938848; Sample description: TOX10124-00; Workorder: 13005778; Analysed content of a.s.: 75.9 %w/w.

Adult *Eisenia fetida* (approximately 8 months old, 4 x 10 animals per test concentration of the treatment group and 8 x 10 animals for the control group) were exposed in an artificial soil (containing 70% industrial quartz sand, 20% kaolinite clay and 10% sphagnum peat) to an untreated control (quartz sand only) and to nominal concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil. The test item was mixed into the soil. The adult earthworms were fed once per week during the test period with approximately 5 g finely ground, air dried animal manure per vessel. During the test period, the temperature was in the range of 18 to 22 °C. The test vessels were kept under a photoperiod: light:dark = 16 h : 8 h with a desired light intensity of 400 - 800 Lux. After 28 days the number of surviving animals and their weight alteration were determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

As minor deviation from the guideline the age of the worms from the synchronised culture used for the study differed not more than 8 weeks (instead of 4 weeks).

Toxic standard: Carbendazim (Carbendazim EC 360 G; 360 g a.s./L): 1.25, 2.5 and 5.0 mg a.s./kg dry weight artificial soil, control: artificial soil moistened with deionised water, solvent control: none.

Dates of experimental work: July 15, 2014 – September 18, 2014

Results:

Table CA 8.4.1- 2: Validity criteria of the study

Validity criteria	Recommended	Obtained
Mortality of the adults in the control	≤ 10 %	0 %
Rate of reproduction of juveniles (earthworms per control vessel)	≥ 30	211, 146, 216, 213, 206, 208, 208, 158
Coefficient of variance of reproduction in the control	≤ 30 %	14.0 %

The validity criteria of the test according to the guideline were fulfilled.

The most recent toxic standard reference test with the reference test item mixed into the artificial soil, was performed from January 10 to July 8, 2014 (kra-Rg-R-Ref 20/14; NON-GLP). No mortality of the adult earthworms was observed 28 days after application. The change of body weight of the adult earthworms of the test concentration of 2.50 and 5.00 mg a.s./kg dry weight soil were statistically significant reduced in comparison to the control. The number of juveniles per test vessel of the three test concentrations of 1.25, 2.50 and 5.00 mg a.s./kg dry weight artificial soil were statistically significant reduced in comparison to the control. EC_{50} for reproduction were calculated to be 1.770 mg a.s./kg dry weight artificial soil, respectively. Confidence limits (95 %) were calculated to be 1.769 – 1.772 mg a.s./kg dry weight artificial soil. The results of the reference test indicated that the test system was sensitive to the reference test item.

Effects on mortality and growth of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days are shown in the following table (values in this table are rounded values).

Table CA 8.4.1- 3: Effect on mortality, biomass and reproduction on *Eisenia fetida*

Test object	*Eisenia fetida*								
Test item	Control	Amidosulfuron WG 75 W							
mg test item/kg dry weight artificial soil	-	18	32	56	100	178	316	562	1000
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [%] *	68.39	63.71	70.08	77.85	67.54	65.67	69.73	70.83	64.80
Standard Deviation	9.27	9.86	5.36	19.93	8.32	13.30	6.98	9.34	8.27
Mean number of offspring per test vessel after 56 days **	195.8	190.5	182.3	185.5	149.0 +	137.0 +	175.5 +	110.3 +	120.0 +
Standard Deviation	27.4	8.2	19.9	29.6	18.1	29.3	36.5	24.0	28.6
Coefficient of variance (%)	14.0	4.3	10.9	16.0	12.1	21.4	20.8	21.8	23.8
% of control	--	97.3	93.1	94.8	76.1	70.0	89.7	56.3	61.3
				Reproduction					
No observed effect concentration (NOEC)				**56** mg test item/kg dry weight soil					

* Williams multiple sequential t-test, two-sided, α = 0.05 (after transformation)

** Williams multiple sequential t-test, one-sided smaller, α = 0.05

\+ Statistical significant compared to the control

Mortality:
After 28 days of exposure no worm died in the control group and no mortality was observed in any test item concentration.

Biomass:
No statistically significant different values for the growth relative to the control were observed.

Therefore, based on biological and statistical significance:
NOEC related to growth: ≥ 100 mg test item/kg dry weight artificial soil
LOEC related to growth: > 100 mg test item/kg dry weight artificial soil

Reproduction:
Statistically significant different values for the number of juveniles per test vessel relative to the control were observed for the four highest test item concentrations.

Therefore, based on biological and statistical significance:
NOEC related to reproduction: 56 mg test item /kg dry weight artificial soil.
LOEC related to reproduction: 100 mg test item /kg dry weight artificial soil

EC_{10} and EC_{20} values of 46.71 (95 % c.l.: 0 – 141.54 mg test item/kg dry weight soil) and 146.57 mg test item/kg dry weight soil with of (95 % c.l.: 2.86 – 410.12 mg test item/kg dry weight soil) have been calculated, respectively.

Conclusions:
Based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for this study is 56 mg test item/kg dry weight artificial soil. Thus, the overall LOEC is determined to be 100 mg test item/kg dry weight artificial soil.

Based on the analysed content of 75.9 % amidosulfuron in the test item the NOEC was re-calculated as 42.5 mg amidosulfuron a.s./kg dry weight artificial soil and the LOEC was re-calculated as 75.9 mg a.s./kg dws.

Studies on the metabolites of amidosulfuron:

Amidosulfuron-desmethyl:

Report: KCA 8.4.1704; [redacted]; 2015; M-529709-01-1
Title: Amidosulfuron-desmetyl (BCS-BB54362): Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil
Report No.: E 312 4703-0
Document No.: M-529709-01-1
Guideline(s): ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004
Guideline deviation(s): none
GLP/GEP: yes

Executive summary:
The purpose of this study was to investigate a NOEC/LOEC for the effects of the metabolite amidosulfuron-desmethyl (BCS-BB54362) on the reproduction (56 days after application), mortality and the biomass development (28 days after application) of the earthworm *Eisenia fetida* using a standardised soil. The test was performed according to the recommendations of the OECD Guideline 222 (2004) and the International Standard ISO 11268-2 (1998).

Ten adult earthworms (*Eisenia fetida*, approximately 3-4 months old) per replicate (4 replicates per test concentration of the treatment group, 8 replicates for the control group) were exposed in an artificial soil (containing 70% industrial quartz sand, 20% kaolinite clay and 10% sphagnum peat) to a

control (water treated) and to nominal concentrations of 10, 18, 32, 56 and 100 mg test item/kg dry weight artificial soil. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration were determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for this study is ≥100 mg test item/kg dry weight artificial soil (equals ≥95.8 mg p.m./kg dws). Thus, the overall LOEC is determined to be > 100 mg test item/kg dry weight artificial soil (equals >95.8 mg p.m./kg dws).

Material and methods:
Test item: Amidosulfuron-desmethyl (BCS-BB54362); Origin batch code: YP 79; Batch code: AE F101630 00 1C97 0001; Certificate No.: AZ 18898; CAS No.: 935867-69-9; LIMS No.: 1324835; Analysed purity: 95.8% w/w.

Adult *Eisenia fetida* (approximately 3 - 4 months old, 4 x 10 animals per test concentration of the treatment group and 8 x 10 animals for the control group) were exposed in an artificial soil (containing 70% industrial quartz sand, 20% kaolinite clay and 10% sphagnum peat) to a control (water treated) and to nominal concentrations of 10, 18, 32, 56 and 100 mg test item/kg dry weight artificial soil. The test item was mixed into the soil. The adult earthworms were fed once per week during the test period with approximately 5 g finely ground, air dried animal manure per vessel. During the test period, the temperature was in the range of 18 to 22 °C. The test vessels were kept under a photoperiod: light:dark = 16 h : 8 h with a desired light intensity of 400 - 800 Lux. After 28 days the number of surviving animals and their weight alteration were determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Toxic standard: Carbendazim (Carbendazim EC 360 G; 360 g a.s./L): 1.25, 2.5 and 5.0 mg a.s./kg dry weight artificial soil, control: artificial soil moistened with deionised water, solvent control: none.

Dates of experimental work: January 13, 2015 – March 16, 2015

Results:

Table CA 8.4.1- 4: Validity criteria of the study

Validity criteria	Recommended	Obtained
Mortality of the adults in the control	≤ 10 %	0 %
Number of juveniles earthworms per control vessels with 10 adults introduced at the start	≥ 30	103, 70, 101, 112, 92, 100, 88, 117
Coefficient of variance of reproduction in the control	≤ 30 %	15.0 %

The validity criteria of the test according to the guideline were fulfilled.

The most recent toxic standard reference test, with the reference test item mixed into the artificial soil, was performed from January 10 to July 8, 2014 (kra-Rg-R-Ref 20/14; NON-GLP). Effects on mortality and growth of the adults after an exposure period of 28 days and the number of offspring after 56 days were determined.
No mortality of the adult earthworms was observed 28 days after application. The change of body weight of the adult earthworms of the test concentration of 2.50 mg and 5.00 mg a.s./kg dry weight soil was statistically significant reduced in comparison to the control (Williams Multiple Sequential t-test, two-sided, $\alpha = 0.05$). The number of juveniles per test vessel of the test concentrations of 1.25, 2.50 and 5.00 mg a.s./kg dry weight artificial soil were statistical significant reduced in comparison to the control (Williams multiple sequential t-test, one-sided smaller, $\alpha = 0.05$). EC_{50} for reproduction were calculated to be 1.770 mg a.s./kg dry weight artificial soil, respectively. The confidence limit (95 %) was calculated to be 1.769 – 1.772 mg a.s./kg dry weight artificial soil.
The results of the reference test item indicated that the test system was sensitive to the reference test item.

Effects on mortality and growth of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days are shown in the following table (values in this table are rounded values).

Table CA 8.4.1- 5: Effect on mortality, biomass and reproduction on *Eisenia fetida*

Test object	*Eisenia fetida*					
Test item	Control	Amidosulfuron-desmethyl (BCS-BB54362)				
mg test item/kg dry weight artificial soil	---	10	18	32	56	100
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0	0
Mean change of body weight (growth) of the adults from day 0 to day 28 [%]	46.61	39.03	34.06	39.73	38.21	41.24
Standard Deviation	7.38	5.19	2.86	3.80	4.22	4.37
Significance (body weight)*	-	-	+	-	-	-
Mean number of offspring per test vessel after 56 days	97.9	96.0	96.0	90.8	98.0	98.8
Standard Deviation	14.7	19.4	19.4	11.0	10.0	13.4
% of control	---	98.1	98.1	92.7	100.1	100.9
Coefficient of variance (%)	15.0	20.3	20.2	12.2	10.2	13.5
Significance **		-	-	-	-	-
NOEC reproduction [mg test item/kg dry weight soil]					**≥100**	
LOEC reproduction[mg test item/kg dry weight soil]					**>100**	

* Dunnett´s Multiple t-test, two-sided, α = 0.05
** Williams multiple sequential t-test, one-sided smaller, α = 0.05
\- not significant
\+ significant

Mortality:
After 28 days of exposure no worm died in the control group and no mortality was observed in any test item concentration.

Effects on growth:
A statistically significant difference for the growth relative to the control was observed in the test concentration of 18 mg test item/kg dry weight artificial soil. The other test concentrations up to and including 100 mg test item/kg dry weight artificial soil showed no statistically significant difference (Dunnett's t-test, two-sided α = 0.05).
No dose response was found within the study.

Therefore, based on biological and statistical significance:
NOEC related to growth: ≥ 100 mg test item/kg dry weight artificial soil
LOEC related to growth: > 100 mg test item/kg dry weight artificial soil

Effects on reproduction:
No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations of 10, 18, 32, 56 and 100 mg test item/kg dry weight artificial soil.
Therefore, based on biological and statistical significance:
NOEC related to reproduction: ≥ 100 mg test item /kg dry weight artificial soil.
LOEC related to reproduction: > 100 mg test item /kg dry weight artificial soil

EC_{10} and EC_{20} cannot be calculated since the maximum difference between the treatment and the control group was only 7.9%.

Conclusions:
Based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for this study is ≥100 mg test item/kg dry weight artificial soil. Thus, the overall LOEC is determined to be >100 mg test item/kg dry weight artificial soil.

Based on the analysed content of 95.8 % amidosulfuron-desmethyl in the test item the NOEC was re-calculated as ≥95.8 mg p.m./kg dws and the LOEC was re-calculated as >95.8 mg p.m./kg dws.

Amidosulfuron-desmethyl-chloropyrimidine:

Report:	KCA 8.4.1/01; [redacted]; 2009; M-359724-01-1
Title:	Amidosulfuron-desmethyl-chloropyrimidine (BCS-C078570): sublethal toxicity to the earthworm Eisenia fetida in artificial soil with 10 % peat
Report No.:	09 10 48 070 S
Document No.:	M-359724-01-1
Guideline(s):	**OECD 222 (2004), ISO 11268-2 (1998)**
Guideline deviation(s):	**none**
GLP/GEP:	**yes**

<u>Note:</u> *This study has been previously submitted to former RMS (Austria) to support the post Annex I process of amidosulfuron. It was evaluated by Austria and is part of the DAR Addendum (Feb. 2011 – Addendum to monograph prepared in the context of post Annex I procedure (new Annex II data)).Upon request of the new RMS Finland, the study has nevertheless been included in the supplemental dossier.*

Executive Summary:
The purpose of this study was to determine the sublethal effects of the metabolite amidosulfuron-desmethyl-chloropyrimidine on reproduction, mortality and growth of the earthworm *Eisenia fetida* by dermal and alimentary uptake using an artificial soil in a laboratory test. The test was performed according to the recommendations of the OECD Guideline 222 (2004) and the International Standard ISO 11268-2 (1998).

Ten adult *Eisenia fetida andrei* (about 3 months old, weight: 310 – 462 mg/worm) per replicate (8 replicates for the control group, 4 replicates per test item concentration) were exposed to 63, 125, 250, 500 and 1000 mg test item/kg soil dry weight containing 69.5 % quartz sand, 20 % kaolin clay, 10 % sphagnum peat and 0.5 % $CaCO3$ at 20.0 – 21.1°C and a photoperiod: light : dark = 16 h : 8 h (570 lx) and were fed with horse manure. An untreated control (quartz sand only) was also included. Mortality and biomass change were determined after 4 weeks and reproduction was determined after 8 weeks. Condition and behaviour were observed weekly.

The test item showed no statistically significantly adverse effects on mortality, growth and reproduction of the earthworm *Eisenia fetida* in artificial soil up to 1000 mg test item/kg soil dry weight, i.e. the highest concentration tested. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 1000 mg test item/kg soil dry weight (equals ≥887 mg p.m./kg dws), and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 1000 mg test item/kg soil dry weight (equals >887 mg p.m./kg dws). The EC_{50} could not be calculated, but it can be concluded that the EC_{50} is higher than 1000 mg test item/kg soil dry weight, the highest tested concentration. The validity criteria of the test according to the guideline were fulfilled.

Materials and Methods:
Test item: Amidosulfuron-desmethyl-chloropyrimidine (BCS-CO78570); Origin Batch No.: BCOO 5766-3-3; Batch code: BCS-CO78570-01-01; Customer Order No.: TOX 08625-00; Analysed purity: 88.7 % w/w; Certificate of analysis No.: AZ 16057.

Ten adult *Eisenia fetida andrei* (about 3 months old, weight: 310 – 462 mg/worm) per replicate (8 replicates for the control group, 4 replicates per test item concentration) were exposed to 63, 125, 250, 500 and 1000 mg test item/kg soil dry weight containing 69.5 % quartz sand, 20 % kaolin clay, 10 % sphagnum peat and 0.5 % $CaCO_3$ at 20.0 – 21.1°C and a photoperiod: light : dark = 16 h : 8 h (570 lx) and were fed with horse manure. An untreated control was also included.

Behaviour (worms on the soil surface) and feeding activity were assessed during each feeding in weekly intervals. After four weeks, the adult worms were removed from the test vessels. The number of surviving worms (adult mortality) and their biomass change was determined, behaviour (including feeding activity) and pathological symptoms were recorded. After 8 weeks the surviving juveniles were counted by manual inspection of the substrate.

Toxic standard: 5 and 10 mg Nutdazim 50 FLOW (Carbendazim SC 500)/kg soil dry weight; control: quartz sand treated, solvent control: none.

Dates of work: August 12, 2009 – October 07, 2009

Results:

Table CA 8.4.1- 6: Validity criteria

Validity criteria	Recommended	Obtained
Mortality of adults in the control after 4 weeks	≤10 %	0 %
Number of juveniles per replicate in the control	≥ 30	68, 83, 102, 116, 94, 92, 101 and 72
Coefficient of variation of reproduction in the control	≤ 30 %	17.7 %

The validity criteria of the test according to the guideline were fulfilled.

In the most recent study (BioChem project No. TER-R 09 10 48 001, dated 17.07.2009), the number of juveniles was reduced by 65 and 92 % (mean number of juveniles = 51 and 11) by the toxic standard Nutdazim 50 FLOW (Carbendazim, SC 500) after 8 weeks of test duration when compared to control (mean number of juveniles = 143). Therefore, the observed effects assure a high sensitivity of the test system.

Table CA 8.4.1- 7: Effects on mortality, growth and reproduction on *Eisenia fetida*

Treatment (mg t.i./kg)	Mean mortality (%)	Mean biomass increase after 4 weeks (%)	Mean number of juveniles (% reduction of reprod.)
Control	0	36.9	91.0 (-)
63	0	37.8	81.0 (-11.0)
125	0	37.5	88.3 (-3.0)
250	0	40.4	93.8 (3.0)
500	2.5	45.1	94.8 (4.1)
1000	0	43.0	101.5 (11.5)
NOEC (body weight, reproduction): ≥ 1000 mg/kg dw			
LOEC (body weight, reproduction): > 1000 mg/kg dw			
LC_{50} > 1000 mg/kg dw			

Mortality:
The test item caused no mortality at concentrations of 63, 125, 250 and 1000 mg test item/kg soil dry weight. Mortality was 2.5 % at 500 mg test item/kg soil dry weight. No mortality (0 %) occurred in the control group.
No effects on behaviour (including feeding activity) of the worms were observed during the test.

Biomass:
The test item caused no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control treatment, i.e. a weight increase of 36.9, 37.8, 37.5, 40.4, 45.1 and 43.0 % in the control group and at concentrations of 63, 125, 250, 500 and 1000 mg test item/kg soil dry weight, respectively.

Reproduction:
No statistically significant effects on the number of juveniles compared to the control group were recorded at any concentration tested.

EC_{10} and EC_{20} cannot be calculated since the maximum difference between the treatment and the control group was only 11.5%.

Conclusions:
Based on the statistical evaluation of these results, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 1000 mg test item/kg soil dry weight, and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 1000 mg test item/kg soil dry weight. The EC_{50} could not be calculated, but it can be concluded that the EC_{50} is higher than 1000 mg test item/kg soil dry weight, the highest tested concentration.

Based on the analysed content of 88.7 % amidosulfuron-desmethyl-chloropyrimidine in the test item the NOEC was re-calculated as ≥887 mg p.m./kg dws and the LOEC was re-calculated as >887 mg p.m./kg dws.

Amidosulfuron-guanidine:

Report:	KCA 8.4.1/02; [redacted]; 2009; M-358183-01-1
Title:	Amidosulfuron-guanidine (BCS-CO41839): Sublethal toxicity to the earthworm Eisenia fetida in artificial soil with 10 % peat
Report No.:	09 10 48 071 S
Document No.:	M-358183-01-1
Guideline(s):	**OECD 222 (2004), ISO 11268-2 (1998)**
Guideline deviation(s):	**none**
GLP/GEP:	**yes**

Report:	KCA 8.4.1/03; [redacted]; 2016; M-554438-01-1
Title:	Calculation of ECx values for report M-359724-01-1
Report No.:	M-554438-01-1
Document No.:	M-554438-01-1
Guideline(s):	**none (calculation only)**
Guideline deviation(s):	**no (calculation only)**
GLP/GEP:	**no**

Note: *This study has been previously submitted to former RMS (Austria) to support the post Annex I process of amidosulfuron. It was evaluated by Austria and is part of the DAR Addendum (Feb. 2011 – Addendum to monograph prepared in the context of post Annex I procedure (new Annex II data)).Upon request of the new RMS Finland, the study has nevertheless been included in the supplemental dossier.*

Executive Summary:
The purpose of this study was to determine the sublethal effects of the metabolite amidosulfuron-guanidine on reproduction, mortality and growth of the earthworm *Eisenia fetida* by dermal and alimentary uptake using an artificial soil in a laboratory test. The test was performed according to the recommendations of the OECD Guideline 222 (2004) and the International Standard ISO 11268-2 (1998).

Ten adult *Eisenia fetida andrei* (about 3 months old, weight: 306 – 464 mg/worm) per replicate (8 replicates for the control group, 4 replicates per test item concentration) were exposed to 63, 125, 250, 500 and 1000 mg test item/kg soil dry weight containing 69.5 % quartz sand, 20 % kaolin clay, 10 % sphagnum peat and 0.5 % CaCO3 at 18.9 – 21.9°C and a photoperiod: light : dark = 16 h : 8 h (580 lx) and were fed with horse manure. An untreated control (quartz sand only) was also included. Mortality and biomass change were determined after 4 weeks and reproduction was determined after 8 weeks. Condition and behaviour were observed weekly.

The test item showed no statistically significantly adverse effects on mortality, growth and reproduction of the earthworm *Eisenia fetida* in artificial soil up to 1000 mg test item/kg soil dry weight, i.e. the highest concentration tested. Therefore, the overall No-Observed- Effect-Concentration (NOEC) was determined to be ≥ 1000 mg test item/kg soil dry weight (equals ≥983 mg p.m./kg dws), and the Lowest-Observed- Effect-Concentration (LOEC) was determined to be > 1000 mg test item/kg soil dry weight (equals >983 mg p.m./kg dws). The EC_{50} could not be calculated, but it can be concluded that the EC_{50} is higher than 1000 mg test item/kg soil dry weight, the highest tested concentration. The validity criteria of the test according to the guideline were fulfilled.

Materials and Methods:
Test item: Amidosulfuron-guanidine (BCS-CO41839); Origin Batch No.: RDL 603-16-20; Batch code: BCS-CO41839-01-01; Customer Order No.: TOX 08626-00; Analysed purity: 98.3 % w/w; Certificate of analysis No.: AZ 16021.

Ten adult *Eisenia fetida andrei* (about 3 months old, weight: 306 – 464 mg/worm) per replicate (8 replicates for the control group, 4 replicates per test item concentration) were exposed to 63, 125, 250, 500 and 1000 mg test item/kg soil dry weight containing 69.5 % quartz sand, 20 % kaolin clay, 10 % sphagnum peat and 0.5 % $CaCO_3$ at 18.9 – 21.9°C and a photoperiod: light : dark = 16 h : 8 h (580 lx) and were fed with horse manure. An untreated control (quartz sand only) was also included. Behaviour (worms on the soil surface) and feeding activity were assessed during each feeding in weekly intervals. 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. After 8 weeks the surviving juveniles were counted by manual inspection of the substrate.

Toxic standard: 5 and 10 mg Nutdazim 50 FLOW (Carbendazim SC 500)/kg soil dry weight; control: quartz sand treated, solvent control: none.

Dates of work: July 31, 2009 – September 25, 2009

Results:
Table CA 8.4.1- 8: Validity criteria

Validity criteria	Recommended	Obtained
Mortality of adults in the control after 4 weeks	≤ 10 %	0 %
Number of juveniles per replicate in the control	≥ 30	79, 67, 65, 86, 70, 58, 97 and 85
Coefficient of variation of reproduction in the control	≤ 30 %	17.2 %

The validity criteria of the test according to the guideline were fulfilled.

In the most recent study (BioChem project No. TER-R 09 10 48 001, dated 17.07.2009), the number of juveniles was reduced by 65 and 92 % (mean number of juveniles = 51 and 11) by the toxic standard Nutdazim 50 FLOW (Carbendazim, SC 500) after 8 weeks of test duration when compared to control (mean number of juveniles = 143). Therefore, the observed effects assure a high sensitivity of the test system.

Table CA 8.4.1- 9: Effects on mortality, growth and reproduction on *Eisenia fetida*

Treatment (mg t.i./kg)	Mean mortality (%)	Mean biomass increase after 4 weeks (%)	Mean number of juveniles (% reduction of reprod.)
Control	0	55.0	75.9 (-)
63	0	57.4	78.3 (3.1)
125	2.5	55.9	71.0 (-6.4)
250	0	49.7	70.5 (-7.1)
500	2.5	53.4	69.0 (-9.1)
1000	0	46.9	60.0 (-20.9)
NOEC (body weight, reproduction): ≥ 1000 mg t.i./kg dw			
LOEC (body weight, reproduction): > 1000 mg t.i./kg dw			
EC_{50} > 1000 mg t.i./kg dw			

t.i. = test item

Mortality:
The test item caused no mortality at concentrations of 63, 250 and 1000 mg test item/kg soil dry weight. Mortality was 2.5 % at 125 and 500 mg test item/kg soil dry weight. No mortality (0 %) occurred in the control group.
No effects on behaviour of the worms were observed during the test. The feeding activity of adult worms was reduced at 1000 mg test item/kg soil dry weight, compared to the control group.

Biomass:
The test item caused no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control treatment, i.e. a weight increase of 55.0, 57.4, 55.9, 49.7, 53.4 and 46.9 % in the control group and at concentrations of 63, 125, 250, 500 and 1000 mg test item/kg soil dry weight, respectively.

Reproduction:
No statistically significant effects on the number of juveniles compared to the control group were recorded at any concentration tested.

The probit analysis of the reproduction data has been conducted by [redacted]; 2016; M-554438-01-1 and gave a suitable fit of the probit curve. An EC_{10} value of 402 mg test item/kg d.w.s. with 95% confidence limits of 60 – 655 mg test item/kg d.w.s. and an EC_{20} value of 1023 mg test item/kg d.w.s. has been derived with corresponding 95% confidence limits of 629 – 7315 mg test item/kg d.w.s. have been determined.

Correcting these EC values for the purity of the test item (98.3%) results in the following values:

EC_{10} (95% CL): 395 (65 - 644) mg p.m./kg d.w.s.
EC_{20} (95% CL): 1006 (618 - 7191) mg p.m./kg d.w.s.

Conclusions:
Based on the statistical evaluation of these results, the overall No-Observed- Effect-Concentration (NOEC) was determined to be ≥ 1000 mg test item/kg soil dry weight, and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 1000 mg test item/kg soil dry weight. The EC_{50} could not be calculated, but it can be concluded that the EC_{50} is higher than 1000 mg test item/kg soil dry weight, the highest tested concentration.

Based on the analysed content of 98.3 % amidosulfuron-guanidine in the test item the NOEC was re-calculated as ≥983 mg p.m./kg dws and the LOEC was re-calculated as >983 mg p.m./kg dws.

Amidosulfuron-ADMP:

Report:	KCA 8.4.1/05; [redacted]; 2013; M-461051-01-1
Title:	AE F092944 (BCS-AA25052): Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil
Report No.:	kra/Rg-R-147/13
Document No.:	M-461051-01-1
Guideline(s):	**OECD 222 (2004), ISO 11268-2 (1998)**
Guideline deviation(s):	**none**
GLP/GEP:	**yes**

Executive Summary:
The purpose of this study was to determine effects of the metabolite amidosulfuron –ADMP (AE F092944) on survival, growth and reproduction of the earthworm *Eisenia fetida* during an exposure in an artificial soil with one test concentration in the 1st run and 5 different test concentrations in the 2nd run.

In the first run adult *Eisenia fetida* (approx. 6 months old, 8 × 10 replicates for control and treatment group) were exposed in artificial soil (with 10 % peat content) to the nominal concentration of 100 mg test item/kg artificial soil dry weight.

In the second run adult *Eisenia fetida* (approx. 5 months old, 8 × 10 replicates for the control group and 4 × 10 replicates per test concentration of the treatment group) were exposed in artificial soil (with 10 % peat content) to nominal concentrations of 5.6, 10, 18, 32 and 56 mg test item/ kg dry weight artificial soil. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined. The test was performed according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004).

Based on the biological and statistical significance of the effects observed on growth and reproduction, the overall No-Observed-Effect-Concentration (NOEC) was determined to be 10 mg test item/ kg dry weight artificial soil (equals 9.98 mg p.m./kg dws). The overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be 18 mg test item/kg dry weight artificial soil (equals 17.96 mg p.m./kg dws). The validity criteria of the test according to the guideline were fulfilled.

Materials and Methods:
Test item: AE F092944 (BCS-AA25052); Batch Code: AE F092944 00 1B99 0002; Origin Batch No.: 23503LR; LIMS-No.: 1034970; Content of a.s. analysed: 99.8 %w/w; Certificate No.: AZ 17077.

In the 1st test run adult *Eisenia fetida* (approx. 6 months old, 8 × 10 replicates for control and treatment group) were exposed in an artificial soil (10 % peat content) to the nominal test concentration of 100 mg test item/kg dry weight artificial soil.

In the 2nd test run adult *Eisenia fetida* (approx. 5 months old, 8 × 10 replicates for the control group and 4 × 10 replicates per test concentration of the treatment group) were exposed in an artificial soil (10 % peat content) to the nominal test concentrations of 5.6, 10, 18, 32 and 56 mg test item/kg dry weight artificial soil. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined.

Toxic standard (Carbendazim EC 360 G): 1.25 – 2.5 – 5.0 mg a.s./kg soil d.w. (corresponds to 3.94 – 7.89 – 15.78 mg test item/ kg soil d.w.); control: quartz sand, solvent control: none.

Dates of experimental work: July 10, 2012 – September 17, 2013 (first run)
April 12, 2013 – June 14, 2013 (second run)

Results:

Table CA 8.4.1- 10: Validity criteria

Validity criteria	Recommended	Obtained 1st run	Obtained 2nd run
Mortality of adults in the control	≤ 10 %	0 %	0 %
Rate of reproduction of juveniles (earthworms per control vessel)	≥ 30	391, 335, 260, 313, 330, 399, 371, 387	246, 350, 278, 228, 285,232, 254, 287
Coefficient of variance of reproduction in the control	≤ 30 %	13.7 %	14.7 %

The validity criteria of the test according to the guideline were fulfilled.

In a separate study (Study No.: Rg-R-Ref 19/12; Report No. kra-Rg-R-Ref 19/12; NON-GLP, performed from September 21 to November 28, 2012) the EC_{10}, EC_{20} and EC_{50} values for reproduction were calculated to be 3.06, 3.22 or 3.54 mg a.s./kg dry weight artificial soil, respectively. Confidence limits (95 %) could not be calculated. The results of the reference test indicated that the test system was sensitive to the reference test item.

Table CA 8.4.1- 11: Effects of AE F092944 on mortality and changes in body weight of the adults of Eisenia fetida after an exposure period of 28 days and the number of offspring per test vessel after 56 days (values in this table are rounded values)

Test object	***Eisenia fetida***							
	1st run		**2nd run**					
Test item	**Control**	**AE F092944**	**Control**	**AE F092944**				
mg test item/kg dry weight artificial soil	-	100	-	5.6	10	18	32	56
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [%] *	31.75	39.10*	13.17	6.14	9.75	17.29	14.30	12.72
Standard Deviation	4.05	7.13	3.31	6.27	2.82	5.52	8.29	2.81
Mean number of offspring per test vessel after 56 days **	348.3	312.5	270.0	271.8	267.8	201.8**	232.3**	223.5**
Standard Deviation	47.8	42.2	39.7	55.2	23.3	19.9	20.6	10.7
Coefficient of variance (%)	13.7	13.5	14.7	20.3	8.7	9.9	8.9	4.8
% of control	-	89.7	-	100.6	99.2	74.7	86.0	82.8
						Reproduction		
NOEC (mg test item/kg dry weight soil)						**10**		

* statistical significance compared to the control (1st run: Student t-test; 2nd run: Williams mult. sequent. t-test, two-sided, $\alpha = 0.05$)

** statistical significance compared to the control (1st run: Student t-test; 2nd run: Williams mult. sequent. t-test, one-sided smaller, $\alpha = 0.05$)

Mortality:
After 28 days of exposure no worms died in the control groups of both test runs and no mortality was observed at any test item concentration.

Effects on growth
Statistically significant different values for the growth relative to the control were observed in the 1st run and the lowest concentration of the 2nd run. Since in all higher concentrations of the test item no significant differences to the control were observed this is considered not to be treatment related.

Therefore, based on biological and statistical significance (for both test runs):
NOEC related to growth: 56 mg test item/kg dry weight artificial soil
LOEC related to growth: 100 mg test item/kg dry weight artificial soil

Effects on reproduction
No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations of 5.6 and 10 mg test item/kg dry weight artificial soil (2nd run). Statistically significant different values for the number of juveniles per test vessel relative to the control were observed in the three highest test concentrations of the 2nd run.

Therefore, based on biological and statistical significance (for both test runs):
NOEC related to reproduction: 10 mg test item/kg dry weight artificial soil
LOEC related to reproduction: 18 mg test item/kg dry weight artificial soil

EC_{10} = 15.35 mg test item/kg dry weight soil (95% confidence limits could not be determined)
EC_{20} = 54.06 mg test item/kg dry weight soil (95% confidence limits could not be determined)

Conclusions:
Overall, based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the NOEC for this study is 10 mg test item/kg dry weight artificial soil. Thus, the overall LOEC is determined to be 18 mg test item/kg dry weight artificial soil.

Based on the analysed content of 98.3 % amidosulfuron-ADMP in the test item the NOEC was re-calculated as 9.98 mg p.m./kg dws and the LOEC was re-calculated as 17.96 mg p.m./kg dws.

AE F094206:

Report: KCA 8.4.1/06; [redacted]; 2015; M-533011-01-1
Title: Amidosulfuron-AE F094206 (BCS-AA25045): Influence on the reproduction of the earthworm Eisenia fetida tested in artificial soil
Report No.: E 312 4706-3
Document No.: M-533011-01-1
Guideline(s): OECD 222 (2004), ISO 11268-2 (1998)
Guideline deviation(s): for deviation see Point 6
GLP/GEP: yes

Executive Summary:
The purpose of this study was to determine the influence of the metabolite amidosulfuron-ADHP (BCS-AA25045) on the reproduction (56 days after application), mortality and the biomass development (28 days after application) of the earthworm *Eisenia fetida* tested in standardised artificial soil. The test was performed according to the International Standard ISO 11268-2 (1998) and the OECD Guideline 222 (2004). As deviation from the guideline, the temperature in the climatic chamber temporarily exceeded the desired temperature of 20 ± 2°C up to 25.3°C. Since nearly no mortality of the adult earthworms and an effect on the reproduction of the juveniles were not observed, it can be stated that this deviation has no influence on the study.

Adult *Eisenia fetida* (approx. 2-3 months old, 8 x 10 animals for the control group (water treated) and 4 x 10 animals per test concentration of the treatment group) were exposed in an artificial soil (with 10 % peat content) to the nominal test concentrations of 1.8, 3.2, 5.6, 10, 18, 32, 56 and 100 mg test item/kg dry weight artificial soil. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days, the number of offspring was determined. A temperature of 20 + 2°C and a light regime of 400 – 800 lux, 16h light, and 8h dark during the conduct of the study were applied.

Based on the biological and statistical significance of the effects observed on growth and reproduction, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥100 mg test item/ kg dry weight artificial soil (equals ≥99.5 mg p.m./kg dws). The overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be >100 mg test item/kg dry weight artificial soil (equals >99.5 mg p.m./kg dws). The validity criteria of the test according to the guideline were fulfilled.

Materials and Methods:
Test item: Amidosulfuron-ADHP; BCS-code: BCS-AA25045; Batch code: AE F094206 00 1C99 0001; Origin batch No.: PW 210/213; Analysed content: 99.5 % w/w; Certificate of analysis No.: AZ 19246.

Ten *Eisenia fetida* (adults, approximately 2-3 months old) per replicate (8 replicates for the control, 4 replicates per test item concentration) were exposed for 28 days in artificial soil (with 10 % peat content) to an untreated deionised water control and to nominal concentrations of 1.8, 3.2, 5.6, 10, 18, 32, 56 and 100 mg test item/kg artificial soil dry weight at 19.4 - 25.3°C and 400 – 800 lux. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. The adults were then removed from the test vessels and the cocoons and juvenile earthworms remained in the test vessels for additional 28 days. Mortality, adverse effects (e.g. abnormal behaviour, lack of movement, rigidity etc.) and growth of the adult worms were assessed after 28 days. After 56 days the number of surviving juveniles was determined.

During the course of the study temporary increases of the temperature in the climatic chamber were observed. Since nearly no mortality of the adult earthworms (2 of 400 inserted were not found after 28 days) and an effect on the reproduction of the juveniles were not observed, it can be stated that this deviation has no influence on the study.

Toxic standard: Carbendazim (360g/L): 1.25, 2.50 and 5.0 mg a.s./kg dry weight artificial soil, control: artificial soil moistened with deionised water, solvent control: none.

Dates of experimental work: February 2, 2015 – April 02, 2015

Results:

Table CA 8.4.1-12: Validity criteria

Validity criteria	Recommended	Obtained
Mortality of adults in the control	≤ 10 %	0 %
Rate of reproduction of juveniles (earthworms per control vessel)	≥ 30	72 to 119
Coefficient of variance of reproduction in the control	≤ 30 %	17.0 %

The validity criteria of the test according to the guideline were fulfilled.

The most recent toxic standard reference test, with the reference test item mixed into the artificial soil, was performed from October 15 to December 11, 2014 (kra-Rg-R-Ref 24/14; NON-GLP). Effects on mortality and growth of the adults after an exposure period of 28 days and the number of offspring

after 56 days were determined. No mortality of the adult earthworms was observed 28 days after application. The change of body weight of the adult earthworms of the test concentration of 5.0 mg a.s./kg dry weight soil was statistically significant reduced in comparison to the control (results of a Williams multiple sequential t-test, two-sided, $\alpha = 0.05$). The number of juveniles per test vessel of the test concentrations 2.5 and 5.0 mg a.s./kg dry weight soil were statistically significant reduced in comparison to the control (results of a Williams multiple sequential t-test, one-sided smaller, $\alpha = 0.05$). EC_{10}, EC_{20} and EC_{50} for reproduction were calculated to be 1.474 (1.331 – 1.587), 1.678 (1.553 – 1.776) and 2.153 (2.080 – 2.213) mg a.s./kg dry weight artificial soil, respectively. Confidence limits (95 %) are given in the brackets. According to the guideline significant effects should be observed between 1 and 5 mg a.s./kg dry weight artificial soil. Thus the results of this reference test indicated that the test system was sensitive to the reference test item.

Table CA 8.4.1- 13: Effects on mortality and growth of adult *Eisenia fetida* after an exposure period of 28 days and the number of offspring per test vessel after 56 days

Test object	*Eisenia fetida*								
Test item	Amidosulfuron-ADHP								
mg test item/kg dry weight artificial soil	Con.	1.8	3.2	5.6	10	18	32	56	100
Mortality of adult earthworms [%] after 28 days	0	5	0	0	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [%]	21.2	21.5	23.9	18.8	23.2	16.1	21.1	30.2	29.9
Standard Deviation	8.6	4.7	8.9	4.5	6.3	2.2	9.1	2.3	4.4
Significance (body weight)*	-	-	-	-	-	-	-	-	-
Mean number of offspring per test vessel after 56 days	94.3	89.8	93.5	94.3	92.5	88.5	95.5	79.5	90.8
Standard Deviation	16.0	23.4	24.5	11.1	9.7	15.9	20.0	30.0	14.1
% of control	---	95.2	99.2	100	98.1	93.9	101	84.4	96.3
Coefficient of variance (%)	17.0	26.1	26.1	11.8	10.5	18.0	20.9	37.7	15.5
Significance (reproduction)**		-	-	-	-	-	-	-	-
Overall NOEC [mg test item/kg dry weight soil]	**≥100**								
Overall LOEC [mg test item/kg dry weight soil]	**>100**								

* (Dunnett's t-test, two-sided, $\alpha = 0.05$)
** (Dunnett's t-test, one-sided smaller, $\alpha = 0.05$)
\- not significant

Mortality:
After 28 days of exposure no earthworms died in the control group and no mortality was observed in the test item concentrations from 3.2 up to 100 mg test item/kg dry weight soil. In the test item group with 1.8 mg test item/kg dry weight soil 5 % of the adult earthworms died.

Effects on growth:
No statistically significant different values for the growth relative to the control were observed in any test item concentrations (Dunnett's t-test, two-sided, $\alpha = 0.05$).

Therefore, based on biological and statistical significance:

NOEC related to growth:	≥ 100 mg test item/kg dry weight artificial soil
LOEC related to growth:	> 100 mg test item/kg dry weight artificial soil

Reproduction:
No statistically significant differences concerning the number of juveniles relative to the control were observed in any test item concentration up to and including 100 mg test item/kg dry weight artificial soil (Dunnett's t-test, one-sided smaller, $\alpha = 0.05$).

Therefore, based on biological and statistical significance:

NOEC related to reproduction:	≥ 100 mg test item/kg dry weight artificial soil
LOEC related to reproduction:	> 100 mg test item/kg dry weight artificial soil

EC_{10} and EC_{20} cannot be calculated since the data do not show a dose response.

Conclusions:
Based on the biological and statistical significance of the effects observed on growth and reproduction, it is concluded, that the overall NOEC for this study is ≥ 100 mg test item/kg dry weight artificial soil. Thus, the overall LOEC is determined to be > 100 mg test item/kg dry weight artificial soil.

Based on the analysed content of 99.5 % amidosulfuron-ADHP in the test item the NOEC was re-calculated as ≥99.5 mg p.m./kg dws and the LOEC was re-calculated as >99.5 mg p.m./kg dws.

CA 8.4.2 Effects on non-target soil meso and macrofauna (other than earthworms)

CA 8.4.2.1 Species level testing

For amidosulfuron and its metabolites amidosulfuron-desmethyl, amidosulfuron-desmethyl-chloropyrimidine, amidosulfuron-guanidine, and amidosulfuron-ADMP, reproductive toxicity studies on *Folsomia candida* were performed. In addition, for amidosulfuron and its metabolites amidosulfuron-desmethyl-chloropyrimidine, amidosulfuron-guanidine, and amidosulfuron-ADMP, reproductive toxicity studies on *Hyposapis aculeifer* were performed.

In the tests with the collembolan species *Folsomia candida* No-Observable-Effect levels ranged from 56 mg/kg dws for the metabolite amidosulfuron-desmethyl-chloropyrimidine to ≥ 759 mg/kg dws for the parent amidosulfuron. In the tests with the soil mite *Hyposapis aculeifer* no effects were observed at the highest tested dose levels when either the parent compound or the metabolites were tested. Resulting NOEC values ranged from ≥ 89 mg p.m./kg dws for its metabolite amidosulfuron-desmethyl-chloropyrimidine to ≥ 1000 mg a.s./kg dws for amidosulfuron. Details of all studies are provided in the following Table CA 8.4.2.1- 1.

Based on the consistent absence of effect to *Hyposapis aculeifer* observed in all studies covering the parent active substance and the two terminal metabolites amidosulfuron-desmethyl-chloropyrimidine and amidosulfuron-guianidine, *Hyposapis aculeifer* was identified to not be the most sensitive species. Thus, it was deemed justified to conclude absence of relevant toxicity of amidosulfuron-desmethyl to the soil mite *Hyposapis aculeifer*.

The metabolite amidosulfuron-biuret was detected as a minor and transient soil metabolite. Maximum occurrence detected in soil was 6.3 %. No potential for persistence of amidosulfuron-biuret is indicated based on its soil half-life.

The chemical structure of amidosulfuron-biuret is very close to the structure of the metabolite amidosulfurone-guanidine, so that similar ecotoxicological properties of both substances may be expected. The latter component, being formed in soil in more relevant quantity and being characterized by longer degradation half-life, has been tested in reproductive toxicity studies on *Folsomia candida* as well as *Hyposapis aculeifer*.

Therefore, for amidosulfuron-biuret no reproductive toxicity testing on *Folsomia candida* and *Hyposapis aculeifer* was deemed required.

Based on the consistent absence of effect to *Folsomia candida* as well as *Hyposapis aculeifer* observed in the studies covering the parent active substance and the intermediate metabolite amidosulfuron-ADMP, it was deemed justified to conclude absence of relevant toxicity of the terminal metabolite amidosulfuron-ADHP to the collembolan species *Folsomia candida* as well as to the soil mite *Hyposapis aculeifer*.

Table CA 8.4.2.1- 1: Reproductive toxicity data of amidosulfuron and metabolites to other non-target macro-organisms presented in this chapter

Test species	Test system	Test duration	Endpoint		Reference
Amidosulfuron					
Hypoaspis aculeifer (soil mite)	reproduction test[1)]	14 d	NOEC	≥ 1000 mg prod./kg dws ≥ 759 mg a.s./kg dws	[redacted]; 2015; M-507488-01-1 KCA 8.4.2.1/01
Folsomia candida (collembolan)	reproduction test[1)]	28 d	NOEC	≥ 1000 mg prod./kg dws ≥ 759 mg a.s./kg dws	[redacted]; 2014; M-506088-01-1 KCA 8.4.2.1/02
Amidosulfuron-desmethyl					
Folsomia candida (collembolan)	reproduction test	28 d	NOEC	8 mg p.m./kg dws	[redacted]; 2016; M-551645-01-1 KCA 8.4.2.1/03
Amidosulfuron-desmethyl-chloropyrimidine					
Hypoaspis aculeifer (soil mite)	reproduction test	14 d	NOEC	≥89 mg p.m./kg dws	[redacted]; 2015; M-507479-01-1 KCA 8.4.2.1/04
Folsomia candida (collembolan)	reproduction test	28 d	NOEC	56 mg p.m./kg dws	[redacted]; 2015; M-524473-01-1 KCA 8.4.2.1/05
Amidosulfuron-guanidine					
Hypoaspis aculeifer (soil mite)	reproduction test	14 d	NOEC	≥ 100 mg p.m./kg dws	[redacted]; 2014; M-503851-01-1 KCA 8.4.2.1/06
Folsomia candida (collembolan)	reproduction test	28 d	NOEC	≥ 100 mg p.m./kg dws	[redacted]; 2014; M-506089-01-1 KCA 8.4.2.1/07

Test species	Test system	Test duration	Endpoint		Reference
Amidosulfuron-ADMP					
Hypoaspis aculeifer (soil mite)	reproduction test	14 d	NOEC	≥99.8 mg p.m./kg dws[2)]	[redacted]; 2013; M-454043-01-1 KCA 8.4.2.1/08
Folsomia candida (collembolan)	reproduction test	28 d	NOEC	≥ 99.8 mg p.m./kg dws[2)]	[redacted]; 2013; M-451142-01-1 KCA 8.4.2.1/09

dws = dry weight soil; prod. = product; a.s. = active substance; p.m. = pure metabolite
[1)] conducted with WG 75 formulation
[2)] corrected to an anlysed purity of 99.8 %

Studies on amidosulfuron:

Note: The following two studies have been conducted using formulation Amidosulfuron WG 75 as a vehicle for delivery of the active substance amidosulfuron to the test system. Since primary intent of the studies is the generation of active substance information, they are summarised and evaluated on document MCA level.

Report: KCA 8.4.2.1/01; [redacted]; 2015; M-507488-01-1
Title: Amidosulfuron WG 75 W: Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No.: LAR-HR-109/14
Document No.: M-507488-01-1
Guideline(s): **OECD 226 (2008)**
Guideline deviation(s): **for deviation see Point 2.5**
GLP/GEP: **yes**

Executive summary:

The purpose of this study was to assess the effect of Amidosulfuron WG 75 W on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment. The test was performed in accordance with the OECD Guideline 226 (2008). As deviation, the pH-values of the artificial soil at the test start were between 5.35 and 5.50 and not in the range 6 ± 0.5 recommended by guideline. No relevance for the study results could be drawn by this deviation.

Ten adult, fertilized, female soil mite (*Hypoaspis aculeifer*) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed for 14 days to an untreated control and to concentrations of 100, 178, 316, 562, 1000 mg test item/kg soil dry weight. After a period of 14 days, the surviving adults and the living juveniles were counted.

The No Observed Effect Concentration (NOEC) for reproduction of *Hypoaspis aculeifer* was determined to be ≥1000 mg test item/kg soil dry weight (equals ≥759 mg a.s./kg dws). The Lowest Observed Effect Concentration (LOEC) for reproduction was determined to be >1000 mg test item/kg soil dry weight (equals >759 mg a.s./kg dws).

Material and methods:

Test item: Amidosulfuron WG 75 W; Sample description: TOX10124-00; Specification No.: 102000000550-03; Batch-ID: EFKE002307; Material No.: 05938848; Master recipe ID: 13005778; Analysed content of a.s: 75.9 % w/w amidosulfuron (AE F075032).

Ten adult, fertilized, female soil mite (*Hypoaspis aculeifer*) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed for 14 days to an untreated control and to concentrations of 100, 178, 316, 562, 1000 mg test item/kg soil dry weight. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry

weight basis): 75 % fine quartz sand, 20 % kaolin clay, 5 % sphagnum peat, air dried and finely ground, and calcium carbonate (CaCO3) for the adjustment to pH to 6.0 ± 0.5. During the test, the *Hypoaspis aculeifer* were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied.

After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Toxic reference: Dimethoate EC 400E G: 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil; control: artificial soil with deionized water, solvent control: none.

Dates of experimental work: October 01, 2014 to October 24, 2014

Results:

Table CA 8.4.2.1- 2: Validity criteria for the untreated control of the study according to OECD Guideline 226

Validity criteria	Recommended by the guideline	Obtained in this study
Mean adult mortality	≤ 20 %	7.1 %
Mean number of juveniles per replicate (with 10 mites introduced)	≥ 50	295.3
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	10.1 %

All validity criteria were met. Therefore this study is valid.

The most recent non-GLP-test (Maria Ivonne Larnaudie Lopez, LAR/HR-O-14/14, March 11, 2014) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil. Dimethoate showed a LC_{50} of 3.51 mg a.s./kg dry weight artificial soil (95 % confidence limits from 3.46 mg a. s./kg dry weight artificial soil to 3.57 mg a. s./kg dry weight artificial soil) for mortality of the adult mites according Probit analysis using maximum likelihood regression.

The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg dry weight artificial soil and accordingly the LOEC is 5.6 mg a.s./kg dry weight artificial soil. Since variances of the data were homogenous Williams-t test α 0.05, one-sided smaller was used. Dimethoate EC 400E G showed an EC_{50} of 5.28 mg a. s./kg dry weight artificial soil (95 % confidence limits from 4.02 mg a. s./kg dry weight artificial soil to 6.47 mg a. s./kg dry weight artificial soil) for reproduction according Probit analysis using maximum likelihood regression. This is in the recommended range of the guideline, indicating that an EC_{50} based on the number of juveniles of 3.0 – 7.0 mg a. s./kg dry weight artificial soil shows that the test organisms are sufficiently sensitive.

Effects on mortality and reproduction of the adults and the number of juveniles per test vessel after an exposure period of 14 days are shown in the following table.

Table CA 8.4.2.1- 3: Effect on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	Amidosulfuron WG 75 W *Hypoaspis aculeifer* Artificial Soil					
mg test item/Kg dry weight artificial soil	% mortality (Adults)	Mean number of juveniles per test vessel ± standard dev.			Reproduction (% of control)	Significance (*)
Control	7.1	295.3	±	29.9		
100	0.0	330.3	±	4.0	111.8	-
178	2.5	328.8	±	9.4	111.3	-
316	0.0	332.3	±	12.7	112.5	-
562	0.0	361.0	±	7.8	122.3	-
1000	0.0	361.0	±	20.8	122.3	-
$NOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)					**≥ 1000**	
$LOEC_{reproduction}$ (mg test item/kg dry weight artificial soil)					**> 1000**	

(*)=William's-t.-test one sided smaller; α=0.05; "-": non-significant; "+": significant

Mortality:
In the control group 7.1 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:
Concerning the number of juveniles statistical analysis (William's-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and any treatment group.

Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 1000 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 1000 mg test item/kg artificial soil dry weight.

EC_{10} and EC_{20} cannot be calculated since the treatment groups did not show adverse effects.

Conclusions:
The No Observed Effect Concentration (NOEC) for reproduction of *Hypoaspis aculeifer* was determined to be ≥1000 mg test item/kg soil. The Lowest Observed Effect Concentration (LOEC) for reproduction was determined to be >1000 mg test item/kg soil.

Based on the analysed content of 75.9 % amidosulfuron in the test item the NOEC was re-calculated as ≥759 mg amidosulfuron a.s./kg dry weight artificial soil and the LOEC was re-calculated as >759 mg a.s./kg dws.

Report: KCA 8.4.2.1/02; [redacted]; 2014; M-506088-01-1
Title: Amidosulfuron WG 75 W: Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No.: FRM-Coll-178/14
Document No.: M-506088-01-1
Guideline(s): OECD 232 (adopted 2009)
Guideline deviation(s): for deviation see Point 2.5
GLP/GEP: yes

Executive summary:
The purpose of this study was to assess the effect of Amidosulfuron WG 75 W on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment. The test was performed in accordance with the OECD Guideline 232 (2009). As deviation, the pH-values of the artificial soil at the test start were between

5.35 and 5.50 and not in the range 6 ± 0.5 recommended by guideline. No relevance for the study results could be drawn by this deviation.

10 collembolans (10-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed during 28 days to a control (water treated) and to the concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg artificial soil dry weight. The assessment of adult mortality and reproduction (number of juveniles) were determined after 28 days.

The No Observed Effect Concentration (NOEC) for reproduction of *Folsomia candida* was determined to be ≥1000 mg test item/kg soil dry weight (equals ≥759 mg a.s./kg dws). The Lowest Observed Effect Concentration (LOEC) for reproduction was determined to be >1000 mg test item/kg soil dry weight (equals >759 mg a.s./kg dws).

Material and methods:
Test item: Amidosulfuron WG 75 W; Sample description: TOX10124-00; Specification No.: 102000000550-03; Batch-ID: EFKE002307; Material No.: 05938848; Workorder: 13005778; Analysed content of a.s: 75.9 % w/w amidosulfuron (AE F075032).

Ten collembolans (10-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to an control (water treated) and to concentrations of 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil containing 75 % fine quartz sand, 20 % kaolin clay, 5 % sphagnum peat, air dried and finely ground, and $CaCO_3$ for the adjustment to pH to 6.0 ± 0.5, at 20 ± 2 °C, 400 - 800 lux, with a photoperiod: light : dark = 16 h : 8 h. Each test vessel of the 8 control and the 4 treatment replicates plus the one for measurement purpose was filled up with 30±1 g wet weight artificial soil. During the study, the collembolans were fed with granulated dry yeast. The assessment of adult mortality and reproduction (number of juveniles) were determined after 28 days.

Toxic reference: 44 - 67 - 100 - 150 - 225 mg boric acid//kg soil dry weight; control: artificial soil moistened with deionised water, solvent control: none.

Dates of experimental work: October 01, 2014 to November 07, 2014

Results:
Table CA 8.4.2.1- 4: Validity criteria for the untreated control of the study according to OECD Guideline 232

Validity criteria	Recommended by the guideline	Obtained in this study
Mean adult mortality	≤ 20 %	5 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1729.8
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	8.3 %

All validity criteria were met. Therefore this study is valid.

The most recent non-GLP-test (FRM-Coll-Ref-24/14, U. Frommholz, March 13, 2014) with the reference item Boric acid was performed at test concentrations 44 – 67 – 100 – 150 and 225 mg Boric acid/kg artificial soil dry weight.

Boric acid showed an EC_{50} of 90 mg test item/kg artificial soil dry weight (95 % confidence limits from 68 mg to 119 mg Boric acid/kg artificial soil dry weight) for reproduction according Probit analysis using maximum likelihood regression. The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial soil dry weight). The $NOEC_{reproduction}$ was calculated to

be <44 mg Boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 44 mg Boric acid/kg artificial soil dry weight according Williams multiple t-test procedure, α = 0.05, one-sided smaller. This shows that the test organisms are sufficiently sensitive.

Effects on mortality of the adults and the number of juveniles per test vessel after an exposure period of 28 days are shown in the following table.

Table CA 8.4.2.1- 5: Effect on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure		Amidosulfuron WG 75 W *Folsomia candida* Artificial soil		
mg test item/kg soil dry weight nominal concentration	Adult mortality (%)	Mean number of juveniles per test vessel ± standard deviation	Reproduction (% of control)	Significance (*)
Control	5.0	1729.8 ± 143.5		
18	7.5	1697.8 ± 165.8	98.2	-
32	0.0	1807.0 ± 54.8	104.5	-
56	0.0	1814.3 ± 56.8	104.9	-
100	22.5	1573.8 ± 87.1	91.0	-
178	17.5	1574.5 ± 245.3	91.0	-
316	0.0	1780.5 ± 97.7	102.9	-
562	5.0	1655.8 ± 259.7	95.7	-
1000	22.5	1606.5 ± 174.3	92.9	-
			Reproduction	
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)			≥1000	
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)			>1000	

The calculations were performed with un-rounded values
(*) = (William's t-test one-sided-smaller, α = 0.05, + = significant, - = not significant)

Mortality:
In the control group 5 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:
Concerning the number of juveniles statistical analysis (William's t-test, one-sided smaller, α = 0.05) revealed no significant difference between control and any treatment group.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥1000 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is >1000 mg test item/kg artificial soil dry weight.

EC_{10} and EC_{20} cannot be calculated since the maximum difference between a treatment group and the control was only 9.0%.

Conclusions:
The No Observed Effect Concentration (NOEC) for reproduction of *Folsomia candida* was determined to be ≥1000 mg test item/kg soil. The Lowest Observed Effect Concentration (LOEC) for reproduction was determined to be >1000 mg test item/kg soil.

Based on the analysed content of 75.9 % amidosulfuron in the test item the NOEC was re-calculated as ≥759 mg amidosulfuron a.s./kg dry weight artificial soil and the LOEC was re-calculated as >759 mg a.s./kg dws.

Studies on the metabolites of amidosulfuron:
Amidosulfuron-desmethyl:

Report:	KCA 8.4.2.1/03; [redacted]; 2016; M-551645-01-1
Title:	Amidosulfuron-desmethyl (BCS-BB54362): Effects on reproduction of the collembola Folsomia candida tested in artificial soil
Report No.:	110161016
Document No.:	M-551645-01-1
Guideline(s):	**OECD 232 (adopted 2009); ISO 11267 (2014)**
Guideline deviation(s):	**none**
GLP/GEP:	**yes**

Executive Summary:
The purpose of this study was to determine the effects of the metabolite amidosulfuron-desmethyl (BCS-BB54362) on mortality and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment. The test was performed as a limit test in accordance with the OECD Guideline 232 (adopted 2009) and the International Standard ISO 11267 (2014).

In the 1st experiment 10 collembolans (9-11 days old) per replicate (8 replicates for the control group and 4 replicates for the single treatment group) were exposed to untreated control and 10, 18, 32, 56 and 100 mg pure metabolite/kg artificial soil dry weight.

In the 2nd experiment 10 collembolans (9-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and 0.8, 1.4, 2.5, 4.5 and 8 mg pure metabolite/kg artificial soil dry weight. After a period of 28 days, adult mortality, behavioural effects and reproduction were determined.

The No Observed Effect Concentration (NOEC) for mortality was determined to be ≥100 mg pure metabolite/kg soil. The Lowest Observed Effect Concentration for mortality was estimated to be greater than 100 mg pure metabolite/kg soil. The NOEC for reproduction was determined to be the concentration of 8 mg pure metabolite/kg soil. The LOEC for reproduction was determined to be the concentration of 10 mg pure metabolite/kg soil.

Materials and Methods:
Test item: Amidosulfuron-desmethyl (BCS-BB54362); Synonym: AE F101630; Batch code: AE F101630 00 1C97 0001; Origin batch No. YP 79; CAS No.: 935867-69-9; LIMS No.: 1324835; Certificate No.: AZ 18898; Analytical findings: 95.8 % w/w.

Two separate experiments were performed with 28-d exposure in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil which was placed into glass vessels before the Collembola were introduced on top of the soil. In the 1st experiment 10 collembolans (9-11 days old) per replicate (8 replicates for the control group and 4 replicates for the single treatment group) were exposed to untreated control (the same amount of untreated fine quartz sand per g substrate as in the test item treated groups was added and moistened with deionised water) and 10, 18, 32, 56 and 100 mg pure metabolite/kg artificial soil dry weight. In the 2nd experiment 10 collembolans (9-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (moistened with deionised water only) and 0.8, 1.4, 2.5, 4.5 and 8 mg pure metabolite/kg artificial soil dry weight. The Collembola were fed with approximately 2 mg dry yeast for each test vessel at the beginning of the test and on day 14. After a period of 28 days, adult mortality, behavioural effects and reproduction were determined. Mortality of adult Collembola, behavioural effects and number of juveniles were used to determine the endpoints. The artificial soil for both runs was prepared according to the guideline containing 74.8 % fine quartz sand, 20 % kaolin clay and 5 % sphagnum peat, air dried and finely ground. 0.2 % Calcium carbonate ($CaCO_3$) was used for the adjustment to pH to 6.0 ± 0.5.

In the 1st experiment the pH was 6.2 at experimental start and 5.9 to 6.3 at experimental end; the water content at experimental start was 22.7 % to 23.3 % (50.5 % to 51.8 % of the maximum water holding capacity) and at experimental end 20.8 % to 22.2 % (46.2 % to 49.4 % of the maximum water holding capacity); temperature was within the range of 18°C to 22°C; the illumination was 16 h light : 8 h dark, light intensity was within the range of 400 to 800 lux. In the 2nd experiment the pH was 5.8 at experimental start and 5.7 at experimental end; the water content at experimental start was 19.6 % to 20.7 % (51.5 % to 54.4 % of the maximum water holding capacity) and at experimental end 15.9 % to 17.4 % (41.9 % to 45.8 % of the maximum water holding capacity); temperature was within the range of 18°C to 22°C; the illumination was 16 h light : 8 h dark, light intensity was within the range of 400 to 800 lux. Each test containers of the 8 control replicates, the 4 treatment replicates (1st experiment) and the 4 treatment replicates (2nd experiment) plus the one container per treatment for measurement purpose was filled up with 30±1 g artificial soil fresh weight.

Statistical evaluation was done for mortality using Fisher´s exact test and for reproduction using Williams t-test.

Toxic reference: 30.5 – 48.8 – 78.1 - 125 - 200 mg boric acid//kg artificial soil dry weight; control (1st experiment): same amount of untreated fine quartz sand per g substrate as the test item groupswas added and moistened with deionised water; control (2nd experiment): moistened with deionised water only; solvent control: none.

Dates of experimental work: November 13, 2015 – March 24, 2016

Results:

Table CA 8.4.2.1- 6: Validity criteria

Validity criteria	Recommended	Achieved	
		1st experiment	2nd experiment
Control mortality	≤ 20 %	9 %	14 %
Control reproduction (juveniles per container)	≥ 100	594 to 729	502 to 716
Coefficient of variation of the control reproduction	≤ 30 %	6.5 %	14.8 %

All validity criteria for the study were met. Therefore this study is valid.

In a separate study (study code 99392016, performed from November to December 2015) the reference item Boric acid showed statistically significant effects on reproduction at concentrations of ≥78.1 mg/kg soil; the EC_{50} for reproduction was calculated to be 94.0 mg/kg soil.

Table CA 8.4.2.1- 7: Effects of amidosulfuron-desmethyl on mortality and reproduction of *Folsomia candida*

Test item **Test object** **Exposure**	**Amidosulfuron-desmethyl** ***Folsomia candida*** **Artificial soil**				
mg pure metabolite/kg soil dry weight	**Mortality (day 28) [%]**	**Significance[1)]**	**Number of juveniles (day 28)**	**Significance[2)]**	**Reproduction in [%] of control (day 28)**
1st experiment					
Control	9	-	649		
10	15	n.s.	553	*	85.3
18	33	*	538	*	82.9
32	13	n.s.	554	*	85.4
56	8	n.s.	550	*	84.8
100	18	n.s.	500	*	77.1
2nd experiment					
Control	14	-	588	-	-
0.8	8	n.s.	697	n.s.	119
1.4	13	n.s.	668	n.s.	114
2.5	10	n.s.	673	n.s.	114
4.5	5	n.s.	652	n.s.	111
8.0	10	n.s.	683	n.s.	116
Endpoints [mg pure metabolite/kg soil dry weight]					
$NOEC_{mortality}$	**> 100**				
$LOEC_{mortality}$	**> 100**				
$NOEC_{reproduction}$	**8**				
$LOEC_{reproduction}$	**10**				

n.s. = not significantly different compared to the control * = significantly different compared to the control
1) Fisher's Exact Test, $\alpha = 0.05$, one-sided greater
2) Williams t-test, $\alpha = 0.05$, one-sided smaller
- not applicable

Mortality

In the 1st experiment only at the concentration of 18 mg pure metabolite/kg soil a statistically significant mortality was observed compared to the control. However this finding was not considered to be treatment related since at all concentrations tested above the mortality was not statistically significantly increased (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).

In the 2nd experiment a mortality of up to 13% was observed, which was not statistically significantly different compared to the control were 14% of the adult Collembolas died (Fisher's Exact test, $\alpha = 0.05$, one-sided greater).

Reproduction

In the 1st experiment the reproduction of the Collembolan exposed to amidosulfuron-desmethyl was statistically significantly different compared to the control at all concentrations tested (Williams t-test, $\alpha = 0.05$, one-sided smaller). No behavioural abnormalities were observed in any of the treatment groups.

In the 2nd experiment the reproduction of the Collembolan exposed to amidosulfuron-desmethyl was not statistically significantly different compared to the control up to and including the highest test concentration of 8 mg pure metabolite/kg soil (Williams t-test one-sided smaller). No behavioural abnormalities were observed in any of the treatment groups.

EC_{10} and EC_{20} cannot be calculated since the data do not show a dose response that is suitable for an ECx calculation.

Conclusions:
In a Collembola reproduction study with amidosulfuron-desmethyl the No Observed Effect Concentration (NOEC) for mortality was determined to be ≥100 mg pure metabolite/kg soil. The Lowest Observed Effect Concentration for mortality was estimated to be greater than 100 mg pure metabolite/kg soil.
The NOEC for reproduction was determined to be the concentration of 8 mg pure metabolite/kg soil.
The LOEC for reproduction was determined to be the concentration of 10 mg pure metabolite/kg soil
The EC values for reproduction could not be determined due to mathematical reasons

Amidosulfuron-desmethyl-chloropyrimidine:

Report:	KCA 8.4.2.1/04; [redacted]; 2015; M-507479-01-1
Title:	Amidosulfuron-desmethyl-chloropyrimidine, sodium salt (BCS-CO78570): Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No.:	LAR-HR-111/14
Document No.:	M-507479-01-1
Guideline(s):	**OECD 226 (2008)**
Guideline deviation(s):	**Deviation none**
GLP/GEP:	**yes**

Executive Summary:
The purpose of this study was to assess the effect of the sodium salt (BCS-CO78570) of the metabolite amidosulfuron-desmethyl-chloropyrimidine (BCS-CO41838) on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment. The test was performed in accordance with the OECD Guideline 226 (2008).

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to control and treatment. A single concentration of 89 mg pure metabolite/kg dry weight artificial soil was tested. After a period of 14 days, the surviving adults and living juveniles were extracted and counted under a binocular.

The No-Observed-Effect-Concentration (NOEC) for reproduction was ≥ 89 mg pure metabolite/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction was > 89 mg pure metabolite/kg dry weight artificial soil. All validity criteria (for the untreated controls) according to the guideline OECD 226 were met.

Materials and Methods:
Test item: Amidosulfuron-desmethyl-chloropyrimidine, sodium salt (BCS-CO78570); Origin batch No.: BCOO5766-3-3; Batch code: BCS-CO78570-01-01; LIMS No.: 1343423; Certificate No.: AZ 19222; Customer order No.: TOX10410-00; Analytical findings: 88.7 % w/w.

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to control and to the concentration of one treatment. The concentration of 89 mg pure metabolite/kg dry weight artificial soil was tested. In each test vessel 20 ± 1 g dry weight artificial soil were weighed in. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days. During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75 % fine quartz sand, 5 % sphagnum peat, air dried and finely ground, 20 % kaolin clay. Calcium carbonate (CaCO3) was used for the adjustment to pH to 6.0 ± 0.5.

After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Toxic reference: (Dimethoate EC 400E G): 1.0 – 1.8 – 3.2 – 5.6 – 10.0 mg dimethoate/kg dry weight artificial soil; control: 5 g quartz sand mixed into pre-moistened 495 g artificial dry weight artificial soil and moistened with 50 mL deionised water, solvent control: none.

Dates of experimental work: August 22, 2014 – September 10, 2014

Results:

Table CA 8.4.2.1- 8: Validity criteria

Validity criteria (control values)	Recommended	Obtained
Mean adult mortality	≤ 20 %	1.40 %
Mean number of juveniles per replicate (with 10 mites introduced)	≥ 50	302.7
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	5.3 %

All validity criteria for the study were met. Therefore this study is valid.

The most recent non-GLP-test (Maria Ivonne Larnaudie Lopez, LAR/HR-O-14/14, March 11, 2014) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil. Dimethoate showed a LC_{50} of 3.51 mg a.s./kg (95 % confidence limits from 3.46 mg a. s./kg to 3.57 mg a. s./kg) for mortality of the adult mites according Probit analysis using maximum likelihood regression. The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were homogenous Williams-t test $\alpha = 0.05$, one-sided smaller was used. Dimethoate EC 400E G showed an EC_{50} of 5.28 mg a. s./kg (95 % confidence limits from 4.02 mg a. s./kg to 6.47 mg a. s./kg) for reproduction according Probit analysis using maximum likelihood regression. This is in the recommended range of the guideline, indicating that an EC_{50} based on the number of juveniles of 3.0 – 7.0 mg a. s./kg dry weight artificial soil shows that the test organisms are sufficiently sensitive.

Table CA 8.4.2.1- 9: Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	Amidosulfuron-desmethyl-chloropyrimidine, sodium salt (BCS-CO78570) *Hypoaspis aculeifer* Artificial soil			
mg pure metabolite/kg dry weight artificial soil	**% mortality (Adults)**	**Mean number of juveniles per test vessel ± standard dev.**	**Reproduction (% of control)**	**Significance (*)**
Control	1.4	302.7 ± 16.0	100	-
89	5.7	290.4 ± 17.2	95.9	-
NOEC (mg pure metabolite/kg dry weight artificial soil)			≥ 89	
LOEC (mg pure metabolite/kg dry weight artificial soil)			> 89	

(*)=Student-t.-test one sided smaller; α=0.05; "-": non-significant; "+": significant

Mortality

In the control group 1.4 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction
Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and the single treatment group. Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 89 mg pure metabolite/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 89 mg pure metabolite/kg artificial soil dry weight.

EC_{10} and EC_{20} cannot be calculated since the study has been conducted as a limit test and the difference between the treatment group and the control was only 4.1%.

Conclusions:
The No-Observed-Effect-Concentration (NOEC) for reproduction is **≥** 89 mg pure metabolite/kg dry weight artificial soil, and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction is **>** 89 mg pure metabolite/ kg dry weight artificial soil.

Report:	KCA 8.4.2.1/05; [redacted]; 2015; M-524473-01-1
Title:	Amidosulfuron-desmethyl-chloropyrimidine, sodium salt (BCS-CO78570): Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No.:	E 314 4678-3
Document No.:	M-524473-01-1
Guideline(s):	**OECD 232 (adopted 2009)**
Guideline deviation(s):	**none**
GLP/GEP:	**yes**

Executive Summary:
The purpose of this study was to assess the effect of the sodium salt (BCS-CO78570) of the metabolite amidosulfuron-desmethyl-chloropyrimidine (BCS-CO18388) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

In the 1st test run 10 collembolans (10-12 days old) per replicate (8 replicates for the control group and 8 replicates for the single treatment group) were exposed to control (water treated) and 89 mg pure metabolite/kg artificial soil dry weight. Since the 1st test run on the test item did not provide a final result, a 2nd test run was performed studying lower test concentrations.

In the 2nd test run 10 collembolans (10-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated) and 5.6, 10, 18, 32 and 56 mg pure metabolite/kg artificial soil dry weight. After a period of 28 days, mortality and reproduction were determined.

The No-Observed-Effect-Concentration (NOEC) for reproduction is 56 mg pure metabolite/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 89 mg pure metabolite/kg dry weight artificial soil. All validity criteria for the untreated control of the study according to the OECD Guideline 232 have been fulfilled.

Materials and Methods:
Test item: Amidosulfuron-desmethyl-chloropyrimidine, sodium salt (BCS-CO78570); Origin batch No.: BCOO5766-3-3; Batch code: BCS-CO78570-01-01; LIMS No.: 1343423 (1st run), 1509780 (2nd run); Certificate No.: AZ 19222 (1st run), AZ 20048 (2nd run); Customer order No.: TOX10410-00 (1st run), TOX10410-01 (2nd run); Analytical findings: 88.7 % w/w (TOX10410-00 and TOX10410-01).

Since the 1st test run on the test item did not provide a final result, a 2nd test run was performed studying lower test concentrations. In the 1st test run 10 collembolans (10-12 days old) per replicate (8 replicates for the control group and 8 replicates for the single treatment group) were exposed to control

(water treated) and 89 mg pure metabolite/kg artificial soil dry weight. In the 2nd test run 10 collembolans (10-12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated) and 5.6, 10, 18, 32 and 56 mg pure metabolite/kg artificial soil dry weight. Both runs at 20 ± 2°C, 400 – 800 lux, 16h light : 8h dark. The artificial soil for both runs was prepared according to the guideline containing 75 % fine quartz sand, 20 % kaolin clay and 5 % sphagnum peat, air dried and finely ground. Calcium carbonate ($CaCO_3$) was used for the adjustment to pH to 6.0 ± 0.5. Each test vessel of the 8 control replicates, the 8 treatment replicates (1st run) and the 4 treatment replicates (2nd run) plus the one for measurement purpose was filled up with 30±1 g wet weight artificial soil. During the test, the collembolans were fed with granulated dry yeast. Mortality and reproduction were determined after 28 days.

Toxic reference: 27 - 37 - 52 - 72 - 100 - 139 - 193 - 269 mg boric acid//kg artificial soil dry weight; control: 5 g quartz sand mixed into pre-moistened 495 g artificial dry weight artificial soil and moistened with 50 mL deionised water, solvent control: none.

Dates of experimental work: August 22, 2014 – February 25, 2015

Results:

Table CA 8.4.2.1- 10: Validity criteria

Validity criteria (untreated control)	Recommended	Obtained	
		1st run	2nd run
Mean adult mortality	≤ 20 %	2.5 %	16.3 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1485.6	1255.3
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	9.2 %	17.1 %

All validity criteria for the study were met. Therefore this study is valid.

The most recent non-GLP-test (FRM-Coll-Ref-26/15, U. Frommholz, March 18, 2015) with the reference item Boric acid was performed at test concentrations 27, 37, 52, 72, 100, 139, 193 and 269 mg boric acid/kg artificial soil dry weight. Boric acid showed an EC_{50} of 77 mg test item/kg artificial soil dry weight (95 % confidence limits from 58 mg to 97 mg boric acid/kg artificial soil dry weight) for reproduction according Weibull analysis using linear maximum likelihood regression. The result is in the recommended range of the guideline (about 100 mg boric acid/kg artificial soil dry weight).
The $NOEC_{reproduction}$ was calculated to be < 27 mg boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 27 mg boric acid/kg artificial soil dry weight according Williams multiple t-test procedure, α = 0.05, one-sided smaller. This shows that the test organisms are sufficiently sensitive.

Table CA 8.4.2.1- 11: Effects of amidosulfuron-desmethyl-chloropyrimidine, sodium salt on mortality and reproduction of *Folsomia candida*

Test item **Test object** **Exposure**	**Amidosulfuron-desmethyl-chloropyrimidine, sodium salt** ***Folsomia candida*** **Artificial soil**			
mg pure metabolite/kg soil dry weight	**Adult mortality (%)**	**Mean number of juveniles per test vessel±SD**	**Reproduction (% of control)**	**Significance (*)**
2nd run				
Control	16.3	1255.3 ± 214.4		
5.6	25.0	1277.5 ± 485.0	101.8	-
10	7.5	1284.5 ± 344.6	102.3	-
18	22.5	1369.5 ± 333.8	109.1	-
32	15.0	1206.0 ± 360.5	96.1	-
56	22.5	1351.0 ± 314.6	107.6	-
1st run				
Control	2.5	1485.6 ± 136.3		
89	6.25	1253.0 ± 194.7	84.3	+
$NOEC_{reproduction}$ (mg pure metabolite/kg soil dry weight)			**56**	
$LOEC_{reproduction}$ (mg pure metabolite/kg soil dry weight)			**89**	

The calculations were performed with un-rounded values
(*) = (Student t-test one-sided-smaller, $\alpha = 0.05$, + = significant, 1st run)
(*) = (William's t-test one-sided-smaller, $\alpha = 0.05$, - = not significant, 2nd run)

Mortality
In the control group 2.5 % (1st run) and 16.3 % (2nd run) of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction
Concerning the number of juveniles statistical analysis (Williams-t test, one-sided smaller, $\alpha = 0.05$) revealed a significant difference between control and the single treatment group of 89 mg pure metabolite/kg artificial soil dry weight in the 1st run. In the 2nd test run William's test, one-sided smaller, $\alpha = 0.05$ revealed no significant difference between control and any treatment group. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 56 mg pure metabolite/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 89 mg pure metabolite/kg artificial soil dry weight.

EC_{10} and EC_{20} cannot be calculated since the maximum difference to the control was only 15.7%.

Conclusions:
The No-Observed-Effect-Concentration (NOEC) for reproduction is 56 mg test item/kg dry weight artificial soil, and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 89 mg test item/ kg dry weight artificial soil.

Amidosulfuron-guanidine:

Report: KCA 8.4.2.1/06; [redacted]; 2014; M-503851-01-1
Title: Amidosulfuron-guanidine (BCS-CO41839): Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No.: LAR-HR-112/14
Document No.: M-503851-01-1
Guideline(s): **OECD 226 (2008)**
Guideline deviation(s): **none**
GLP/GEP: **yes**

Executive Summary:
The purpose of this study was to assess the effect of the metabolite amidosulfuron-guanidine (BCS-CO41839) on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in an artificial soil comparing control and treatment. The test was performed in accordance with the OECD Guideline 226 (2008).

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and the treatment group) were exposed to control and one treatment. The concentration of 100 mg pure metabolite/kg dry weight artificial soil was tested. After a period of 14 days, the surviving adults and living juveniles were extracted and counted under a binocular.

The No-Observed-Effect-Concentration (NOEC) for reproduction was ≥ 100 mg pure metabolite/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction was > 100 mg pure metabolite/kg dry weight artificial soil. All validity criteria (for the untreated controls) according to the guideline were met.

Materials and Methods:
Test item: Amidosulfuron-guanidine (BCS-CO41839); BCS code: BCS-CO41839; Batch code: BCS-CO41839-01-03; Origin Batch No.: GSE 61576-5-20; LIMS No.: 1414227; Customer order No.: TOX10434-00; purity: 98.0 %w/w.

Ten adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and for the treatment group) were exposed to control and to the concentration of one treatment. The concentration of 100 mg pure metabolite/kg dry weight artificial soil was tested. In each test vessel 20 ± 1 g dry weight artificial soil were weighed in. The *Hypoaspis aculeifer* were of a uniform age not differing more than three days (28 days after start of egg laying). During the test, they were fed with cheese mites bred on brewer's yeast. During the study a temperature of 20 ± 2 °C and light regime of 400 – 800 Lux, 16 h light : 8 h dark was applied. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75 % fine quartz sand, 5% sphagnum peat, air dried and finely ground, 20 % kaolin clay. Calcium carbonate ($CaCO_3$) was used for the adjustment to pH to 6.0 ± 0.5.

After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen Apparatus. Extracted mites were collected in a fixing solution (20 % ethylene glycol, 80 % deionised water; 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Toxic reference: (Dimethoate EC 400E G): 1.0 – 1.8 – 3.2 – 5.6 – 10.0 mg dimethoate/kg dry weight artificial soil; control: 5 g quartz sand mixed into pre-moistened 495 g artificial dry weight artificial soil and moistened with 50 mL deionised water, solvent control: none.

Dates of experimental work: August 22, 2014 – September 11, 2014

Results:
Table CA 8.4.2.1- 12: Validity criteria

Validity criteria (control values)	Recommended	Obtained
Mean adult female mortality	≤ 20 %	1.4 %
Mean number of juveniles per replicate (with 10 mites introduced)	≥ 50	305.1
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	5.4 %

All validity criteria for the study were met. Therefore this study is valid.

The most recent non-GLP-test (Maria Ivonne Larnaudie Lopez, LAR/HR-O-14/14, March 11, 2014) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.

Dimethoate showed a LC_{50} of 3.51 mg a.s./kg (95 % confidence limits from 3.46 mg a. s./kg to 3.57 mg a. s./kg) for mortality of the adult mites according Probit analysis using maximum likelihood regression.

The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were homogenous Williams-t test $\alpha = 0.05$, one-sided smaller was used. Dimethoate EC 400E G showed an EC_{50} of 5.28 mg a. s./kg (95 % confidence limits from 4.02 mg a. s./kg to 6.47 mg a. s./kg) for reproduction according Probit analysis using maximum likelihood regression.

This is in the recommended range of the guideline, indicating that an EC_{50} based on the number of juveniles of 3.0 – 7.0 mg a. s./kg dry weight artificial soil shows that the test organisms are sufficiently sensitive.

Table CA 8.4.2.1- 13: Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item **Test object** **Exposure**	**Amidosulfuron-guanidine (BCS-CO41839)** ***Hypoaspis aculeifer*** **Artificial soil**			
mg pure metabolite/kg dry weight artificial soil	**% mortality (Adults)**	**Mean number of juveniles per test vessel ± standard dev.**	**Reproduction (% of control)**	**Significance (*)**
Control	1.4	305.1 ± 16.3	100	-
100	2.5	304.5 ± 22.9	99.8	-
$NOEC_{reproduction}$ (mg pure metabolite/kg dry weight artificial soil)			≥100	
$LOEC_{reproduction}$ (mg pure metabolite/kg dry weight artificial soil)			>100	

(*)=Student-t.-test one sided smaller; α=0.05; "-": non-significant; "+": significant

Mortality
In the control group 1.4 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction
Concerning the number of juveniles statistical analysis (Student-t test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and the treatment group.

Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg pure metabolite/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg pure metabolite/kg artificial soil dry weight.

EC_{10} and EC_{20} cannot be calculated since the study has been conducted as a limit test and the difference between the treatment group and the control was only 0.2%.

Conclusions:
The No-Observed-Effect-Concentration (NOEC) for reproduction is **≥** 100 mg pure metabolite/kg dry weight artificial soil, and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction is **>** 100 mg pure metabolite/kg dry weight artificial soil.

Report:	KCA 8.4.2.1/07; [redacted]; 2014; M-506089-01-1
Title:	Amidosulfuron-guanidine (BCS-CO41839): Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No.:	FRM-Coll-180/14
Document No.:	M-506089-01-1
Guideline(s):	**OECD 232 (2009)**
Guideline deviation(s):	**none**
GLP/GEP:	**yes**

Executive Summary:
The purpose of this study was to assess the effect of the metabolite amidosulfuron-guanidine (BCS-CO41839) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil, by comparing control and treatment.

10 collembolans (10 - 12 days old) per replicate (8 replicates for the control group and 8 replicates for the single treatment group) were exposed to control (water treated) and 100 mg pure metabolite/kg artificial soil dry weight. After a period of 28 days, mortality and reproduction were determined.
The No-Observed-Effect-Concentration (NOEC) for reproduction is ≥ 100 mg pure metabolite/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is > 100 mg pure metabolite/kg dry weight artificial soil. All validity criteria for the untreated control of the study according to the OECD Guideline 232 have been fulfilled.

Materials and Methods:
Test item: Amidosulfuron-guanidine (BCS-CO41839); Analytical findings: 98.0 % w/w; Origin batch no.: GSE 61576-5-20; customer order no.: TOX-No. 10434-00; LIMS no.: 1414227; Batch code: BCS-CO41839-01-03.

10 collembolans (10 - 12 days old) per replicate (8 replicates for the control group and 8 replicates for the single treatment group) were exposed to control (water treated) and to the single concentration of 100 mg pure metabolite/kg dry weight artificial soil containing 75 % fine quartz sand, 20 % kaolin clay, 5 % sphagnum peat, air dried and finely ground and calcium carbonate ($CaCO_3$) for the adjustment to pH to 6.0 ± 0.5, at 20 ± 2°C, 400 - 800 lux, with a photoperiod: light : dark = 16 h : 8 h. Each test vessel of the 8 control and the 8 treatment replicas plus the one for measurement purpose was filled up with 30±1 g wet weight artificial soil. During the test, the collembolans were fed with granulated dry yeast. Mortality and reproduction were determined after 28 days.

Toxic reference: 44 - 67 - 100 - 150 - 225 mg boric acid/kg soil dry weight; control: 5 g quartz sand mixed into pre-moistened 495 g artificial dry weight artificial soil and moistened with 50 mL deionised water, solvent control: none.

Dates of experimental work: August 22, 2014– September 23, 2014

Results:

Table CA 8.4.2.1- 14: Validity criteria

Validity criteria (untreated control)	Recommended	Obtained
Mean adult mortality	≤ 20 %	4.3 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1082.7
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	18.5 %

All validity criteria for the study were met. Therefore this study is valid.

The most recent non-GLP-test (FRM-Coll-Ref-24/14, U. Frommholz, March 13, 2014) with the reference item boric acid was performed at test concentrations 44 – 67 – 100 – 150 and 225 mg boric acid/kg artificial soil dry weight. Boric acid showed an EC_{50} of 90 mg test item/kg artificial soil dry

weight (95 % confidence limits from 68 mg to 119 mg boric acid/kg artificial soil dry weight) for reproduction according Probit analysis using maximum likelihood regression. The result is in the recommended range of the guideline (about 100 mg boric acid/kg artificial soil dry weight). The $NOEC_{reproduction}$ was calculated to be <44 mg Boric acid/kg artificial soil dry weight and accordingly the $LOEC_{reproduction}$ is 44 mg Boric acid/kg artificial soil dry weight according Williams multiple t-test procedure, $\alpha = 0.05$, one-sided smaller. This shows that the test organisms are sufficiently sensitive.

Table CA 8.4.2.1- 15: Effects of amidosulfuron-guanidine (BCS-CO41839) on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	Amidosulfuron-guanidine (BCS-CO41839) *Folsomia candida* Artificial soil			
mg pure metabolite/kg soil dry weight nominal concentration	Adult mortality (%)	Mean number of juvenile per test vessel ± SD	Reproduction (% of control)	Significance (*)
Control	4.3	1082.7 ± 200.0		
100	17.5	1056.5 ± 318.2	97.6	-
			Reproduction	
$NOEC_{reproduction}$ (mg pure metabolite/kg soil dry weight)			**≥100**	
$LOEC_{reproduction}$ (mg pure metabolite /kg soil dry weight)			**>100**	

The calculations were performed with un-rounded values
SD = Standard deviation
(*) = (Student's t-test one-sided-smaller, $\alpha = 0.05$, + = significant, - = not significant)

Mortality
In the control group 4.3 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction
Concerning the number of juveniles statistical analysis (Student's t-test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control and the single treatment group.

Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥100 mg pure metabolite/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is >100 mg pure metabolite/kg artificial soil dry weight.

EC_{10} and EC_{20} cannot be calculated since the study has been conducted as a limit test and the difference between the control and the treatment group was only 2.4%.

Conclusions:
The No-Observed-Effect-Concentration (NOEC) for reproduction is **≥** 100 mg pure metabolite/kg dry weight artificial soil, and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction is **>** 100 mg pure metabolite/kg dry weight artificial soil.

Amidosulfuron-ADMP:

Report:	KCA 8.4.2.1/08; [redacted]; 2013; M-454043-01-1
Title:	AE F092944 (BCS-AA25052): Effects on the reproduction of the predatory mite Hypoaspis aculeifer
Report No.:	13 10 48 044 S
Document No.:	M-454043-01-1
Guideline(s):	**OECD 226 (2008)**
Guideline deviation(s):	**none**
GLP/GEP:	**yes**

Executive Summary:
The purpose of this study was to determine potential effects of the metabolite amidosulfuron-ADMP (AE F092944) on the mortality and the reproductive output of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) as a representative of soil micro-arthropods during a test period of 14 days.

10 adult soil mites (females) per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to 100 mg test item/kg soil dry weight. Two weeks after start of exposure, the number of juveniles and surviving parental mites was determined. The test was performed as a limit test in accordance with the OECD Guideline 226 (2008).

The overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg soil dry weight (equals ≥ 99.8 mg p.m./kg dws). The Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg soil dry weight (equals > 99.8 mg p.m./kg dws). The validity criteria for the control group of the study were accomplished.

Materials and Methods:
Test item. AE F092944 (BCS-AA25052); Batch code: AE F092944 00 1B99 0002; Origin Batch No.: 23503LR; CAS No.: 36315-01-2; LIMS No.: 1034970; analysed purity: 99.8 % w/w; certificate No.: AZ 17077.

Per test vessel 10 adult soil mites (females) were exposed to untreated control and to 100 mg test item/kg dry weight of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$, at 19.5 – 21.5 °C and a photoperiod: light : dark = 16 h : 8 h (580 lx) and were fed every 2 days with *Tyrophagus putrescentiae* (SCHRANK). Mortality and reproduction were determined after 14 days of exposure.

Toxic standard (Dimethoate EC 400): 4.10 – 5.12 – 6.40 – 8.00 – 10.00 mg a.s./kg soil d.w.; control: quartz sand, solvent control: none.

Dates of work: January 15, 2013 – February 04, 2013

Results:
Table CA 8.4.2.1- 16: Validity criteria

Validity criteria (for the control group)	Recommended	Obtained
Mean mortality of adult females	≤ 20 %	7.5 %
Mean number of juveniles per replicate	≥ 50	263.9
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	16.4 %

All validity criteria for the study were met.

In a separate study (BioChem project No. R 13 10 48 001 S, dated February 04, 2013), the EC_{50} (reproduction) of the reference item, Dimethoate EC 400, was calculated to be 6.64 mg a.s./kg soil dry weight. The results of the reference test demonstrate sensitivity of the test system.

Table CA 8.4.2.1- 17: Effects of AE F092944 on mortality and reproduction of *Hypoaspis aculeifer*

Test item Test object Exposure	AE F092944 *Hypoaspis aculeifer* Artificial soil	
	Adult mortality	Reproduction
	(mg test item/kg soil d.w.)	
NOEC	≥ 100	≥ 100
LOEC	> 100	> 100
EC_{10}	-	-
EC_{20}	-	-
LC_{50}/EC_{50}	> 100	> 100
95 % confidence limit	-	-

Table CA 8.4.2.1- 18: Effects of AE F092944 on mortality of parental collembolans and on number of juvenile collembolans

Endpoint	**AE F92944** (mg metabolite/kg soil d.w.)	
	control	**100**
Mortality of soil mites after 14 days (%)	7.5	8.8
Mean number of juveniles after 14 days	263.9	244.3
CV %	16.4	17.4
Reproduction (% to control)	100	93

No statistically significant differences compared to the control were calculated (Chi² 2x2 Test for mortality, α = 0.05; Student t-test for reproduction; α = 0.05)
CV: coefficient of variation, d.w.: dry weight (of artificial soil)
Calculations were done using non-rounded values
Percent reproduction: $(R_t / R_c) * 100$ %
R_t = mean number of juvenile mites in the treated group(s)
R_c = mean number of juvenile mites in the control group

In the control group and in the test item treatment group a parental mortality of 7.5 % and 8.8 %, respectively, could be observed at the end of the 14-day exposure period.

Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 263.9 in the control and 244.3 in the test item treatment group.

The test item caused no statistically significantly adverse effects on adult mortality (Chi² 2x2 Test, α = 0.05, one-sided greater) and reproduction (Student t-test, α = 0.05, one-sided smaller) of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg test item/kg soil dry weight.

EC_{10} and EC_{20} cannot be calculated since the study has been conducted as a limit test and the difference between the control and the treatment group was only 7%.

Conclusions:
The test item AE F092944 showed no statistically significantly adverse effects on adult mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg test item/kg soil dry weight. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg soil dry weight, and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg soil dry weight.

Based on the analysed content of 99.8 % amidosulfuron-ADMP in the test item the NOEC was re-calculated as ≥99.8 mg p.m./kg dry weight artificial soil and the LOEC was re-calculated as >99.8 mg p.m./kg dws.

Report:	KCA 8.4.2.1/09; [redacted]; 2013; M-451142-01-1
Title:	AE F092944 (BCS-AA25052): Effects on the reproduction of the collembolan Folsomia candida
Report No.:	13 10 48 045 S
Document No.:	M-451142-01-1
Guideline(s):	**OECD 232 (2009), ISO 11267 (1999)**
Guideline deviation(s):	**none**
GLP/GEP:	**yes**

Executive Summary:

The purpose of this study was to determine potential effects of the metabolite amidosulfuron-ADMP (AE F092944) on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days.

10 juvenile collembolans (9-12 days old) per replicate (8 replicates for the control group and 8 replicates for each treatment group) were exposed to untreated control and to 100 mg test item/kg soil dry weight. After 4 weeks the number of offspring (juveniles) and surviving parental collembolans was counted. The test was performed as a limit test in accordance with the OECD Guideline 232 (adopted 2009) and the International Standard ISO 11267 (1999).

The overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg soil dry weight (equals ≥ 99.8 mg p.m./kg dws). The Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg soil dry weight (equals > 99.8 mg p.m./kg dws). The validity criteria for the control group of the study were accomplished.

Materials and Methods:

Test item: AE F092944 (BCS-AA25052); Substance code: AE F092944; Batch code: AE F092944 00 1B99 0002; Origin Batch No.: 23503LR; CAS No.: 36315-01-2; LIMS No.: 1034970; analysed purity: 99.8 % w/w; certificate No.: AZ 17077.

10 juvenile collembolans (9-12 days old) per test vessel were exposed to untreated control and to 100 mg test item/kg dry weight of soil containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$, at 19.1 – 20.7 °C and a photoperiod: light : dark = 16 h : 8 h (580 lx) and were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

Toxic standard: 44 – 67 – 100 – 150 – 225 mg boric acid/kg soil d.w.; control: quartz sand, solvent control: none.

Dates of work: February 01, 2013 – March 01, 2013

Results:

Table CA 8.4.2.1- 19: Validity criteria

Validity criteria (for the control group)	Recommended	Obtained
Mean adult mortality	≤ 20 %	2.5 %
Mean number of juveniles per replicate	≥ 100	563
Coefficient of variation (mean number of juveniles per replicate)	< 30 %	7.6 %

All validity criteria for the study were met.

In a separate study (BioChem project No. R 12 10 48 003 S, dated May 24, 2012), the EC_{50} (reproduction) of the reference item boric acid was calculated to be 104 mg/kg soil dry weight. The results of the reference test demonstrate the sensitivity of the test system.

Table CA 8.4.2.1- 20: Effects of AE F092944 on mortality and reproduction of *Folsomia candida*

Test item Test object Exposure	AE F092944 *Folsomia candida* Artificial soil	
	Adult mortality	Reproduction
	(mg test item/kg soil d.w.)	
LOEC	> 100	> 100
NOEC	≥ 100	≥ 100
LC_{50}/EC_{50} 95 % confidence limit	> 100 -	> 100 -

Table CA 8.4.2.1- 21: Effects of AE F092944 on mortality of parental collembolans and on number of juvenile collembolans

Endpoint	**AE F092944 (mg test item/kg soil d.w.)**	
	control	**100**
Mortality of parental collembolans after 4 weeks (%)	2.5	2.5
Mean number of juveniles after 4 weeks	563	580
CV %	7.6	14.3
Reproduction (% to control)	100	103

No statistically significant differences compared to the control were calculated for mortality (Fisher's Exact Binomial Test, α = 0.05, one-sided greater) and reproduction (Student-t-test, α = 0.05, one-sided smaller)
CV: coefficient of variation, d.w.: dry weight (of artificial soil)
Calculations were done using unrounded values
Percent reproduction: (Rt / Rc) * 100 %
Rt = mean number of juveniles observed in the treated groups
Rc = mean number of juveniles observed in the control group

The test item caused 2.5 % parental mortality at a concentration of 100 mg test item/kg soil d.w. 2.5 % parental mortality was also observed in the control.

No statistically significant effect (Fisher's Exact Binomial Test, α = 0.05, one-sided greater) on parental mortality was found for the concentration tested.

No effects on behaviour of the collembolans were observed during the test.

The mean number of juvenile springtails counted four weeks after introduction of the parental collembolans into the test vessels was on average 563 in the control and 580 at 100 mg test item/kg soil d.w. No statistically significant effects (Student-t-test, α = 0.05, one-sided smaller) on the number of juveniles compared to the control group were found at 100 mg test item/kg soil d.w.

The No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg dry weight.

EC_{10} and EC_{20} cannot be calculated since the study has been conducted as a limit test and the reproduction was not affected in the treatment group (103% relative to the control).

Conclusions:
The test item AE F092944 (BCS-AA25052) showed no statistically significant adverse effects on adult mortality and reproduction of the collembolan *Folsomia candida* in artificial soil at 100 mg test

item/kg soil d.w. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 100 mg test item/kg soil d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 100 mg test item/kg soil d.w.

Based on the analysed content of 99.8 % amidosulfuron-ADMP in the test item the NOEC was re-calculated as ≥99.8 mg p.m./kg dry weight artificial soil and the LOEC was re-calculated as >99.8 mg p.m./kg dws.

CA 8.5 Effects on nitrogen transformation

For amidosulfuron and its metabolites amidosulfuron-desmethyl, amidosulfuron-desmethyl-chloropyrimidine, amidosulfuron-guanidine, amidosulfuron-biuret, amidosulfuron-ADMP, and amidosulfuron-ADHP, studies on the effect on soil nitrogen transformation were performed. In none of the studies unacceptable effects were found at the highest tested dose level which ranged from 0.10 mg p.m./kg dws to 0.8 mg a.s./kg dws. An overview of all studies is provided in the following table.

Table CA 8.5- 1: Effect data of amidosulfuron and metabolites on soil nitrogen transformation presented in this chapter

Test species	Test system	Test duration	Endpoint	Reference
Amidosulfuron				
Soil microflora	inhibition of nitrogen transformation	28 d	**no unacceptable effects**	**≥ 0.8 mg a.s./kg dws**	[redacted]; 1987; M-119378-01-2 KCA 8.5 /01
Amidosulfuron-desmethyl				
Soil microflora	inhibition of nitrogen transformation	28 d	**no unacceptable effects**	**≥ 0.29 mg p.m./kg dws**	[redacted]; 2015; M-527883-01-1 KCA 8.5 /11
Amidosulfuron-desmethyl-chloropyrimidine				
Soil microflora	inhibition of nitrogen transformation	28 d	**no unacceptable effects**	**≥ 0.39 mg p.m./kg dws**	[redacted]; 2009; M-359509-01-1 KCA 8.5/06
Amidosulfuron-guanidine				
Soil microflora	inhibition of nitrogen transformation	28 d	**no unacceptable effects**	**≥ 0.29 mg p.m./kg dws**	[redacted]; 2009; M-359398-01-1 KCA 8.5/07
Amidosulfuron-biuret				
Soil microflora	inhibition of nitrogen transformation	28 d	**no unacceptable effects**	**≥ 0.30 mg p.m./kg dws**	[redacted]; 2014; M-504115-01-1 KCA 8.5 /08
Amidosulfuron-ADMP				
Soil microflora	inhibition of nitrogen transformation	28 d	**no unacceptable effects**	**≥0.137 mg p.m./kg dws**	[redacted]; 2013; M-453511-01-1 KCA 8.5/09
Amidosulfuron-ADHP				
Soil microflora	inhibition of nitrogen transformation	28 d	**no unacceptable effects**	**≥ 0.10 mg p.m./kg dws**	[redacted]; 2015; M-541593-01-1 KCA 8.5/10

dws = dry weight soil; a.s. = active substance; p.m. = pure metabolite
Bold letters: Values considered relevant for risk assessment in the MCP document

Studies on amidosulfuron:

Report:	KCA 8.5/01; [redacted]; [redacted]; [redacted]; 1987; M-119378-01-2
Title:	Investigating the effect of Hoe 075032 substance technical Code: Hoe 075032 OH ZC96 0001 on ammonification and nitrification of horn meal nitrogen
Report No.:	A40575
Document No.:	M-119378-01-2
Guideline(s):	**BBA guideline, VI, 1-1 (1987)**
Guideline deviation(s):	**not specified**
GLP/GEP:	**no**

The study reports on a soil nitrogen transformation test with amidosulfuron technical a.s.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. An EU agreed endpoint of 4 % effect at day 28 at 0.8 mg a.s./kg d.w.soil (0.6 kg a.s/ha) was derived based on this test.

Studies on the metabolites of amidosulfuron:

Amidosulfuron-desmethyl:

Report:	KCA 8.5/11; [redacted]; 2015; M-527883-01-1
Title:	Amidosulfuron-desmethyl (BCS-BB54362): Effects on the activity of soil microflora (Nitrogen transformation test)
Report No.:	15 10 48 034 N
Document No.:	M-527883-01-1
Guideline(s):	**OECD 216 (adopted 2000).**
Guideline deviation(s):	**none**
GLP/GEP:	**yes**

Executive summary:
The purpose of this study was to determine the effects of the metabolite amidosulfuron-desmethyl (BCS-BB54362) on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.06 mg test item/kg soil dry weight and 0.30 mg test item/kg soil dry weight. Application rates were equivalent to 0.045 kg test item/ha and 0.225 kg test item/ha. Lucerne meal was added to the soil (concentration in soil 0.5 %) to stimulate nitrogen transformation. Amidosulfuron-desmethyl caused a temporary inhibition of the daily nitrate rate at the tested concentrations of 0.06 mg test item/kg dry soil and 0.30 mg test item/kg dry soil at time interval 7-14 days after application. However, no adverse effects of amidosulfuron-desmethyl on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 28 days after application (time interval 14-28). Differences from the control of +1.0 % (test concentration 0.06 mg test item/kg dry soil) and -2.7 % (test concentration 0.30 mg test item/kg dry soil, equals 0.29 mg p.m./kg dry weight soil) were measured at the end of the 28-day incubation period (time interval 14-28). Amidosulfuron-desmethyl caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period.

Material and methods:
Test item: Amidosulfuron-desmethyl; BCS Code: BCS-BB54362; Substance type: metabolite; Batch code: AE F101630 00 1C97 0001; Origin batch code: YP 79; CAS No.: 935867-69-9; LIMS No.: 1324835; Analysed purity: 95.8% w/w; Certificate of analysis-No.: AZ 18898.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.06 mg test item/kg soil dry weight and 0.30 mg test item/kg soil dry weight. Application rates were equivalent to 0.045 kg test item/ha and 0.225 kg 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).

Dates of work: March 18, 2015 to April 15, 2015

Results:
Validity Criteria:
The coefficients of variation in the control (No_3-N) were maximum 10.6 % and thus fulfilled the demanded range (≤ 15 %).

In a separate study (conducted from 06.01.2014 to 03.02.2015) the reference item Dinoterb caused an effect of +39.1 %, +62.5 % and +112.0 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 6.80 mg, 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application (time interval 14-28) and thus demonstrates the sensitivity of the test system.

Nitrogen transformation:
The test item amidosulfuron-desmethyl caused a temporary inhibition of the daily nitrate rate at the tested concentrations of 0.06 mg test item/kg dry soil and 0.30 mg test item/kg dry soil at time interval 7-14 days after application.

However, no adverse effects of amidosulfuron-desmethyl on nitrogen transformation in soil could be observed at both tested concentrations at the end of the test, 28 days after application (time interval 14-28). Differences from the control of +1.0 % (test concentration 0.06 mg test item/kg dry soil) and -2.7 % (test concentration 0.30 mg test item/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Table CA 8.5- 2: Effects on nitrogen transformation in soil after treatment with amidosulfuron-desmethyl

Time Interval (days)	Control			0.06 mg test item/kg soil dry weight equivalent to 0.045 kg test item/ha				0.30 mg test item/kg soil dry weight equivalent to 0.225 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	6.35	±	1.21	5.63	±	1.07	**-11.3** n.s.	5.93	±	0.26	**-6.6** n.s.
7-14	-0.49	±	0.92	-0.90	±	0.46	**-85.3** n.s.	-1.21	±	0.08	**-149.0** n.s.
14-28	3.48	±	0.25	3.51	±	0.13	**+1.0** n.s.	3.38	±	0.22	**-2.7** n.s.

The calculations were performed with unrounded values
1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Conclusions:
Amidosulfuron-desmethyl 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 0.30 mg test item/kg dry soil

(equals 0.29 mg p.m./kg dws based on the analysed content of 95.8 % amidosulfuron-desmethyl in the test item), which are equivalent to application rates up to 0.225 kg test item/ha.

Amidosulfuron-desmethyl-chloropyrimidine:

Report:	KCA 8.5/06; [redacted]; 2009; M-359509-01-1
Title:	Metabolite Amidosulfuron-desmethyl-chloropyrimidine: Determination of effects on nitrogen transformation in soil
Report No.:	FRM-N-128/09
Document No.:	M-359509-01-1
Guideline(s):	**OECD 216 (adopted 2000).**
Guideline deviation(s):	**for minor deviations see Point 2.2**
GLP/GEP:	**yes**

Note: *This study has been previously submitted to former RMS (Austria) to support the post Annex I process of amidosulfuron. It was evaluated by Austria and is part of the DAR Addendum (Feb 2011 – Addendum to monograph prepared in the context of post Annex I procedure (new Annex II data)).Upon request of the new RMS Finland, the study has nevertheless been included in the supplemental dossier.*

Executive Summary:
The objective of this study was to determine the effects of the metabolite amidosulfuron-desmethyl-chloropyrimidine on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

A loamy sand soil (according to DIN 'mittel lehmiger Sand', texture: 10.4 % clay, 17.4 % silt, 72.2 % sand, 1.57 % org. carbon content) was exposed for 28 days to 0.04 and 0.44 mg test item/kg soil dry weight. Application rates were equivalent to 0.033 and 0.334 kg test item/ha. Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation. Between 0 and 22 % difference to the control in Nitrate were measured in the treatment groups in the weekly time intervals. During the 28-day test, 0.04 mg and 0.44 mg test item/kg dry weight soil (equals 0.035 and 0.39 mg p.m./kg dws, respectively) had no relevant influence on nitrogen transformation in a loamy sand soil supplemented with Lucerne-grass-green meal. Even though both test concentrations revealed a statistically significant difference to the control at the end of the study, the deviation from the control was still below the threshold value recommended by the guideline. In none of the time intervals analysed during the 28 day exposure the difference in the daily nitrate-N rates exceeded the trigger value of 25 %.

Materials and Methods:
Test item: Amidosulfuron-desmethyl-chloropyrimidine, sodium salt; Short name: BCS-CO78570; LIMS No.: 0922452; Batch code: BCS-CO78570-01-01; Origin Batch No.: BCOO 5766-3-3; TOX-No.: 08625-00; analysed content: 88.7 % w/w.

A loamy sand soil (according to DIN 'mittel lehmiger Sand', texture: 10.4 % clay, 17.4 % silt, 72.2 % sand, 1.57 % org. carbon content) was exposed for 28 days to 0.04 and 0.44 mg test item/kg soil dry weight. Application rates were equivalent to 0.033 and 0.334 kg test item/ha. This quantities were determined by taking the field rate and the 10-fold rate of the parent compound (0.030 and 0.30 kg a.s/ha) and converting this quantities into the molecular weight equivalent of metabolite. Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation. Soil samples of 300 g dry weight per incubation flask were used. Three replicates were prepared per treatment. Sodium chloride was used as a reference standard in the tests. The soil was held in the dark at 20 ± 2 °C and about 40-50 % of the maximum water holding capacity (WHC_{max}). Immediately after treatment and after 7, 14 and 28 days, the soil in each jar was mixed by shaking. Samples (10 g dry soil) were extracted with KCl, NO_2^- and NO_3^- and were determined using a Flow Analyser.

Dates of work: August 27, 2009 – October 07, 2009

Results:

Validity Criteria:

The coefficient of variation in the control at the end of the study was 2 %. Therefore the validity criteria for the study, which requires a coefficient of variation ≤ 15 % in the control, was fulfilled.

In separate tests (non-GLP) the reference standard sodium chloride was used. In these tests with the agricultural soil, 16 g NaCl/kg dry weight soil had a distinct and long-term (> 28 days) influence on microbial mineralization of nitrogen.

Nitrogen transformation:

During the 28-day test, 0.04 mg and 0.44 mg test item/kg dry weight soil had no relevant influence on nitrogen transformation in a loamy sand soil supplemented with Lucerne-grass-green meal. Even though both test concentrations revealed a statistically significant difference to the control at the end of the study, the deviation from the control was still below the threshold value recommended by the guideline. In none of the time intervals analysed during the 28 day exposure the difference in the daily nitrate-N rates exceeded the trigger value of 25 %.

Table CA 8.5- 3: Effects on nitrogen transformation in soil after treatment with amidosulfuron-desmethyl-chloropyrimidine

Time Interval (days)	Control			0.04 mg test item/kg soil dry weight equivalent to 0.033 kg test item/ha				0.44 mg test item/kg soil dry weight equivalent to 0.334 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	0.55	±	0.04	0.42	±	0.11	**22** n.s.w.	0.60	±	0.31	**10** n.s.w.
7-14	3.51	±	0.17	3.65	±	0.24	**4** n.s.	3.53	±	0.17	**0** n.s.
14-28	1.80	±	0.02	1.69	±	0.04	**6** *	1.68	±	0.07	**6** *

1) Rate: Nitrate-N in mg/kg dry weight soil/time interval/day, mean of 3 replicates and standard deviation

* = Statistically significant difference to the control (Student-t Test, two-sided, α = 0.05).

n.s. = No statistically significant difference to the control (Student-t Test, two-sided, α = 0.05).

n.s.w. = No statistically significant difference to the control (Welch-t Test for inhomogeneous variances, two-sided, α = 0.05).

Conclusion:

During the 28-day test, 0.04 mg and 0.44 mg test item/kg dw soil (equals 0.035 and 0.39 mg p.m./kg dws, respectively based on the analysed content of 88.7 % amidosulfuron-desmethyl-chloropyrimidine in the test item) had no relevant influence on nitrogen transformation in a loamy sand soil supplemented with Lucerne-grass-green meal. In none of the time intervals analysed during the 28 day exposure the difference in the daily nitrate-N rates exceeded the trigger value of 25 %.

Amidosulfuron-guanidine:

Report:	KCA 8.5/07; [redacted]; 2009; M-359398-01-1
Title:	Metabolite Amidosulfuron-guanidine: Determination of effects on nitrogen transformation in soil
Report No.:	FRM-N-129/09
Document No.:	M-359398-01-1
Guideline(s):	**OECD 216 (adopted 2000).**
Guideline deviation(s):	**for minor deviations see Point 2.2**
GLP/GEP:	**yes**

Note: *This study has been previously submitted to former RMS (Austria) to support the post Annex I process of amidosulfuron. It was evaluated by Austria and is part of the DAR Addendum (Feb. 2011 – Addendum to monograph prepared in the context of post Annex I procedure (new Annex II data)).Upon request of the new RMS Finland, the study has nevertheless been included in the supplemental dossier.*

Executive Summary:

The objective of this study was to determine the effects of the metabolite amidosulfuron-guanidine on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

A loamy sand soil (according to DIN 'mittel lehmiger Sand', texture: 10.4 % clay, 17.4 % silt, 72.2 % sand, 1.57 % org. carbon content) was exposed for 28 days to 0.03 and 0.29 mg test item/kg soil dry weight. Application rates were equivalent to 0.022 and 0.222 kg test item/ha. Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation. Between 1 and 13 % difference to the control in Nitrate were measured in the treatment groups in the weekly time intervals. During the 28-day test, in a soil supplemented with lucerne-grass-green-meal, it was found that 0.03 mg and 0.29 mg test item/kg dry weight soil (equivalent to 0.022 kg and 0.222 kg test item/ha) had no relevant influence on nitrogen transformation in a loamy sand. In none of the time intervals analysed during the 28 day exposure the difference in the daily nitrate-N rates exceeded the trigger value of 25 %.

Materials and Methods:

Test item: Amidosulfuron-guanidine; Short name: BCS-CO41839; LIMS No.: 0920454; Batch code: BCS-CO41839-01-01; Origin Batch No.: RDL 603-16-20; TOX-No.: 08626-00; analysed content: 98.3 % w/w.

A loamy sand soil (according to DIN 'mittel lehmiger Sand', texture: 10.4 % clay, 17.4 % silt, 72.2 % sand, 1.57 % org. carbon content) was exposed for 28 days to 0.03 and 0.29 mg test item/kg soil dry weight. Application rates were equivalent to 0.022 and 0.222 kg test item/ha. This quantities were determined by taking the field rate and the 10-fold rate of the parent compound (0.030 and 0.30 kg a.s/ha) and converting this quantities into the molecular weight equivalent of metabolite. Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation. Soil samples of 300 g dry weight per incubation flask were used. Three replicates were prepared per treatment. Sodium chloride was used as a reference standard in the tests. The soil was held in the dark at 20 ± 2 °C and about 40-50 % of the maximum water holding capacity (WHC_{max}). Immediately after treatment and after 7, 14 and 28 days, the soil in each jar was mixed by shaking. Samples (10 g dry soil) were extracted with KCl, NO_2^- and NO_3^- and were determined using a Flow Analyser.

Dates of work: August 27, 2009 – October 08, 2009

Results:

Validity Criteria:

The coefficient of variation in the control at the end of the study was 5 %. Therefore the validity criterium for the study, which requires a coefficient of variation ≤ 15 % in the control, was fulfilled.

In separate tests (non-GLP) the reference standard sodium chloride was used. In these tests with the agricultural soil, 16 g NaCl/kg dry weight soil had a distinct and long-term (> 28 days) influence on microbial mineralization of nitrogen.

Nitrogen transformation:

During the 28-day test, 0.03 mg and 0.29 mg of the Metabolite amidosulfuron-guanidine/kg dry weight soil had no relevant influence on nitrogen transformation in a loamy sand soil supplemented with Lucerne-grass-green meal. In none of the time intervals analysed during the 28 day exposure the difference in the daily nitrate-N rates exceeds the trigger value of 25 %.

Table CA 8.5- 4: Effects on nitrogen transformation in soil after treatment with amidosulfuron-guanidine

Time Interval (days)	Control			0.03 mg test item/kg soil dry weight equivalent to 0.022 kg test item/ha				0.29 mg test item/kg soil dry weight equivalent to 0.222 kg test item/ha			
	Nitrate-N[1]			Nitrate-N[1]			% difference to control	Nitrate-N[1]			% difference to control
0-7	0.92	±	0.13	0.89	±	0.29	**3** n.s.	0.94	±	0.08	**3** n.s.
7-14	3.32	±	0.09	3.76	±	0.17	**13** *	3.70	±	0.07	**11** *
14-28	1.81	±	0.18	1.80	±	0.15	**1** n.s.	1.74	±	0.09	**4** n.s.

1) Rate: Nitrate-N in mg/kg dry weight soil/time interval/day, mean of 3 replicates and standard deviation
* = Statistically significant difference to the control (Student-t Test, two-sided, α = 0.05).
n.s. = No statistically significant difference to the control (Student-t Test, two-sided, α = 0.05).

Conclusion:

During the 28-day test, in a soil supplemented with lucerne-grass-green-meal (5 g/kg), it was found that 0.03 mg and 0.29 mg test item/kg dry weight soil (equivalent to 0.022 kg and 0.222 mg test item/ha) had no relevant influence on nitrogen transformation in a loamy sand. In none of the time intervals analysed during the 28 day exposure the difference in the daily nitrate-N rates exceeds the trigger value of 25 %.

Amidosulfuron-biuret:

Report: KCA 8.5/08; [redacted]; 2014; M-504115-01-1
Title: Amidosulfuron-biuret (BCS-CQ51287): Effects on the activity of soil microflora (Nitrogen transformation test)
Report No.: 14 10 48 086 N
Document No.: M-504115-01-1
Guideline(s): **OECD 216 (adopted 2000)**
Guideline deviation(s): **none**
GLP/GEP: **yes**

Executive Summary:

The purpose of this study was to determine the effects of the metabolite amidosulfuron-biuret (BCS-CQ51287) on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

A loamy sand soil (DIN 4220) was exposed for 28 d to concentrations of 0.06 and 0.32 mg test item/kg soil dry weight (equals 0.056 and 0.30 mg p.m./kg dws). Application rates were equivalent to 0.048 and 0.240 kg test item/ha. Lucerne meal was added to the soil (concentration in soil 0.5 %) to stimulate nitrogen transformation. No adverse effects of amidosulfuron-biuret on nitrogen transformation in soil could be observed in both test concentrations (0.06 mg/kg dry soil and 0.32 mg/kg dry soil) after 28 days. Differences from the control of -13.8 % (test concentration 0.06 mg/kg dry soil) and +9.1 % (test concentration 0.32 mg /kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28). Amidosulfuron-biuret caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period.

Material and methods:
Test item: Amidosulfuron-biuret (BCS-CQ51287); BCS-code: BCS-CQ51287; Batch code: BCS-CQ51287-01-02; Origin batch No.: GSE61653-3-3; Customer Order No.: TOX-No.: 10517-00; LIMS No.: 1421391; Analysed purity: 93.6 % w/w; Certificate No.: AZ 19475.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.06 and 0.32 mg test item/kg soil dry weight. Application rates were equivalent to 0.048 and 0.240 kg 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).

Dates of work: October 10, 2014 – November 10, 2014

Results:
Validity Criteria:
The coefficients of variation in the control (NO_3-N) were maximum 2.8 % and thus fulfilled the demanded range (≤ 15 %).

In a separate study the reference item Dinoterb (BioChem study code: R 14 10 48 001 N, conducted from 08.01.2014 to 05.02.2014) caused a stimulation of nitrogen transformation of +101.8 % and +172.8 % (required ≥ 25 %) at 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, 28 days after application (time interval 14-28) and thus demonstrates the sensitivity of the test system.

Nitrogen transformation:
No adverse effects of amidosulfuron-biuret on nitrogen transformation in soil could be observed at both test concentrations (0.06 mg/kg dry soil and 0.32 mg /kg dry soil) during the 28-day experiment. Differences from the control of -13.8 % (test concentration 0.06 mg/kg dry soil) and +9.1 % (test concentration 0.32 mg /kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Table CA 8.5- 5: Effects on nitrogen transformation in soil after treatment with amidosulfuron-biuret

Time Interval (days)	Control			0.06 mg test item/kg soil dry weight equivalent to 0.048 kg test item/ha				0.32 mg test item/kg soil dry weight equivalent to 0.240 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	4.25	±	0.16	4.20	±	0.34	**-1.1** [n.s.]	4.40	±	0.16	**+3.7** [n.s.]
7-14	1.62	±	0.09	1.50	±	0.41	**-7.9** [n.s.]	1.43	±	0.23	**-11.7** [n.s.]
14-28	1.20	±	0.14	1.04	±	0.18	**-13.8** [n.s.]	1.31	±	0.31	**+9.1** [n.s.]

The calculations were performed with unrounded values
[1)] Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
[n.s.] = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Conclusions:
Amidosulfuron-biuret caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured 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 0.32 mg test item/kg soil dry weight (equals 0.30 mg p.m./kg dws based on the analysed content of 93.6 % amidosulfuron-biuret in the test item), which are equivalent to application rates up to 0.240 kg test item/ha.

Amidosulfuron-ADMP:

Report: KCA 8.5.09; [redacted]; 2013; M-453511-01-1
Title: AE F092944 (BCS-AA25052): Effects on the activity of soil microflora (Nitrogen transformation test)
Report No.: 13 10 48 018 N
Document No.: M-453511-01-1
Guideline(s): **OECD 216 (adopted 2000)**
Guideline deviation(s): **none**
GLP/GEP: **yes**

Executive Summary:
The purpose of this study was to determine the effects of the metabolite amidosulfuron-ADMP (AE F092944) on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

A loamy sand soil (DIN 4220) was exposed for 28 d to concentrations of 0.028 and 0.137 mg test item/kg soil dry weight. Application rates were equivalent to 0.021 and 0.103 kg test item/ha. Lucerne meal was added to the soil (concentration in soil 0.5 %) to stimulate nitrogen transformation. No adverse effects of AE F092944 (BCS-AA25052) on nitrogen transformation in soil could be observed in both test concentrations (0.028 mg/kg dry soil and 0.137 mg/kg dry soil) after 28 days. Differences from the control of +7.9 % (test concentration 0.028 mg/kg dry soil) and +9.2 % (test concentration 0.137 mg /kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28). AE F092944 (BCS-AA25052) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 28-day incubation period.

Material and methods:
Test item: AE F092944 (BCS-AA25052); BCS-code: BCS-AA25052; Batch code: AE F092944 00 1B99 0002; Origin batch No.: 23503LR; CAS No.: 36315-01-2; LIMS No.: 1034970; Analysed purity: 99.8 % w/w; certificate of analysis-No.: AZ 17077.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.028 and 0.137 mg test item/kg soil dry weight. Application rates were equivalent to 0.021 and 0.103 kg test item/ha. Determination of the nitrogen transformation (NO_3-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined using the Autoanalyser (BRAN+LUEBBE) at different sampling intervals (0, 7, 14 and 28 days after treatment).

Dates of work: January 17, 2013 – February 14, 2013

Results:

Validity Criteria:

The coefficients of variation in the control (NO_3-N) were maximum, 5.1 % and thus fulfilled the demanded range (≤15 %).

In a separate study the reference item Dinoterb (BioChem study code: R 13 10 48 001 N) caused a stimulation of nitrogen transformation of +33.7 % and +42.6 % at 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively,28 days after application and thus demonstrates the sensitivity of the test system.

Nitrogen transformation:

No adverse effects of AE F092944 (BCS-AA25052) on nitrogen transformation in soil could be observed at both test concentrations (0.028 mg/kg dry soil and 0.137 mg /kg dry soil) after 28 days. Differences from the control of +7.9 % (test concentration 0.028 mg/kg dry soil) and +9.2 % (test concentration 0.137 mg /kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

Table CA 8.5- 6: Effects on nitrogen transformation in soil after treatment with AE F092944

Time Interval (days)	Control			0.028 mg test item/kg soil dry weight equivalent to 0.021 kg test item/ha				0.137 mg test item/kg soil dry weight equivalent to 0.103 kg test item/ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	3.16	±	0.29	3.23	±	0.05	**+2.3** [n.s.]	3.35	±	0.09	**+5.9** [n.s.]
7-14	1.30	±	0.15	1.26	±	0.24	**-3.3** [n.s.]	1.26	±	0.33	**-3.3** [n.s.]
14-28	0.93	±	0.04	1.00	±	0.14	**+7.9** [n.s.]	1.02	±	0.15	**+9.2** [n.s.]

The calculations were performed with unrounded values

[1)] Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation

[n.s.] = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Conclusions:

AE F092944 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured 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 0.137 mg test item/kg soil dry weight, which are equivalent to application rates up to 0.103 kg test item/ha.

Amidosulfuron-ADHP:

Report:	KCA 8.5/10; [redacted]; 2015; M-541593-01-1
Title:	Amidosulfuron-AE F094206 (BCS-AA25045): Effects on the activity of soil microflora (nitrogen transformation test)
Report No.:	15 10 48 053 N
Document No.:	M-541593-01-1
Guideline(s):	**OECD 216 (adopted 2000)**
Guideline deviation(s):	**none**
GLP/GEP:	**yes**

Executive Summary

The purpose of this study was to determine the effects of the metabolite amidosulfuron-ADHP (AE F094206) on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. The test was performed in accordance with OECD guideline 216 (2000) by measuring the nitrogen turnover.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.02 mg test item/kg soil dry weight and 0.10 mg test item/kg soil dry weight. Application rates were equivalent to 0.015 kg test item/ha and 0.077 kg 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).

No adverse effects of amidosulfuron-ADHP on nitrogen transformation in soil could be observed at both test concentrations (0.02 mg test item/kg soil dry weight and 0.10 mg test item/kg soil dry weight) during the 28-day experiment. Differences from the control of +3.8 % (test concentration 0.02 mg test item/kg soil dry weight) and +7.2 % (test concentration 0.10 mg test item/kg soil dry weight) were measured at the end of the 28-day incubation period (time interval 14-28). Amidosulfuron-ADHP 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.

Material and methods:

Test item: Amidosulfuron-ADHP (AE F094206); BCS-code: BCS-AA25045; Batch code: AE F094206 00 1C99 0001; Origin batch No.: PW 210/213; LIMS No.: 1401264; CAS No.: 56-09-7; Analysed purity: 99.5 % w/w; Certificate of analysis No.: AZ 19246.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.02 mg test item/kg soil dry weight and 0.10 mg test item/kg soil dry weight. Application rates were equivalent to 0.015 kg test item/ha and 0.077 kg test item/ha. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH4-nitrogen, NO3- and NO2-nitrogen were determined by an Autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment).

Dates of experimental work: July 09, 2015 – August 06, 2015

Results:

Validity criteria:

The coefficients of variation in the control for NO_3-N were maximum 9.0 % and thus fulfilled the demanded range (≤ 15 %).

In a separate study the reference item Dinoterb caused stimulations of nitrogen transformation of +39.1 %, +62.5 % and +112.0 % at 6.80 mg, 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application (time interval 14-28).

<u>Nitrogen transformation:</u>
No adverse effects of amidosulfuron-AE F094206 (BCS-AA25045) on nitrogen transformation in soil could be observed at both test concentrations (0.02 mg test item/kg soil dry weight and 0.10 mg test item/kg soil dry weight) during the 28-day experiment. Differences from the control of +3.8 % (test concentration 0.02 mg test item/kg soil dry weight) and +7.2 % (test concentration 0.10 mg test item/kg soil dry weight) were measured at the end of the 28-day incubation period (time interval 14-28).

Table CA 8.5- 7: Effects on nitrogen transformation in soil after treatment with amidosulfuron-ADHP

Time interval (days)	Control			0.02 mg test item/kg soil dw, equivalent to 15 g/ha dw				0.10 mg test item/kg soil dw, equivalent to 77 g/ha dw			
	Nitrate [1)]			Nitrate [1)]			% deviation to control	Nitrate [1)]			% deviation to control
0-7	4.58	±	0.65	4.51	±	0.16	**-1.5**[n.s.]	4.81	±	0.37	**+5.2**[n.s.]
7-14	2.13	±	0.36	2.50	±	0.44	**+17.7**[n.s.]	2.16	±	0.19	**+1.3**[n.s.]
14-28	1.56	±	0.08	1.62	±	0.09	**+3.8**[n.s.]	1.67	±	0.39	**+7.2**[n.s.]

The calculations were performed with unrounded values
1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

Conclusion
Amidosulfuron-ADHP 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 0.10 mg test item/kg soil dry weight, which are equivalent to application rates up to 0.077 kg test item/ha (corresponding to 0.077 kg pure metabolite/ha).

****

<u>Supportive information:</u> In the new European dossier format/data requirements there is no data point that corresponds to soil carbon transformation studies. Nevertheless, four studies are mentioned here as supportive information, since they are contained in the baseline dossier and in the List of Endpoints from the first EU review. In context of application for EU approval renewal of amidosulfuron, these studies are superseded since soil carbon transformation is no longer assessed under Regulation 1107/2009. Nitrogen transformation tests are now available for all soil metabolites considered relevant for risk assessment.

Report: KCA 8.5/02; [redacted]; [redacted]; [redacted]; 1988; M-120507-01-2
Title: Investigating the effect of Hoe 075032 - substance technical Code: Hoe 075032 OH ZC96/0001 on aerobic soil respiration
Report No.: A40574
Document No.: M-120507-01-2
Guideline(s): **BBA guideline, VI, 1-1 (1987)**
Guideline deviation(s): **not specified**
GLP/GEP: **no**

The study reports on a soil carbon transformation test with amidosulfuron technical a.s.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered not acceptable, due to several deficiencies and deviations from the guideline. No EU agreed endpoint was derived based on this test.

Report:	KCA 8.5/03; [redacted]; 1998; M-143359-01-1
Title:	Amidosulfuron (prov. approved ISO)+ mefenpyr-diethyl (draft ISO)+ AEF115008 water dispersible granule 12.5 + 12.5 + 1.25 % Code: AE F075032 08 WG26 A201 Effects on soil microbial activity (short-term respiration)
Report No.:	A59697
Document No.:	M-143359-01-1
Guideline(s):	**BBA guideline, VI, 1-1 (1990)**
Guideline deviation(s):	**not specified**
GLP/GEP:	**yes**

The study reports on a soil carbon transformation test with a product formulation containing 12.6% (w/w) amidosulfuron, 1.33% (w/w) iodosulfuron-methyl-sodium, and 12.9% (w/w) safener mefenpyr-diethyl, different to the representative formulation.

Based on bridging considerations, it was concluded that amidosulfuron has no unacceptable effect on soil respiration up to a concentration of 0.20 mg a.s./kg soil (corresponding to 151 g a.s./ha).

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. No EU agreed endpoint was derived based on this test.

Report:	KCA 8.5/04; [redacted]; 2004; M-236878-01-1
Title:	Amidosulfuron AE F075032 Effects on soil microflora - carbon turnover Interpretation of findings from document A40574
Report No.:	C040314
Document No.:	M-236878-01-1
Guideline(s):	
Guideline deviation(s):	**not specified**
GLP/GEP:	**no**

The statement provides supportive discussion to studies KCA 8.5/03 and KCA 8.5/04, and concludes that in spite of the deficiencies of study KCA 8.5/03 the data generated reflects the safety with regard to soil carbon turnover, which is also backed up by the results of study KCA 8.5/04.

Nevertheless, the conduct of a new study was announced, which is found described below under KCA 8.5/05.

Report:	KCA 8.5/05; [redacted]; 2004; M-182622-01-1
Title:	Amidosulfuron tech. (AE F075032 00 1D99 0013): Determination of effects on carbon transformation in soil
Report No.:	LKC 04.35/04
Document No.:	M-182622-01-1
Guideline(s):	**OECD/OCDE Guideline No.217 (adopted 2000)**
Guideline deviation(s):	**none**
GLP/GEP:	**yes**

The study reports on a soil carbon transformation test with amidosulfuron technical a.s.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (Addendum of August 2007).

The study was considered acceptable. An EU agreed endpoint of 13 % effect at day 28 at 0.06 mg a.s./kg d.w.soil (0.045 kg a.s/ha) and 22 % effect at day 28 at 0.3 mg a.s./kg d.w.soil (0.225 kg a.s/ha)[18] was derived based on this test.

CA 8.6 Effects on terrestrial non-target higher plants

CA 8.6.1 Summary of screening data

For amidosulfuron, greenhouse screening was performed on a number of higher plant species including crops, broadleaf and grass weeds (KCA 8.6.1 /01). As expected for a sulfonyl urea herbicide, the compound showed herbicidal activity for several plants, in both pre- and post-emergence applications. The tests indicated selective control in particular of a spectrum of broadleaf plants.

Soil metabolites amidosulfuron-desmethyl, amidosulfuron-desmethyl-chloropyrimidine, amidosulfuron-guanidine, amidosulfuron-biuret, amidosulfuron-ADMP, and amidosulfuron-ADHP were screened for herbicidal activity in greenhouse assays. None of the components revealed an herbicidal effect comparable to that of the parent active substance. For some of these components, this information will be required for assessing the potential relevance of these metabolites in groundwater, cf. Document N4.

Details of all studies are provided in the following table.

[18] citation of text in brackets corrected for typo, original LoE reads “(mg 0.045a.s/ha)” and “(mg 0.225a.s/ha)”

Table CA 8.6.1- 1: Screening data for effect of amidosulfuron and selected metabolites to higher terrestrial plants

Test design	Test species	Ecotoxicological endpoint	Reference
Amidosulfuron			
Greenhouse, seedling emergence and growth, 26-28 d	Crop plants (7[1,2] species) Broadleaf plants (10[1]-12[2]- species) Grass plants (8[1,2] species)	Amidosulfuron is active pre-emergence as well as post-emergence to broadleaf plants. At low dosage of 20 g a.s./ha only very few broadleaf plants are effectively damaged after post-emergence use. Grasses are highly tolerant even to a high rate of 300 grams. After pre-emergence application, amidosulfuron shows still activity on some broadleaf plants at a low dosage of 20g a.s./ha while low herbicide activity is seen against other broadleaf plants and all grass species.	[redacted]; 1999; M-187775-01-1 KCA 8.6.1 /01
Amidosulfuron-desmethyl, formulated as WP05			
Greenhouse, growth, 28 d	Weed species (4[1] species)	After post-emergence application, amidosulfuron-desmethyl was found devoid of biological activity on the range of tested weed species.	[redacted]; 2005; M-244564-01-1 KCA 8.6.1 /02
Amidosulfuron-desmethyl-chloropyrimidine, formulated as WP05			
Greenhouse, growth, 28 d	Weed species (8[1] species)	After post-emergence application, amidosulfuron-desmethyl-chloropyrimidine showed no biological activity on the range of weed species tested under standard glasshouse screening conditions.	[redacted]; 2009; M-363717-01-1 KCA 8.6.1 /06
Amidosulfuron-guanidine, formulated as WP05			
Greenhouse, growth, 28 d	Weed species (8[1] species)	After post-emergence application, amidosulfuron-guanidine showed no biological activity on the range of weed species tested under standard glasshouse screening conditions.	[redacted]; 2009; M-363717-01-1 KCA 8.6.1 /06
Amidosulfuron-biuret, formulated as WP05			
Greenhouse, growth, 28 d	Weed species (8[1] species)	After post-emergence application, amidosulfuron-biuret showed no biological activity on the range of weed species tested under standard glasshouse screening conditions.	[redacted]; 2010; M-369645-01-1 KCA 8.6.1 /07
Amidosulfuron-ADMP			
Greenhouse, primary pre- and post emergence screening	Weed species (6 species)	In primary pre- and post-emergence screening, this metabolite was almost herbicidally inactive against a wide range of grass and broad-leaved plant species at 2500 g technical substance / ha.	[redacted]; 1999; M-185253-01-1 KCA 8.6.1/08
Amidosulfuron-ADHP, formulated as WP05			
Greenhouse, growth, 28 d	Weed species (4[1] species)	After post-emergence application, amidosulfuron-ADHP showed no biological activity on the range of weed species.	[redacted]; 2004; M-244563-01-1 KCA 8.6.1 /03
Amidosulfuron - Lysimeter leachate			

Test design	Test species	Ecotoxicological endpoint	Reference
Greenhouse, growth, 14 d	*Helianthus annuus*	After post-emergence application, no phytotoxicity and no growth inhibition of percolates from lysimeters treated with 49 g a.s./ha and 54 g a.s./ha to sunflower were observed. The tested percolate samples contained the highest not-identified radioactivity concentrations of the respective year in the lysimeter study. The percolate samples contained no amidosulfuron as the parent substance only appeared at the beginning of the first year in the lysimeter experiment.	[redacted]; [redacted]; 1992; M-138080-01-2 KCA 8.6.1 /04
AE 1569309 [not a confirmed soil metabolite of amidosulfuron], formulated as WP05			
Greenhouse, growth, 28 d	Weed species (4[1] species)	After post-emergence application, AE 1569309 had no effect on three of the tested weed species. Slight visual effects on *Sinapis arvensis* were attributed to natural biological variance.	[redacted]; 2005; M-247760-01-1 KCA 8.6.1.3

[1] post-emergent application of the test item
[2] pre-emergent application of the test item

Studies on amidosulfuron:

Report:	KCA 8.6.1/01; [redacted]; 1999; M-187775-01-1
Title:	Effectivity of the herbicide Amidosulfuron (AE F075032) on higher plant species as applied under greenhouse conditions
Report No.:	C004348
Document No.:	M-187775-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a greenhouse screening test for herbicidal activity of amidosulfuron (formulated as WP20) on a number of crops, broadleaf and grass weeds. After post-emergence treatment, the greenhouse trials demonstrated activity on some broadleaf plants as expected for a narrow spectrum herbicide.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered as non-essential, additional information only - no EU agreed endpoint was derived from this test.

Studies on the metabolites of amidosulfuron:

Amidosulfuron-desmethyl:

Report:	KCA 8.6.1/02; [redacted]; 2005; M-244564-01-1
Title:	Evaluation of the biological activity of AE F101630, a metabolite of amidosulfuron
Report No.:	C046651
Document No.:	M-244564-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a glasshouse test under standardized conditions to determine the biological activity of metabolite amidosulfuron-desmethyl (formulated as WP05 as a vehicle for application) in comparison to a commercial formulation of amidosulfuron (WG75). It was concluded that the metabolite had no biological activity on a range of weed species.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. No EU agreed endpoint was derived from this test.

Amidosulfuron-desmethyl-chloropyrimidine & Amidosulfuron-guanidine:

Report:	KCA 8.6.1/06; [redacted]; 2009; M-363717-01-1
Title:	Evaluation of the post emergence biological activity of amidosulfuron-desmethyl-chloropyrimidine (BCS-CO78570) and amidosulfuron- guanidine (BCS-CO41839) metabolites of amidosulfuron (AE F075032)
Report No.:	PP09039
Document No.:	M-363717-01-1
Guideline(s):	**not specified**
Guideline deviation(s):	**not specified**
GLP/GEP:	**no**

***Note:** This study has been previously submitted to former RMS (Austria) to support the post Annex I process of amidosulfuron. It was evaluated by Austria and is part of the DAR Addendum (Feb. 2011 – Addendum to monograph prepared in the context of post Annex I procedure (new Annex II data)).Upon request of the new RMS Finland, the study has nevertheless been included in the supplemental dossier.*

Executive Summary:
This test was conducted to determine the post-emergence biological activity of Amidosulfuron-desmethyl-chlorpyrimidine (BCS-CO78570) and Amidosulfuron-guanidine (BCS-CO41839), both metabolites of Amidosulfuron (AE F075032). The study was conducted under standardized glasshouse conditions using a WP05 formulation of the metabolite in comparison with a WP05 formulation of the parent amidosulfuron. Seeds of the weed species (EPPO code) *Galium aparine* (GALAP), *Matricaria chamomilla* (MATCH), *Sinapis arvensis* (SINAR), *Stellaria media* (STEME), *Helianthus annuus* (HELAN), *Brassica napus* (BRSNW); *Capsella bursa-pastoris* (CAPBP), *Papaver rhoeas* (PAPRH) were planted in pots and post-emergence applications of the metabolites amidosulfuron-desmethyl-chloropyrimidine and amidosulfuron-guanidine were made at rates of 31.66, 15.83, 7.91, 3.96 and 1.98 g a.s./ha for amidosulfuron-desmethyl-chloropyrimidine and 22.19, 11.09, 5.56, 2.77 and 1.39 g a.s./ha for amidosulfuron-guanidine. Furthermore, the parent amidosulfuron was applied at rates of 30, 15, 7.5, 3.75 and 1.88 g a.s./ha. Effects were assessed visually four weeks after application. It could be shown, that neither amidosulfuron -desmethyl-chloropyrimidine nor amidosulfuron-guanidine, both metabolites of amidosulfuron, had any post-emergence biological activity on the range of weeds tested under standard glasshouse screening conditions.

Materials and Methods:

Test materials:
Amidosulfuron-desmethyl-chloropyrimidine, formulated as WP05; Batch no.: BCS-CO78570; Certificate of Analysis: AZ 16057; TOX-No.: 08625-00;

Amidosulfuron-gaunidine, formulated as a WP05; Batch No.: BCS-CO41839; Certificate of Analysis: AZ 16021; TOX-No: 08626-00;

Amidosulfuron (AE F075032 00 1D99 0013); Batch No.: COIL70090; Certificate of Analysis: AZ 14145.

Test species: 8 weed species (EPPO code): *Galium aparine* (GALAP), *Matricaria chamomilla* (MATCH), *Sinapis arvensis* (SINAR), *Stellaria media* (STEME), *Helianthus annuus* (HELAN), *Brassica napus* (BRSNW); *Capsella bursa-pastoris* (CAPBP), *Papaver rhoeas* (PAPRH).

Jiffy pots (8 cm diameter) were filled to within 2 cm of the top with a silt-loam soil (20% sand, 57% silt, 23% clay, pH 6.8 and 1.4% organic matter). Seeds of the weed species (listed above) were sown into these pots and covered with 0.5 to 1 cm of the same soil mixed 1 to 1 with sharp sand. For each weed species 2 replicates were used in the test. The sowing density was selected based on prior

experience to provide approximately 60-70% soil cover by the plants at application timing. After sowing the pots were placed into a glasshouse set 20°C+/-2°C at day and 12°C+/-2°C at night and watered according to need. High pressure sodium lamps (400W) were used to augment daylight during cloudy conditions and to extend the day length to 14 hours.

The parent amidosulfuron and the two metabolite samples were dissolved in deionized water and diluted to obtain the required dose rates. Amidosulfuron was applied at 30, 15, 7.5, 3.75 and 1.88 g a.s./ha. The metabolites amidosulfuron-desmethyl-chloropyrimidine and amidosulfuron-guanidine were applied at rates of 31.66, 15.83, 7.91, 3.96 and 1.98 g a.s./ha for amidosulfuron-desmethyl-chloropyrimidine and 22.19, 11.09, 5.56, 2.77 and 1.39 g a.s./ha for amidosulfuron-guanidine. The post-emergence applications (28 July 2009) were made using a high precision track-sprayer with a spray volume of 300 L/ha equipped with a flat fan nozzle. Application was carried out at BBCH 12-13 of the weeds. Four weeks after application (25 August 2009) the treated plants were visually assessed for injury compared with the untreated control plants. The assessments were on a percentage basis (0 = no effects, 100 = complete kill).

Results:
The results of the visual assessments are presented as means from the 2 replicates in the following table.

Table CA 8.6.1- 2: Percent weed control after post-emergence application of amidosulfuron and two metabolites

	[g a.s./ha]	BRSNW	CAPBP	GALAP	HELAN	MATCH	PAPRH	SINAR	STEME	Average
Amidosulfuron	30	58	90	75	93	86	60	93	0	69
	15	45	80	58	89	78	50	86	0	61
	7.5	33	70	35	88	53	45	79	0	50
	3.75	10	60	30	33	23	30	65	0	31
	1.88	0	40	0	0	0	0	60	0	13
Amidosulfuron -desmethyl-chloropyrimidine	31.66	0	0	0	0	0	0	0	0	0
	15.83	0	0	0	0	0	0	0	0	0
	7.92	0	0	0	0	0	0	0	0	0
	3.96	0	0	0	0	0	0	0	5	0
	1.98	0	0	0	0	0	0	0	0	0
Amidosulfuron -guanidine	22.19	0	0	0	0	0	0	0	0	0
	11.10	0	0	0	0	0	0	0	0	0
	5.55	0	0	0	0	0	0	0	0	0
	2.77	0	0	0	0	0	0	0	0	0
	1.39	0	0	0	0	0	0	0	0	0

Amidosulfuron demonstrated good control of 4 of the 8 tested species with medium control of GALAP and PAPRH and only weak/ no control of BRSNW and STEME. A clear dose response was observed on all species except STEME where no control was seen. Neither metabolite showed any biological activity at all.

Conclusion:
In a direct comparison study, it could be shown, that neither amidosulfuron -desmethyl-chloropyrimidine nor amidosulfuron-guanidine, both metabolites of amidosulfuron, had any post-emergence biological activity on the range of weeds tested under standard glasshouse screening conditions.

Amidosulfuron-biuret:

Report:	KCA 8.6.1/07; [redacted]; 2010; M-369645-01-1
Title:	Evaluation of the post-emergence biological activity of amidosulfuron- biuret (BCS-CQ51287) metabolite of amidosulfuron (AE F075032)
Report No.:	PP10016
Document No.:	M-369645-01-1
Guideline(s):	**not specified**
Guideline deviation(s):	**not specified**
GLP/GEP:	**no**

Note: *This study has been previously submitted to former RMS (Austria) to support the post Annex I process of amidosulfuron. It was evaluated by Austria and is part of the DAR Addendum (Feb. 2011 – Addendum to monograph prepared in the context of post Annex I procedure (new Annex II data)).Upon request of the new RMS Finland, the study has nevertheless been included in the supplemental dossier.*

Executive Summary:
The test was conducted to determine the post-emergence biological activity of amidosulfuron-biuret, a metabolite of amidosulfuron. The study was conducted under standardized glasshouse conditions using a WP05 formulation of the metabolite in comparison with a WP05 formulation of the parent amidosulfuron. *Galium aparine* (GALAP), *Matricaria chamomilla* (MATCH), *Sinapis arvensis* (SINAR), *Stellaria media* (STEME), *Helianthus annuus* (HELAN), *Brassica napus* (BRSNW); *Capsella bursa-pastoris* (CAPBP), *Papaver rhoeds* (PAPRH) were planted in pots and post-emergence applications of the metabolite amidosulfuron-biuret were made at rates of 22.27, 11.14, 5.57, 2.78 and 1.39 g a.s./ha. Furthermore, the parent amidosulfuron was applied at rates of 30, 15, 7.5, 3.75 and 1.88 g a.s./ha. Effects were assessed visually four weeks after application. It could be shown, that amidosulfuron-biuret, a metabolite of amidosulfuron, had no post-emergence biological activity on the range of weeds tested under standard glasshouse screening conditions.

Materials and Methods:
Test material: Amidosulfuron-biuret (BCS-CQ51287), formulated as WP05; Purity 97.5%; Batch code: BCS-CQ51287-01-01; Origin batch: BCOQ 6067-2-7; Certificate of Analysis: AZ 16572; TOX-No.: 08956-00.

Amidosulfuron (AE F075032), formulated as WP05; Purity 99.3%; Batch code: AE F075032 00 1D99 0013; Origin batch: COIL70090; Certificate of Analysis: AZ 14145.

Test species: 8 plant species: *Galium aparine* (GALAP), *Matricaria chamomilla* (MATCH), *Sinapis arvensis* (SINAR), *Stellaria media* (STEME), *Helianthus annuus* (HELAN), *Brassica napus* (BRSNW); *Capsella bursa-pastoris* (CAPBP), *Papaver rhoeas* (PAPRH).

Jiffy pots (8 cm diameter) were filled (30 March 2010) to within 2 cm of the top with a silt-loam soil (20% sand, 57% silt, 23% clay, pH 6.8 and 1.4 % organic matter). Seeds of the weed species (listed above) were sown into these pots and covered with 0.5 to 1 cm of the same soil mixed 1 to 1 with sharp sand. For each weed species 2 replicates were used in the test. The sowing density was selected based on prior experience to provide approximately 60-70% soil cover by the plants at application timing. After sowing the pots were placed into a glasshouse set 20°C+/-2°C day and 12°C+/-2°C night and watered via flood irrigation according to need. High pressure sodium lamps (400W) were used to augment daylight during cloudy conditions and to extend the day length to 14 hours.

The parent amidosulfuron and the metabolite sample were dissolved in deionised water and diluted to obtain the required dose rates. Amidosulfuron was applied at 30, 15, 7.5, 3.75 and 1.88 g a.s./ha. The metabolite amidosulfuron-biuret was applied at rates of 22.27, 11.14, 5.57, 2.78 and 1.39 g a.s./ha. The

post-emergence applications (22 April 2010) were made using a high precision track-sprayer with a spray volume of 300 L/ha equipped with a flat fan nozzle. Application was carried out at BBCH 12-13 of the weeds. Four weeks after application (18 May 2010) the treated plants were visually assessed for injury compared with the untreated control plants. The assessments were on a percentage basis (0 = no effects, 100 = complete kill).

Results:
The results of the visual assessments are presented as means from the 4 replications in the following table.

Table CA 8.6.1- 3: Percent weed control after post-emergence application of amidosulfuron and the metabolite amidosulfuron-biuret

	[g a.s./ha]	BRSNW	HELAN	CAPBP	GALAP	MATCH	PAPRH	SINAR	STEME	Average
Amidosulfuron	30	85	95	95	90	90	60	100	75	86
	15	82	93	92	80	80	40	95	55	77
	7.5	77	90	88	80	70	25	95	35	70
	3.75	60	77	78	45	40	0	90	10	50
	1.88	40	68	75	25	20	0	30	0	32
Amidosulfuron-biuret	22.27	0	0	0	0	0	0	0	0	0
	11.14	0	0	0	0	0	0	0	0	0
	5.57	0	0	0	0	0	0	0	0	0
	2.78	0	0	0	0	0	0	0	0	0
	1.39	0	0	0	0	0	0	0	0	0

Amidosulfuron demonstrated good control of 6 of the 8 tested species with medium/weak control of PAPRH and STEME. A clear dose response was observed on all species. The metabolite amidosulfuron-biuret showed no biological activity at all.

Conclusion:
In a direct comparison study it could be shown that Amidosulfuron-biuret, a metabolite of Amidosulfuron, had no post-emergence biological activity on the range of weeds tested under standard glasshouse screening conditions.

Amidosulfuron-ADMP:

Report: KCA 8.6.1/08[redacted]; 1999; M-185253-01-1
Title: Evaluation of herbicidal potential of metabolites of AE F130060
Report No.: C002929
Document No.: M-185253-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: no

The study reports on primary screening tests for herbicidal activity of a number of components related to active substance mesosulfuron-methyl (AE F130060), a further sulfonylurea class herbicide. Amongst the tested compounds was compoment Amidosulfuron-ADMP (AE F092944), a metabolite shared between both active substances.

In primary pre- and post-emergence screening, this metabolite was found almost herbicidally inactive against a wide range of grass and broad-leaved plant species at the highly exaggerated test rate of 2500 g technical substance / ha.

Table CA 8.6.1- 4: Herbicidal activity of the metabolite Amidosulfuron-ADMP (AE F092944)

		% Activity					
Compound	g /ha	CHYSE	AVESA	LOLMU	ECHCG	SINAL	STEME
AE F092944	2500	30	0	20	0	40	40

Amidosulfuron-ADHP:

Report: KCA 8.6.1/03; [redacted]; 2004; M-244563-01-1
Title: Evaluation of the biological activity of AE F094208, a metabolite of amidosulfuron
Report No.: C046650
Document No.: M-244563-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **no**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a glasshouse test under standardized conditions to determine the biological activity of metabolite amidosulfuron-ADHP (formulated as WP05 as a vehicle for application) in comparison to a commercial formulation of amidosulfuron (WG75). It was concluded that the metabolite had no biological activity on a range of weed species.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. No EU agreed endpoint was derived from this test.

Lysimeter leachate:

Report: KCA 8.6.1/04; [redacted]; [redacted]; 1992; M-138080-01-2
Title: Phytotoxicity test using leachates from lysimeter studies with Hoe 075032-14C
Report No.: C032687
Document No.: M-138080-01-2
Guideline(s): **BBA: IV, 7**
Guideline deviation(s): **not specified**
GLP/GEP: **yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a bioassay testing the effect to sunflower (*Helianthus annuus*) of percolates containing the maximum amount of radioactive residues from a lysimeter treated with ^{14}C-amidosulfuron. No phytoxicity and no growth inhibition were observed.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. No EU agreed endpoint was derived from this test.

Other components:

Report:	KCA 8.6.1/05; [redacted]; 2005; M-247760-01-1
Title:	Evaluation of the biological activity of AE 1569309, a metabolite of amidosulfuron
Report No.:	C047020
Document No.:	M-247760-01-1
Guideline(s):	**not specified**
Guideline deviation(s):	**not specified**
GLP/GEP:	**no**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a glasshouse test under standardized conditions to determine the biological activity of component AE 1569309 (formulated as WP05 as a vehicle for application) in comparison to a commercial formulation of amidosulfuron (WG75). It was concluded that the metabolite had no biological activity on a range of weed species.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered acceptable. No EU agreed endpoint was derived from this test.

Note: Results of this study are considered of no relevance for approval renewal. New study information on the route of degradation in aerobic soil of amidosulfuron (cf. KCA 7.1.1.1/09) led to the conclusion of erroneous previous structure assignment for the chromatographic peak formerly assigned to AE 1569309, now corrected to be metabolite amidosulfuron-desmethyl-chloropyrimdine.

CA 8.6.2 Testing on non-target plants

No studies on the pure active substance were generated. Tests on non-target plants are product related information and as such found reported in document MCP for the representative formulation.

CA 8.7 Effects on other terrestrial organisms (flora and fauna)

For amidosulfuron, a screening study on entomology species was performed, details are provided in the following table.

Table CA 8.7- 1: Effect data of amidosulfuron WP 20 to entomology screening species presented in this chapter

Test design	Test species	Ecotoxicological endpoint	Reference
Amidosulfuron, formulated as WP 20			
different treated stages (eggs, larvae, adults, all stages)	*Spodoptera littoralis, Oncopeltus fasciatus, Aphis fabae, Calandra granaria, Epilachna varivestis, Tetranychus urticae, Panonychus ulmi, Blattella germanica, Musca domestica, Aedes aegypti*	The test item is not effective on any tested species.	[redacted]; 1999; M-134263-01-1 KCA 8.7 /01

Report: KCA 8.7/01; [redacted]; 1999; M-134263-01-1
Title: Effectivity of the herbicide amidosulfuron (AE F075032) on entomology screening species.
Report No.: A53599
Document No.: M-134263-01-1
Guideline(s): **not specified**
Guideline deviation(s): **not specified**
GLP/GEP: **no**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on a screening test for biological activity of amidosulfuron (formulated as WP20 as a vehicle for application) on 10 entomology species in different development stages (*Spodoptera littoralis*, *Oncopeltus fasciatus*, *Aphis fabae*, *Calandra granaria*, *Epilachna varivestis*, *Tetranychus urticae*, *Panonychus ulmi*, *Blattella germanica*, *Musca domestica*, *Aedes aegypti*). The test item was reported to not be effective on any of the test organisms.

The study was evaluated in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

The study was considered as non-essential, additional information only - no EU agreed endpoint was derived from this test.

CA 8.8 Effects on biological methods for sewage treatment

For amidosulfuron, one study with activated sludge has been conducted. Based on these test results, the DAR (2006) evaluation (B9.10) concluded it is unlikely that amidosulfuron influences methods of sewage treatment.

Table CA 8.8- 1: Effect data of amidosulfuron to activated sludge presented in this chapter

Test species	Test design	Ecotoxicological endpoint	Reference
Amidosulfuron			
Activated sludge	Respiration inhibition, 3 h, static (OECD 209)	Activated sludge, inhibition of respiratory activity : 3-h EC_{20} 700 mg/L 3-h EC_{50} > 1000 mg/L 3-h EC_{80} > 1000 mg/L	[redacted]; 1991; M-130851-01-2 KCA 8.8. /01

Report: KCA 8.8/01; [redacted]; 1991; M-130851-01-2
Title: Testing of Hoe 075032 for bacterial toxicity in a respiration inhibition test with activated sludge in accordance with the OECD Guideline 209 for testing of chemicals in a respiration inhibition test with activated sludge (04 April 1991)
Report No.: C002512
Document No.: M-130851-01-2
Guideline(s): **OECD: 209**
Guideline deviation(s): **for deviation see results, Point 4.1**
GLP/GEP: **yes**

[Study submitted and evaluated for the first inclusion of amidosulfuron on Annex I]

The study reports on an activated sludge bacterial toxicity test with parent active substance amidosulfuron.

The study was rated valid in the EU review for the first inclusion of amidosulfuron on Annex I, a study review is found in the previous DAR (2006).

An EU agreed endpoint of EC_{50} > 1000 mg a.s./L was derived from this test.

CA 8.9 Monitoring data

No monitoring data have been created by the notifier since no additional data was deemed necessary to complete risk assessments. No relevant and reliable monitoring studies were found in the required literature searches of the peer reviewed open literature.