

Draft Renewal Assessment Report  
under Regulation (EC) 1107/2009



**FORAMSULFURON**  
Active substance and formulation data

**Volume 3**  
**Annex B.9.**  
**Ecotoxicology**

Rapporteur Member State: Finland  
Co- Rapporteur Member State: Slovakia

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**Version History**

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## B.9. ECOTOXICOLOGY DATA

**CO-RMS SLOVAKIA HAS EVALUATED THE ECOTOXICOLOGICAL DATA, BUT HAS WORKED CLOSELY WITH THE RMS FINLAND.**

Foramsulfuron was included into Annex I of Council Directive 91/414/EEC with Commission Directive 2003/23/EC (25 March 2003) which requires Member States pay particular attention to the protection of aquatic plants and that risk mitigation measures should be applied, where appropriate. Foramsulfuron (FSN) is a herbicidally active sulfonyl urea. Foramsulfuron is used as a selective post-emergence herbicide in maize for the control of grass and dicotyledonous weed species after germination of the weeds. Foramsulfuron acts on the target weeds both via foliar uptake (major route) and from soil uptake (minor route). The representative formulation in the Annex I inclusion was Equip OD 45 (22.5 g/L foramsulfuron and 22.5 g/L of the safener isoxadifen-ethyl). This same formulation is also the representative formulation in this process of renewal.

Proposed maximum use of foramsulfuron within the European Union is summarised in Table B.9-1.

**Table B.9-1: Proposed maximum use of foramsulfuron within the European Union**

Crop	Timing of application (range)	Number of Applications	Application interval (days)	Maximum label rate (range) L/ha	Maximum application rate individual treatment (ranges) g/ha	
					foramsulfuron	isoxadifen-ethyl
Maize	BBCH 12 -18	1	-	2.6	60	60
Maize	BBCH 12 -18	2	7	1.3	30	30

This section contains only summaries of ecotoxicological studies on the active substance foramsulfuron (AE F130630), its metabolites and formulation Equip OD 45 which were not available at the time of the first Annex I inclusion of foramsulfuron and were therefore not evaluated during the first EU review of this compound. All studies, which were already submitted by Bayer CropScience for the first Annex I inclusion are contained in the Monograph and its Addendums evaluated by RMS Germany. For all new studies a detailed study summary is provided.

For a better overview, study endpoints resulting from the evaluation process of Annex I inclusion are presented in this document, together with the information whether or not this endpoint was listed in the List of Endpoints in the Review Report (SANCO/10324/2002-Final).

In the beginning of each section summary tables are provided including very short summaries of the studies evaluated during Annex I inclusion and of studies submitted for the purpose of renewal. To distinguish the studies, the old ones have been presented in grey and the new ones with black.

Due to changes in triggers for metabolites to be further assessed as well as due to new studies on the route of degradation in various environmental compartments, additional metabolites are proposed to be included in the residue definition for the risk assessment (see Table B.9-2). Accordingly, studies have been prepared to describe the ecotoxicological profile of these metabolites in the relevant environmental compartment.



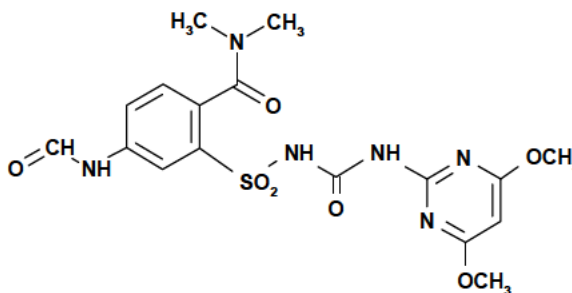
**Table B.9-2: Definition of the residue for risk assessment**

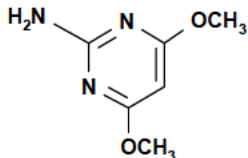
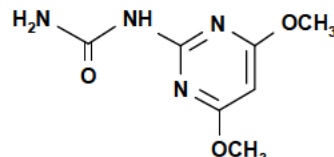
Compartment	Compound / Code	
Soil	Foramsulfuron AE F092944 AE F130619 AE F153745	
Groundwater	Foramsulfuron AE F092944 AE F130619 AE F153745	
Surface water	Foramsulfuron AE F092944 AE F130619 AE F153745 AE 0338795 AE F099095 4-Amino-N-methylbenzamide 4-Formamido-N-methylbenzamide Foramsulfuron sulfamic acid	
Plant material	Foramsulfuron	

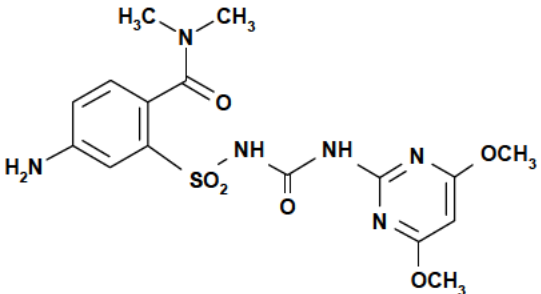
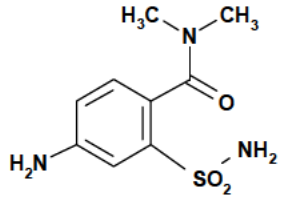
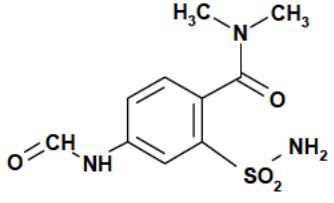
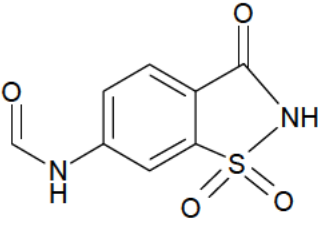
\*Justification for the residue definition for risk assessment is provided in Vol 1, Level 2 Section 2.8.5.

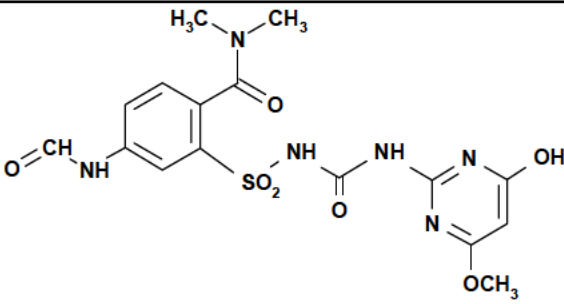
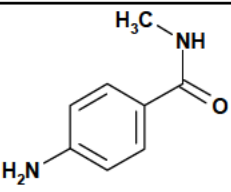
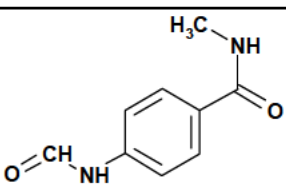
Molecular structures, names and codes of the active substance and metabolites occurring in environmental compartments are shown in the table below.

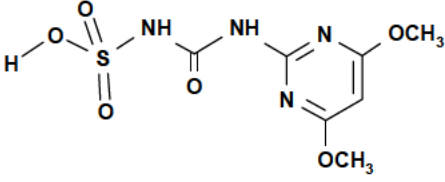
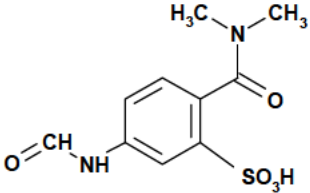
**Table B.9-3: Molecular structures, names and codes of the active substance and metabolites occurring in environmental compartments**

	Report name Structure IUPAC name CAS name [CAS registry number]	Molecular formula molar mass Other names / codes
a.s.	<p><b>Foramsulfuron (parent substance)</b></p>  <p>N,N-dimethyl-2-[3-(4,6-dimethoxypyrimidin-2-yl)ureidosulfonyl]-4-formylaminobenzamide (IUPAC) 2-[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-formylaminobenzamide (CAS) CAS no: 173159-57-4</p>	<p>C<sub>17</sub> H<sub>20</sub> N<sub>6</sub> O<sub>7</sub> S 452.49 g/mol</p> <p><b>Foramsulfuron</b> (common name) AE F130360 BCS-AH47626</p>

	<b>Report name</b> <b>Structure</b> <b>IUPAC name</b> <b>CAS name</b> <b>[CAS registry number]</b>	<b>Molecular formula</b> <b>molar mass</b> <b>Other names / codes</b>
M01	<b>AE F092944</b>  2-amino-4,6-dimethoxypyrimidine (IUPAC) 4,6-Dimethoxy-2-pyrimidinamine (CAS) CAS no: 36315-01-2	$C_6H_9N_3O_2$ 155.16 g/mol  AE F092944 BCS-AA25052 Foramsulfuron-pyrimidinamine ADMP K-1782 Metabolite E
M02	<b>AE F099095</b>  4,6-dimethoxypyrimidin-2-ylurea (IUPAC) (4,6-dimethoxy-2-pyrimidinyl)urea (CAS) CAS no: 151331-81-6	$C_7H_{10}N_4O_3$ 198.18 g/mol  AE F099095 BCS-AB40283 Foramsulfuron-urea 05537 DMPU Metabolite B

	<b>Report name</b> <b>Structure</b> <b>IUPAC name</b> <b>CAS name</b> <b>[CAS registry number]</b>	<b>Molecular formula</b> <b>molar mass</b> <b>Other names / codes</b>
M03	<b>AE F130619</b>  4-amino-2-[3-(4,6-dimethoxypyrimidin-2-yl)ureidosulfonyl]-N,N-dimethylbenzamide (IUPAC) 4-amino-2-[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-N,N-dimethylbenzamide (CAS) CAS no: 190520-75-3	$C_{16}H_{20}N_6O_6S$ 424.48 g/mol  AE F130619 BCS-AU59648 Foramsulfuron-amine
M04	<b>AE F148003</b>  4-amino-N,N-dimethyl-2-sulfamoylbenzamide (IUPAC) 4-amino-2-(aminosulfonyl)-N,N-dimethyl-benzamide (CAS) CAS no: 190521-44-9	$C_9H_{13}N_3O_3S$ 243.31 g/mol  AE F148003 BCS-AU73987
M05	<b>AE F153745</b>  4-formylamino-N,N-dimethyl-2-sulfamoylbenzamide (IUPAC) 2-(aminosulfonyl)-4-(formylamino)-N,N-dimethyl-benzamide (CAS) CAS no: 173159-94-9	$C_{10}H_{13}N_3O_4S$ 271.32 g/mol  AE F153745 BCS-AU80017
M06	<b>AE 0014940</b> 	$C_8H_6N_2O_4S$ 226.21 g/mol

	Report name Structure IUPAC name CAS name [CAS registry number]	Molecular formula molar mass Other names / codes
	N-(1,1-dioxido-3-oxo-2,3-dihydro-1,2-benzothiazol-6-yl)formamide (IUPAC) 6-formamido-1,2-benzisothiazol-3(2H)-one 1,1-dioxide (IUPAC) CAS no: NA	AE 0014940 BCS-AW41697
<b>M07</b>	<b>AE 0338795</b>	
	 <p>4-formylamino-2-[3-(4-hydroxy-6-methoxypyrimidin-2-yl)ureidosulfonyl]-N,N-dimethylbenzamide (IUPAC) CAS no: NA</p>	<p>C<sub>16</sub> H<sub>18</sub> N<sub>6</sub> O<sub>7</sub> S 438.42 g/mol</p> <p>AE 0338795 BCS-AW78711 4-(formylamino)-2-[[[(4-hydroxy-6-methoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-N,N-dimethylbenzamide</p>
<b>M08</b>	<b>4-Amino-N-methylbenzamide</b>	
	 <p>4-amino-N-methylbenzamide (IUPAC) benzamide, 4-amino-N-methyl (CAS) CAS no: 6274-22-2</p>	<p>C<sub>8</sub> H<sub>10</sub> N<sub>2</sub> O 150.18 g/mol</p> <p>AMB BCS-CV29520</p>
<b>M09</b>	<b>4-Formamido-N-methylbenzamide</b>	
	 <p>4-formamido-N-methylbenzamide (IUPAC) CAS no: NA</p>	<p>C<sub>9</sub> H<sub>10</sub> N<sub>2</sub> O<sub>2</sub> 178.19 g/mol</p> <p>FMB BCS-CW90756</p>

	Report name Structure IUPAC name CAS name [CAS registry number]	Molecular formula molar mass Other names / codes
<b>M10</b>	<b>Foramsulfuron sulfamic acid</b>	
	 <p>[4,6-dimethoxypyrimidin-2-yl]carbamoyl]sulfamic acid (IUPAC) Sulfamic acid, N-[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]- (CAS) CAS no: 591747-53-4</p>	<p>C<sub>7</sub> H<sub>10</sub> N<sub>4</sub> O<sub>6</sub> S 278.24 g/mol</p> <p>BCS-AW41401</p>
<b>M11</b>	<b>Sulfonic acid</b>	
	 <p>2-(dimethylcarbamoyl)-5-formamidobenzenesulfonic acid (IUPAC) CAS no: NA</p>	<p>C<sub>10</sub> H<sub>12</sub> N<sub>2</sub> O<sub>5</sub> S 272.28 g/mol</p> <p>BCS: n.a. Foramsulfuron-sulfonic acid</p>

## B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES

### B.9.1.1. Effects on birds

#### B.9.1.1.1. Acute oral toxicity to Birds

Two acute toxicity studies on non-related bird species, bobwhite quail and mallard duck, were performed with foramsulfuron and one study with the product Equip OD 45. These studies were evaluated within the process of Annex I inclusion and were considered acceptable by the RMS Germany. These studies were not re-evaluated since the studies were performed according to the US EPA 71-1 which is in line with the OECD test guideline No. 223: Avian Acute Oral Toxicity Test. The highest tested dose level in all studies was 2000 mg/kg bw. No mortality occurred. Brief details of the studies are provided in the Table B.9.1.1.1-1. Short summaries of the studies are provided thereafter.

Summaries of the studies are provided below.

**Table B.9.1.1.1-1: Avian acute oral toxicity data**

Test species	Substance	Test design	Ecotoxicological endpoint	Reference
Bobwhite quail	Foramsulfuron	acute, oral	LD <sub>50</sub> > 2000 <sup>1)</sup> LD <sub>50</sub> extrapol 3776 <sup>2)</sup> mg as/kg bw	1998 M-143541-01-1 KCA 8.1.1.1 /01
Mallard duck	Foramsulfuron	acute, oral	LD <sub>50</sub> > 2000 <sup>1)</sup> LD <sub>50</sub> extrapol 3776 <sup>2)</sup> mg as/kg bw	1997 M-142752-01-1 KCA 8.1.1.1 /02
Bobwhite quail	Equip OD 45	acute, oral	LD <sub>50</sub> >2000 mg/kg bw <sup>3)</sup> LD <sub>50</sub> extrapol	M; 2007; M-192635-01-1 KCA 10.1.1.1/01;

**Bold letters:** Values considered relevant for risk assessment

<sup>1)</sup> 10 birds per group

<sup>2)</sup> LD<sub>50</sub> extrapolated according to EFSA GD Birds & Mammals (2009) by applying a factor of 1.888 to the top dose in case 10 animals have been tested and no mortality occurred

<sup>3)</sup> mg product/kg bw

#### B.9.1.1.1.1. The oral toxicity of foramsulfuron to Bobwhite quail

<b>Report:</b>	KCA 8.1.1.1 /01 1998;M-143541-01
<b>Title:</b>	Code: Hoe 130360 00 ZC98 0001 - Bobwhite quail acute oral toxicity study
<b>Report No:</b>	A59886
<b>Document No(s):</b>	Report includes Trial Nos.: 96.0762 TOX96116 M-143541-01-1
<b>Guidelines:</b>	USEPA (=EPA): 71-1; Deviation not specified
<b>GLP/GEP:</b>	Yes

- ☐ **Test substance:** technical foramsulfuron
- ☐ **Purity:** 98.4 %
- ☐ **Test species:** Bobwhite quail (*Colinus virginianus*)
- ☐ **Age:** ca. 5 m
- ☐ **Birds per treatment:** 5 M + 5 F
- ☐ **Administration:** intubation
- ☐ **Solvent / vehicle:** methylcellulose
- ☐ **Dose levels:** 0/2000 mg/kg
  
- ☐ **Conclusion:** no effects up to the top dose  
LD<sub>50</sub>: >2000 mg/kg bw

Lowest lethal dose: >2000 mg/kg bw  
 NOED: 2000 mg/kg bw

- ☐ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.1.1.1.2. Acute oral toxicity of foramsulfuron to Mallard duck

<b>Report:</b>	<u>KCA 8.1.1.1 /02; [REDACTED] 1997;M-142752-01</u>
<b>Title:</b>	Hoe 130360 (AE F130360); Code: Hoe 130360 00 ZC98 0001 - Mallard duck acute oral toxicity study
<b>Report No:</b>	A59045
<b>Document No(s):</b>	Report includes Trial Nos.: 96.0763 TOX96280 <u>M-142752-01-1</u>
<b>Guidelines:</b>	USEPA (=EPA): E 71-1; Deviation not specified
<b>GLP/GEP:</b>	yes

- ☐ **Test substance:** technical foramsulfuron  
☐ **Purity:** 98.4 %  
☐ **Test species:** Mallard duck (*Anas platyrhynchos*)  
☐ **Age:** ca. 4 m  
☐ **Birds per treatment:** 5 M + 5 F  
☐ **Administration:** intubation  
☐ **Solvent / vehicle:** methylcellulose  
☐ **Dose levels:** 0/2000 mg/kg
- ☐ **Conclusion:** no effects up to the top dose  
 LD<sub>50</sub>: >2000 mg/kg bw  
 Lowest lethal dose >2000 mg/kg bw  
 NOED: 2000 mg/kg bw

- ☐ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.1.1.1.3. Acute oral toxicity of AE F130360+AE F122006, 22.5+22.5 g/L flowable oil to Bobwhite quail

<b>Report:</b>	<u>KCA 10.1.1.1/01; [REDACTED] 2007; M-192635-01-1</u>
<b>Title:</b>	Bobwhite quail acute oral toxicity (LD50); AE F130360+AE F122006, 22.5+22.5 g/L flowable oil; Code AE F130360 01 1K05 A3
<b>Report No:</b>	M-192635-01-1, T0X/00/262-51, BBA-Ref.-No.:AVS2000-79
<b>Document No(s):</b>	
<b>Guidelines:</b>	USEPA (=EPA): E 71-1; The test substance was referred to as AE F130360 01 1K05 A3 throughout the protocol and raw data. Four birds were individually housed during the study due to aggressive behaviour. Neither of these deviations was considered to have affected the integrity or outcome of the study.
<b>GLP/GEP:</b>	yes

- ☐ **Test substance:** formulation AE F130360 + AE F122006, 22.5+22.5 g/L (Equip)  
 foramsulfuron 22.5 g/L + safener 22.5 g/L  
☐ **Test species:** Bobwhite quail (*Colinus virginianus*)  
☐ **Age:** ca. 25 m  
☐ **Birds per treatment:** 5 M + 5 F  
☐ **Administration:** intubation

- ☐ **Solvent / vehicle:** none  
☐ **Dose levels:** 0/500/1000/2000 mg/kg  
  
☐ **Conclusion:** no effects up to the top dose  
 LD<sub>50</sub>: >2000 mg/kg bw  
 Lowest lethal dose: >2000 mg/kg bw  
 NOED: 2000 mg/kg bw  
  
☐ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

### B.9.1.1.2. Short-term dietary toxicity to birds

Two short-term dietary studies on non-related bird species, bobwhite quail and mallard duck, were performed. These studies were evaluated within the process of Annex I inclusion and were considered acceptable by the RMS Germany. These studies were not re-evaluated since the studies were performed according to the US EPA 71-2 which is in line with OECD test guideline No. 205: Avian Dietary Toxicity Test. The lowest LC<sub>50</sub> was determined to be > 5000 ppm corresponding to an LDD<sub>50</sub> of > 985 mg a.s./kg bw/d. Details of the studies are provided briefly in the Table B.9.1.1.2.-1. Short summaries of the studies are provided thereafter.

**Table B.9.1.1.2-1: Avian short-term dietary toxicity data of foramsulfuron**

Test species	Test design	Ecotoxicological endpoint	Reference
Bobwhite quail	5-day dietary	LC <sub>50</sub> > 5000 <sup>1)</sup> ppm ≡ LDD <sub>50</sub> > 985 mg as/kg bw/d	1998 M-147825-01-1 KCA 8.1.1.2 /01
Mallard duck	5-day dietary	LC <sub>50</sub> > 5000 <sup>1)</sup> ppm ≡ LDD <sub>50</sub> > 1792 mg as/kg bw/d	1998 M-147826-01-1 KCA 8.1.1.2 /02

<sup>1)</sup> 10 birds per group

#### B.9.1.1.2.1. Short-term dietary toxicity of foramsulfuron to *Bobwhite* quail

<b>Report:</b>	KCA 8.1.1.2 /01 1998;M-147825-01
<b>Title:</b>	Bobwhite quail dietary LC50 study Code: AE F130360 00 1C98 0001
<b>Report No:</b>	A67441
<b>Document No(s):</b>	Report includes Trial Nos.: 96.0781 Tox96117 M-147825-01-1
<b>Guidelines:</b>	OECD: 205; USEPA (=EPA): E 71-2; Deviation not specified
<b>GLP/GEP:</b>	yes

- ☐ **Test substance:** technical foramsulfuron  
☐ **Purity:** 98.0 %  
☐ **Test species:** Bobwhite quail (*Colinus virginianus*)  
☐ **Age:** 14 d  
☐ **Birds per treatment:** 10 unsexed  
☐ **Solvent / vehicle:** none  
☐ **Exposure period:** 5 d  
☐ **Conc. levels (nom.):** 0/0/312.5/625/1250/2500/5000 ppm  
☐ **Conc. levels (meas.):** 0/100/100/100/98/99 %  
☐ **Dose levels:** 0/57/105/198/435/985 mg/kg bw/d  
  
☐ **Conclusion:** no effects up to the top concentration  
 LC50: >5000 ppm



Lowest lethal conc.: >5000 ppm  
 NOEC: 5000 ppm

- ☐ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.1.1.2.2. Short-term dietary toxicity of foramsulfuron to Mallard duck

<b>Report:</b>	KCA 8.1.1.2 /02 [REDACTED] 1998;M-147826-01
<b>Title:</b>	Mallard duck dietary LC50 study Code: AE F130360 00 1C98 0001
<b>Report No:</b>	A67442
<b>Document No(s):</b>	Report includes Trial Nos.: 96.0780 Tox96118 M-147826-01-1
<b>Guidelines:</b>	OECD: 205; USEPA (=EPA): E 71-2; Deviation not specified
<b>GLP/GEP:</b>	yes

- ☐ **Test substance:** technical foramsulfuron
- ☐ **Purity:** 98.0 %
- ☐ **Test species:** Mallard duck (*Anas platyrhynchos*)
- ☐ **Age:** 10 d
- ☐ **Birds per treatment:** 10 unsexed
- ☐ **Solvent / vehicle:** none
- ☐ **Exposure period:** 5 d
- ☐ **Conc. levels (nom.):** 0/0/312.5/625/1250/2500/5000 ppm
- ☐ **Conc. levels (meas.):** 0/91/96/97/91/89 %
- ☐ **Dose levels:** 0/115/234/452/975/1792 mg/kg bw/d
  
- ☐ **Conclusion:** no effects up to the top concentration  
 LC50: >5000 ppm  
 Lowest lethal conc.: >5000 ppm  
 NOEC: 5000 ppm

- ☐ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.1.1.3. Sub-chronic toxicity and reproduction to birds

Two reproductive studies on non-related bird species, bobwhite quail and mallard duck, were performed. These studies were evaluated within the process of Annex I inclusion and were considered acceptable by the RMS Germany. These studies were not re-evaluated since the studies were performed according to the US EPA 71-4 which is in line with OECD test guideline No. 206: Avian Dietary Toxicity Test. The lowest NOEL was determined to be  $\geq 104$  mg a.s./kg bw/d. Details of the studies are provided briefly in the Table B.9.1.1.3.-1. Short summaries of the studies are provided thereafter.

**Table B.9.1.1.3-1: Avian reproductive toxicity data of foramsulfuron**

Test species	Test design	Ecotoxicological endpoint			Reference
Bobwhite quail	21-weeks feeding chronic, reproduction	NOEC ≡ NOEL	$\geq 1000$ $\geq 104^*$	ppm mg as/kg bw/d	[REDACTED] 1999 M-194248-01-1 KCA 8.1.1.3 /01
Mallard duck	21-weeks feeding chronic, reproduction	NOEC ≡ NOEL	$\geq 1000$ $\geq 132$	ppm mg as/kg bw/d	[REDACTED] 1999 M-194250-01-1 KCA 8.1.1.3 /02

**Bold letters:** Values considered relevant for risk assessment

\* Calculated test substance intake is presented in the study report ([M-194248-01-1](#))

#### B.9.1.1.3.1. Toxicity and reproduction study with foramsulfuron on Northern bobwhite quail

<b>Report:</b>	<a href="#">KCA 8.1.1.3 /01</a> ; [REDACTED] <a href="#">1999;M-194248-01</a>
<b>Title:</b>	Northern Bobwhite quail dietary reproduction study AE F130360 Code: AE F130360 00 1C97 0002
<b>Report No:</b>	C006593
<b>Document No(s):</b>	Report includes Trial Nos.: TOX96125 <a href="#">M-194248-01-1</a>
<b>Guidelines:</b>	<b>OECD: 206; USEPA (=EPA): FIFRA 71-4; Deviation not specified</b>
<b>GLP/GEP:</b>	yes

- ☐ **Test substance:** technical foramsulfuron
- ☐ **Purity:** 97.3 %
- ☐ **Test species:** Bobwhite quail (*Colinus virginianus*)
- ☐ **Age:** 35 w
- ☐ **Birds per treatment:** 16 pairs
- ☐ **Solvent / vehicle:** none
- ☐ **Exposure period:** 20 w
- ☐ **Conc. levels (nom.):** 0/40/200/1000 ppm
- ☐ **Conc. levels (meas.):** 0/94.4/97.8/100.5 %
- ☐ **Dose levels:** 0/4.0/21.4/104 mg/kg bw/d
  
- ☐ **Conclusion:** no effects up to the top concentration  
**NOEC: 1000 ppm**
  
- ☐ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.1.1.3.2. Toxicity and reproduction study with foramsulfuron on Mallard duck

<b>Report:</b>	<a href="#">KCA 8.1.1.3 /02</a> ; [REDACTED] <a href="#">1999;M-194250-01</a>
<b>Title:</b>	Mallard duck dietary reproduction study AE F130360 Code: AE F130360 00 1C97 0002
<b>Report No:</b>	C006594
<b>Document No(s):</b>	Report includes Trial Nos.: TOX96127 <a href="#">M-194250-01-1</a>
<b>Guidelines:</b>	<b>OECD: 206; USEPA (=EPA): FIFRA 71-4; Deviation not specified</b>
<b>GLP/GEP:</b>	yes

- ☐ **Test substance:** technical foramsulfuron
- ☐ **Purity:** 97.3 %
- ☐ **Test species:** Mallard duck (*Anas platyrhynchos*)
- ☐ **Age:** 23 w
- ☐ **Birds per treatment:** 16 pairs
- ☐ **Solvent / vehicle:** none
- ☐ **Exposure period:** 20 w
- ☐ **Conc. levels (nom.):** 0/40/200/1000 ppm
- ☐ **Conc. levels (meas.):** 0/94.4/97.8/100.5 %
- ☐ **Dose levels:** 0/4.9/26.2/132 mg/kg bw/d
  
- ☐ **Conclusion:**

Offspring body weight at day 14 was slightly but statistically significant lower than the study control; however, as the weight was within the range of the controls of four contemporary studies the deviation was not considered as a treatment related effect.

NOEC: 1000 ppm

**□ Comment (Co-RMS and RMS):**

The difference observed was very slight,  $264 \pm 21$  g for the control group vs.  $244 \pm 19$  g for the 1000 ppm a.i. treatment group. The difference is 7.5% which could be considered as no biologically relevant (lower than 20%). According data from the laboratory the mean value for the 1000 ppm a.i. treatment group was comparable to the control values from four other contemporary mallard reproduction studies ( $246 \pm 21$ g,  $257 \pm 33$ g,  $246 \pm 27$ g and  $249 \pm 20$ g), the difference observed was considered not to be related to treatment. Taken into account aforementioned informations the NOEC value of 1000 ppm can be taken as the outcome from this study.

#### B.9.1.1.4. Metabolites

No major metabolites identified in plants.

#### B.9.1.2. Effects on terrestrial vertebrates other than birds

##### B.9.1.2.1. Acute oral toxicity to mammals

##### B.9.1.2.1.1. Acute oral toxicity of foramsulfuron to Rat

The acute oral toxicity of foramsulfuron and the product Equip OD 45 was determined on male and female rats. These studies were evaluated within the process of Annex I inclusion and were considered acceptable by the RMS Germany. These studies were not re-evaluated since the studies were performed either according to the US EPA 71-4 which is in line with OECD test guideline No 401: Acute Oral Toxicity or according to it. The LD50 values were greater than 5000 mg/kg bodyweight for the active substance and for the product. Details of the studies are provided briefly in the Table B. 9.1.2.1.1-1. Summaries of the studies are provided thereafter.

**Table 9.1.2.1.1-1: Mammalian acute oral toxicity data of foramsulfuron**

Test species	Substance	Test design	Ecotoxicological endpoint	Reference
Rat	Foramsulfuron	acute, oral	LD <sub>50</sub> > 5000 <sup>1)</sup> mg as/kg bw	1997 M-141959-01-1 KCA 5.2.1 /01
Rat	Equip OD 45	acute, oral	LD <sub>50</sub> > 5000 <sup>1, 2)</sup> mg/kg bw	1999; M-192928-01-1 KCP 7.1.1 /01;

**Bold letters:** Values considered relevant for risk assessment

<sup>1)</sup> 10 rats per group, no mortality occurred <sup>2)</sup> mg product/kg body bw

Report:	KCA 5.2.1 /01; 1997; M-141959-01-1
Title:	Rat acute oral toxicity.
Report No:	M-141959-01-1, T0X96110, A58267
Document No(s):	
Guidelines:	OECD TG 401, adopted 24 February 1987 (Limit Test)
GLP/GEP:	yes

- Test substance:** Foramsulfuron, batch no. H 2037, purity: 98.4% (w/w); formulated at a concentration of 50% w/v in 1% w/v aqueous methylcellulose and administered at a dose volume of 10 ml/kg bw
- Purity:** 98.4 %

- ❑ **Test species:** Hsd/Ola: Sprague-Dawley (CD) rats, bw (Day 1): 228–278 g,  
Source [REDACTED]
- ❑ **Administration:** Intubation
- ❑ **Solvent / vehicle:** methylcellulose
- ❑ **Dose levels:** 5000 mg/kg

❑ **Material and Methods:**

A group of five male and five female Sprague-Dawley rats was given foramsulfuron as a single oral dose by gavage of 5000 mg/kg bw following overnight fasting.

Animals were observed soon after dosing and at frequent intervals for the remainder of Day 1 (day of dosing). Thereafter they were observed twice daily for 15 days. Individual body weights were recorded just prior to dosing and once weekly thereafter. All animals were sacrificed and examined externally and internally (abdominal and thoracic cavities) for macroscopic abnormalities on Day 15, the end of the observation period.

❑ **Findings:**

There were no deaths during the study. Treatment-related clinical signs were seen in all the animals included piloerection (seen within 5 minutes of dosing), hunched posture and white, soft to liquid faeces. Recovery was complete in all cases by Day 4. A slightly low body weight gain was recorded on Day 15 for all males and 3/5 females. No abnormalities were detected in any animal at the necropsy on Day 15.

- ❑ **Conclusion:** The acute lethal oral dose (LD50) of foramsulfuron to rats was > 5000 mg/kg bw
- ❑ **Comment (Co-RMS and RMS):** No comments. Study considered acceptable.

#### B.9.1.2.1.2. Acute oral toxicity of AE F130360+AE F122006, 22.5+22.5 g/L oil flowable to Rat

<b>Report:</b>	KCP 7.1.1 /01; [REDACTED];1999; M-192928-01-1
<b>Title:</b>	Rat acute oral toxicity; AE F130360 + AE F122006, 22.5 +22.5 g/l Oil flowable, Code: AE F130360 01 1K05 A304
<b>Report No:</b>	M-141959-01-1, T0X96110, A58267
<b>Document No(s):</b>	
<b>Guidelines:</b>	OECD TG 401, adopted 24 February 1987 (Limit Test)
<b>GLP/GEP:</b>	yes

- ❑ **Test substance:** AE F130360 01 1K05 A304, batch number KD945/990301
- ❑ **Purity:** AE F130360: 2.42% w/w; AE F122006: 2.44% w/w
- ❑ **Test species:** Hsd: Sprague-Dawley (CD) rats
- ❑ **Administration:** Intubation
- ❑ **Solvent / vehicle:** None
- ❑ **Dose levels:** 5000 mg/kg
- ❑ **Material and Methods:**

Sprague Dawley CD rats, approximately 8 to 11 weeks old and weighing from 237 to 259 g (males) and from 205 to 215 g (females) at the time of treatment, were used. They were acclimatised for 11 days prior to dosing and caged by sex in groups of 5.

Groups of 5 male and 5 female fasted rats were given a single oral dose, by gavage, of 5000 mg/kg body weight of Equip, as supplied i.e. undiluted, at a dose volume of 5.26 ml/kg. The dose level was chosen after review of preliminary study findings. Animals were observed at least twice daily for mortality. Observations for clinical signs of toxicity were carried out soon after dosing, at frequent intervals on the remainder of the day of dosing (Day 1), twice daily for 14 days and once at Day 15, the day of termination. Individual body weights were recorded prior to dosing and at Days 8 and 15 (or death). All animals were killed at Day 15 and examined for macroscopic abnormalities. This consisted

of opening the thorax and abdominal cavities and the cranial cavity in 8 of the animals. The macroscopic appearance of all organs and tissues was recorded.

**□ Findings:**

At 5000 mg/kg, 1/5 males and 1/5 females died within a few minutes of dosing.

The principal clinical signs of toxicity observed in both sexes included piloerection, hunched posture, waddling/unsteady gait, lethargy, walking on toes and pallid extremities. Less commonly observed signs were partially closed eyelids and thin and/or ungroomed appearance. The onset of signs (piloerection and hunched posture) was within 10 minutes of dosing, and recovery of surviving rats was complete by Day 9.

Body weight gain was unaffected in the majority of animals, although one female showed a low gain at Day 15 compared with its cagemates.

Macroscopic examination of decedents revealed some minimal darkened tissue and prominent blood vessels in the lungs. Examination of 6 out of 8 animals killed at termination revealed thickening of the stomach wall accompanied, in 2 females, by gaseous distension of the duodenum. There were no findings in the remaining two animals.

**Table B.9-1.1.1.2-1: Mortality and clinical signs in rats given a single oral dose of Equip**

Parameter	Dose Level (mg/kg)	
	5000	5000
	Males	Females
<b>Mortality:</b>	1/5	1/5
<b>Clinical signs:</b>		
Piloerection	4/5	4/5
Hunched posture	4/5	4/5
Waddling/unsteady gait	4/5	4/5
Lethargy	4/5	4/5
Walking on toes	1/5	4/5
Pallid extremities	2/5	4/5
Partially closed eyelids	3/5	0/5
Thin appearance	1/5	2/5
Ungroomed appearance	1/5	2/5

- **Conclusion:** The acute oral LD<sub>50</sub> for Equip in both male and female Sprague Dawley rats was >5000 mg/kg body weight, the highest international limit dose.
- **Comment (Co-RMS and RMS):** No comments. Study considered acceptable.

#### **B.9.1.2.2. Long-term and reproduction toxicity to mammals**

A two-generation reproductive toxicity study on male and female rats was performed. In addition, teratogenicity effects were studied on rat and rabbit. All these studies were evaluated within the process of Annex I inclusion and were considered acceptable by the RMS Germany. These studies were not re-evaluated since the studies were performed according to the OECD test guidelines No. 416 Two-Generation Reproduction Toxicity Test and OECD 414: Prenatal Development Toxicity Study.

In two-generation study the NOEL was determined to be 15 000 ppm. In developmental studies the NOEL for teratogenic effects was 1000 mg a.s./kg bw for rat and 500 mg a.s./kg bw, the highest tested concentrations. Detailed summaries of the studies are provided.

**Table B.9.1.2.2-1: Summary of reproductive toxicity studies**

Study and dose levels	Target	NOEL/ NOAEL	LOAEL	Effects	Reference
Rat 2-generation study 0–100–1225–15000 ppm	Parental + reproductive tox.	15000 ppm m: 1038 mg/kg bw/d f: 1430 mg/kg bw/d	–	No effects observed	██████ 1999 M-187748-01-1 KCA 5.6.1 /01
Rat teratogenicity 0–5–71–1000 mg/kg bw/d	Maternal + developmental toxicity	1000 mg/kg bw/d	–	No effects observed	██████ 1997 M-147435-01 KCA5.6.2 /02
Rabbit teratogenicity 0–5–50–500 mg/kg bw/d	Maternal toxicity	50 mg/kg bw/d	500 mg/kg bw/d	↓ body weight gain, ↓ food intake, reddish urine	██████ 1997 M-143157-01 KCA 5.6.2 /01
	Developmental toxicity	500 mg/kg bw/d	–	No effects observed	██████ 1997 M-147441-01 KCA 5.6.2 /03

**Bold letters:** Values considered relevant for risk assessment

<sup>1)</sup> Geometric mean of male and female

#### B.9.1.2.2.1. Toxicity and reproduction study with foramsulfuron on Rat

<b>Report:</b>	<u>KCA 5.6.1 /01: [REDACTED] 1999; M-187748-01-1</u>
<b>Title:</b>	Rat dietary two-generation reproductive toxicity study
<b>Report No:</b>	<b>M-187748-01-1, 96123, T0X/99262-35</b>
<b>Document No(s):</b>	
<b>Guidelines:</b>	<b>OECD guideline 416, adopted 26 May 1983</b>
<b>GLP/GEP:</b>	yes

- |  |  |
|--|--|
| <input type="checkbox"/> <b>Test substance:</b>      | Foramsulfuron, a blend of batch numbers H2075/1-H2075/4 + 1/97, containing 96.1% w/w of active substance |
| <input type="checkbox"/> <b>Purity:</b>              | 96.1 %   |
| <input type="checkbox"/> <b>Test species:</b>        | Sprague Dawley CrI:CD (SD)BR rats  |
| <input type="checkbox"/> <b>Source:</b>              | [REDACTED]   |
| <input type="checkbox"/> <b>Conc. levels (nom.):</b> | 0/100/1225/15000 ppm   |
| <input type="checkbox"/> <b>Dose levels:</b>         | 0/7/82/1038 mg/kg bw/d for males<br>10/115/1430 mg/kg bw/d for females                                   |

## □ Material and Methods:

Groups of 30 male and 30 female FO and F1 Sprague Dawley Crl:CD rats, received dietary concentrations of either 0, 100, 1225 or 15000 ppm of technical foramsulfuron continuously throughout the study. Animals were housed individually except during pairing and lactation.

F0 and F1 animals were treated for at least 70 days prior to pairing (one male and one female from same dose group); treatment was continued throughout pairing, gestation and lactation up to termination (following weaning of pups on post-natal Day 22). Treatment commenced when the F0 animals were 6 weeks of age and commenced at weaning, post-natal Day 22, for the F1 generation when one male and one female from each of the F0 generation litters were selected to form this generation. Mated females were allowed to litter. Pups were individually identified and the litters in excess of 8 pups were adjusted to this maximum as appropriate on post-natal Day 4. At weaning, an additional one male and one female pup per litter were selected for detailed necropsy and organ weights. Surplus F1 and F2 pups were sacrificed and necropsied on post-natal Day 21.



Parent animals were subjected to detailed necropsy after their pups had been weaned and selected organs were weighed. Spermatogenetic endpoints (sperm motility, morphology and numbers) were recorded for all F0 and F1 males. Histopathology was conducted on the reproductive and target organs of 10 males and 10 females from the control and highest dose levels of each generation.

All F0 and F1 animals were observed twice daily for clinical signs and behaviour. Body weights and food consumption were recorded weekly prior to pairing. Females were weighed on Days 0, 4, 7, 10 and 20 of gestation and on Days 1, 4, 7, 14 and 21 of lactation. Male body weights were recorded weekly. Food consumption of females and their litters were recorded weekly. Vaginal smears were taken for 21 days prior to pairing, during pairing until mating occurred and at necropsy. Litters were examined twice daily for pup mortality, clinical signs and behaviour. Pups were weighed individually and sexed on post-natal Days 0/1, 4, 7, 14 and 21. Balanopreputial separation and vaginal opening were monitored in the F1 generation animals.

#### □ Findings:

Mean achieved intakes of the test substance for the combined generations were 7, 82 and 1038 mg/kg bw/d for males and 10, 115 and 1430 mg/kg bw/d for females at 100, 1225 and 15000 ppm.

There were no treatment-related findings, including no effects on reproductive parameters (fertility, mating, days between pairing and mating, gestation, parturition, litter size sex ratios, pup mortality), parental toxicity (body weight and body weight gain, food consumption, clinical condition, macroscopic pathology), neonatal toxicity (body weights and clinical condition), or on markers of endocrine function (oestrous cycling, balanopreputial separation, vaginal opening, spermatogenetic function and capacity).

#### □ Conclusion:

Dietary administration of up to 15000 ppm of foramsulfuron to rats for two successive generations did not cause any parental, neonatal or reproductive toxicity.

The No Observed Adverse Effect Level (NOAEL), and No Observed Effect Level (NOEL) of reproductive toxicity and parental toxicity was 15000 ppm, equivalent to an achieved mean daily intake of 1038 mg/kg bw/d for FO and F1 males combined and 1430 mg/kg bw/d for FO and F1 females combined (1234 mg/kg bw/d for the study overall).

□ **Comment (Co-RMS and RMS):** No comments. Study considered acceptable.

#### B.9.1.2.2.2. Toxicity and developmental toxicity study with foramsulfuron on Rat

<b>Report:</b>	KCA5.6.2 /02; [REDACTED] 1997;M-147435-01
<b>Title:</b>	Rat oral development toxicity (teratogenicity) study Code: Hoe 130360 00 ZC98 0001
<b>Report No:</b>	M-147435-01-1, 96.0760, A67035, TOX95390
<b>Document No(s):</b>	
<b>Guidelines:</b>	EU (=EEC): 88/302; JMAF: 4200; OECD: 414; USEPA (=EPA): 83-3; Deviation not specified
<b>GLP/GEP:</b>	yes

- **Test substance:** Foramsulfuron, Hoe 130360 (AE F130360), technical, batch No. H 2037
- **Purity:** 98.4 % w/w
- **Test species:** Rat / Wistar / female, strain: Hoe: WISKf(SPF71) Wistar
- **Source:** [REDACTED]
- **Dose levels:** 0/5/71/1000 mg/kg bw/d

#### □ Material and Methods:

Groups of 23 mated female Wistar rats received technical HOE 130360 (AE F130360) by oral gavage once daily at the dose levels of 0, 5, 71 or 1000 mg/kg body weight from day 7 -16 of pregnancy (day 0: day of mating, day 1: day of sperm detection) and were sacrificed on day 21 of pregnancy.

Animals were observed daily for mortality and clinical signs of toxicity. Body weight and food consumption were determined regularly throughout the study.

At necropsy the dams were examined for macroscopically visible changes. Gravid uterus weight was recorded. The uterus was opened and the number of live and dead fetuses and the number of conceptuses undergoing resorption were determined. Body weights, crown-rump lengths, sex ratios of the fetuses and placental weights were determined and external, visceral and skeletal examinations of the fetuses performed.

**□ Findings:**

There was no mortality.

No clinical signs were observed in the animals treated with the test compound.

Body weights and food consumption were not affected by the administration of the test compound.

No compound-related effects were observed at necropsy of the animals.

Gravid uterus weights, crown-rump lengths, litter size, sex ratios, foetal and placental weights remained unaffected by the administration of the test compound. There was no increase in the number of early or late conceptuses undergoing resorption.

Morphological examination of the fetuses did not reveal any compound-related effect.

**□ Conclusion:**

Oral administration of HOE 130360 (AE F130360) to the pregnant rat up to and including 1000 mg/kg body weight, the international regulatory limit dose, did not cause maternal toxicity or embryofetal toxicity.

Hoe 130360 (AE F130360) was not teratogenic in the rat.

With regard to the present study the 'No Observed Effect Level' (NOEL), and hence the NOAEL, was 1000 mg/kg/day, for both maternal and developmental toxicity.

**□ Comment (Co-RMS and RMS):** No comments. Study considered acceptable.

#### B.9.1.2.2.3. Toxicity and developmental toxicity study with foramsulfuron on Rabbit

<b>Report:</b>	<u>KCA 5.6.2 /01;</u> [REDACTED] <u>1997; M-143157-01</u>
<b>Title:</b>	Hoe 130360 (AE F130360); Code: Hoe 130360 00 ZC98 0001 - Rabbit oral developmental toxicity (teratogenicity) range finding study
<b>Report No:</b>	M-143157-01-1, 96.0564, A 59486, TOX95391
<b>Document No(s):</b>	
<b>Guidelines:</b>	EU (=EEC): 88/302; JMAF: 4200; OECD: 414; USEPA (=EPA): F 83-3; Deviation not specified
<b>GLP/GEP:</b>	yes

- Test substance:** Foramsulfuron, Hoe 130360 (AE F130360), batch No. H 2037
- Purity:** 98.4 % (w/w)
- Test species:** Rabbit/Himalayan/female,(Chbb:HM(SPF) Kleinrusse (Himalayan))
- Source:** [REDACTED]
- Dose levels:** 500/1000 mg/kg bw/d

**□ Material and Methods:**

Groups of four mated female Himalayan rabbits received Hoe 130360 (AE F130360) as a preparation in 1% (w/v) methyl cellulose in deionised water by oral gavage once daily at the dose levels of 500 or 1000 mg/kg body weight, respectively, from day 6 -18 of pregnancy (day 0: day of sperm detection). The dose volume was 5 ml/kg body weight in each case, corresponding to concentrations of 100 and 200 mg/ml.

All animals were observed daily for clinical signs whilst body weight and food consumption were recorded at regular intervals. The animals were sacrificed on day 29 of pregnancy. The uterus was opened and the number of live and dead fetuses and the number of conceptuses undergoing resorption were determined.



#### □ Findings:

There were no mortalities. Daily administration of both dosages was tolerated by the dams without clinical signs of intoxication.

There was an initial loss of body weight from day 6 -13 in the 1000 mg/kg body weight group. Body weight gain was also impaired at 500 mg/kg body weight, but to a lesser degree.

Food consumption was decreased during the treatment period in both dose groups, showing a dose-dependency.

No abnormalities were observed at necropsy of the dams. Foetal body weights were slightly lower at 1000 mg/kg body weight. One dam had six slightly small fetuses. Two fetuses of another dam showed bent forepaws. Because of the low group size the toxicological significance of this finding is unclear.

No effects on embryofoetal development were seen at 500 mg/kg.

#### □ Conclusion:

Based on the results of this study, 500 mg Hoe 130360 (AE F130360) / kg body weight per day would be a suitable high dose for the main study.

□ **Comment (Co-RMS and RMS):** No comments. Study considered acceptable as preliminary study.

#### B.9.1.2.2.4. Toxicity and developmental toxicity study with foramsulfuron on Rabbit

<b>Report:</b>	<u>KCA 5.6.2 /03: [REDACTED];1997; M-147441-01</u>
<b>Title:</b>	Code: Hoe 130360 00 ZC98 0001 - Rabbit oral developmental toxicity (teratogenicity) study
<b>Report No:</b>	M-147441-01, 96.0761, A 67041, TOX95392
<b>Document No(s):</b>	
<b>Guidelines:</b>	EU (=EEC): 88/302; JMAF: 4200; OECD: 414; USEPA (=EPA): F 83-3; Deviation not specified
<b>GLP/GEP:</b>	yes

- **Test substance:** Foramsulfuron, Hoe 130360 (AE F130360), technical batch No. H 2037
- **Purity:** 98.4 % (w/w)
- **Test species:** Rabbit/Himalayan/female.(Chbb:HM(SPF) Kleinrusse (Himalayan))
- **Source:** [REDACTED]
- **Dose levels:** 0/5/50/500 mg/kg bw/d

#### □ Material and Methods:

Groups of 15 mated female Himalayan rabbits received Hoe 130360 (AE F130360) by oral gavage once daily at the dose levels of 0, 5, 50 or 500 mg/kg body weight from day 6-18 of pregnancy (day 0: day of sperm detection) and were sacrificed on day 29 of pregnancy.

Animals were observed daily for mortality and clinical signs of toxicity. Body weight and food consumption were determined regularly throughout the study.

At necropsy the dams were examined for macroscopically visible changes. Gravid uterus weight was recorded. The uterus was opened and the number of live and dead fetuses and the number of conceptuses undergoing resorption were determined. Body weights, crown-rump lengths, sex ratios of the fetuses and placental weights were determined and external, visceral and skeletal examinations of the fetuses performed.

#### □ Findings:

There was no mortality.

Reddish coloured urine was observed for 1 to 3 days between days 10 and 12 in six animals and from day 15 - 17 in one animal of the high dose group. No compound-related clinical signs were observed in

the animals from the other groups. One animal from the control and high dose group each had only implantation sites (total litter loss) at caesarean section.

Body weight gain and food consumption were decreased in the animals from the high dose group during the treatment period. Body weight change and food consumption remained unaffected by the administration of the test compound in the females from the low and the intermediate dose group.

No compound-related effects were observed at necropsy of the animals.

Gravid uterus weights, foetal body weights, crown-rump lengths, sex ratios as well as placental weights remained unaffected by the administration of the test compound. Likewise, there were no differences between the numbers of early and late conceptuses undergoing resorption and dead foetuses in the animals from the control and treatment groups. Survival rate of the foetuses over 24 hours was not impaired.

No compound-related effects were detected by morphological examination of the foetuses.

□ **Conclusion:**

Oral administration of Hoe 130360 (AE F130360) to the pregnant rabbit at 500 mg/kg body weight caused maternal toxicity, as shown by slightly reduced body weight gain and food consumption. Embryofoetal toxicity was not observed.

Administration of Hoe 130360 (AE F130360) at doses of 5 and 50 mg/kg body weight was tolerated by the dams and their conceptuses without signs of toxicity.

Hoe 130360 (AE F130360) was not teratogenic in rabbits.

With regard to the present study the **No Observed Effect Level (NOEL)** for maternal toxicity was **50 mg/kg/day** and for developmental toxicity was **500 mg/kg/day**.

□ **Comment (Co-RMS and RMS):** No comments. Study considered acceptable.

#### **B.9.1.2.2.5. Addendum to toxicity and developmental toxicity study with foramsulfuron on Rabbit**

<b>Report:</b>	<u>KCA 5.6.2 /04; [REDACTED]; 2000; M-199311-01</u>
<b>Title:</b>	1st Addendum to Report number TOX/98/262-25 Rabbit oral developmental toxicity (teratogenicity) study: Provision of historical control body weight data as requested by the EU Code: Hoe 130360 00 ZC98 0001
<b>Report No:</b>	<b>M-199311-01-1, C 010603</b>
<b>Document No(s):</b>	
<b>Guidelines:</b>	<b>Deviation not specified</b>
<b>GLP/GEP:</b>	<b>no</b>

#### **B.9.1.3. Effects of active substance bioconcentration in prey of birds and mammals**

According to EFSA Guidance Document on Risk Assessment for Birds and Mammals, 2009 substances with a log  $K_{ow}$  greater than 3 have potential for bioaccumulation. Foramsulfuron log  $K_{ow}$  is < 3 (log  $K_{ow}$  value 1.44 at pH 2; 0.78 at pH 7) indicating that a secondary poisoning risk assessment is not necessary. The log  $K_{ow}$  values for foramsulfuron metabolites are as following: AE F130619 (log  $K_{ow}$  = 1.57 (pH 6)); AE 0338795 (log  $K_{ow}$  = - 2.33 (pH 7)); AE F092944 (log  $K_{ow}$  = 0.92); AE F153745 (log  $K_{ow}$  = - 0.62) indicating that a secondary poisoning risk assessment is not necessary. Also no biomagnification is expected to occur.

#### **B.9.1.4. Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)**

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

#### **B.9.1.5. Potential for endocrine disruption**

Following EU regulation 1107/2009, an assessment has to be provided concerning potential endocrine

disrupting properties of the active substance concerned.

WHO/IPCS (2002)<sup>1</sup> provided the currently widely accepted definition “*An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations.*” An adverse effect has been defined also by WHO/IPCS (2009)<sup>2</sup>: “*Change in the morphology, physiology, growth, development, reproduction, or, life span of an organism, system, or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences.*” Both definitions were used as the basis for evaluating the potential impact of foramsulfuron to wildlife presented below.

#### Wild Mammals:

Potential endocrine activity and potential population relevant effects of foramsulfuron on mammals were studied in 90-d, chronic, and multi-generation studies in rats, 90-d and chronic studies in mice, 90-d and 1-year studies in dogs, and in teratology studies in rats and rabbits. In none of these studies any observations of effects were noticed that could be related to primary endocrine activity.

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

#### Birds:

The population relevant effects of foramsulfuron on birds were studied in reproductive toxicity studies on bobwhite quail and mallard ducks. For both species 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 either species, it is concluded that there are no population relevant adverse effects of foramsulfuron. No additional studies seem necessary.

#### 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 exist, 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.

**Conclusion:** Neither in mammals, nor birds were any indications for adverse endocrine activity observed. Therefore further special testing for endocrine disrupting behaviour is not warranted.

□ **Comment (Co-RMS and RMS): Agreed.**

## **B.9.2. EFFECT ON AQUATIC ORGANISMS**

Aquatic organisms have been tested with the active ingredient and the metabolites included in the residue definition for aquatic risk assessment (see Vol 1, Level 2 Section 2.8.5). Due to the fact that *Lemna* is by far the most sensitive standard aquatic organism to the parent compound, metabolite testing was confined to this species in most cases, with two exceptions: AE F092944 and AE F099095. These are common metabolites with one or more sulfonyl urea herbicides. Tests with further aquatic species have been performed in context of risk assessments for other parent compounds. Although for the risk assessment of foramsulfuron these studies on further species are not considered essential, they are provided here for sake of completeness.

### **B.9.2.1. Acute toxicity to fish**

For foramsulfuron three acute toxicity studies on three different fish species were performed. These studies were evaluated within the process of Annex I inclusion and were considered acceptable by the RMS Germany. These studies were not re-evaluated since the studies were performed according to the OECD test guideline No. 203 Fish, Acute Toxicity Test. The tested dose level in all studies was 100 mg a.s./L. No sublethal effects and only

<sup>1</sup> WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2002. Global Assessment of the State-of-the- science of Endocrine Disruptors. WHO/PCS/EDC/02.2, 180 pp. o

WHO/IPCS (World Health Organization/International Programme on Chemical Safety), 2009. Principles and Methods for the Risk Assessment of Chemicals in Food. Environmental Health Criteria 240. 689 pp.

random mortality (in one study only) were observed in the treatment, resulting in an LC<sub>50</sub> of >100 mg a.s./L. For the formulation Equip OD 45 two acute toxicity studies on two different fish were performed. The 96-LC<sub>50</sub> was 14 mg /L for rainbow trout and 7.8 mg/L for bluegill sunfish.

For the purpose of renewal one acute study on rainbow trout was conducted with test doses ranging from 18 to 1000 mg/L for the metabolite AE F092944. The study is evaluated in this dRAR and resulted in a 96-hour-LC<sub>50</sub> value of 254 mg/L.

Details of all studies are provided briefly in the **Table B.9.2.1-1**. Summaries of the studies are provided thereafter.

**Table B.9.2.1-1: Acute toxicity data of foramsulfuron and metabolite to fish**

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
<b>Foramsulfuron-sodium</b>				
<i>Oncorhynchus mykiss</i> (rainbow trout)	static acute	96 h	LC <sub>50</sub> > 100	1997 A57725 1997 A57751 (Amendment) <u>M-141405-02-1</u> KCA 8.2.1 /01
<i>Lepomis macrochirus</i> (bluegill sunfish)	static acute	96 h	LC <sub>50</sub> > 100	1997 A57726 1997 A57752 (Amendment) <u>M-141406-02-1</u> KCA 8.2.1 /02
<i>Cyprinodon variegatus</i> (sheephead minnow)	static acute	96 h	LC <sub>50</sub> > 100	1998 A59901 <u>M-143551-01-1</u> KCA 8.2.1 /03
<b>Equip OD 45</b>				
<i>Oncorhynchus mykiss</i> (rainbow trout)	static renewal acute	96 h	LC <sub>50</sub> 14 <sup>1)</sup>	T. J.; 2000; M-238518-02 KCP 10.2.1 /01
<i>Lepomis macrochirus</i> (bluegill sunfish)	static renewal acute	96 h	LC <sub>50</sub> 7.8 <sup>1)</sup>	T. J.; 2000 M-238517-02 KCP 10.2.1 /02
<b>AE F092944</b>				
<i>Oncorhynchus mykiss</i> (rainbow trout)	static acute	96 h	LC <sub>50</sub> 254	1993 A50396 <u>M-131422-01-1</u> KCA 8.2.1 /04

**Bold letters:** Values considered relevant for risk assessment

<sup>1)</sup> mg product/L

### Studies on foramsulfuron

#### **B.9.2.1.1. Acute toxicity of foramsulfuron to rainbow trout (*Oncorhynchus mykiss*) (static renewal)**

<b>Report:</b>	KCA 8.2.1 /01; [REDACTED] 1997;M-141405-02; Amended: 1997-06-05
<b>Title:</b>	96 hour acute toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , in a static renewal system AE F130360 technical 98.6 % w/w Code: AE F130360 00 1C98 0001
<b>Report No:</b>	A57725
<b>Document No:</b>	M-141405-02-1
<b>Guidelines:</b>	OECD: 203; USEPA (=EPA): E 72-2; Deviation not specified
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final): EC<sub>50</sub> > 100 mg/L.
- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

#### **B.9.2.1.2. Acute toxicity of foramsulfuron to the bluegill sunfish (*Lepomis macrochirus*) (static renewal)**

<b>Report:</b>	KCA 8.2.1 /02; [REDACTED] 1997; M-141406-02-1; Amended: 1997-06-05
<b>Title:</b>	AE F130360; technical 98.6 percent w/w; Code: AE F130360 00 1C98 0001 - 96 hour acute toxicity to the bluegill sunfish, <i>Lepomis macrochirus</i> , in a static renewal system
<b>Report No:</b>	A57726
<b>Document No:</b>	M-141406-02-1
<b>Guidelines:</b>	OECD: 203; USEPA (=EPA): E 72-2; Deviation not specified
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final): EC<sub>50</sub> > 100 mg/L.
- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

#### **B.9.2.1.3. Acute toxicity of foramsulfuron to the Sheepshead minnow (*Cyprinodon variegatus*) (static)**

<b>Report:</b>	KCA 8.2.1 /03; [REDACTED] 1998; M-143551-01
<b>Title:</b>	96 hour acute toxicity to the Sheepshead minnow ( <i>Cyprinodon variegatus</i> ) in a static system AE F130360 technical 94.2% w/w Code: AE F130360 00 1C94 0001
<b>Report No:</b>	A59901
<b>Document No:</b>	M-143551-01-1
<b>Guidelines:</b>	OECD: 203; USEPA (=EPA): E 72-3; Deviation not specified
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).  
LC<sub>50</sub> (96 h) > 100 mg as/L
- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

Studies on Equip OD 45**B.9.2.1.4. Acute toxicity of the product AE F130360 + AE F122006: AE F130360 01 1K05 A304 to the rainbow trout, *Oncorhynchus mykiss* (static renewal)**

<b>Report:</b>	KCP 10.2.1 /01; [REDACTED] 2000; M-238518-02;
<b>Title:</b>	Static renewal toxicity with the rainbow trout, <i>Oncorhynchus mykiss</i> : AE F130360 + AE F122006: AE F130360 01 1K05 A304
<b>Report No:</b>	B002796
<b>Document No:</b>	M-238518-02;
<b>Guidelines:</b>	OECD: 203; USEPA (=EPA): E 72-3; Deviation not specified
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** Exposure of rainbow trout resulted in a 96-hour LC<sub>50</sub> of 14 mg /L AE F130360 + AE F122006 oil flowable, with a 95% confidence interval of 11 to 18 mg/L. The 96-hour no observed effect concentration is 3.9 mg /L AE F130360 + AE F122006 oil flowable, based upon nominal concentrations and the number of live, unaffected rainbow trout.
- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

**B.9.2.1.5. Acute toxicity of the product AE F130360 + AE F122006: AE F130360 01 1K05 A304 to the bluegill sunfish, *Lepomis macrochirus* (static renewal)**

<b>Report:</b>	KCP 10.2.1 /02; [REDACTED] 2000; M-238517-02
<b>Title:</b>	Static renewal toxicity with the bluegill sunfish, <i>Lepomis macrochirus</i> : AE F130360 + AE F122006: AE F130360 01 1K05 A304
<b>Report No:</b>	B002795
<b>Document No:</b>	M-238517-02
<b>Guidelines:</b>	OECD: 203; USEPA (=EPA): E 72-3; Deviation not specified
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** Exposure of bluegill sunfish resulted in a 96-hour LC<sub>50</sub> of 7.8 mg./L AE F130360 + AE F122006 oil flowable, with a 95% confidence interval of 6.8 to 9.1 mg/L. The 96- hour no observed effect concentration is 3.9 mg/L AE F130360 + AE F122006 oil flowable, based upon nominal concentrations and the number of live, unaffected bluegill sunfish.
- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

Toxicity of metabolite AE F092944**B.9.2.1.6. Acute toxicity of metabolite AE F092944 to the Rainbow trout (*Oncorhynchus mykiss*) (static)**

<b>Report:</b>	KCA 8.2.1 /04; [REDACTED] 1993; M-131422-01
<b>Title:</b>	Hoe 092944 - substance, technical (Hoe 092944 00 ZD99 0001) Effect to <i>Oncorhynchus mykiss</i> (Rainbow trout) in a Static-Acute Toxicity Test (method OECD)
<b>Report No:</b>	A50396
<b>Document No:</b>	M-131422-01-1
<b>Guidelines:</b>	OECD: 203 (1984); Deviation not specified
<b>GLP/GEP:</b>	yes

- ❑ **Summary:**

The aim of the study was to determine the acute effects of metabolite AE F092944 (2-amino-4,6-dimethoxypyrimidine; code: AE F092944 00 ZD99 0001; purity > 99.0%) to rainbow trout (*Oncorhynchus mykiss*). *Oncorhynchus mykiss* (5 months old) were exposed in a static system over a period of 96 hours to nominal concentrations of 18, 32, 56, 100, 180, 320, 560, and 1000 mg/L. In addition a water control was tested. Mortality and sublethal behavioural effects were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. The 96-hour-LC<sub>50</sub> was 254 mg/L (95% confidence limits 202 - 317 mg/L), the 96-hour-NOEC was determined to be 100 mg/L.

❑ **Materials and Methods:**

<b>Test item:</b>	Hoe 092944 – substance, technical
<b>Identification code:</b>	Hoe 092944 00 ZD99 0001
<b>Common name:</b>	2-amino-4,6-dimethoxypyrimidine
<b>Analysed purity:</b>	> 99 % w/w
<b>Analytical certificate No.:</b>	AZ 04888

**Treatments**

**Nominal concentrations:** 18, 32, 56, 100, 180, 320, 560, and 1000 mg/L; in addition a water control was tested

**Test water:** Test water was a well aerated water mixture of 60% filtered tap water and 40% deionized water passed through sand and activated charcoal filters.

**Analysis of test concentrations:** yes

**Analytical verification of the test item concentrations samples:** At days 0, 2 and 4 from systems exposed to concentrations of 18, 100 and 1000 mg/L

**Analytical method:** High-performance liquid chromatography (HPLC)

**Test animals**

**Species:** *Oncorhynchus mykiss* WALBAUM 1792

**Age:** 5 months old

**An average weight at the start of testing:** 3.03 g (mean of ten organisms)

**An average length at the start of testing:** 5.83 cm (mean of ten organisms)

**The static biological loading:** 0.15 g/L or 0.29 cm/L

**Test design**

**Test containers:** The test was conducted in 300 L stainless steel tanks, which were chemically clean, containing 200 L of test water. The vessels had a length of 70 cm, a width of 60 cm and a height of 68 cm (approximate values of internal dimensions). The depth of test water lay between 37.1 and 44.5 cm at the beginning and at the end of testing. Each vessel (stainless steel tanks; 300 L) served as one replicate filled with 200 L.

**No of fish per replicate:** 10

**No of replicate per treatment level:** 1

**Exposure regime:** static

**Duration:** 96 hours

**Environmental conditions**

**Illumination and climatic conditions:** By wide spectrum fluorescent lights during a 16 hour daylight photoperiod. The test temperature of 16±1°C was kept by aircondition

❑ **Results:**

Validity Criteria:

The validity criterion of control mortality less than 10% and the validity criterion of oxygen saturation above 60% are fulfilled.

Analytical findings:

Biological results are reported as nominal. Detailed analytical results are presented in the **Table B.9.2.1.6.-1**.

**Table B.9.2.1.6.-1: Nominal and measured concentrations of AE F092944**

Nominal test concentrations	18 mg/L	100 mg/L	1000 mg/L
Nominal a.s. (mg/L)	17.82	99	990
Day 0	18.012	48.796	494.1
Day 2	18.257	104.4	879.8
Day 4	17.929	102.5	-
<b>Mean a.s.</b>	<b>18.07</b>	<b>85.25</b>	<b>686.95</b>
% recovery day 0	101.1	49.3	49.9
% recovery day 2	102.5	105.5	88.9
% recovery day 4	100.6	103.5	-
<b>% recovery mean</b>	<b>101.4</b>	<b>86.1</b>	<b>69.4</b>

Biological findings:

Mortality was observed as listed in **Table B.9.2.1.6.-2** below.

**Table B.9.2.1.6.-2: Effect of metabolite AE F092944 on mortality of *Oncorhynchus mykiss***

Exposure time	24 h	48 h	72 h	96 h	
Test level mg / L	no. of dead	no. of dead	no. of dead	no. of dead	no. of dead
Control	0	0	0	0	0
18	0	0	0	0	0
32	0	0	0	0	0
56	0	0	0	0	0
100	0	0	0	0	0
180	0	0	1	1	10
320	5	6	8	8	80
560	10	10	10	10	100
1000	10	10	10	10	100

Biological endpoints derived:

From the results presented in **Table B.9.2.1.6.-2** above the following biological endpoints can be derived:

**96-hour-figures:**

highest concentration with no effect (NOEC): 100 mg/L

LC<sub>50</sub>: 254 mg/L (95% confidence limits 202 – 317 mg/L)

- ❑ **Conclusion:** The acute effect of AE F092944 (2-amino-4,6-dimethoxypyrimidine; AE 092944 00 ZD99 0001) on rainbow trout (*Oncorhynchus mykiss*) can be quantified as a 96-hour-LC<sub>50</sub> of 254 mg/L (95% confidence limits 202 – 317 mg/L). The highest concentration with no observed mortality and no sublethal behavioural effects can be set to 100 mg/L.
- ❑ **Comment (Co-RMS and RMS):** The end point for rainbow trout has been based on nominal concentrations even though the measured concentration in the highest treatment group is below 80 % of the nominal. Since the toxicity of AE F092944 is not driving the risk assessment there is no need for recalculation of the end point based on the measured concentrations. Therefore the study is acceptable.

## **B.9.2.2. Long-term and chronic toxicity to fish**



One prolonged toxicity test on rainbow trout was performed with foramsulfuron and one with the product Equip OD 45. These studies were evaluated within the process of Annex I inclusion and were considered acceptable by the RMS Germany. These studies were not re-evaluated since the studies were performed according to the OECD test guideline No. 204 Fish, Prolonged Toxicity Test: 14-Day Study. The maximum tested dose level with foramsulfuron was 100 mg a.s./L with rainbow trout and no relevant treatment related effects were observed at the maximum dose level, resulting in a NOEC of 100 g a.s./L. The NOEC of nominal concentration of 1.8 mg./L was obtained with the Equip OD 45.

For the purpose of renewal an early life stage toxicity study with fathead minnow was submitted. The study is evaluated in this dRAR and no relevant treatment related effects were observed at the maximum dose level, resulting in a NOEC of 10.5 mg a.s./L.

Details of all studies are provided briefly in the **Table B.9.2.2.-1**. Summaries of the studies are provided thereafter.

**Table B.9.2.2.-1: Chronic toxicity data of foramsulfuron to fish presented**

Test species	Substance	Test system	Test duration	Endpoint [mg as/L]	Reference
<i>Oncorhynchus mykiss</i> (rainbow trout)	Foramsulfuron	chronic	28 d	NOEC 100	1999 C004117 M-187354-01-1 KCA 8.2.2.1 /01
<i>Oncorhynchus mykiss</i> (rainbow trout)	Equip OD 45	chronic		NOEC 1.8 <sup>1)</sup>	2000; M-238492-02 KCP 10.2.2 /01
<i>Pimephales promelas</i> (fathead minnow)	Foramsulfuron	Early Life Stage flow-through	35 d	NOEC 10.5	2004 B004606 M-241508-01-1 KCA 8.2.2.1 /02

**Bold letters:** Values considered relevant for risk assessment

<sup>1)</sup> mg product/L

#### **B.9.2.2.1. Prolonged toxicity to the rainbow trout, *Oncorhynchus mykiss*, in a flow through system**

##### Study on foramsulfuron

Report:	KCA 8.2.2.1 /01; 1999;M-187354-01
Title:	Prolonged toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , in a flow through system AE F130360 technical 95.8 % w/w Code: AE F130360 00 1C96 0002
Report No:	C004117
Document No:	M-187354-01-1
Guidelines:	OECD: 204; Deviation not specified
GLP/GEP:	yes

- ❑ **Conclusion:** Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):

NOEC = 100 mg/L

- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

##### Study on Equip OD 45

**B.9.2.2.2. Prolonged toxicity of the product AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L: AE F130360 01 1K05 A304 to the rainbow trout, *Oncorhynchus mykiss* (a flow through system)**

<b>Report:</b>	KCP 10.2.2 /01; [REDACTED] 2000; M-238492-02
<b>Title:</b>	Prolonged toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , in a flow through system: AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L: AE F130360 01 1K05 A304
<b>Report No:</b>	B002764
<b>Document No:</b>	M-238492-02
<b>Guidelines:</b>	OECD: 204; Deviation not specified
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** Based on growth of fish during this study, the NOEC (No Observed Effect Concentration) is the nominal concentration of 1.8 mg/L and the LOEC (Lowest Observed Effect Concentration) is the nominal concentration of 3.0 mg/L. The 28-day EC<sub>20</sub> for average specific growth rate, calculated using non-linear regression, was 2.0 mg/L (95% CL: 1.7 to 2.5 mg/L).
- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

**B.9.2.2.3. Fish early life stage toxicity test**

<b>Report:</b>	KCA 8.2.2.1 /02; [REDACTED] 2004; M-241508-01
<b>Title:</b>	Early Life Stage Toxicity of Foramsulfuron (AE F130360) Technical to the Fathead Minnow ( <i>Pimephales promelas</i> ) Under Flow-Through Conditions
<b>Report No:</b>	B004606
<b>Document No(s):</b>	Report includes Trial Nos.: EBFSX001 (A3841201) M-241508-01-1
<b>Guidelines:</b>	OECD: 210; USEPA (=EPA): 72-4, OPPTS 850.1400; Deviation not specified
<b>GLP/GEP:</b>	yes

❑ **Summary**

The effects of foramsulfuron on fathead minnow (*Pimephales promelas*) embryos and larvae were evaluated in a 35 days (30 days post-hatch) toxicity test under flow-through conditions. The nominal test item concentrations were 0.63, 1.25, 2.50, 5.00 and 10.0 mg a.s./L (corresponding to 0.69, 1.23, 2.72, 5.01 and 10.5 mg a.s./L mean measured concentrations over the course of the study). In addition, a dilution water control was tested. Four replicates were used for each test item concentration and the control. Thirty five embryos were impartially selected and distributed to each of 24 embryo incubation cups, one of which was then suspended in each test aquarium per exposure concentration and the control. On the morning of day 33, during the diluter routine check, it was observed that one syringe pump was not operating. The diluter could have only been operating under these conditions for a maximum of 16 hours, and corrective action was quickly taken to insure that the diluter was operating correctly as soon as possible. This relatively brief deviation from nominal concentrations occurred at a late stage of the study and most likely had no impacts on the study results. Observations were made on the survival of organisms at hatch and on the survival and growth (dry weight, total length) of larvae after 30 days of post-hatch exposure. Observations of abnormal behaviour, abnormal physical changes and mortality were recorded daily by visually inspecting the organisms in each growth chamber. Effects were determined based on the mean measured concentrations of the test substance.

With regard to survival of fathead minnows, no statistically significant differences between treatment rates and control were detected. This applies both to the hatching period (5 days) and to the post-hatch exposure period (30 days). At test termination (30 days post-hatch), no statistically significant differences of mean total length and mean dry weight between treatment rates and control were found.

In conclusion, no treatment related effects occurred in the early life stage exposure of the fathead minnow to foramsulfuron technical at the tested concentrations. The NOEC was 10.5 mg a.s./L and the LOEC was > 10.5 mg a.s./L for all endpoints.

#### □ Materials and Methods

<b>Test material:</b>	Foramsulfuron Technical
<b>Purity:</b>	97.3%
<b>Batch number:</b>	AAIR04430
<b>CAS No.:</b>	173159-57-4
<b>Test Solutions:</b>	A 0.257 g a.i./L stock solution was prepared by adding approximately 9.1 g of foramsulfuron (adjusted for purity of 97.3% a.i.) to a 35-liter quantity of dilution water in glass aquarium. The contents were then mixed using two mechanical mixers for at least 4 hours. No visible precipitates were noted.
<b>Test Object</b>	Fathead Minnow eggs
<b>Source:</b>	The adult fathead minnows (Lot #OC080503) used to produce the eggs were received from Osage Catfisheries, Inc. in Osage Beach. This lot was maintained in the laboratory under flow-through conditions in soft blended water. The fish were healthy and no treatments for disease were administered. Thirty-eight liter aquaria were used for the breeding culture. Each aquarium contained 2 male and 6 female fathead minnows, and three spawning substrates. Eggs were removed from the substrates daily and were collected for the test on the morning of test initiation. On that day, the collected eggs were sorted and any eggs appearing to be nonviable were not used in the test. All eggs used in the test were < 24 hours old and between the 2-cell stage and gastrulation, as determined by careful observation under a stereomicroscope.
<b>Tested concentrations:</b>	The nominal test item concentrations were 0.63, 1.25, 2.50, 5.00 and 10.0 mg a.s./L (corresponding to 0.69, 1.23, 2.72, 5.01 and 10.5 mg a.s./L mean measured concentrations over the course of the study)
<b>No. of replicates:</b>	4 replicate exposure chambers per concentration; each chamber contained one egg cup. Egg cups were positioned on an oscillating rocker arm assembly to facilitate the circulation of test solution in the egg cups.
<b>Environmental condions</b>	
<b>Test chambers:</b>	8-liter glass aquaria containing approximately 7 liters of test solution with a water depth of 25.5 cm.
<b>Temperature:</b>	25 ± 1 °C
<b>Photoperiod:</b>	16-hours light and 8-hours dark
<b>The light intensity:</b>	The light intensity was adjusted to 50 to 100 footcandles during the light phase. Light intensity ranged from 57 to 88 foot-candles (611 to 950 lux) and averaged 77 foot candles (833 lux) as measured by a Sper Scientific Model 840020 light meter.
<b>Exposure:</b>	35 Day, flow-through (ELS)

#### □ Principle of the test:

The exposure of fathead minnow (*Pimephales promelas*) embryos and larvae to foramsulfuron was initiated with fertilised embryos. Thirty five embryos were impartially selected and distributed to each of 24 embryo incubation cups, one of which was then suspended in each quadruplicate test aquarium per exposure concentration and the control. The nominal test item concentrations were 0.63, 1.25, 2.50, 5.00 and 10.0 mg a.s./L (corresponding to 0.69, 1.23, 2.72, 5.01 and 10.5 mg a.s./L mean measured concentrations over the course of the study). In addition, a dilution water control was tested. Four replicates were used for each test item concentration and the control. Dead embryos were counted daily

until hatching was complete. Hatching was complete on exposure day 5 at which all viable eggs had hatched.

Calculations of percentage survival of organisms at hatch were based on the number of live larvae and embryos per incubation cup after hatching was complete, compared to the number of embryos per cup on test day 0. To initiate the post-hatch larval exposure, 20 live larvae were impartially selected from the surviving larvae in each incubation cup on test day 5 and placed into their respective exposure aquaria.

Behaviour and appearance of larvae were observed and recorded daily and larval survival was analysed on study day 5 and study day 35. Effects were determined based on the mean measured concentrations of the test substance.

The control and the high, middle and low test concentrations were each sampled once and analysed for foramsulfuron concentrations prior to the start of the definitive exposure.

During the in-life phase of the definitive study, water samples were removed from the test solutions on days 0, 7, 14, 21, 27, 33 and 35 for analysis of foramsulfuron.

## □ Results

### Analytical findings:

Results of the analyses (HPLC) of the test solutions during the in-life phase of the study (days 0, 7, 14, 21, 27, 33 and 35) demonstrated that mean measured concentrations of foramsulfuron were generally consistent between replicate solutions and sampling intervals. The concentration range established was generally consistent with the expected concentration gradient (i.e. 50% dilutions between treatment levels). Based on the results of the weekly solution analyses, the exposure solutions were defined as 0.69, 1.23, 2.72, 5.01 and 10.5 mg a.s./L (i.e. mean measured concentrations over the course of the study).

### Biological findings:

During hatching period survival of fathead minnows in the five treatment levels (0.69 – 10.5 mg a.s./L) ranged from 81 to 92%, hence it was similar to the survival of the control organisms. No statistically significant differences between treatment groups and control were found. At the end of the post-hatch exposure period (30 days) the survival rates of larvae exposed to the five concentrations of foramsulfuron (0.69 – 10.5 mg a.s./L) ranged from 81% to 94% and, thus, they were in the same range as survival rates of the pooled control larvae. Again, no statistically significant differences between treatment groups and control could be revealed (Table B.9.2.2.3.-1).

Growth data (total length and dry weight) were determined at test termination (30 days post-hatch). The mean total length and dry weight of larvae exposed to the five treatment levels (0.69 – 10.5 mg a.s./L) ranged from 21.0 to 22.2 mm and 38.6 to 46.0 mg, respectively. Growth in these treatment levels was statistically comparable to the control data, no significant differences were found. Based on these data, it was suggested that exposure to foramsulfuron concentrations up to 10.5 mg a.s./L did not adversely affect larval growth (Table B.9.2.2.3.-1).

**Table B.9.2.2.3.-1: Survival of fish at hatch (test day 5) and survival, total length and dry weight of fathead minnow (*Pimephales promelas*) larvae after 30 days post-hatch exposure to foramsulfuron**

Mean measured concentration [mg a.s./L]	Survival of organism at hatch* [%]	30 days post-hatch		
		Larval survival* [%]	Total length [mm]	Dry weight [g]
Control	88	84	21.1	42.6
0.69	85	93	21.0	38.6
1.23	92	81	21.9	45.6
2.72	83	93	21.9	44.3
5.01	85	89	21.7	42.0
10.5	81	94	22.2	46.0

\* Mean values of four replicates

- ❑ **Conclusions:** In conclusion, no treatment related effects occurred in the early life stage exposure of the fathead minnow to foramsulfuron technical at the tested concentrations. The NOEC was 10.5 mg a.s./L and the LOEC was > 10.5 mg a.s./L for all endpoints.
- ❑ **Comment (Co-RMS and RMS):** Study is considered acceptable.

#### B.9.2.2.4. Fish full life cycle test

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

#### B.9.2.2.5. Bioconcentration in fish

Due to the low  $P_{OW}$  foramsulfuron has no potential for bioconcentration.

#### B.9.2.3. Potential for endocrine disruption

Based on the definition of the WHO/IPCS on endocrine disruption presented in Point B.9.1.5 following results concerning relevant adverse effects of foramsulfuron on fish are presented below.

##### Fish

Population relevant effects of foramsulfuron on fish were studied in an early life-stage test (ELS). No effects were seen at the highest tested concentration of 10 mg/L. No further testing is indicated to evaluate the endocrine disrupter potential of foramsulfuron to fish.

- ❑ **Comment (Co-RMS and RMS):** There were no indications for adverse endocrine activity observed in fish. Therefore further special testing for endocrine disrupting behaviour is not warranted.

#### B.9.2.4. Acute toxicity to aquatic invertebrates

One acute toxicity study on *Daphnia magna* was performed with foramsulfuron and the product Equip OD 45. These studies were evaluated within the process of Annex I inclusion and were considered acceptable by the RMS Germany. These studies were not re-evaluated since the studies were performed according to the OECD test guideline No. 202 *Daphnia* sp. Acute Immobilisation Test. In the test with foramsulfuron no mortality occurred at the tested dose level of 100 mg a.s./L, resulting in a NOEC of 100 mg a.s./L and an  $EC_{50}$  >100 mg a.s./L. In the test with Equip OD 45 a 48-hour  $EC_{50}$  of 6.9 mg product/L was obtained.

For the purpose of renewal one acute study on *Daphnia magna* was conducted with the metabolite AE F092944. This study is evaluated in this dRAR. The tested dose level ranged from 10 to 560 mg/L, the determined  $EC_{50}$  was 233 mg/L.

Details of all studies are provided briefly in the Table B.9.2.4.-1. Summaries of the studies are provided thereafter.

**Table B.9.2.4.-1: Acute toxicity data of foramsulfuron and metabolite to *Daphnia magna***

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
<b>Foramsulfuron-sodium</b>				
<i>Daphnia magna</i> (water flea)	static acute	48 h	$EC_{50}$ > 100	Stachura & Ruff, (1997) A57724 & A57750 (Amendment) M-141404-02-1 KCA 8.2.4.1 /01
<b>Equip OD 45</b>				
<i>Daphnia magna</i>	static acute	48 h	$EC_{50}$ 6.9 <sup>1)</sup>	Boeri, R. L.; Ward, T.



Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
(water flea)				J.; 2000 M-238519-02; Amended: 1997-06-05 KCP 10.2.1 /03
<b>AE F092944</b>				
<i>Daphnia magna</i> (water flea)	static acute	48 h	EC <sub>50</sub> <b>223</b>	Heusel, 1993 A50353 <u>M-131382-01-1</u> KCA 8.2.4.1 /02

**Bold letters:** Values considered relevant for risk assessment

<sup>1)</sup> mg product/L

#### Study on foramsulfuron

##### B.9.2.4.1. Acute toxicity of foramsulfuron to *Daphnia magna* (static renewal test)

Report:	<u>KCA 8.2.4.1 /01</u> ; Stachura, B. J.; Ruff, D. F.;1997; M-141404-02; Amended: 1997-06-05
Title:	AE F130360; technical 98.4 percent w/w; Code: AE F130360 00 1C98 0001 - The 48 hour acute toxicity to <i>Daphnia magna</i> , in a static renewal system
Report No:	A57724
Document No:	<u>M-141404-02-1</u>
Guidelines:	<b>OECD: 202; USEPA (=EPA): E 72-2;Deviation not specified</b>
GLP/GEP:	yes

- ❑ **Conclusion:** Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final): EC<sub>50</sub> = 100 mg/L\*

\* In the Study it is noted in the conclusion: "The 48 hour EC<sub>50</sub> of AE F130360 technical to *Daphnia magna* could not be determined under the conditions of this study. The no observed effect concentration (NOEC) was 100 mg/L."

- ❑ **Comment (Co-RMS and RMS)** Therefore the end point should be corrected to an EC<sub>50</sub> value of > 100 mg/L. Refer to original EU review. No new data or assessments of the study are provided.

#### Study on the Equip OD 45

##### B.9.2.4.2. Acute toxicity of the product AE F130360 + AE F122006: AE F130360 01 1K05 A304 to *Daphnia magna* (static renewal test)

Report:	<u>KCP 10.2.1 /03</u> ; Boeri, R. L.; Ward, T. J.; 2000; M-238519-02; Amended: 1997-06-05
Title:	Static renewal toxicity test with the Daphnid, <i>Daphnia magna</i> : AE F130360 + AE F122006: AE F130360 01 1K05 A304
Report No:	B002797
Document No:	M-238519-02
Guidelines:	<b>OECD: 202; USEPA (=EPA): E 72-2;Deviation not specified</b>
GLP/GEP:	yes

- ❑ **Conclusion:** Exposure of daphnids resulted in a 48-hour EC<sub>50</sub> of 6.9 mg/L AE F130360 + AE F122006 oil flowable, with a 95% confidence interval of 6.0 to 10 mg/L. The 48-hour no observed effect concentration is 3.6 mg/L AE F130360 + AE F122006 oil flowable, based upon nominal concentrations and the number of live, mobile daphnids.

- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

## Study on the metabolite AE F092944

### B.9.2.4.3. Acute toxicity of metabolite AE F092944 to *Daphnia magna* (static test)

<b>Report:</b>	KCA 8.2.4.1 /02;Heusel, R.;1993;M-131382-01
<b>Title:</b>	Hoe 092944 - substance, technical (Hoe 092944 00 ZD99 0001) Effect to <i>Daphnia magna</i> (waterflea) in a Static -Acute Toxicity Test (method OECD)
<b>Report No:</b>	A50353
<b>Document No:</b>	M-131382-01-1
<b>Guidelines:</b>	OECD: 202 (1984); Deviation not specified
<b>GLP/GEP:</b>	yes

#### □ Summary

The aim of the study was to determine the acute effects of AE F092944 (2-amino-4,6-dimethoxypyrimidine; code: AE F092944 00 ZD99 0001; purity > 99.0%) to *Daphnia magna*.

*Daphnia magna* (< 24 hour old neonates) were exposed in a static system over a period of 48 hours to nominal concentrations of 10, 18, 32, 56, 100, 180, 320, and 560 mg/L (corresponding to analytically verified concentrations of 100.4%). In addition a water control and solvent control was tested.

Immobilisation and sublethal behavioural effects were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. The 48-hour-EC<sub>50</sub> was 223 mg/L (95% confidence limits 180 – 320 mg/L), the 48-hour-NOEC was determined to be 32 mg/L.

#### □ Materials and Methods

<b>Test item:</b>	Hoe 092944 – substance, technical
<b>Identification code:</b>	Hoe 092944 00 ZD99 0001
<b>Common name:</b>	2-amino-4,6-dimethoxypyrimidine
<b>Analysed purity:</b>	> 99 % w/w
<b>Analytical certificate No.:</b>	AZ 04888
<b>Solvent:</b>	Acetone of analytical grade was used as a solvent with 0.1 ml per liter test water.
<b>Tested species:</b>	<i>Daphnia magna</i> (Waterflea)
<b>Source of test animals:</b>	own culture
<b>Age of test animals:</b>	1 – 24 hours
<b>Test design</b>	
<b>Nominal test concentrations:</b>	untreated control, 10, 18, 32, 56, 100, 180, 320, and 560 mg/L.
<b>Length of study:</b>	48 hours
<b>Test vessels:</b>	The test was conducted in 300 mL glass jars, which were chemically clean, containing 200 mL of test water. The jars had a diameter of 95 mm and a height of 50 mm (approximate values of internal dimensions). The depth of test water lay between 30 and 31 mm at the start and at the end of testing. The jars were covered with glass plates during the test.
<b>Test water:</b>	Test water was an artificial mineral medium M4 (Elendt 1990), slightly modified. To prevent precipitations during preparation of medium, the chemicals were given into 500 – 800 mL deionized water and then filled up to 1000 mL.
<b>Illumination and climatic conditions:</b>	The maintenance room as well as the test room were illuminated by wide spectrum fluorescent lights during a 16 hour daylight photoperiod. To keep the test temperature at 20 ± 1°Celsius the jars stood in a regulated water bath in an air-conditioned room.

#### □ Principle of the test:

*Daphnia magna* (< 24 hour old neonates) were exposed to AE F 092944 (2-amino-4,6-dimethoxy-pyrimidine; code: AE F092944 00 ZD99 0001; purity > 99.0%) in a static system over a period of 48 hours. Nominal concentrations were 10, 18, 32, 56, 100, 180, 320, and 560 mg/L. In addition a water control and solvent control was tested. Each vessel (glass jar; 300 mL) served as one replicate filled with 200 mL artificial mineral medium M4 (Elendt 1990), slightly modified. 10 daphnids were used per replicate. Biological loading rate was 20 mL/animal. The test was conducted with 2 replicates per treatment level. Immobilisation of daphnids, intoxication symptoms and physical-chemical water parameters were assessed.

For analytical verification of the test item concentrations samples were taken at 0 and 48 hours from 10 mg/L concentrations. High-performance liquid chromatography (HPLC) was used as analytical method.

#### □ Results:

##### Validity Criteria:

The validity criterion of control mortality less than 10% is fulfilled. The validity criterion of oxygen saturation above 60% is fulfilled.

##### Analytical findings:

Analytical verification of test solutions revealed measured concentrations of 100.4% of nominal calculated as arithmetic mean. Biological results are reported as nominal. Detailed analytical results are presented in the **Table B.9.2.4.3.-1**.

**Table B.9.2.4.3.-1: Nominal and measured concentrations of AE F092944**

Nominal Concentration	Concentration (mg /L)	Day 0 (New)		Day 2 (Old)		mean	
		Measured (mg a i./L)	Percent Nominal	Measured (mg a i./L)	Percent Nominal	Measured (mg a.i./L)	Percent Nominal
10 mg/L	9.9	9.849	98.5	10.237	102.4	10.043	100.4

##### Biological findings:

No sublethal behavioural changes were observed. Observations on immobilisation and sublethal intoxication symptoms are summarized in **Table B.9.2.4.3.-2**.

**Table B.9.2.4.3.-2: Immobilization symptoms of *Daphnia magna***

Nominal Test Concentration mg/L	Number of Immobilised Daphnids	
	24 h	48 h
Control	0	0
Solvent control	0	0
10	0	0
18	0	0
32	0	0
56	0	4
100	0	3
180	2	4
320	17	19
560	20	20

#### □ Conclusion:

##### Biological endpoints derived:

From the results presented in the **Table B.9.2.4.3.-2** above the following biological endpoints can be derived:



**24-hour-figures:**EC<sub>50</sub>: 247 mg/L (95% confidence limits 215 – 283 mg/L)**48-hour-figures:**

NOEC: 32 mg/L

EC<sub>50</sub>: 223 mg/L (95% confidence limits 180 – 320 mg/L)

□ **Comment (Co-RMS and RMS):** Study considered acceptable.

**B.9.2.4.4. Acute toxicity to an additional aquatic invertebrate species**

Foramsulfuron has no insecticidal activity and no effects on *Daphnia magna* have been observed. Therefore no additional testing with aquatic invertebrate species is needed.

**B.9.2.5. Long-term and chronic toxicity to aquatic invertebrates****B.9.2.5.1. Reproductive and development toxicity to *Daphnia magna***

One reproductive toxicity study on *Daphnia magna* was performed with active substance and one with the product. These studies were evaluated within the process of Annex I inclusion and were considered acceptable by the RMS Germany. These studies were not re-evaluated since the studies were performed according to the OECD No. 211: *Daphnia magna* Reproduction Test. The active substance showed no chronic effects on the survival, growth or reproduction of the water flea at a concentration of 100 mg/L. In the test with Equip OD 45 a 21 day NOEC of 0.4 mg/L was obtained.

Details of all studies are provided briefly in the Table B.9.2.5.1.-1. Summaries of the studies are provided thereafter.

**Table B.9.2.5.1.-1: Reproductive toxicity data of foramsulfuron to *Daphnia magna***

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
<b>Foramsulfuron</b>				
<i>Daphnia magna</i> (water flea)	chronic	21 d	NOEC > 100	Young & Ruff, 1999 B002180 M-237962-01-2 KCA 8.2.5.1 /01
<b>Equip OD 45</b>				
<i>Daphnia magna</i> (water flea)	chronic	21 d	NOEC 0.4 <sup>1)</sup>	Young, B. M.; Ruff, D. F.; 2000 M-238488-02 KCP 10.2.2 /02

**Bold letters:** Values considered relevant for risk assessment;

<sup>1)</sup> mg product/L

**B.9.2.5.1.1. Effects of foramsulfuron on life-cycle of the water flea (*Daphnia magna*) (a static renewal)**

<b>Report:</b>	<u>KCA 8.2.5.1 /01; Young, B. M.; Ruff, D. F.; 1999; M-237962-01</u>
<b>Title:</b>	Effects on life-cycle of the water flea ( <i>Daphnia magna</i> ) in a static renewal system AE F130360 technical 95.8% w/w
<b>Report No:</b>	B002180
<b>Document No(s):</b>	Report includes Trial Nos.: CF99W537 <u>M-237962-01-2</u>
<b>Guidelines:</b>	OECD: 211; USEPA (=EPA): 72-4(b); Deviation not specified
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** The active substance showed no chronic effects on the survival, growth or reproduction of the water flea at a concentration of 100 mg/L. Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final):  
NOEC > 100 mg/L.
- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

#### Study on Equip OD 45

#### **B.9.2.5.1.2. Effects of the product AE F130360 + AE F122006, oil flowable 22.5 + 22.5 g/L: AE F130360 01 1K05 A304 on life-cycle of the water flea (*Daphnia magna*) (static renewal)**

<b>Report:</b>	KCP 10.2.2 /02; Young, B. M.; Ruff, D. F.; 2000; M-238488-02
<b>Title:</b>	Effects on life-cycle of the water flea ( <i>Daphnia magna</i> ) in a static renewal system: AE F130360 + AE F122006, oil flowable 22.5 + 22.5 g/L; AE F130360 01 1K05 A304
<b>Report No:</b>	B002760
<b>Document No(s):</b>	Report includes Trial Nos.: CF99W540; M-238488-01-2; M-238488-02
<b>Guidelines:</b>	USEPA (=EPA): 72-4(b); Deviation not specified
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** In a static renewal exposure system for 21 days, AE F1 30360 + AE F1 22006 oil flowable has chronic effects on the growth and reproduction of the water flea, *D. magna*, at a concentration of 0.8 mg/L, but no effects at a concentration of 0.4 mg/L.  
NOEC 0.4 mg/L
- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

#### **B.9.2.5.2. Reproductive and development toxicity to an additional aquatic invertebrate species**

Foramsulfuron has no insecticidal activity and no chronic effects on *Daphnia magna* have been observed. No additional chronic testing with aquatic invertebrate species is needed.

#### **B.9.2.5.3. Development and emergence in Chironomus species**

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

#### **B.9.2.5.4. Sediment dwelling organisms**

Foramsulfuron is water soluble but it is found in the sediment in amounts > 20 % of the applied amount in water/sediment studies (see Vol 3 CA Section B-8: Tables B.8.4.2.3-19 and B.8.4.2.3-21) . Commission regulation (EU) No 283/2013 (Point 8.2.5.4) states that when accumulation of an active substance in aquatic sediment is indicated or predicted by environmental fate studies, the impact on a sediment-dwelling organism shall be assessed. The Applicant has not submitted any toxicity study on sediment dwelling organisms. However, the chronic toxicity to daphnia is very low (NOEC >100 mg/L). In addition, the active substance is an herbicide and is therefore not predicted to affect sediment dwelling organisms and therefore it is considered that a toxicity study on sediment dwelling organisms is not needed.

#### **B.9.2.6. Effects on algal growth**

Potential effects of foramsulfuron on algal growth were investigated with four different algae species, a green alga, a blue-green alga and a freshwater and a marine diatom. These studies were evaluated within the process of Annex I inclusion and were considered acceptable by the RMS Germany. These studies were not re-evaluated

since the studies were performed according to the OECD test guideline No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. The blue-green alga *Anabaena flos-aquae* were found to be, by a factor of 10, more sensitive than other algae species. The EC<sub>50</sub> of foramsulfuron for this species is 8.1 mg a.s./L. Potential effects of product Equip OD 45 on algal growth were also investigated with green algae and the study was evaluated within the process of Annex I inclusion and was considered acceptable by the RMS Germany. The study resulted in a 96-hour E<sub>r</sub>C<sub>50</sub> greater than 5.0 mg/L, the highest tested concentration.

For the purpose of renewal toxicity studies were performed according to the OECD 201 with green algae with metabolites AE F092944 and AE F0999095. In both cases the EC<sub>50</sub> values were above the highest tested dose level (EC<sub>50</sub> > 560 and 100 mg/L, respectively) – and also clearly above the respective EC<sub>50</sub> for green algae of the parent compound.

Details of all studies are provided briefly in the Table B.9.2.6-1. Summaries of the studies are provided thereafter.

**Table B.9.2.6-1: Growth effect data of Equip OD 45, foramsulfuron and its metabolites to algae**

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
<b>Foramsulfuron-sodium</b>				
<i>Pseudokirchneriella subcapitata</i> (syn. <i>Selenastrum capricornutum</i> ) (green alga)	growth inhibition	72 h	E <sub>r</sub> C <sub>50</sub> <sup>1)</sup> 75.0	Christ & Ruff, 1998 A59926 <u>M-143574-01-1</u> KCA 8.2.6.1 /01
		96 h	E <sub>r</sub> C <sub>50</sub> <sup>1)</sup> 86.2	
<i>Navicula pelliculosa</i> (diatom)	growth inhibition	72 h /96 h	E <sub>r</sub> C <sub>50</sub> <sup>1)</sup> > 112	Young & Ruff, 1999 C002422 <u>M-184469-01-1</u> KCA 8.2.6.2 /01
<i>Anabaena flos-aquae</i> (blue-green algae)	growth inhibition	72 h	E <sub>r</sub> C <sub>50</sub> <sup>1)</sup> 8.1	Christ & Ruff, 1999 C003699 <u>M-186627-01-1</u> KCA 8.2.6.2 /02
		96 h	E <sub>r</sub> C <sub>50</sub> <sup>1)</sup> 8.1	
<i>Skeletonema costatum</i> (marine diatom)	growth inhibition test	72 h /96 h	E <sub>r</sub> C <sub>50</sub> > 105	Young & Ruff, 1999 C002436 <u>M-184494-01-1</u> KCA 8.2.6.2 /03
<b>AE F092944</b>				
<i>Desmodesmus subspicatus</i> (syn. <i>Scenedesmus subspicatus</i> ) (green alga)	growth inhibition	72 h	E <sub>r</sub> C <sub>50</sub> > 560	Heusel, 1993 A50395 <u>M-131421-01-1</u> KCA 8.2.6.1 /02
<b>AE F099095</b>				
<i>Pseudokirchneriella subcapitata</i> (green alga)	growth inhibition	72 h	E <sub>r</sub> C <sub>50</sub> > 100	Dorgerloh, 2005 <u>M-254084-01-1</u> KCA 8.2.6.1 /03
<b>Equip OD 45</b>				
<i>Selenastrum capricornutum</i>	growth inhibition	96 h	E <sub>r</sub> C <sub>50</sub> > 5.0 <sup>2)</sup>	Boeri, R. L.; Ward, T. J.; 2000 M-238520-02 KCP 10.2.1 /04;

**Bold letters:** Values considered relevant for risk assessment in the MCP document

<sup>1)</sup> Since the new aquatic GD<sup>2</sup> focusses on endpoints based on growth rates the old E<sub>b</sub>C<sub>50</sub> figures were omitted from the table above. - <sup>2)</sup> mg product/L

<sup>2</sup> 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

## Studies on foramsulfuron

### Effects on growth of green algae

#### **B.9.2.6.1. Effect of foramsulfuron to green alga (*Pseudokirchneriella subcapitata*)**

<b>Report:</b>	KCA 8.2.6.1 /01;Christ, M. T.; Ruff, D. F.;1998;M-143574-01
<b>Title:</b>	Effect to <i>Pseudokirchneriella subcapitata</i> (green alga) in a growth inhibition test AE F130360 technical 94.2% w/w
<b>Report No:</b>	A59926
<b>Document No:</b>	M-143574-01-1
<b>Guidelines:</b>	OECD: 201; USEPA (=EPA): 40 CFR Part 160; Deviation not specified
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

72 h ErC<sub>50</sub> = 75.0 mg as/L

96 h ErC<sub>50</sub> = 86.2 mg as/L

- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

### Effects on growth of an additional algal species

#### **B.9.2.6.2. Effect of foramsulfuron to freshwater diatom (*Navicula pelliculosa*)**

<b>Report:</b>	KCA 8.2.6.2 /01;Young, B. M.; Ruff, D. F.;1999;M-184469-01
<b>Title:</b>	Effect to <i>Navicula pelliculosa</i> (freshwater diatom) in a growth inhibition test AE F130360 technical 94.6% w/w Code: AE F130360 00 1C94 0001
<b>Report No:</b>	C002422
<b>Document No:</b>	M-184469-01-1
<b>Guidelines:</b>	OECD: 201; USEPA (=EPA): 122-2;Deviation not specified
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

72 h /96 h ErC<sub>50</sub> > 112 mg a.s./L

- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

#### **B.9.2.6.3. Effect of foramsulfuron to blue-green alga (*Anabaena flos-aquae*)**

<b>Report:</b>	KCA 8.2.6.2 /02;Christ, M. T.; Ruff, D. F.;1999;M-186627-01
<b>Title:</b>	Effect to <i>Anabaena flos-aquae</i> (blue-green alga) in a growth inhibition test technical 94.6% w/w Code: AE F130360 00 1C94 0001
<b>Report No:</b>	C003699
<b>Document No:</b>	M-186627-01-1
<b>Guidelines:</b>	OECD: 201; USEPA (=EPA): 123-1;Deviation not specified
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** The new aquatic guidance document (EFSA 20134) only regards endpoints based on growth rates as relevant. Therefore the biomass based endpoint of E<sub>b</sub>C<sub>50</sub> = 3.3 mg/L

according to the Review Report for foramsulfuron (SANCO/10324/2002-Final) has to be revised by  $E_rC_{50}$  of 8.1 mg/L to the new list of end points.

- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

#### B.9.2.6.4. Effect of foramsulfuron to Marine Diatom (*Skeletonema costatum*)

<b>Report:</b>	<u>KCA 8.2.6.2 /03;Young, B. M.; Ruff, D. F.;1999;M-184494-01</u>
<b>Title:</b>	Effect to <i>Skeletonema costatum</i> (Marine Diatom) in a growth inhibition test AE F130360 technical 94.6 % w/w Code: AE F130360 00 1C94 0001
<b>Report No:</b>	C002436
<b>Document No:</b>	<u>M-184494-01-1</u>
<b>Guidelines:</b>	<b>OECD: 201; USEPA (=EPA): 122-2;Deviation not specified</b>
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

72 h /96 h  $E_rC_{50}$  > 105 mg as/L

- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

#### Studies on the metabolites of foramsulfuron

For metabolites AE F092944 and AE F099095 aquatic toxicity studies on green algae, *Pseudokirchneriella subcapitata* or *Desmodesmus subspicatus*, were performed.

#### B.9.2.6.5. Effect of the metabolite AE F092944 to green alga (*Scenedesmus subspicatus*)

<b>Report:</b>	<u>KCA 8.2.6.1 /02;Heusel, R.;1993;M-131421-01</u>
<b>Title:</b>	Hoe 092944 - substance, technical (Hoe 092944 00 ZD99 0001) Effect to <i>Scenedesmus subspicatus</i> (Green alga) in a Growth Inhibition Test (method OECD)
<b>Report No:</b>	A50395
<b>Document No:</b>	<u>M-131421-01-1</u>
<b>Guidelines:</b>	<b>OECD: 201 (1984); Deviation not specified</b>
<b>GLP/GEP:</b>	yes

##### ❑ **Summary**

The aim of the study was to determine the effects of AE F092944 (2-amino-4,6-dimethoxypyrimidine; code: AE 092944 00 ZD99 0001; purity > 99.0%) to *Scenedesmus subspicatus*.

Cultures of *Scenedesmus subspicatus* with an initial cell density of 10 000 cells/mL were exposed in a static system over a period of 72 hours to nominal concentrations of 10, 18, 32, 56, 100, 180, 320, and 560 mg/L. In addition a water control and a solvent control were tested.

24, 48 and 72 hour growth rate based on cell density and visual assessment of potential cell deformations were used to determine the endpoints. Based on analytical findings the biological endpoints are reported as nominal figures. The 96-hour- $E_rC_{50}$  was > 560 mg/L, the 96-hour-NOEC was determined to be 56 mg/L.

##### ❑ **Materials and Methods**

**Test material:**

Hoe 092944 technical

<b>Purity:</b>	> 99.0%
<b>Code:</b>	Hoe 092944 00 ZD99 0001
<b>Analytical certificate No.:</b>	AZ 04888
<b>Nominal test concentrations:</b>	untreated control, solvent control (0.1 mL acetone/L medium), 10, 18, 32, 56, 100, 180, 320, and 560 mg/L.
<b>Length of study:</b>	72 hours
<b>Tested species:</b>	<i>Scenedesmus subspicatus</i> (Green alga)
<b>Source of organisms:</b>	Own culture
<b>Solvent:</b>	Acetone of analytical grade was used as a solvent with 0.1 mL per liter medium
<b>Test vessels:</b>	The test was conducted in 300 mL Erlenmeyer flasks, which were filled with 100 mL medium and closed with cotton stoppers.
<b>Illumination and climatic conditions:</b>	The flasks for pre-culture and main study were standing in a water bath regulated to $25 \pm 2$ °C on an electric shaker with a constant motion of 100cycles/minute. The cultures were illuminated constantly using white spectrum fluorescent lamps of the universal white-type L25 and a quantum flux density of $180 \pm 20$ /E.n <sub>r</sub> 2 .s~ 1 (mean and standard deviation of 30 measuring points). The test was performed without adjustment of pH.
<b>Analyses of test:</b>	Substance concentration
<b>Chemical analyses of the test substance concentration:</b>	Chemical analyses of the test substance concentration in the test water was made in a separate stability test after 0, and 72 hours test duration from the nominal concentration of 18 mg/L by HPLC-analysis

#### □ Principle of the test:

Green alga (*Scenedesmus subspicatus*) was exposed to 2-amino-4,6-dimethoxypyrimidine (code: AE 092944) in a static system over a period of 72 hours. Nominal concentrations were 10, 18, 32, 56, 100, 180, 320, and 560 mg/L. In addition, a water control and a solvent control were tested. Each vessel (Erlenmeyer flasks; 300 mL) served as one replicate filled with 100 mL test solution. At test initiation the cell density was 10 000 cells/mL. The test was conducted with 3 replicates per treatment level. In the controls 6 replicates were tested.

For analytical verification samples were taken at 0 and 72 hours from test solutions with 18 mg/L. High-performance liquid chromatography (HPLC) was used as analytical method.

Growth rates, observation on cell abnormalities and physical-chemical water parameters were assessed.

#### □ Results:

##### Validity criteria:

The validity criterion of cell density increase > 16x in the control is fulfilled. The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35% and the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata* and *Desmodesmus subspicatus*. These criteria were fulfilled.

##### Analytical findings:

Analytical verification of test solutions revealed measured concentrations of AE F092944, calculated as an arithmetic mean. Biological results are reported as nominal. Detailed analytical results are presented in the **Table B.9.2.6.5.-1**.

**Table B.9.2.6.5.-1: Nominal and measured concentrations of AE F092944**

Nominal concentration	Concentration (mg /L)	Day 0 (New)		Day 3 (Old)		Mean	
		Measured (mg a.i./L)	Percent Nominal	Measured (mg a.i./L)	Percent Nominal	Measured (mg a.i./L)	Percent Nominal
18 mg/L	17.82	17.5	98.2	17.11	96.0	17.31	97.1

Biological findings:

Observations on growth rates are presented in the **Table B.9.2.6.5.-2**. No cell abnormalities were observed.

**Table B.9.2.6.5.-2: Effect of AE F092944 on growth-inhibition of *Scenedesmus subspicatus***

Nominal treatment level (mg/L)	% inhibition according to mean area under the growth curve after 72 h	% inhibition according to mean growth rate after 72 h
Control	-	-
Solvent control	-0.02	2.5
32	-1.9	0.8
56	-3.6	0.6
100	-7.4	0.2
180	22.4	7.9
320	37.4	11.2
560	67.6	26.7

**□ Conclusion:**Biological endpoints derived:

From the results presented in the **Table B.9.2.6.5.-2** above the following biological endpoints can be derived:

**72-hour-figures (growth rate):**

EC<sub>50</sub> - area under the growth curve: 403 mg/L (95% confidence limits 320 – 560 mg/L)  
 EC<sub>50</sub> - growth rate: > 560 mg/L  
 NOEC: 56 mg/L

**□ Comment (Co-RMS and RMS):** Study considered acceptable.**B.9.2.6.6. Effect of the metabolite AE F099095 to green alga (*Pseudokirchneriella subcapitata*) (static test)**

<b>Report:</b>	<u>KCA 8.2.6.1 /03:Dorgerloh, M.:2005:M-254084-01</u>
<b>Title:</b>	<i>Pseudokirchneriella subcapitata</i> - growth inhibition test with AE F099095 00 1B99 0001
<b>Report No:</b>	EBMMX092
<b>Document No:</b>	<u>M-254084-01-1</u>
<b>Guidelines:</b>	<b>Draft Proposal for Updating OECD Guideline 201: "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" (Feb. 18, 2004);none</b>
<b>GLP/GEP:</b>	yes

**□ Summary:**

The aim of this study was to determine the influence of metabolite AE F099095 on exponentially growing *Pseudokirchneriella subcapitata* (freshwater microalgae, formerly known as *Selenastrum capricornutum*)

expressed as NOEC; LOEC and ECx for growth rate of algal biomass (cells per volume). The study was designed to meet OECD criteria. *Pseudokirchneriella subcapitata* were exposed in a chronic multigeneration test for 3 days under static exposure conditions to the nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg p.m. (pure metabolite)/L in comparison to an untreated control. Three replicate vessels per test level and six replicate vessels for the control were used. Cell numbers per volume (as a surrogate for biomass per volume) were estimated photometrically at day 1, 2 and 3 of the exposure period. To detect possible cell deformations, samples were examined under a microscope. Based on analytical findings, the biological endpoints are reported as nominal figures. The (0 – 72 h)-ErC<sub>50</sub> was > 100 mg p.m./L, the (0 – 72 h)-NOErC was determined to be 25 mg p.m./L.

#### □ Material and methods:

<b>Test item:</b>	AE F099095 00 1B99 0001
<b>Batch No.:</b>	KR363/364
<b>Purity:</b>	99.6 % w/w
<b>Certificate of analysis-No.:</b>	AZ 10810
<b>Analytical reference-No.:</b>	0305473
<b>Test organism</b>	
<b>Species:</b>	<i>Pseudokirchneriella subcapitata</i> , formerly named <i>Selenastrum capricornutum</i> , strain SAG 61.81
<b>Origin:</b>	Collection of Algal Cultures, Inst. for Plant Physiology, University of Gottingen, Nikolausberger Weg 18, 37077 Gottingen, Germany
<b>Method of cultivation:</b>	200 µL of a 7 – 9 days old stock culture was transferred into a 250 ml cotton plugged Erlenmeyer flask containing 50 ml of nutrient medium once every week. Stock cultures of algae were kept at 23 ± 2°C with 16 h light/day. All operations were conducted under sterile conditions to handle an axenic algae culture.
<b>Test system</b>	
<b>Stock solution:</b>	210.6 mg test item (=210 mg p.m.) ad 2100 g nutrient medium were prepared immediately prior to test initiation. The stock solution was well agitated for 2 x 10 minutes on a magnetic stirrer and for 2 x 10 minutes in an ultra sonic bath before further use. An adequate amount of the stock solution was transferred to a dilution series to obtain the concentration levels used in the study.
<b>Test concentrations:</b>	0 (control), 6.25, 12.5, 25, 50, and 100 mg p.m./L (nominal initial of the pure metabolite)
<b>Test item application:</b>	The test item was applied into the test medium on day 0.
<b>Test medium (exposure):</b>	Mixture of nutrient medium, inoculated algae cells, added test item
<b>Test duration:</b>	3 days
<b>Test volume:</b>	150 mL test medium per replicate
<b>No. of replicates:</b>	3 replicate vessels per test level (6 replicate vessels per control) and, if needed, additional test vessels for solvent control (6 replicates) and for analytical determination
<b>Test vessels:</b>	The test vessels (300 mL-Erlenmeyer flasks) labelled with the study number, series number, and concentration of the test item were sealed with cotton wool or cellulose plugs and placed in a growth incubator.
<b>Inoculum:</b>	To ensure that the algae used as inoculum were exponentially growing, an inoculum pre-culture was prepared 2 – 4 days before the start of the test and cultivated under the same conditions as in the main test. In order to reach an initial cell density of 10 000 cells/mL in the test medium at the beginning of the 72 hours exposure period of the main test, adequate dilution of the pre-culture was done with nutrient medium.
<b>Density of inoculum:</b>	10 000 cells/mL
<b>Temperature:</b>	nominally 23 ± 2°C
<b>Light intensity:</b>	nominally mean: 4440 – 8880 lux; variation within the test: ±15% lux



- Light regime:** The exposure of individual flasks to permanent light was made more uniform by randomised repositioning after each observation day.
- Aeration:** Test vessels were placed on a tablet rotating 100 rpm to prevent sedimentation of the cells without additional aeration.

□ **Principle of the test:**

*Pseudokirchneriella subcapitata* (freshwater microalgae, formerly known as *Selenastrum capricornutum*), strain SAG 61.81 were exposed in a chronic multigeneration test for 3 days under static exposure conditions to the nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg p.m./L (pure metabolite)/L in comparison to control(s). The pH values ranged from 7.7 to 8.5 in the controls and the incubation temperature ranged from 22.4°C to 23.4°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6619 lux. Quantitative amounts of AE F099095 were measured in all treatment groups and in the control(s) on day 0 and day 3 of the exposure period.

□ **Results:**

Validity Criteria:

The test conditions met all validity criteria, given by the mentioned guideline. The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35% and the coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7% in tests with *Pseudokirchneriella subcapitata*. These criteria were fulfilled.

Analytical findings:

The analytical findings of AE F099095 in the treatment levels found on day 0 were 96 to 102 % of nominal (average 98.0 %) (Table B.9.2.6.6.-1). On day 3 analytical findings of 96 to 103 % of nominal (average 99.6 %) were found. All results are based on nominal test concentrations (Table B.9.2.6.6.-2).

**Table B.9.2.6.6.-1: Concentrations of metabolite AE F099095 in the test solutions at day 0**

Nominal Concentration in mg p.m./L	Day 0			
	Actual Concentration (mg AE F099095/L)			%
	1. Determination	2. Determination	Average	
Control	<0.0110	<0.0110	<0.0110	-
6.25	6.06	5.92	5.99	96
12.5	12.0	12.3	12.2	97
25	25.5	25.4	25.4	102
50	49.4	48.7	49.1	98
100	97.0	96.1	96.5	97
			<b>Mean</b>	<b>98.0</b>

**Table B.9.2.6.6.-2: Concentrations of metabolite AE F099095 in the test solutions at day 3**

Nominal Concentration in mg p.m./L	Day 3			
	Actual Concentration (mg AE F099095/L)			%
	1. Determination	2. Determination	Average	
Control	<0.0110	<0.0110	<0.0110	-
6.25	6.14	6.32	6.23	100
12.5	12.0	12.1	12.0	96
25	25.0	24.9	25.0	100
50	51.8	50.7	51.3	103
100	98.6	99.2	98.9	99
			<b>Mean</b>	<b>99.6</b>

Biological findings:

Observations on growth rates are presented in the **Table B.9.2.6.6.-3**.

**Table B.9.2.6.6.-3: Inhibitory effects**

Nominal initial Concentration (mg p m./L)	Cell Number after 72 h (means) per mL	(0-72h)-Average Specific Growth Rates (days-1)	Inhibition of Average Specific Growth Rate (%)	Doubling time of algae cells (days)
Control	812,000	1.466	--	0.473
6.25	751,000	1.439	1.8	0.482
12.5	806,000	1.463	0.2	0.474
25	788,000	1.455	0.7	0.476
50	727,000	1.428	2.5	0.485
100	660,000	1.396	4.7	0.497

test initiation with 10,000 cells/mL

- ❑ **Conclusion:** The (0 – 72h)-ErC<sub>50</sub> for AE F099095 is > 100 mg pure metabolite /L and the (0 – 72h)-NOErC is 25 mg pure metabolite /L (based on nominal initial concentrations).

- ❑ **Comment (Co-RMS and RMS):** Study considered acceptable.

#### Study on Equip OD 45

#### **B.9.2.6.7. Effect of the product AE F130360 + AE F122006: AE F130360 01 1K05 A304 to the freshwater alga, *Selenastrum capricornutum***

<b>Report:</b>	<u>KCP 10.2.1 /04; Boeri, R. L.; Ward, T. J.; 2000; M-238520-02</u>
<b>Title:</b>	Growth and reproduction toxicity test with the freshwater alga, <i>Selenastrum capricornutum</i> : AE F130360 + AE F122006: AE F130360 01 1K05 A304
<b>Report No:</b>	B002798
<b>Document No:</b>	M-238520-02
<b>Guidelines:</b>	OECD: 201
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** Exposure of the freshwater alga, *Selenastrum capricornutum*, to AE F130360 + AE F122006 oil flowable resulted in a 96-hour ErC<sub>50</sub> greater than 5.0 mg/L, the highest tested concentration, when calculated using the growth rate and a 96-hour E<sub>b</sub>C<sub>50</sub> of 3.5 mg/L (95% confidence interval = 3.1 to 3.9 mg/L) when calculated using the area under the growth curve. The 96-hour NOEC is 1.3 mg/L AE F130360 + AE F122006 oil flowable when calculated using both the growth rate and the area under the growth curve.
- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

#### **B.9.2.7. Effects on aquatic macrophytes**

Potential effects of foramsulfuron, metabolites AE F153745, AE 0338795 and formulations of foramsulfuron (2 different) on *Lemna gibba* growth were investigated. These studies were evaluated within the process of Annex I inclusion and were considered acceptable by the RMS Germany. These studies were not re-evaluated since the studies were performed with different test methods which, however, are in line with OECD test guideline No. 221: *Lemna* sp. Growth Inhibition Test.

Besides *Lemna gibba*, also *Myriophyllum spicatum* was tested as a second macrophyte species. In addition, an outdoor growth inhibition study was performed with a total of ten species representing different taxonomic groups. Since *Lemna gibba* turned out to be the most sensitive species to foramsulfuron, higher-tier studies (recovery, peak exposure, long-term exposure) were performed with this species. These studies are evaluated in this dRAR.

Studies investigating the toxicity to *Lemna gibba* were also performed for all metabolites of the residue definition for risk assessment in surface water. It was found that one metabolite, AE F130619, has a similar activity to Lemna as the parent compound, while all other metabolites turned out to be non-toxic to these organisms. These studies are also evaluated in this dRAR.

Details of studies on foramsulfuron and metabolites are provided briefly in the **Table B.9.2.7.-1** and on formulations of foramsulfuron in the **B.9.2.7.-2**. Summaries of the studies are provided thereafter.

**Table B.9.2.7.-1: Effect data of foramsulfuron and metabolites to aquatic macrophytes**

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
<b>Foramsulfuron-sodium</b>				
<i>Lemna gibba</i> (duck weed)	growth inhibition, static	7 d	ErC <sub>50</sub> <sup>1)</sup> 1.01 µg/L	Christ & Ruff, 1998 A67514 Christ, 1999 C002148 (Amendment) M-147891-02-1 KCA 8.2.7 /01
<i>Lemna gibba</i> (duck weed)	growth inhibition + recovery	7 d + 14 d	NOEC 5 µg/L	Dorgerloh, 2005 MO-05-007405 M-250268-01-1 KCA 8.2.7 /05
<i>Lemna gibba</i> (duck weed)	growth inhibition, peak exposure	1 d + 6 d	ErC <sub>50</sub> <sup>1)</sup> > 56.7 µg/L	Bruns, 2013 EBFSN003 M-462569-01-1 KCA 8.2.7 /06
<i>Lemna gibba</i> (duck weed)	growth inhibition, mimicking exposure of outdoor study	42 d	ErC <sub>50</sub> <sup>1)</sup> 1.18 µg/L	Bruns, 2013 EBFSL014 M-464150-01-1 KCA 8.2.7 /08
Aquatic macrophytes (10 species)	growth inhibition + recovery	2 d + 5.5 weeks	NOEC (6 weeks) 0.1 µg/L NOEC (48 h peak) 4.1 µg/L	Kirkwood, 2012 EBFSL012 M-429538-01-1 KCA 8.2.7 /07
<i>Myriophyllum spicatum</i> (aquatic plant)	growth inhibition	14 d	EC <sub>50</sub> > 84 µg/L	Banman et al., 2012 EBFSL004 M-431270-01-1 KCA 8.2.7 /09
<b>AE F153745</b>				
<i>Lemna gibba</i> (duck weed)	growth inhibition	7 d	EC <sub>50</sub> > 100	Christ & Ruff, 2000 B002765 M-240924-01-2 KCA 8.2.7 /02
<b>AE 0338795</b>				
<i>Lemna gibba</i> (duck weed)	growth inhibition	7 d	ErC <sub>50</sub> <sup>1)</sup> 27.2	Christ & Ruff, 2000 B002774 M-238498-01-2 KCA 8.2.7 /03
<b>AE F092944</b>				
<i>Lemna gibba</i> (duck weed)	growth inhibition	7 d	ErC <sub>50</sub> <sup>1)</sup> > 100	Sowig, 2002 C003865 M-186916-01-1 KCA 8.2.7 /10
<b>AE F099095</b>				
<i>Lemna gibba</i>	growth inhibition	7 d	ErC <sub>50</sub> <sup>1)</sup> > 100	Dorgerloh, 2005

Test species	Test system	Test duration	Endpoint [mg as/L]	Reference
(duck weed)				EBMMX091 <u>M-254496-01-1</u> KCA 8.2.7 /11
<b>AE F130619</b>				
<i>Lemna gibba</i> (duck weed)	growth inhibition	7 d	$E_rC_{50}^{1)}$ <b>0.889 µg/L</b>	Bruns, 2013 EBFSL011 <u>M-452669-01-1</u> KCA 8.2.7 /12
<b>4-Amino-N-methylbenzamide</b>				
<i>Lemna gibba</i> (duck weed)	growth inhibition	7 d	$E_rC_{50}^{1)}$ > 10	Bruns, 2013 EBFSN010 <u>M-464163-01-1</u> KCA 8.2.7 /13
<b>4-Formylamido-N-methylbenzamide</b>				
<i>Lemna gibba</i> (duck weed)	growth inhibition	7 d	$E_rC_{50}^{1)}$ > 10	Bruns, 2013 EBFSN011 <u>M-464321-01-1</u> KCA 8.2.7 /14
<b>Foramsulfuron-sulfamic acid</b>				
<i>Lemna gibba</i> (duck weed)	growth inhibition	7 d	$E_rC_{50}$ > 10	Hoffmann, 2013 EBFSN012 <u>M-464386-01-1</u> KCA 8.2.7 /15

**Bold letters:** Values considered relevant for risk assessment

<sup>1)</sup> Since the new aquatic GD<sup>3</sup> focusses on endpoints based on growth rates the old  $E_bC_{50}$  figures were omitted from the table above.

**Table B.9.2.7.-2: Effect data of formulations of foramsulfuron to aquatic macrophytes**

Test species	Test system	Test duration	Endpoint [µg /L]	Reference
<b>Equip OD 45</b>				
<i>Lemna gibba</i> (duck weed)	growth inhibition, static	7 d	$E_rC_{50}^{1)}$ <b>1.01 µg/L</b> NOEC 0.225 µg/L	Boeri, R.; Wyskiel, D.; Ward, T.; 2000 M-238581-01 KCP 10.2.1 /05
<i>Lemna gibba</i> (duck weed)	growth inhibition, static	7 d	$E_rC_{50}^{1)}$ 1.56 µg a.s./L <sup>2)</sup> NOEC >3.08 µg a.s./L <sup>3)</sup>	Banman, C. S.; Hoffmann, J. M.; Lam, C. V.; 2008 M-296352-01- KCP 10.2.1 /09;
<i>Lemna gibba</i> (duck weed)	growth inhibition, static	7 d	$E_rC_{50}^{1)}$ 1.1 µg a.s./L NOEC 0.2 µg a.s./L	Hoberg, J. R.; 2002 M-240877-01 KCP 10.2.1 /08
<b>Formulation AE F130360 + AE F122006 + AE F115008</b>				
<i>Lemna gibba</i> (duck weed)	growth inhibition static renewal	7 d	$E_rC_{50}^{1)}$ 2.8 µg/L NOEC 1.0 µg/L	Madsen, T. J.; Bussard, J. B.; 2000 M-238567-01 KCP 10.2.1 /06
<i>Lemna gibba</i> (duck weed)	growth inhibition static renewal	7 d	$E_rC_{50}^{1)}$ 4-8 µg/L NOEC 1.0 µg/L	Madsen, T. J.; Bussard, J. B.; 2000

<sup>3</sup> 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 [µg /L]	Reference
				M-238536-01 KCP 10.2.1 /07

<sup>1)</sup> Since the new aquatic GD<sup>4</sup> focusses on endpoints based on growth rates the old E<sub>b</sub>C<sub>50</sub> figures were omitted from the table above.

<sup>2)</sup> Based on frond number

<sup>3)</sup> Based on dry weight

### Studies on foramsulfuron

#### B.9.2.7.1. Effects of the active substance foramsulfuron on aquatic plant duckweed (*Lemna gibba*)

<b>Report:</b>	<u>KCA 8.2.7 /01; Christ, M. T.; Ruff, D. F.:1998; M-147891-02; Amended: 1999-04-20</u>
<b>Title:</b>	Effect to <i>Lemna gibba</i> (duckweed), in a growth inhibition test AE F130360 technical 96.1% w/w Code: AE F130360 00 1C96 0002
<b>Report No:</b>	A67514
<b>Document No(s):</b>	Report includes Trial Nos.: CF98W507 <u>M-147891-02-1</u>
<b>Guidelines:</b>	USEPA (=EPA): 122-2; Deviation not specified
<b>GLP/GEP:</b>	yes

#### □ Conclusions:

Since the new aquatic guidance document (EFSA 2013) only regards endpoints based on growth rates as relevant, the biomass based endpoint of EC<sub>50</sub> = 0.00065 mg/L according to the Review Report for foramsulfuron (SANCO/10324/2002-Final) has to be revised and replaced by 0.00101 mg/L.

#### □ Comment (Co-RMS and RMS):

Refer to original EU review. No new data or assessments of the study are provided.

#### B.9.2.7.2. Exposure and recovery test with foramsulfuron (tech.) on *Lemna gibba* G3

<b>Report:</b>	<u>KCA 8.2.7 /05; Dorgerloh, M.:2005; M-250268-01</u>
<b>Title:</b>	<i>Lemna gibba</i> G3 Exposure and recovery test with Foramsulfuron (tech.) (code: AE F130360 00 1D97 0001)
<b>Report No:</b>	EBFSX010
<b>Document No:</b>	<u>M-250268-01-1</u>
<b>Guidelines:</b>	OECD 221 " <i>Lemna</i> sp. Growth Inhibition Test" Revised Proposal for a New Guideline (April 2004); none
<b>GLP/GEP:</b>	yes

#### □ Summary:

Aim of this study was to determine the effects of the test item foramsulfuron on exponentially growing *Lemna gibba* G3 after static exposure of 7 days. *Lemna* cultures were cultivated for 7 days at 0.625, 1.25, 2.50, 5.00, 10.0, and 20.0 µg a.s./L under static conditions. In addition an untreated control was tested. 3 replicates were used per treatment level (12 fronds/replicate). Plant frond numbers and total frond area of plants were recorded after 0, 2 or 3, 4 or 5, and 7 days. Growth and growth inhibition in percent were calculated. In the second part of the study aliquots were transferred into freshly prepared

<sup>4</sup> 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 medium without the test item and the growth rates during the recovery phase were measured. Furthermore, recovery of visual effects of treated plants was evaluated. The growth rates for frond numbers and total front area fully recovered for all test levels (up to 20 µg a.s./L) within the first phase of the recovery period (day 7 – 14). The previously treated plants fully recovered up to the treatment level of 5 µg a.s./L within the second phase of the recovery period (day 14 – 21).

□ **Material and methods**

<b>Test item:</b>	Foramsulfuron (AE F130360) a.s.
<b>Batch No.:</b>	AAIR04430
<b>CAS No.:</b>	173159-59-4
<b>Analysed content of a.s.:</b>	97.3 % w/w
<b>Certificate No.:</b>	AZ 11043
<b>Test organism</b>	
<b>Species:</b>	<i>Lemna gibba</i> , strain G3
<b>Origin:</b>	Dr. Janet Slovin, Horticulture Crops Quality Laboratory, U.S. Department of Agriculture, Beltsville, MD 20705, U.S.A.
<b>Method of cultivation:</b>	Stock cultures are maintained in glass dishes filled with growth medium under illumination of 6.50 – 10.0 klx and temperature of 24 ± 2°C for a minimum of three weeks. Transfers are made regularly into fresh medium to provide 7 – 10 days old colonies as test inoculum. All operations were conducted under sterile conditions to sustain an axenic <sup>1</sup> algae culture.
<b>Growth medium:</b>	The medium (20X-AAP), freshly prepared
<b>Test system for the exposure phase</b>	
<b>Stock solution:</b>	123.6 mg foramsulfuron ad 500 mL growth medium prepared immediately prior to the test. The stock solution was well agitated on a magnetic stirrer for 15 minutes and treated in an ultrasonic bath for 10 minutes before further use. An adequate amount of the stock solution was transferred to a dilution series to obtain the concentration levels used in the study.
<b>Test concentrations:</b>	0 (control), 0.625, 1.25, 2.50, 5.00, 10.0, and 20.0 µg a.s./L
<b>Selection of test levels:</b>	The range of test concentrations was selected based on pre experiments in order to reach a significant growth inhibition in the lowest treatment level and ErCx (to cover preferably the range up to 75% growth rate inhibition).
<b>Exposure conditions:</b>	Static
<b>Test item application:</b>	The test item was applied into the freshly prepared test medium on day 0.
<b>Test medium (exposure):</b>	Mixture of nutrient medium and added test item
<b>Test duration:</b>	7 days
<b>Test volume:</b>	200 mL test medium per replicate
<b>No. of replicates:</b>	3 replicate vessels per test level and 3 replicate vessels per control
<b>Test vessels:</b>	Glass dishes were used with a diameter of 10 cm and a height of 6 cm (total volume of approx. 470 mL). These test vessels were covered with lids of glass to permit gas exchange and illumination under sterile conditions. All dishes were labelled with study number, a vessel number, and test concentration were placed in a growth incubator.
<b>Inoculum:</b>	To ensure that the plants used as inoculum are exponentially growing, an inoculum pre-culture will be prepared 7 – 10 days before the start of the test and cultivated under the same conditions as in the main test.
<b>Density of inoculum:</b>	9 to 12 fronds per vessel
<b>Temperature:</b>	nominally 24 ± 2°C
<b>Light intensity:</b>	nominally 6.50 – 10.0 klx (±15% variation from the mean)
<b>Light regime:</b>	The flasks were exposed to permanent light and repositioned in randomised order after each observation day.
<b>Test system for the recovery phase</b>	
<b>Exposure conditions:</b>	static (renewal of test medium after 7 days to prevent starving)
<b>Test duration:</b>	2 x 7 days

<b>Test volume:</b>	200 mL test medium per replicate
<b>No. of replicates:</b>	3 replicate vessels per test level and 3 replicate vessels per control
<b>Test vessels:</b>	Glass dishes were used with a diameter of 10 cm and a height of 6 cm (total volume of approx. 470 mL). These test vessels were covered with lids of glass to permit gas exchange and illumination under sterile conditions. All dishes were labelled with study number, a vessel number, and test concentrations were placed in a growth incubator.
<b>Inoculum:</b>	The plants were taken from the treated groups of the exposure phase. If possible, 3 plants with 4 fronds or 4 plants with 3 fronds were rinsed with deionised water and transferred into vessels with freshly prepared growth medium without test medium.
<b>Temperature:</b>	nominally $24 \pm 2^\circ\text{C}$
<b>Light intensity:</b>	nominally 6.50 – 10.0 klx ( $\pm 15\%$ variation from the mean)
<b>Light regime:</b>	The flasks were exposed to permanent light and repositioned in randomised order after each observation day.

#### Observations

Following observations were made on days 2, 5, and 7 of the exposure phase and on days 2, 5, 7, 9, 12, and 14 of the recovery phase: observation of changes in plant development (e.g. frond size, appearance, necrosis, chlorosis or gibbosity, colony break-up or loss of buoyancy, root length, morphology or breakdown)

#### □ Principle of the test:

3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed under static conditions (7-day-exposure phase of the study) to the nominal concentrations of 0.625, 1.25, 2.50, 5.00, 10.0, and 20.0 µg a.s./L in comparison to control to reach a graduated inhibition of growth around the expected  $\text{ErC}_{50}$ . In the second part of the study (14-day recovery phase (post exposure phase)), aliquots (12 fronds/replicate) were transferred (by rinsing with deionised water) into freshly prepared test medium without foramsulfuron. As the recovery phase lasted 14 days, the growth medium was renewed on day 7 and the culture was re-started with 12 fronds of the recovery phase to prevent starvation. The pH values ranged from 7.4 to 8.8 in the controls and the incubation temperature ranged from  $23.3^\circ\text{C}$  to  $24.4^\circ\text{C}$  (measured in an additional incubated glass vessel) over the whole period of testing, at a continuous illumination of 7.29 klx.

Foramsulfuron was quantitatively measured in all freshly prepared test levels on day 0 and, additionally, in all aged test levels on day 7 of the exposure period. Additional measurements for foramsulfuron were done for all test levels at day 2 of the recovery phase, to show that no unintended transfer of the test item into the recovery phase occurred.

#### □ Results

##### Validity Criteria:

The test conditions met all validity criteria, given by the mentioned guideline. Mean doubling time (d) of frond number in the control: 2.0; mean growth rate  $\mu$  [1/d] (0→7 d) was 0.353; static conditions.

##### Analytical findings:

Analytical measurements for AE F130360 found in all freshly prepared test levels on day 0 in reference to nominal concentrations ranged between 89% and 113% (average 104 %). In aged test levels on day 7 there were analytical findings between 94% and 112% (average 103 %) of nominal. As expected in samples taken on day 2 of the post exposure period, the test item was not detectable in any test level including the control. All reported results are based on nominal initial values of the active substance during the exposure period (Table B.9.2.7.2.-1).

**Table B.9.2.7.2.-1: Concentrations of AE F130360 in the test solutions**

Day	Nominal concentration [µg a.s./L]	Detection 1 [µg a.s./L]	Detection 2 [µg a.s./L]	Mean [µg a.s./L]	% of nominal
0	Control	< 0.070	< 0.070	< 0.070	--
7		< 0.070	< 0.070	< 0.070	--

Day	Nominal concentration [µg a.s./L]	Detection 1 [µg a.s./L]	Detection 2 [µg a.s./L]	Mean [µg a.s./L]	% of nominal
9*		< 0.070	< 0.070	< 0.070	--
0	0.625	0.694	0.714	0.704	113
7		0.705	0.630	0.667	107
9*		< 0.070	< 0.070	< 0.070	--
0	1.25	1.24	1.32	1.28	102
7		1.39	1.42	1.40	112
9*		< 0.070	< 0.070	< 0.070	--
0	2.50	2.46	2.65	2.55	102
7		2.29	2.39	2.34	94
9*		< 0.070	< 0.070	< 0.070	--
0	5.00	5.09	5.25	5.17	103
7		5.00	5.13	5.06	101
9*		< 0.070	< 0.070	< 0.070	--
0	10.00	8.84	8.94	8.89	89
7		9.58	10.4	10.0	100
9*		< 0.070	< 0.070	< 0.070	--
0	20.00	22.9	22.2	22.6	113
7		21.4	20.6	21.0	105
9*		< 0.070	< 0.070	< 0.070	--

lowest standard solution (concentration multiplied with the dilution factor of 1.25) of foramsulfuron used for determination: 0,070 µg/L

\*day 2 post exposure

#### Biological findings:

Inhibitory effects and observed intoxication symptoms are summarized in **Table B.9.2.7.2.-2**.

**Table B.9.2.7.2.-2: Inhibitory effects and intoxication symptoms**

nominal µg a.s./L	7 day - % inhibition growth rate frond #	7 days - % inhibition growth rate frond area	first day when full recovery acc. growth was observed	first day when full recovery acc. symptoms was observed
0.625	49.0	52.0	12	16
1.25	70.3	69.1	9	16
2.5	74.9	75.7	9	16
5	78.1	80.3	12	19
10	83.6	84.0	12	
20	78.7	85.1	12	

The following observations were made: small fronds, deformed fronds and fronds clustered.

- ❑ **Conclusions:** The growth rates for frond number and total frond area fully recovered for all test levels (up to 20 µg a.s./L) within the first phase of the recovery period (study days 7 – 14).

Fronds fully recovered from all visual effects (reduction of size, deformation, decolouration and necrosis) up to 5µg/L (formerly used test level) after 14 days.

**NOEC = 5 µg a.s./L**

- ❑ **Comment (Co-RMS and RMS):** Study considered acceptable.

#### **B.9.2.7.3. Growth inhibition test with foramsulfuron under peak exposure conditions on *Lemna gibba* G3**



<b>Report:</b>	<u>KCA 8.2.7 /06;Bruns, E.;2013;M-462569-01</u>
<b>Title:</b>	<i>Lemna gibba</i> G3 - Growth inhibition test with foramsulfuron (tech) (AE F 130360) under peak exposure conditions
<b>Report No:</b>	EBFSN003
<b>Document No:</b>	<u>M-462569-01-1</u>
<b>Guidelines:</b>	<b>EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4400; Plants were firstly washed and then dipped into clean media. All plants were transferred.</b>
<b>GLP/GEP:</b>	yes

#### □ Summary

The aim of the study was to determine the effects of foramsulfuron (code: AE F130360; purity 97.3 %) on the growth of duck weed (*Lemna gibba* G3) after a 24-hour peak exposure. Cultures of *Lemna gibba* with an initial frond density of 12 fronds per vessel were exposed in a static system over a period of 7 days (1 day exposure; 6 days recovery) to nominal concentrations of 0.50, 1.10, 2.42, 5.32, 11.7, 25.8 and 56.7 µg a.s./L (corresponding to analytically verified concentrations of 115 % (mean at day 0) and 114 % (mean in aged solutions at day 1). In addition a water control was tested.

Frond numbers and total frond area at each occasion were used to determine the endpoints. Based on analytical findings, the biological endpoints are reported as nominal figures. The EC<sub>50</sub> regarding growth inhibition was > 56.7 µg a.s./L for both, frond number and dry weight. The NOEC for growth during the period between day 2 and day 7 was determined to be 2.42 µg a.s./L.

#### □ Materials and Methods

**Test item:** foramsulfuron tech. (AE F130360)  
**Analysed content of active substance:** foramsulfuron tech. (AE F130360): 97.3 % w/w  
**Specified by origin batch no:** ELIR004294  
**Specification number:** 102000011654  
**Tox No.:** 09600-00  
**Visual appearance:** white powder

#### Treatments

**Test concentrations:** Nominal concentrations were 0.50, 1.10, 2.42, 5.32, 11.7, 25.8 and 56.7 µg a.s./L. In addition a water control was tested.

**Analysis of test concentrations:**  
 in all freshly prepared test levels on day 0 and 1, and additionally in all aged levels on day 1 and 7 of the exposure period

#### Test organism:

**Species:** Duck weed (*Lemna gibba*), strain G3  
**Source:** Dr. Janet Slovin, Horticulture Crops Quality Laboratory, U.S. Department of Agriculture, Beltsville, MD 20705, U.S.A.

**Method of cultivation:** Stock cultures are maintained in glass dishes filled with nutrient medium under illumination of 6500 – 10000 lux and a temperature of 24 ± 2°C. Transfers into fresh nutrient medium are made regularly every 7 – 10 days. All operations were conducted under sterile conditions to sustain an axenic culture.

**Nutrient medium:** the 20X AAP medium, freshly prepared

#### Test system

**Stock solution:** 50.0 mg of the test substance ad 500 mL nutrient medium was prepared immediately prior to the test. An adequate amount of the stock solution was transferred to a dilution series to obtain the concentration levels used in the study.

**Test concentrations:** 0 (control), 0.50, 1.10, 2.42, 5.32, 11.7, 25.8 and 56.7 µg a.s./L  
**Selection of test levels:** The range of test concentrations was selected based on pre experiments in order to define the NOErC, LOErC and ErCx (to cover preferably the range up to 75% growth rate inhibition).

**Exposure conditions:** peak exposure

<b>Exposure regime:</b>	a static system
<b>Test item application:</b>	The test item was applied into the freshly prepared test medium on day 0 after a 24 h peak, the plants were transferred into clean media.
<b>Test medium (exposure):</b>	Mixture of nutrient medium and added test item
<b>Test duration:</b>	7 days (1 day exposure; 6 days recovery)
<b>Test volume:</b>	200 mL test medium per replicate
<b>No. of replicates:</b>	3 replicate vessels per test level and 3 replicate vessels per control
<b>Number plants/replicate:</b>	at test initiation the number of fronds was 12 fronds per vessel
<b>Test vessels:</b>	Glass dishes were used with a diameter of 10 cm and a height of 6 cm (total volume of approx. 470 mL). These test vessels were covered with lids of glass to permit gas exchange and illumination under sterile conditions to the greatest possible extent. All dishes labelled with study number, a vessel number, and test concentrations were placed in a growth incubator. Each vessel (glass dishes; 470 mL) served as one replicate filled with 200 mL 20xAAP with an initial pH of $7.5 \pm 0.1$
<b>Inoculum:</b>	Stock cultures are maintained in glass dishes filled with nutrient medium under illumination of 6500 – 10000 lux and a temperature of $24 \pm 2^\circ\text{C}$ . Transfers into fresh nutrient medium are made regularly every 7 – 10 days. All operations were conducted under sterile conditions to sustain an axenic culture.
<b>Density of inoculum:</b>	12 fronds per vessel
<b>Environmental conditions</b>	
<b>Temperature:</b>	mean $25.1^\circ\text{C}$ (range: $25.0^\circ\text{C}$ to $25.3^\circ\text{C}$ )
<b>pH:</b>	7.5 to 8.4 during peak exposure (day 1) and 7.5 to 8.9 during recovery period
<b>Lighting:</b>	mean 6633 lux (range: 6501 to 6739 lux)

#### □ Results:

##### Analytical results:

Analytical verification of test solutions revealed measured concentrations of 115 % (mean at day 0) and 114 % (mean in aged solutions at day 1) calculated as arithmetic mean. Based on these analytical findings, the biological endpoints are reported as nominal figures (Table B.9.2.7.3.-1).

**Table B.9.2.7.3.-1: Concentrations of AE F130360 in test solutions**

Nominal test levels [ $\mu\text{g a.s./L}$ ]	measured day 0		measured day 1 (aged media)		measured day 7 (old)	
	$\mu\text{g a.s./L}$	% nominal	$\mu\text{g a.s./L}$	% nominal	$\mu\text{g a.s./L}$	% nominal
control	<0.051	-	<0.051	-		
0.5	0.587	117	0.566	113	<0.051	n.a.
1.1	1.28	116	1.26	114	<0.051	n.a.
2.42	2.94	122	2.97	123	<0.051	n.a.
5.32	6.38	120	6.22	117	<0.051	n.a.
11.7	12.9	110	13.1	112	<0.051	n.a.
25.8	28.5	111	28.3	110	<0.051	n.a.
56.7	62.6	110	61.1	108	<0.051	n.a.
	<b>Mean</b>	<b>115%</b>		<b>114%</b>		

##### Biological results:

Growth inhibition is presented in Table B.9.2.7.3.-2.

**Table B.9.2.7.3.-2: Survey of biological findings**

Nominal test levels [ $\mu\text{g a.s./L}$ ]	Final frond no. (replicate means, day 7)	Final total frond area of plants (replicate means) [ $\text{mm}^2$ ] day 7	% inhibition (growth rate for frond no.)	% inhibition (growth rate for total frond area of plants)
control	209.7	1643.7	--	--
0.5	214	1568.7	-0.8	1.2
1.1	180.3	1331.7	5.2	9
2.42	131.3	989.0	16.2	16.3
5.32	106.7	792.0	23.6	23.2
11.7	107.3	783.3	23.3	26.5
25.8	94.0	662.0	28.1	34.1
56.7	84.7	599.0	31.7	35.6

- % inhibition: increase in growth relative to the control

The validity criterion of a doubling time less than 60 hours (2.5 days) in the control is fulfilled.

Observed visual effects on *Lemna gibba*: no visual effects observed.

**□ Conclusion:**

From the results presented in **Table B.9.2.7.3.-2** above the following biological endpoints can be derived:

**7-day-figures (growth rate frond number):**

highest concentration with no effect (NOEC) (day 0-7):	0.5 $\mu\text{g a.s./L}$
highest concentration with no effect (NOEC) (day 2-7):	2.42 $\mu\text{g a.s./L}$
EC <sub>50</sub> :	> 56.7 $\mu\text{g a.s./L}$

**7-day-figures (growth rate frond area):**

highest concentration with no effect (NOEC) (day 0-7):	0.5 $\mu\text{g a.s./L}$
highest concentration with no effect (NOEC) (day 2-7):	2.42 $\mu\text{g a.s./L}$
EC <sub>50</sub> :	> 56.7 $\mu\text{g a.s./L}$

The EC<sub>50</sub> regarding growth inhibition was > 56.7  $\mu\text{g a.s./L}$  for both, frond number and dry weight. The NOEC for growth between day 2 and 7 was determined to be 2.42  $\mu\text{g a.s./L}$ . After a 24-hour peak exposure up to 2.42  $\mu\text{g a.s./L}$  the growth rate of duck weed does not differ significantly from an untreated control. Therefore, this NOEC can be regarded as relevant for the risk assessment.

- Comment (Co-RMS and RMS):** Study considered acceptable.

**B.9.2.7.4. *Lemna gibba* G3 - Prolonged growth inhibition test with foramsulfuron (AE F130360) with stepwise decreasing concentrations over an 6 week test duration**

<b>Report:</b>	<u>KCA 8.2.7 /08;Bruns, E.:2013;M-464150-01</u>
<b>Title:</b>	<i>Lemna gibba</i> G3 - Prolonged growth inhibition test with foramsulfuron (AE F130360) with stepwise decreasing concentrations over an 6 week test duration
<b>Report No:</b>	EBFSL014
<b>Document No:</b>	<u>M-464150-01-1</u>
<b>Guidelines:</b>	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4400; none
<b>GLP/GEP:</b>	yes

## □ Summary

The aim of the study was to determine the long-term influence over a total period of six weeks of the test item foramsulfuron 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 objective of this study was to obtain 6-week endpoints for *Lemna gibba* by mimicking the outdoor-concentrations under laboratory conditions. These endpoints are directly comparable to endpoints obtained from the outdoor-pond study (KCA 8.2.7 /07; Kirkwood, A.; 2012; M-429538-01-1).

## □ Materials and Methods

**Test item:** foramsulfuron tech. (AE F130360)

**Analysed content of active substance:** foramsulfuron tech. (AE F130360): 97.3 % w/w

**Specified by origin batch no:** ELIR004294

**Specification number:** 102000011654

**Tox No.:** 09600-00

### Test organism

**Species:** *Lemna gibba*, strain G3

**Origin:** Dr. Janet Slovin, Horticulture Crops Quality Laboratory, U.S. Department of Agriculture, Beltsville, MD 20705, U.S.A.

**Method of cultivation:** Stock cultures are maintained in glass dishes filled with nutrient medium under illumination of 6500 – 10000 lux and a temperature of  $24 \pm 2^{\circ}\text{C}$ . Transfers into fresh nutrient medium are made regularly every 7 – 10 days. All operations were conducted under sterile conditions to sustain an axenic culture.

**Growth medium:** The 20X AAP medium

### Test system

**Stock solution:** Week 1: 50.1 mg of foramsulfuron ad 500 mL growth medium was prepared immediately prior to the test.  
Week 2: 50.0 mg of foramsulfuron ad 500 mL growth medium was prepared immediately prior to the test.  
Week 3: 50.1 mg of foramsulfuron ad 500 mL growth medium was prepared immediately prior to the test.  
Week 4: 50.0 mg of foramsulfuron ad 500 mL growth medium was prepared immediately prior to the test.  
Week 5: 50.1 mg of foramsulfuron ad 500 mL growth medium was prepared immediately prior to the test.  
Week 6: 50.1 mg of foramsulfuron ad 500 mL growth medium was prepared immediately prior to the test.

The stock solutions were well agitated on a magnetic stirrer for approximately 100 – 130 minutes and sonicated for approximately 15 – 30 minute before further use. An adequate amount of the stock solution was transferred to a dilution series to obtain the concentration levels used in the study.

**Test concentrations:** Week 1: 0.20, 0.40, 0.80, 1.60 and 3.20 µg a.s. /L from day 0 to day 7.  
Week 2: 0.156, 0.312, 0.624, 1.25 and 2.50 µg a.s. /L from day 7 to day 14 (78.1% of the concentrations of week 1)  
Week 3: 0.121, 0.241, 0.483, 0.965 and 1.93 µg a.s. /L from day 14 to day 21 (60.3% of the concentrations of week 1)  
Week 4: 0.108, 0.216, 0.432, 0.864 and 1.73 µg a.s. /L from day 21 to day 28 (54.0% of the concentrations of week 1)

	Week 5: 0.097, 0.193, 0.387, 0.774 and 1.55 µg a.s. /L from day 28 to day 35 (48.4% of the concentrations of week 1)
	Week 6: 0.081, 0.161, 0.322, 0.644 and 1.29 µg a.s. /L from day 35 to day 42 (40.3% of the concentrations of week 1)
<b>Selection of test levels:</b>	The range of test concentrations was selected based on concentrations determined in an 6-week outdoor pond study to simulate the dissipation of the test substance over a period of 6-weeks.
<b>Exposure conditions:</b>	static- renewal
<b>Test item application:</b>	The test item was applied into the freshly prepared test medium on day 0, 7, 14, 21, 28, 35 and 42.
<b>Test medium (exposure):</b>	Mixture of nutrient medium and added test item
<b>Test duration:</b>	6 times 7 days
<b>Test volume:</b>	200 mL test medium per replicate
<b>No. of replicates:</b>	3 replicate vessels per test level and 3 replicate vessels per control.
<b>Test vessels:</b>	Glass dishes were used with a diameter of 10 cm and a height of 6 cm (total volume of approx. 470 mL). These test vessels were covered with lids of glass to permit gas exchange and illumination under sterile conditions to the greatest possible extent. All dishes, labelled with the study number, a vessel number, and the test concentrations were placed in a growth incubator.
<b>Inoculum:</b>	To ensure that the plants used as inoculum are exponentially growing, an inoculum pre-culture will be prepared 7 – 10 days before the start of the test and cultivated under the same conditions as in the main test.
<b>Density of inoculum:</b>	12 fronds per vessel
<b>Temperature:</b>	24°C ± 2°C
<b>Light intensity:</b>	6500 – 10000 lux ± 15% variation from the mean
<b>Light regime:</b>	The flasks were exposed to permanent light and repositioned in randomised order after each observation.
<b>Observations</b>	Visual observations were made on study days 2, 4, and 7.
<b>Measurements:</b>	
<b>Temperature:</b>	All test vessels were placed under isothermal conditions and the temperature was determined by a continuous measurement in one additional incubated glass vessel filled with the same amount of de-ionised water as in the test vessels. Temperature was recorded hourly by a data logger.
<b>pH-values:</b>	The pH-values were measured on day 0 and 7 of every week in all test levels and the control by an electronic pH meter.
<b>Light:</b>	The light was measured at least once during the test using an electric luxmeter.
<b>Biomass quantification:</b>	Counting of fronds and determination of total frond area was carried out using the LemnaTec Scanalyzer machine, validated for such measurements.
<b>Test item analysis:</b>	Samples were analysed for the actual concentration of foramsulfuron present in all freshly prepared test levels at the start day and in all aged test levels after 7 days of the exposure period. Aliquots for freshly prepared test levels for start-day-sample analyses were sampled from the prepared volume of each test treatment level. For sampling of aged test media, after removing of plant material from the test vessels after the 7 day test period, the contents of all replicate vessels were combined, and the pH was determined. The combined test solutions were then submitted for analyses.
<b>Validity criteria:</b>	For the test to be valid, the following performance criteria should be met: the frond number in the control must increase by a factor of 7 corresponding to a doubling time (TD) of about 2.5 days.

❑ **Principle of the test:**

3 x 12 fronds of *Lemna gibba* G3 per test concentration were exposed in a chronic multi-generation test for six times 7 days under static exposure conditions to the nominal concentrations of the following listed concentrations. The concentrations were derived from the analytical results of an outdoor pond-study (*Lemna gibba* could not be tested under outdoor-conditions).

The objective of this study was to obtain 6-week endpoints for *Lemna gibba* by mimicking the outdoor-concentrations under laboratory conditions.

After each week, preferably 12 fronds were transferred into the respective following concentration (e.g. fronds from the samples of 3.20 µg/L, the highest concentration of week 1, were transferred into the replicates of 2.50 µg/L, the highest concentration of week 2, fronds from the test concentration of 0.20 µg/L, the lowest concentration of week 1, were transferred into the replicates of 0.080 µg/L, the lowest concentration of week 2, etc. In cases where the number of fronds after a 7-day period was below 12 due to damages caused by the tested substance only the remaining fronds were transferred.

**Table B.9.2.7.4.-1: Intended concentrations per week and treatment level**

nominal initial test levels foramsulfuron [µg /L]	week 1	week 2	week 3	week 4	week 5	week 6
% of week 1*	100	78.1	60.3	54.0	48.4	40.3
0.20	0.20	0.156	0.121	0.108	0.097	0.081
0.40	0.40	0.312	0.241	0.216	0.193	0.161
0.80	0.80	0.624	0.483	0.432	0.387	0.322
1.60	1.60	1.25	0.965	0.864	0.774	0.644
3.20	3.20	2.50	1.93	1.73	1.55	1.29

\* Percentage figures in this row were obtained from analytical measurements in the outdoor pond study. The intended concentrations were derived as the respective percentages of each nominal initial concentration.

#### □ Results:

##### Environmental conditions:

Temperature varied between 23.8 and 24.4°C. pH varied between 7.5 and 7.7 at the start of each 7-day period and between 8.5 and 9.2 at the end of each 7-day period. Mean light intensity was 8038 Lux.

##### Analytical results:

Analytical findings of foramsulfuron are presented in **Table B.9.2.7.4.-2**.

**Table B.9.2.7.4.-2: Analytical findings of foramsulfuron**

	day 0	day 7
week1	103 and 114 % (average 109 %)	108 and 116 % (average 111 %)
week 2	105 and 114 % (average 110 %)	110 and 135 % (average 123 %)
week 3	160 and 165 % (average 162 %)	159 and 182 % (average 171 %)
week 4	105 and 108 % (average 106 %)	107 and 121 % (average 115 %)
week 5	108 and 160 % (average 126 %)	108 and 161 % (average 130 %)
week 6	104 and 106 % (average 106 %)	106 and 116 % (average 109 %)

According to the objective of this study the endpoints were referred to nominal initial test concentrations and not to weekly treatment levels. As in weeks three and five the analytical recovery was > 120% concentrations were expressed as mean measured, while in the other weeks nominal figures were used.

##### Biological results:

Weekly inhibition with regard to the mean growth rates of frond numbers are presented in **Table B.9.2.7.4.-3**.



**Table B.9.2.7.4.-3: Weekly inhibition with regard to the mean growth rates of frond numbers**

nominal initial test levels	% inhibition of mean growth rate of frond numbers					
Foramsulfuron [ $\mu\text{g/L}$ ]	week 1	week 2	week 3	week 4	week 5	week 6
% of week 1	100	78.1	60.3	54.0	48.4	40.3
control	--	--	--	--	--	--
0.20	9.3	-2.0	3.9	5.0	3.5	10.2
0.40	25.0	6.2	12.7	10.2	8.0	10.3
0.80	49.3	47.9	53.8	35.5	30.8	16.5
1.60	57.6	69.2	80.2	75.4	84.3	76.9
3.20	64.6	75.6	95.2	89.2	98.2	99.3
NOEC	<0.20	0.20	0.199	<0.20	0.257	<0.20
EC <sub>50</sub>	1.22	1.08	0.813	1.03	0.579	<b>1.18</b>

Weekly inhibition with regard to the mean growth rates of frond areas are presented in **Table B.9.2.7.4.-4**.

**Table B.9.2.7.4.-4: Weekly inhibition with regard to the mean growth rates of frond areas**

nominal initial test levels	% inhibition of mean growth rate of frond area					
foramsulfuron [ $\mu\text{g/L}$ ]	week 1	week 2	week 3	week 4	week 5	week 6
% of week 1	100	78.1	60.3	54.0	48.4	40.3
control	--	--	--	--	--	--
0.20	6.4	1.0	1.2	2.0	3.3	9.3
0.40	24.1	15.0	8.5	3.7	6.6	6.1
0.80	54.2	61.7	61.8	24.4	20.5	12.0
1.60	64.7	86.4	91.9	96.3	79.8	75.9
3.20	74.4	92.2	100.4	118.2	95.9	98.0
NOEC	<0.20	0.2	0.199	0.80	0.257	n.d.
EC <sub>50</sub>	0.960	0.712	0.728	0.972	0.644	<b>1.23</b>

#### □ Conclusion:

The six week exposure of *Lemna gibba* to foramsulfuron led to decreasing effects when the dissipation of foramsulfuron in a static water-sediment system is mimicked.

Based on initial nominal concentrations the following 6-week endpoints can be derived (**Table B.9.2.7.4.-5**):

**Table B.9.2.7.4.-5: Endpoints obtained after the 6-week test period**

6-week end point	mean growth rate	
	effect on frond no. [ $\mu\text{g a.s./L}$ ]	effect on total frond area of plants [ $\mu\text{g a.s./L}$ ]
EC <sub>50</sub> (CI 95%)	<b>1.18</b> (0.746 – 1.77)	<b>1.23</b> (0.903 – 1.56)
EC <sub>20</sub> (CI 95%)	0.830 (0.200 – 1.10)	0.901 (0.429 – 1.13)
EC <sub>10</sub> (CI 95%)	0.691 (0.0901 – 0.956)	0.691 (0.277 – 0.998)
LOEC	<0.20	<0.20
NOEC	<0.20	<0.20

n.d.: not determined due to mathematical reasons or inappropriate data

- **Comment (Co-RMS and RMS):** study is acceptable. However, there is no clear decrease in the growth rate of frond numbers (EC<sub>50</sub> values of 1.22  $\mu\text{g/L}$  and 1.18  $\mu\text{g/L}$  in week 1 and 6, respectively).

### B.9.2.7.5. Outdoor growth inhibition and recovery of aquatic plants exposed to foramsulfuron WG 50 percent

<b>Report:</b>	KCA 8.2.7 /07;Kirkwood, A.;2012;M-429538-01
<b>Title:</b>	Outdoor growth inhibition and recovery of aquatic plants exposed to foramsulfuron WG 50 percent
<b>Report No:</b>	EBFSL012
<b>Document No:</b>	M-429538-01-1
<b>Guidelines:</b>	not applicable; not specified
<b>GLP/GEP:</b>	yes

#### □ Summary

The objective of the study was to evaluate the toxicity of foramsulfuron WG 50% to ten aquatic plants in small, outdoor, replicated ponds under natural atmospheric conditions. Plants were placed in the ponds for a 1 to 4 week acclimation period prior to continuous exposure to nominal (initial measured) concentrations of 0.10, 0.25, 0.63, 1.6, 3.9, 9.8, 24 and 61 µg a.s./L (0.10, 0.25, 0.65, 1.6, 3.9, 9.7, 24 and 65 µg a.s./L). A 2-day exposure followed by a 5.5-week recovery phase was also conducted concurrently at nominal (initial measured) concentrations of 1.6 (1.6) and 3.9 (4.1) µg a.s./L for all ten species. In addition a deionized water control was tested. During the test duration of six weeks for *Nymphaea odorata*, the emergence of tubers was low in all ponds and the biomass collected at test termination was highly variable. Due to the inconsistency in emergence and growth, statistical analysis was not performed for *N. odorata*.

For all species exposed in the outdoor ponds and all biological endpoints measured, there were no significant differences when the 2-day peak exposures (e.g., 1.6 and 4.1 µg a.s./L initial measured concentrations) were compared to the untreated controls. However, there were statistical differences in the endpoints for some species when the 2-day peak exposures were compared to the respective treatment levels with 6-week exposure. The overall NOEC for the 2-day peak exposure followed by a 5.5 week growth in untreated water is 3.9 µg a.i./L (nominal) 4.1 µg a.s./L (initially measured).

#### □ Materials and Methods

##### Test Material

<b>Test item:</b>	Foramsulfuron WG 50%
<b>Batch No.:</b>	2011-001810
<b>CAS No.:</b>	173159-57-4
<b>Analysed purity:</b>	52.2 % w/w
<b>Expiry date:</b>	15 April 2012

##### Test organisms

<b>Test species:</b>	The selected plant species were chosen because they represent a wide range of freshwater aquatic habitats and they represent both monocotyledon and dicotyledon plants and one fern.
<b>Monocotyledon:</b>	Water weed ( <i>Elodea canadensis</i> ) Sago pondweed ( <i>Stuckenia pectinata</i> , formerly <i>Potamogeton pectinatus</i> ) Reed sweetgrass ( <i>Glyceria maxima</i> ) Arrowhead weed ( <i>Sagittaria latifolia</i> )
<b>Dicotyledon:</b>	Water lily ( <i>Nymphaea odorata</i> ) Coontail weed ( <i>Ceratophyllum demersum</i> ) Variable milfoil ( <i>Myriophyllum heterophyllum</i> ) Water mint ( <i>Mentha aquatic</i> ) Fanwort ( <i>Cabomba caroliniana</i> )
<b>Fern:</b>	Water fern ( <i>Salvinia minima</i> )
<b>Test duration:</b>	1- to 4- week acclimation; 6 week outdoor exposure
<b>Test end points:</b>	<b>Nine Macrophytes:</b> Reduction in mean shoot length, mean shoot dry weight and growth rates for mean shoot length and mean shoot dry weight, as applicable.



	<b>Salvinia minima:</b> Reduction in leaf density, leaf dry weight and growth rates for leaf density and leaf dry weight
<b>Dilution water:</b>	Town of Wareham fortified well water, hardness approximately 160 mg/L as CaCO <sub>3</sub>
<b>Treatments</b>	
<b>Nominal test concentrations:</b>	0.10, 0.25, 0.63, 1.6, 3.9, 9.8, 24, 61 µg a.i./L continuous exposure and 2-day Peak 1.6 and Peak 3.9 µg a.i./L for all 10 species
<b>Initial measured concentrations:</b>	0.10, 0.25, 0.65, 1.6, 3.9, 9.7, 24, 65 and Peak 1.6 and Peak 4.1 µg a.i./L
<b>Stock and Exposure Solution Preparation:</b>	A 30 mg/L primary stock solution was prepared by adding 1.1494 g of foramsulfuron WG 50% (0.6000 g as active ingredient) to 20 L of deionized water in a glass aquarium. This stock solution was stirred using 3 Teflon®-coated stir bars and 3 magnetic stir plates, and was observed to be cloudy with visible undissolved test substance. The stock solution remained cloudy with visible undissolved test substance following continuous mixing during dosing. Each replicate test solution was individually prepared. A 3760 mL volume of untreated deionized water was added to the 1850-L of pond water in each control pond.
<b>Pond design and construction:</b>	<p>Thirty-two, square, 3000-L, outdoor, freshwater ponds (inside dimensions 230 cm x 230 cm x 60 cm deep) were constructed by stacking 15 cm x 15 cm x 240 cm pressure-treated timbers. The frames were lined with liners designed for use in aquatic horticulture. Each pond contained a 5 cm layer of sandy loam soil to serve as sediment. The percent sand:silt:clay of the soil was determined to be 75:19:6%, respectively, the percent organic matter was 5.2% and the pH was 6.9. Each pond was filled with approximately 1850 liters (35 cm depth) of unchlorinated well water and fortified in hardness to approximately 160 mg/L as CaCO<sub>3</sub>. The ponds received full sunlight throughout the day. The covers were temporarily installed over the ponds when heavy rain was forecast, in order to prevent major dilution of the test solutions.</p> <p>Each rooted species was planted in an appropriately sized plastic pot. The sandy loam, mixed in a 1:1 ratio by volume with commercial potting soil (e.g., Sun-Gro Coir® Metro Mix 560), was added as the substrate to the pots. Slow-release pelleted fertilizer (Scotts Osmocote Plus™, 15-9-12) was added to mid-depth of the soil in each pot and the soil surface was covered with masonry sand. <i>Ceratophyllum</i>, which does not typically root in sediment, was placed in plastic mesh bags to contain the shoots, and the bag was anchored to the sediment with a small stone. The floating water fern, <i>Salvinia minima</i>, was placed in two, 30 cm diameter floating corrals to contain the plants.</p> <p>Plants were placed in the ponds for a 1 to 4 week acclimation period prior to exposure to the test substance (<b>Table B.9.2.7.5.-1</b>).</p>

**Table B.9.2.7.5.-1: Survey of species-specific characteristics of methods**

Plant Species	Pot Diameter (cm)	Number Plants per Pot	Number Pots Per Pond	Total Number Plants per Pond
<i>Elodea canadensis</i>	20	5	3	15
<i>Stuckenia pectinata</i>	20	5	3	15
<i>Glyceria maxima</i>	30	3	3	9
<i>Sagittaria latifolia</i>	30	3	3	9
<i>Nymphaea odorata</i>	30	1	5	5
<i>Ceratophyllum demersum</i>	mesh bag	5	3	15
<i>Myriophyllum heterophyllum</i>	20	5	3	15
<i>Mentha aquatica</i>	30	5	3	15
<i>Cabomba caroliniana</i>	20	5	3	15
<i>Salvinia minima</i>	30 cm corral	20 (leaves)	2	40

Additional pots were planted and placed in two additional ponds containing the same water, and sediment, to serve as replacement plants for any plants that did not grow normally during the acclimation phase.

**Replicates:**

Four replicates were established for the control and the two lowest concentrations (0.10 and 0.25 µg a.i./L). Three replicates were established for the two intermediate levels (0.63 and 1.6 µg a.i./L) while two replicates were established for the four highest concentrations (3.9, 9.8, 24 and 61 µg a.i./L). In addition, three replicate ponds were established for each of the two peak dose treatments (1.6 and 3.9 µg a.i./L). The peak dose treatments approximated expected environmental concentrations.

**Analytical verification:**

A minimum of four water column samples were removed at test initiation and during weeks 2, 4 and 6 (test termination) from each pond. The sampling device collected a vertical water column sample from near the sediment to the water surface. The water samples for an individual pond were combined into the 20 L bucket assigned to that pond. A subsample of the composite sample was removed for analysis of foramsulfuron concentration. Additionally, a second set of water samples was collected from each composite sample and held frozen for future analysis, if necessary. The peak dose pond solutions were also collected and analysed on day 3, one day after the water exchange, to characterize remaining residues of foramsulfuron. Water samples were also collected from each pond during weeks 1, 3 and 5, and stored frozen for future analysis, if necessary.

**Analytical method:**

Exposure, control and QC samples were analysed for foramsulfuron using a liquid chromatography/mass spectrometry (LC/MS/MS) procedure based on methodology validated at Smithers Viscient. The method validation study was conducted prior to the initiation of the test and established an average recovery of 90.7% ± 1.84% for foramsulfuron from pond water. The QC acceptance range was set at 80 to 120%. Conditions and procedures used throughout the analysis of exposure solutions and QC samples during this study were similar to those used in the method validation study.

**Testing Procedures**

**Initial Length and Dry Weight Measurements:**

On the day of test substance application mean shoot length and mean shoot dry weight data was collected for each species. These values represented the initial shoot length and shoot dry weight

values later used to calculate growth rates from test initiation to test termination.

#### Health Monitoring:

The outdoor health observations were performed on submerged, emergent and floating plants during weeks 2, 4 and 6. An additional observation period at week one was conducted for *Salvinia* due to its rapid growth rate. In addition, the plants in the peak dose ponds were also observed on exposure days 2 and 7. Visual observations such as chlorosis, leaf curl and necrosis were recorded. Effects observed were rated as percentage effect against the control plants. The number of *Nymphaea odorata* leaves emerged from the water surface was counted weekly. Flowering was noted when observed for all plant species tested. Additionally, plants were inspected daily for caterpillars or other insects that may graze or damage the plants, and the insects were removed if observed. General observations of the ponds were made weekly (e.g., water clarity, algal blooms). Additionally, plant cover was mapped on day 0 and at test termination (week 6). Water lost from the ponds due to evaporation was replaced when necessary in order to maintain the pond depth within 10% of the required depth, 35 cm. When filamentous algae were observed, it was noted and the algae carefully removed from the ponds.

#### □ Results:

##### Environmental conditions:

The environmental conditions maintained throughout the test period were within acceptable limits for the growth and survival of the test species. Total rainfall during the exposure period was 22.5 cm. Due to the use of temporary covers approximately 9.25 cm of rainfall was prevented from entering the ponds on several occasions between 9 June and 9 July 2011. The remaining rainfall entering the ponds (e.g., 13.25 cm) generally replenished water evaporated during the study. Water in the ponds did not evaporate more than 10% of the initial depth (e.g., 35 cm).

##### Analytical results:

Initial measured concentrations ranged from 99 to 110% of nominal concentrations and defined the treatment levels as 0.10, 0.25, 0.65, 1.6, 3.9, 9.7, 24 and 65 µg a.i./L (Table B.9.2.7.5.-2). Initial measured concentrations of the Peak 1.6 and Peak 3.9 µg a.i./L treatments were both 100% of nominal concentrations and defined the treatment levels as 1.6 and 4.1 µg a.i./L (Table B.9.2.7.5.-3).

**Table B.9.2.7.5.-2: Measured concentrations of foramsulfuron (µg a.i./L) in pond water with static exposure over 6-weeks**

Nominal Conc. (µg a.i./L)	Day 0	% Nom.	Day 14	% Nom.	Day 28	% Nom.	Day 41	% Nom.
0.1	0.10	100	0.061	61.25	0.047	47.25	0.0383	38.25
0.25	0.25	100	0.145	58.25	0.115	46	0.0833	33.5
0.63	0.65	100	0.3733	59.67	0.3133	49.0	0.2133	34.0
1.6	1.6	100	0.9467	59.33	0.7367	46.0	0.5133	32.3
3.9	3.9	100	2.35	60.50	1.95	50.5	1.3	33.5
9.8	9.7	99	6.0	62.0	5.0	51.0	3.25	30.5
24	24	100	14.5	61.0	12.0	50.0	7.4	31.0
61	65	110	38.5	62.5	31.5	52.0	19.0	31.0

**Table B.9.2.7.5.-3: Measured concentrations of foramsulfuron ( $\mu\text{g a.i./L}$ ) in pond water with peak exposure over 2-days**

Nominal Conc. ( $\mu\text{g a.i./L}$ )	Day 0	% Nom.	Day 3	% Nom.	Day 14	% Nom.	Day 28	% Nom.	Day 41	% Nom.
Peak 1.6	1.6	100	0.0707	4.43	0.07	4.37	0.057	3.57	0.0423	2.67
Peak 3.9	4.1	100	0.2267	5.8	0.2033	5.2	0.170	4.37	0.1167	2.97

**Biological results:**

The exposure concentrations in the following text are expressed as initial measured concentrations. The  $\text{EC}_{50}$  values and NOEC values were calculated using nominal and initial measured concentrations for each species, with the exception of *Nymphaea odorata*. Growth inhibition was observed as listed in Table B.9.2.7.5.-4 and Table B.9.2.7.5.-5 below.

**Table B.9.2.7.5.-4: 6-week NOEC and  $\text{EC}_{50}$ -figures ( $\mu\text{g a.i./L}$ ) for nine aquatic macrophytes tested in the outdoor ponds based on nominal concentrations**

	Shoot Length		Week 6 Growth Rate Based on Mean Shoot Length		Week 6 Mean Shoot Dry Weight		Week 6 Growth Rate Based on Dry Weight	
	NOEC	$\text{EC}_{50}$ (95% CL) <sup>a</sup>	NOEC	$\text{EC}_{50}$ (95% CL)	NOEC	$\text{EC}_{50}$ (95% CL)	NOEC	$\text{EC}_{50}$ (95% CL)
<i>Elodea canadensis</i>	NC <sup>b</sup>	NC	NC	NC	0.10	1.5 (0.94-2.1)	0.10	1.5 (0.97-2.1)
<i>Stuckenia pectinata</i>	NC	NC	NC	NC	3.9	8.1 (6.9-9.5)	3.9	7.7 (6.5-9.0)
<i>Glyceria maxima</i>	24	>61 (NA <sup>c</sup> )	24	38 (25-53)	61	>61 (NA <sup>c</sup> )	61	60 (46-NA <sup>d</sup> )
<i>Sagittaria latifolia</i>	1.6	>61 (NA <sup>c</sup> )	1.6	2.3 (1.9-2.9)	3.9	5.7 (2.1-8.3)	3.9	4.6 (2.5-7.5)
<i>Ceratophyllum demersum</i>	NC	NC	NC	NC	61	>61 (NA <sup>c</sup> )	61	21 (9.0-NA <sup>d</sup> )
<i>Myriophyllum heterophyllum</i>	24	>61 (NA <sup>c</sup> )	24	>61 (NA <sup>c</sup> )	61	44 (34-54)	61	41 (31-50)
<i>Mentha aquatica</i>	61	>61 (NA <sup>c</sup> )	61	>61 (NA <sup>c</sup> )	61	>61 (NA <sup>c</sup> )	61	>61 (NA <sup>c</sup> )
<i>Cabomba caroliniana</i>	61	>61 (NA <sup>c</sup> )	61	>61 (NA <sup>c</sup> )	61	>61 (NA <sup>c</sup> )	61	>61 (NA <sup>c</sup> )
	Week 6 Mean Leaf Density		Week 6 Growth Rate Based on Leaf Density		Week 6 Mean Leaf Dry Weight		Week 6 Growth Rate Based on Leaf Dry Weight	
	NOEC	$\text{EC}_{50}$ (95% CL)	NOEC	$\text{EC}_{50}$ (95% CL)	NOEC	$\text{EC}_{50}$ (95% CL)	NOEC	$\text{EC}_{50}$ (95% CL)
<i>Salvinia minima</i>	1.6 <sup>e</sup>	2.8 (0.16-3.4)	1.6	5.5 (5.0-5.8)	1.6 <sup>e</sup>	2.8 (1.4-3.3)	1.6 <sup>e</sup>	2.8 (1.8-3.3)

<sup>a</sup> CL = Confidence level.

<sup>b</sup> NC = Not calculated and not a required endpoint for this species. Due to the constant branching or the fact that stems could not be associated with an individual plant, plant lengths were not measured.

<sup>c</sup> NA = Not applicable.  $\text{EC}_{50}$  value was empirically estimated, therefore 95% confidence limits could not be calculated.

<sup>d</sup> Corresponding 95% confidence interval could not be calculated.

<sup>e</sup> Due to substantial % inhibition at the higher treatment levels, the 1.6  $\mu\text{g a.i./L}$  treatment was used as a conservative NOEC value.

Note: Due to the inconsistency in emergence and growth, statistical analysis was not performed for *Nymphaea odorata*.



**Table B.9.2.7.5.-5: 6-week NOEC and EC<sub>50</sub>-figures (µg a.s./L) for nine aquatic macrophytes tested in the outdoor ponds based on initial measured concentrations**

	Week 6 Mean Shoot Length		Week 6 Growth Rate Based on Mean Shoot Length		Week 6 Mean Shoot Dry Weight		Week 6 Growth Rate Based on Dry Weight	
	NOEC	EC <sub>50</sub> (95% CL <sup>a</sup> )	NOEC	EC <sub>50</sub> (95% CL)	NOE C	EC <sub>50</sub> (95% CL)	NOEC	EC <sub>50</sub> (95% CL)
<i>Elodea canadensis</i>	NC <sup>b</sup>	NC	NC	NC	0.10	1.5 (0.96-2.2)	0.10	1.5 (0.94-2.1)
<i>Stuckenia pectinata</i>	NC <sup>b</sup>	NC	NC	NC	3.9	8.0 (6.8-9.5)	3.9	7.7 (6.7-9.0)
<i>Glyceria maxima</i>	24	>65 (NA <sup>c</sup> )	24	39 (26-57)	65	>65 (NA <sup>c</sup> )	65	64 (48-NA <sup>d</sup> )
<i>Sagittaria latifolia</i>	1.6 <sup>e</sup>	>65 (NA <sup>c</sup> )	1.6	2.3 (1.8-2.9)	3.9	5.7 (2.1-8.1)	3.9	4.6 (2.5-7.8)
<i>Ceratophyllum demersum</i>	NC <sup>b</sup>	NC	NC	NC	65	>65 (NA <sup>c</sup> )	65	21 (7.7-NA <sup>d</sup> )
<i>Myriophyllum heterophyllum</i>	24	>65 (NA <sup>c</sup> )	24	>65 (NA <sup>c</sup> )	65	46 (35-57)	65	43 (31-52)
<i>Mentha aquatica</i>	65	>65 (NA <sup>c</sup> )	65	>65 (NA <sup>c</sup> )	65	>65 (NA <sup>c</sup> )	65	>65 (NA <sup>c</sup> )
<i>Cabomba caroliniana</i>	65	>65 (NA <sup>c</sup> )	65	>65 (NA <sup>c</sup> )	65	>65 (NA <sup>c</sup> )	65	>65 (NA <sup>c</sup> )
	Week 6 Mean Leaf Density		Week 6 Growth Rate Based on Leaf Density		Week 6 Mean Leaf Dry Weight		Week 6 Growth Rate Based on Leaf Dry Weight	
	NOEC	EC <sub>50</sub> (95% CL)	NOEC	EC <sub>50</sub> (95% CL)	NOE C	EC <sub>50</sub> (95% CL)	NOEC	EC <sub>50</sub> (95% CL)
<i>Salvinia minima</i>	1.6 <sup>e</sup>	2.8 (0.33-3.4)	1.6	5.5 (4.9-5.8)	1.6 <sup>e</sup>	2.8 (1.8-3.3)	1.6 <sup>e</sup>	2.8 (1.6-3.2)

<sup>a</sup> CL = Confidence level.

<sup>b</sup> NC = Not calculated and not a required endpoint for this species. Due to the constant branching or the fact that stems could not be associated with an individual plant, plant lengths were not measured.

<sup>c</sup> NA = Not applicable. EC<sub>50</sub> value was empirically estimated, therefore 95% confidence limits could not be calculated.

<sup>d</sup> Corresponding 95% confidence interval could not be calculated.

<sup>e</sup> Due to substantial % inhibition at the higher treatment levels, the 1.6 µg a.i./L treatment was used as a conservative NOEC value.

Note: Due to the inconsistency in emergence and growth, statistical analysis was not performed for *Nymphaea odorata*.

During the exposure phase for *Nymphaea odorata*, the emergence of tubers was low in all ponds and the biomass collected at test termination was highly variable. Due to the inconsistency in emergence and growth, statistical analysis was not performed for *N. odorata*.

For all species exposed in the outdoor ponds and all biological endpoints measured, there were no significant differences when the 2-day peak exposures (e.g., 1.6 and 4.1 µg a.s./L initial measured concentrations) were compared to the untreated controls. However, there were statistical differences in the endpoints for some species when the 2-day peak exposures were compared to the respective treatment levels with 6-week exposure. The overall NOEC for the 2-day peak exposure followed by a 5.5 week growth in untreated water is 3.9 µg a.s./L (nominal) 4.1 µg a.s./L (initially measured).

#### ❑ Conclusion:

The initial measured concentrations of foramsulfuron in the treated ponds closely approximated the desired nominal concentrations indicating each pond was dosed correctly. After six weeks of exposure, the concentrations of foramsulfuron declined to approximately 30 to 40% of the nominal concentrations. The 6-week geometric mean measured concentrations ranged from 54 to 58% of nominal concentration, indicating continuous, measurable concentrations of foramsulfuron were present in all treatments throughout the six week exposure. The peak dose exposure ponds which were renewed with untreated water on day 2, resulted in a > 90% reduction in test concentrations on day 3 and slowly declined for the remaining five weeks of testing.

Seven of the ten aquatic plants exposed to foramsulfuron WG 50% in outdoor ponds indicated sensitivity in reduced plant biomass or morphological abnormalities over the range of concentrations tested. Based on initial measured concentrations and the lowest NOEC and EC<sub>50</sub> values, 0.10 µg a.s./L and 1.5 µg a.s./L, respectively, water weed (*Elodea canadensis*) was the most sensitive plant tested. Based on a comparison of the EC<sub>50</sub> values for the nine species tested in outdoor plants, the most sensitive to least sensitive species rank as follows: *Elodea canadensis* < *Sagittaria latifolia* < *Salvinia minima* < *Stuckenia pectinata* < *Ceratophyllum demersum* < *Glyceria maxima* < *Myriophyllum heterophyllum* < *Mentha aquatica* < *Cabomba caroliniana*. The EC<sub>50</sub> values ranged from 1.5 µg a.s./L to > 65 µg a.i./L.

Recovery was observed in the peak dose ponds which underwent a 2-day exposure followed by a 5.5-week recovery period for the following species: *Elodea canadensis*, *Salvinia minima*, and *Sagittaria latifolia*, based on statistical comparisons of the continuous dose vs. peak dose data. Recovery could not be assessed for the remaining six species, since they were generally unaffected at the continuous dose and equivalent peak dose concentrations. The overall NOEC for the 2-day peak exposure followed by a 5.5 week growth in untreated water is 3.9 µg a.s./L (nominal) 4.1 µg a.s./L (initially measured).

In general, the health and survival of the control plants for each species indicated the exposure systems were appropriate for use. Additionally, the results demonstrated that the plant species selected were appropriate to detect responses to the test substance.

#### ❑ Comment (Co-RMS and RMS): Study considered acceptable.

#### B.9.2.7.6. Toxicity of foramsulfuron technical to the aquatic macrophyte, *Myriophyllum spicatum*

<b>Report:</b>	KCA 8.2.7 /09;Banman, C. S. Alexander, T. M.; Lam, C. V.;2012;M-431270-01
<b>Title:</b>	Toxicity of foramsulfuron technical to the aquatic macrophyte, <i>Myriophyllum spicatum</i>
<b>Report No:</b>	EBFSL004
<b>Document No:</b>	M-431270-01-1
<b>Guidelines:</b>	OCSPP Guideline Number 850.SUPP; not specified
<b>GLP/GEP:</b>	yes

#### ❑ Summary

The objective of this study was to determine the dose-response effect of foramsulfuron to the rooted aquatic macrophyte, *Myriophyllum spicatum*, over an exposure period of 14 days under static conditions.

5 plants (thinned to 3 shoots on day 0) per replicate (3 replicate test vessels per treatment group) were exposed to nominal (geometric mean measured) concentrations of control (< LOQ), 1.0 (1.1), 3.0 (3.4), 9.0 (10), 27 (30) and 81 (84) µg a.s./L. Effects on yield for total shoot length, total plant wet weight and total plant dry weight were determined on a per plant basis, based on the growth of each plant during the 14 day growth intervals. Toxicity values were calculated based on mean measured concentrations. The statistical NOEC, LOEC and EyC<sub>50</sub> for all endpoints were 84, > 84 and > 84 µg a.s./L, respectively.

#### ❑ Materials and Methods

**Test item:** Foramsulfuron (technical)  
**Batch code:** AE F130360-01-01  
**Origin Batch No.:** ELIR004130  
**CAS No.:** 173159-57-4; Customer Order No: Tox-No: 09032-00; LIMS No.: 1014240  
**Analysed purity:** 97.6%  
**Certificate No.:** AZ 16624  
**Test organism:** *Myriophyllum spicatum*  
**Source:** Environmental Sciences Laboratory of DuPont Company, Newark, Delaware, USA

#### Experimental Parameters

**Exposure:** 14 day  
**Test System:** static  
**Number of Replicates:** 3 at each test level  
**Aeration Used:** No  
**Nominal test concentrations:** Control, 1.0, 3.0, 9.0, 27 and 81 µg a.s./L  
**Mean Measured Test Concentrations:** Control (<LOQ), 1.1, 3.4, 10, 30, and 84 µg a.s./L  
**Solvent Carrier:** None  
**Analyte Measured:** Foramsulfuron  
**Interval of Analytical Verification:** Days 0, 7 and 14  
**Photoperiod:** 16 hours light, 8 hours dark  
**Light Intensity:** 9270 to 12,330 lux (mean = 10,443 lux)  
**Test Vessel Size:** Shoots within a replicate are planted in sediment within a 650 mL borosilicate glass crystallization dish housed in a 4-L glass beaker  
**Test Vessel Fill Volume:** 3.5-L  
**Test/Culture Media:** Hard Processed Water (blended spring and R.O. water)  
**Temperature Range (study duration):** 19.37 to 20.51 °C  
**pH Range:** 7.9 to 9.9  
**Dissolved Oxygen (D.O.) Range:** 8.0 to 14.9 mg/L  
**Interval of pH and Dissolved Oxygen measurements:** Days -7, 0, 7 and 14

#### □ Principle of the test:

Following a seven day acclimation period, *Myriophyllum spicatum* shoots were exposed to a control (< LOQ) and to nominal (mean measured) concentrations of 1.0 (1.1), 3.0 (3.4), 9.0 (10), 27 (30), and 81 (84) µg a.s./L for 14 days under static conditions. Mean measured concentrations are determined based on results of the recoveries from days 0, 7, and 14 sampling and ranged from 104 to 113% of the nominal concentration. The toxicity values were calculated based on these mean measured concentrations.

The test system consisted of three replicate test vessels per treatment group. Each replicate contained five plants for a total of 15 plants per group. Dissolved oxygen content and pH value were measured on days -7, 0, 7 and 14. Visual observations were conducted on a daily basis.

Following 14 days of exposure, all plants were removed from the test system. Length of the main shoot and all side shoots was measured, wet weights were measured, and following drying of plants for at least 72 hours, dry weight measurements were collected. All test vessels were contained in an environmentally controlled study area.

Yield (NOEC, LOEC and EC<sub>50</sub>) of total shoot lengths, total plant wet weight and total plant dry weight were the parameters measured in the study.

#### □ Results:

##### Validity Criteria:

Not applicable, higher tier study.

##### Analytical findings:

The concentration of the test item was stable within the test vessels during the 14 day exposure period (within 20% of initial measured concentrations).



Biological findings:

Active growth of the control plants during the 14 day exposure period was demonstrated by an average total shoot length yield of approximately 33.5 cm. Plants in the control vessels and all treatment groups appeared normal throughout the study. At study termination roots and shoots appeared normal in the controls. In the 1.1, 3.4, 10, and 30 µg a.s./L treatment groups, the plant shoots appeared normal, but brown tips on the roots were observed on 15 of 36 plants within various test replicates throughout these treatment groups. In the 84 µg a.s./L treatment group; six plants, throughout all test replicates, were observed as having brown tips on the roots and six plants, throughout all test replicates, were observed to have brown terminal buds on the side shoots. However, growth data for all plants was included in the data analysis.

Total shoot length growth rate:

Shoot length yield was analysed at test termination on study day 14. Data analysis showed no statistically significant difference, in comparison to the control data, in any of the treatment levels. Percent inhibitions as compared to the control group were 15.1, 13.1, -1.7, 19.6 and 13.6% for the 1.1, 3.4, 10, 30, and 84 µg a.s./L test groups, respectively.

Total plant wet weight growth rate:

Total plant wet weight yield was analysed at test termination on study day 14. Data analysis showed no statistically significant difference, in comparison to the control data, in any of the treatment levels. Percent inhibitions, as compared to the control group, were 2.1, -10.5, -18.5, 2.1, and 10.1% for the 1.1, 3.4, 10, 30, and 84 µg a.s./L test groups, respectively.

Total plant dry weight growth rate:

Plant dry weight yield was analysed at test termination on study day 14. Data analysis was performed utilizing a one tailed test, one sided distribution which will not capture the differences; showing no statistically significant difference, in comparison to the control data, in any of the treatment levels. Percent inhibitions, as compared to the control group, were -7.5, -18.4, -18.0, -32.7, and -20.3% for the 1.1, 3.4, 10, 30, and 84 µg a.s./L test groups, respectively.

**Table B.9.2.7.6.-1: Toxicity to *Myriophyllum spicatum***

Test Substance	Foramsulfuron technical		
Test Object	<i>Myriophyllum spicatum</i>		
Exposure	14 Day – Static Exposure		
Endpoint Units	(µg a.i./L)		
Endpoint results	Day 14 Shoot Length Yield	Day 14 Wet Weight Yield	Day 14 Dry Weight Yield
Highest Concentration Without an Effect (NOEC)	84	84	84
Lowest Concentration With an Effect (LOEC)	> 84	> 84	> 84
EyC <sub>50</sub>	> 84	> 84	> 84

- ❑ **Conclusion:** Statistical analysis of the growth data of shoot length, wet weight and dry weight yield indicated no statistical differences from the controls. The statistical NOEC, LOEC and EyC<sub>50</sub> for all endpoints were 84, > 84 and > 84 µg a.s./L, respectively.

- ❑ **Comment (Co-RMS and RMS):** Study considered acceptable.

### Studies on the metabolites of foramsulfuron

#### **B.9.2.7.7. Studies on the metabolite AE F153745**

<b>Report:</b>	<u>KCA 8.2.7 /02; Christ, M. T.; Ruff, D. F.;2000;M-240924-01</u>
<b>Title:</b>	Effect to <i>Lemna gibba</i> (duckweed) in a growth inhibition test: AE F153745 technical 97.8% w/w
<b>Report No:</b>	B002765
<b>Document No(s):</b>	Report includes Trial Nos.: CF99W565 <u>M-240924-01-2</u>
<b>Guidelines:</b>	<b>USEPA (=EPA): 123-2; Deviation not specified</b>
<b>GLP/GEP:</b>	<b>yes</b>

- ❑ **Conclusion:** Based on the lack of both phytotoxic effects and inhibition of frond growth, the NOEC is 100 mg/L and the LOEC is > 100 mg/L. Due to the nature of the data, the  $E_rC_{50}$  (Specific Growth Rate) and  $E_bC_{50}$  (Area Under The Curve) could not be calculated under the conditions of this study. In a static renewal exposure system for 7 days, AE F1 53745 technical has no phytotoxic or growth effects to the aquatic plant, *Lemna gibba*, up to 100 mg/L.
- ❑ **Comment (Co-RMS and RMS):** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).  $EC_{50}$  is > 100 mg/L.

#### **B.9.2.7.8. Studies on the metabolite AE 0338795**

<b>Report:</b>	<u>KCA 8.2.7 /03; Christ, M. T.; Ruff, D. F.;2000;M-238498-01</u>
<b>Title:</b>	Effect to <i>Lemna gibba</i> (duckweed) in a growth inhibition test: AE 0338795 technical 90.2 percent w/w: AE 0338795 00 1C90 0001
<b>Report No:</b>	B002774
<b>Document No(s):</b>	Report includes Trial Nos.: CF99W566 <u>M-238498-01-2</u>
<b>Guidelines:</b>	<b>USEPA (=EPA): 123-2; Deviation not specified</b>
<b>GLP/GEP:</b>	<b>yes</b>

- ❑ **Conclusion:** The 7 Day  $E_rC_{50}$  (growth rate) and  $E_bC_{50}$  (biomass) values ( $\pm$  95% CL), as determined by non-linear regression and the moving average method, were calculated as 27.2 mg/L (18.8 to 39.5) and 14.8 mg/L (12.4 to 16.7), respectively.
- ❑ **Comment (Co-RMS and RMS):** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).  $E_rC_{50}$  = 27.2 mg/L.

#### **B.9.2.7.9. Studies on the metabolite AE F092944**

<b>Report:</b>	<u>KCA 8.2.7 /10; Sowig, P.; Weller, O.;2000;M-186916-01</u>
<b>Title:</b>	Duckweed ( <i>Lemna gibba</i> G3) growth inhibition test AE F092944 (metabolite of ethoxysulfuron and amidosulfuron) substance technical Code: AE F092944 00 1C99 0001
<b>Report No:</b>	C003865
<b>Document No:</b>	<u>M-186916-01-1</u>
<b>Guidelines:</b>	<b>ASTM: E 1415-91; OECD: Draft June 1998; USEPA (=EPA): J § 123-2; Deviation not specified</b>
<b>GLP/GEP:</b>	<b>yes</b>

## □ Summary

The objective of this test was conducted to determine the effect of the metabolite AE F092944 on a higher freshwater plant under semi-static conditions according to draft OECD guideline, US-EPA Pesticide Assessment Guidelines J 123-2 and according to ASTM E 1415-91 guideline under GLP.

Triplicate *Lemna* cultures with an initial frond number of 12 fronds per replicate were exposed to the test substance in 20X-AAP medium at five nominal treatment levels (i.e. 10, 18, 32, 56 and 100 mg/L). Growth and abnormal appearance of fronds in each replicate were determined on test days 3, 5 and 7.

Analyses of freshly prepared water for AE F092944 resulted in concentrations ranging from 94.0% to 103.2% of nominal values. Analyses of aged water for AE F092944 at experimental termination resulted in concentrations ranging from 93.9% to 102.6% of nominal values. Therefore, nominal treatment levels of AE F092944 are reported in this study.

The concentration of the test substance leading to a 50% inhibition of the growth regarding frond numbers ( $\mu$ ) in comparison to the untreated control ( $ErC_{50}$ ) after 7 days test duration was nominal >100 mg/L. The concentration of the test substance leading to a 50% inhibition of the growth regarding biomass (dry weight) increase ( $\Delta b$ ) in comparison to the untreated control ( $EbC_{50}$ ) after 7 days test duration was nominal >100 mg/L. Intoxication symptoms were not observed.

A significant inhibition of growth both related on frond number or total biomass increase was not observed at a significance level of  $\alpha = 0.05$  at any treatment level.

The no observed effect concentration (NOEC), defined as no significant growth inhibition and no changes in plant appearance and development, was set to nominal 100 mg/L.

## □ Materials and Methods

**Test item:** AE F092944

**Code:** AE F092944 00 1C99 0001

**Analysed content:** 99.8 % w/w

**Certificate No.:** AZ 06326

**Solvent:** The test substance was dissolved in nutrient medium.

**Test organisms**

Species: *Lemna gibba* G3

Supplier: Plant Hormone Laboratory, United States Department of Agriculture (USDA), Beltsville, MD

**Test vessels:** The test was conducted in 300 mL Erlenmeyer-flasks, which were filled with 150 ml medium and closed with pressed paper stoppers.

**Test substance analysis:** Chemical analyses were conducted on day 0, 3 and 5 (fresh water) and on day 3, 5 and 7 (aged water) of all concentration level by chromatographic determination.

**Illumination and climatic conditions:**

The cultures were illuminated constantly using wide spectrum fluorescent lamps of the universal white-type L25 and a light intensity of 59.7 (min. 59,2; max. 60,2; standard deviation: 0.3)  $\mu E \cdot m^{-2} \cdot s^{-1}$ . The flasks for pre-culture and study were randomly placed in a water bath regulated from 23 to 25°C. The test was performed with an adjustment of the pH.

**Treatment levels:** The final project CE99/023 was performed with the nominal test substance concentrations of 10, 18, 32, 56 and 100 mg/L. Additionally an untreated control was tested. Three replicates were prepared each of the treated groups and the control. Separate vessels were prepared for chemical analysis of the test substance.

## □ Principle of the test:

Three replicates of *Lemna gibba* G3 per test concentration with 12 fronds were exposed for 7 days under semi-static conditions to the nominal concentrations of 10, 18, 32, 56 and 100 mg/L in comparison to untreated control. Separate vessels were prepared for chemical analysis of the test substance. The test media were analysed for chemical and physical parameters (pH, temperature, oxygen content and conductivity) on day 0, 3, 5 and 7.

Although the freshly prepared test water was adjusted to pH 7.5 there was a deviation to pH 8.6 to 9.0 in the aged test water. The temperature ranged from 24.5°C to 25.0°C at a constant light intensity of 59.7  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ .

Growth and abnormal appearance of fronds in each replicate were determined on test days 3, 5 and 7.

#### □ Results:

##### Validity Criteria:

The validation results and chromatograms demonstrate sufficient reliability of the method for the desired application: The lowest concentration level is above the LOQ and all concentrations of the analyte solution prepared for HPLC are within the linearity range. The repeatability precision is sufficient expressed by a mean CV of duplicate determinations < 20 % for all concentration levels. The accuracy is within 80 – 120 % recovery with a CV < 20 %. The specificity of the method is sufficient: The chromatograms display no matrix interference > LOQ of the determined compound and their identity is established by co-chromatography with the corresponding certified reference substance

The study fulfilled the biological validity criteria. Mean doubling time (d) of frond number in the control was < 2.5 (1.878, 1.847 and 1.839) and the mean growth rate (d-1) was 0.374 under semi-static conditions.

##### Analytical findings:

Analyses of freshly prepared water for AE F092944 resulted in concentrations ranging from 94.0% to 103.2% of nominal values. Analyses of aged water for AE F092944 at experimental termination resulted in concentrations ranging from 93.9% to 102.6% of nominal values. Therefore, nominal treatment levels of AE F092944 are reported in this study (Table 9.2.7.9.-1).

**Table 9.2.7.9.-1: Analytical findings in test solutions**

Nominal concentration [ $\mu\text{g a.s./L}$ ]	Day	Fresh water		Aged water	
		[mg test item/L]	% of nominal	[mg test item/L]	% of nominal
Control	0	0.00	96.7*	0.00	97.5*
	3	0.00	97.5*	0.00	98.0*
	5	0.00	98.6*	0.00	99.3*
	Mean	0.00	97.6*	0.00	98.3*
	Variability	--	--	--	--
10.00	0	9.98	100.00	9.67	96.9
	3	9.38	94.00	9.37	93.9
	5	10.23	102.5	9.39	94.1
	Mean	9.86	98.8	9.48	95.0
	Variability	1.09	--	1.03	--
18.00	0	17.41	96.9	17.79	99.0
	3	17.63	98.1	17.76	98.9
	5	18.35	102.2	17.80	99.1
	Mean	17.80	99.1	17.79	99.0
	Variability	1.05	--	1.00	--
32.00	0	30.73	96.2	30.75	96.3
	3	32.30	101.1	31.16	97.6
	5	32.12	100.6	31.01	97.1
	Mean	31.72	99.3	30.97	97.0
	Variability	1.05	--	1.01	--
56.00	0	54.66	97.8	55.56	99.4
	3	55.84	99.9	52.57	94.1
	5	55.69	99.7	55.06	98.5
	Mean	55.40	99.1	54.40	97.3
	Variability	1.02	--	1.06	--
100.00	0	98.44	98.6	102.41	102.6
	3	103.02	103.2	99.34	99.5

Nominal concentration [µg a.s./L]	Day	Fresh water		Aged water	
		[mg test item/L]	% of nominal	[mg test item/L]	% of nominal
	5	98.71	98.9	98.45	98.6
	Mean	100.06	100.3	100.07	100.3
	Variability	1.05	--	1.04	--

\* Concurrent recovery rate of laboratory fortifications prepared

The test results are within 80 – 120% of the nominal concentration and the variability is < 1.5.

#### Biological findings:

Mean values of absolute and percentual growth inhibition compared to the solvent control are presented in **Table 9.2.7.9.-2**.

The concentration of the test substance leading to a 50% inhibition of the growth regarding frond numbers ( $\mu$ ) in comparison to the untreated control ( $ErC_{50}$ ) after 7 days test duration was nominal >100 mg/L.

The concentration of the test substance leading to a 50% inhibition of the growth regarding biomass (dry weight) increase ( $\Delta b$ ) in comparison to the untreated control ( $EbC_{50}$ ) after 7 days test duration was nominal >100 mg/L.

**Table 9.2.7.9.-2: Mean values of absolute and percentual growth inhibition compared to the solvent control**

treatment level (mg/L)	mean growth rate (d-1)	percentual inhibition of growth rate	mean increase in biomass (mg)	percentual inhibition of biomass increase
untreated control	0.374	0.00	19.4	0.00
10	0.373	0.31	20.3	-4.46
18	0.369	1.26	19.8	-2.06
32	0.370	1.03	19.3	0.69
56	0.387	-3.48	21.7	-11.84
100	0.377	-0.81	21.2	-8.92

No intoxication symptoms were observed.

A significant inhibition of growth both related on frond number or total biomass increase was not observed at a significance level of  $\alpha = 0.05$  at any treatment level (**Table 9.2.7.9.-3**).

The no observed effect concentration (NOEC) defined as no significant growth inhibition and no changes in plant appearance and development was set to nominal 100 mg/L.

**Table 9.2.7.9.-3: Comparison of specific growth rates ( $\mu$ ), doubling times ( $T_d$ ) and biomass increase ( $\Delta b$ ) after 7 days test duration with DUNCAN's Multiple Range Test at a significance level of  $\alpha = 0.05$ .**

Concentration in mg/L	growth rate $\mu$ (d-1)		doubling time (d)		change of biomass $\Delta b$ (mg)	
untreated control	0.374	A	1.854	A	19.4	A
10	0.373	A	1.861	A	20.3	A
18	0.369	A	1.879	A	19.8	A
32	0.370	A	1.879	A	19.3	A
56	0.387	A	1.795	A	21.7	A
100	0.377	A	1.840	A	21.2	A

Concentrations with the same letter within each column are not significantly different.

#### ❑ Conclusion:

The concentration of AE F092944 leading to a 50% inhibition of the growth regarding frond numbers ( $\mu$ ) in comparison to the untreated control ( $ErC_{50}$ ) after 7 days test duration was nominal  $>100$  mg/L.

The concentration of test substance leading to a 50% inhibition of the growth regarding biomass (dry weight) increase ( $\Delta b$ ) in comparison to the untreated control ( $EbC_{50}$ ) after 7 days test duration was nominal  $>100$  mg/L.

A significant inhibition at a significance level of  $\alpha = 0.05$  of growth both related on both frond number and biomass increase was not observed up to a nominal concentration of 100 mg/L, which was the highest tested treatment level.

The no observed effect concentration (NOEC), defined as no significant growth inhibition and no changes in plant appearance and development, was nominal 100 mg/L.

#### ❑ Comment (Co-RMS and RMS): study is acceptable.

### B.9.2.7.10. Studies on the metabolite AE F099095

<b>Report:</b>	<u>KCA 8.2.7 /11;Dorgerloh, M.;2005;M-254496-01</u>
<b>Title:</b>	<i>Lemna gibba</i> G3 - growth inhibition test with AE F099095 under static conditions (Code: AE F099095 00 1B99 0001)
<b>Report No:</b>	EBMMX091
<b>Document No:</b>	<u>M-254496-01-1</u>
<b>Guidelines:</b>	OECD 221 " <i>Lemna</i> sp. Growth Inhibition Test" Revised Proposal for a New Guideline (April 2004);only minor (see temperature measurements) not influencing the outcome of this study negatively
<b>GLP/GEP:</b>	yes

#### ❑ Summary

The aim of the study was to determine the influence of AE F099095 on exponentially growing *Lemna gibba* G3 expressed as NOEC, LOEC and EC<sub>x</sub> for growth rate of both response variables, frond number and total frond area of plants. The pH values ranged from 7.4 to 8.5 in the control and the incubation temperature ranged from 23.4°C to 26.2°C (measured in one additional incubated glass vessel filled with the same amount of de-ionised water as in the test vessels) over the whole period of testing at a continuous illumination of 7.03 klx. The measured values for the temperature ranged within typical tolerances of calibrated measuring devices and showed only slight deviations from defined guideline recommendations. This did not influence the outcome of the study negatively.

Plant frond numbers and total frond area of plants are 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<sub>50</sub>) was determined where possible. The overall  $ErC_{50}$  for AE F099095 was  $> 100$  mg/L and the NOEC was  $< 100$  mg/L.

#### ❑ Materials and Methods

**Test item:** AE F099095, (code: AE F099095 00 1B99 0001)

**Purity:** 99.6 %

**Specified by batch-no.:** KR363/364

**Certificate of analysis:** AZ 10810

#### **Test organism**

**Species:** *Lemna gibba*, strain G3

**Origin:** Dr. Janet Slovin, Horticulture Crops Quality Laboratory, U.S. Department of Agriculture, Beltsville, MD 20705, U.S.A.

- Method of cultivation:** Stock cultures are maintained in glass dishes filled with growth medium under illumination of 6.50 – 10.0 klx and temperature of  $24 \pm 2^\circ\text{C}$  for a minimum of three weeks.  
Transfers are made regularly into fresh medium to provide 7 – 10 days old colonies as test inoculum. All operations were conducted under sterile conditions to sustain an axenic culture.
- Growth medium:** The medium (20X – AAP)
- Test system**
- Stock solution:** 201.4 mg of the test item (purity: 99.6 %) ad 2000 mL test medium, prepared immediately prior to the test. The solution was well agitated on a magnetic stirrer for 15 minutes and treated in an ultrasonic bath for 5 minutes before further use. An adequate amount of the stock solution was transferred to a dilution series to obtain the concentration levels used in the study.
- Test concentrations:** 0 (control) and 100 mg pure metabolite/L
- Selection of test levels:** The limit concentration was selected based on pre experiments to show that the  $\text{ErC}_{50}$  will be above 100 mg/L.
- Eposure conditions:** static
- Test item application:** The test item was applied into the freshly prepared test medium on day 0.
- Test medium (exposure):** Mixture of nutrient medium and added test item.
- Test duration:** 7 days
- Test volume:** 200 mL test medium per replicate
- No. of replicates:** 6 control and 6 treatment vessels (limit test)
- Test vessels:** Glass dishes were used with a diameter of 10 cm and a height of 6 cm (total volume of approx. 470 mL). These test vessels were covered with lids of glass to permit gas exchange and illumination under sterile conditions to the greatest possible extent. All dishes labelled with study number, a vessel number, and test concentrations were placed in a growth incubator.
- Inoculum:** To ensure that the plants used as inoculum are exponentially growing, an inoculum pre-culture will be prepared 7 – 10 days before the start of the test and cultivated under the same conditions as in the main test.
- Density of inoculum:** 9 to 12 fronds per vessel
- Temperature:** nominally  $24 \pm 2^\circ\text{C}$
- Light intensity:** nominally 6.50 – 10.0 klx ( $\pm 15\%$  variation from the mean)
- Light regime:** The flasks were exposed to permanent light and repositioned in randomised order after each observation.
- Observations:** Following observations were made on study days 2, 5, and 7: observation of changes in plant development (e.g. frond size, appearance, necrosis, chlorosis or gibbosity, colony break-up or loss of buoyancy, root length, morphology or breakdown)
- Test item analysis:** Samples were analysed for the actual concentration of AE F099095 present in all freshly prepared test levels on day 0 and in all aged test levels on day 7 of the exposure period. Aliquots for freshly prepared test levels for day 0 analyses were sampled from the prepared volume of each test treatment level. For sampling of aged test media, after removing of plant material from the test vessels on day 7 the contents of all three replicate vessels were combined, and the pH was measured. The combined test solutions were then submitted for analyses.

❑ **Principle of the test:**

3 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 the nominal concentration of 100 mg pure metabolite/L in comparison to control. The pH values ranged from 7.4 to 8.5 in the control and the incubation temperature ranged from  $23.4^\circ\text{C}$  to  $26.2^\circ\text{C}$  (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 7.03 klx.



Quantitative amounts of AE F099095 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.

#### □ Results:

##### Validity Criteria:

Test conditions met all validity criteria, given by the mentioned guideline. The frond number increased in the control by a factor of 7.3 within 7 days corresponding to a doubling time (Td) of about 2.5 days, respectively. Mean growth rate  $\mu$  [1/d] (0→7 d) in control was 0.282; under static conditions.

##### Analytical findings:

The analysed quantity of AE F099095 in the treatment level found on day 0 was 102 % of nominal. On day 7 an amount of 107 % of nominal was found.

All reported results are based on nominal initial values of the pure metabolite.

**Table 9.2.7.10.-1: Measured concentrations of AE F099095 in test solutions**

Day	Nominal concentration [mg/L]	Actual concentration of AE F099095			
		Detection 1 [mg/L]	Detection 2 [mg/L]	Mean [mg/L]	% of nominal
0	Control	< 0.01102	< 0.01102	< 0.01102	--
7		< 0.01102	< 0.01102	< 0.01102	--
0	100.000	100.950	103.126	102.038	102
7		106.563	106.656	106.609	107

Lowest standard solution of AE F099095 used for determination: < 0.01102 mg/L

##### Growth rate:

Results for the effects of the static 7 day growth inhibition test are listed in the **Table 9.2.7.10.-2** below.

**Table 9.2.7.10.-2: Survey of biological findings and the derived inhibitions of growth rate**

Nominal test levels [mg/L]	Final frond no. (replicate means, day 7)	Total frond area of plants (replicate means) [mm <sup>2</sup> ]	% inhibition	
			Average growth rate for frond no.	Average growth rate for total frond area of plants
control	87	705	--	--
100	72	572	9.7	7.9

##### Observed visual effects:

Observed visual effects are listed in the **Table 9.2.7.10.-3** below.

**Table 9.2.7.10.-3: Survey of visual effects**

Test level [mg/L]	Observations
Control	no visual effects observed
100	

The results based on nominal concentrations of the test item are shown in the **Table 9.2.7.10.-4** below.

**Table 9.2.7.10.-4: Survey of 7-day endpoints for AE F099095**

End point (0-7 day)	Effect on frond no. [mg/L]	Effect on total frond area of plants [mg/L]
ErC <sub>50</sub>	> 100	> 100
LOErC	100	100
NOErC	< 100	< 100

**□ Conclusion:**

The overall ErC<sub>50</sub> for AE F099095 was > 100 mg/L in this study.

The NOEC (< 100 mg/L) was based on statistical analysis.

**□ Comment (Co-RMS and RMS):** study is acceptable.

**B.9.2.7.11. Studies on the metabolite AE F130619 on *Lemna gibba* G3 (static)**

<b>Report:</b>	<u>KCA 8.2.7 /12;Bruns, E.;2013;M-452669-01</u>
<b>Title:</b>	<i>Lemna gibba</i> G3 - Growth inhibition test with AE F130619 (metabolite of foramsulfuron) under static conditions
<b>Report No:</b>	EBFSL011
<b>Document No:</b>	<u>M-452669-01-1</u>
<b>Guidelines:</b>	EU Council Directive 91/414/EEC; OECD Guideline 221 <i>Lemna</i> sp. Growth Inhibition Test (March 23, 2006); none
<b>GLP/GEP:</b>	yes

**□ Summary:**

The aim of this study was to determine the effects of AE F130619 on exponentially growing *Lemna gibba* G3 exposed under defined conditions for 7 days. 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 lowest NOErC was 0.179 µg p m./L.

**□ Materials and methods:**

<b>Test Material:</b>	AE F130619
<b>Chemical name:</b>	4-amino-2-[3-(4,6-dimethoxypyrimidin-2-yl)ureidosulfonyl]-N,N-dimethylbenzamide
<b>Lot/Batch No:</b>	origin batch no.: SES 10641-3-3, Batch Code: AE F 130619-01-01
<b>Actual content of active ingredient:</b>	94 %
<b>Description:</b>	off white powder
<b>Stability of test compound:</b>	+5 ±5 °C
<b>Expiry date:</b>	4 December 2012
<b>Density</b>	Not applicable
<b>Treatments</b>	
<b>Test concentrations:</b>	control; solvent control; nominal concentrations of 0.179, 0.572, 1.84, 5.86 and 18.7 µg p m./L
<b>Solvent:</b>	Dimethylformamid (DMF)
<b>Vehicle and/or positive control:</b>	None reported
<b>Analysis of test concentrations:</b>	Yes, analysis of AE F130619 was conducted at the start and the end of testing from all tested concentration using HPLC-MS/MS.
<b>Test organisms</b>	
<b>Species:</b>	<i>Lemna gibba</i> G3
<b>Source:</b>	Bayer CropScience AG

**Original supplier:**

Development -Environmental Safety - Testing  
40789 Monheim, Germany, The plant material was transferred to the laboratory on June 27, 2002 and kept since then.

**Test vessels:**

Dr. Janet Slovin, Horticulture Crops Quality Laboratory, U.S. Department of Agriculture, Beltsville, MD 20705, U.S.A.

**Test medium:**

Glass dishes with a diameter of 10 cm and a height of 6 cm (total volume of approx. 470 mL), covered with lids of glass to permit gas exchange and illumination

**Replication:**

20X AAP-Growth Medium

**Initial frond number:**

Three vessels for the control and each test concentration

**Exposure regime:**

12 fronds per vessel

**Duration:**

static

**Environmental conditions**

7 days

**Temperature:**

23.9 – 25.3°C

**pH:**

7.5 – 9.0

**Lighting:**

Continuous illumination, mean of 8914 lux (min. 8800; max. 9120)

**□ Principle of the test:**

3 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 the nominal concentrations of 0.179, 0.572, 1.84, 5.86 and 18.7 µg p.m./L in comparison to controls. The pH values ranged from 7.5 to 9.0 in the controls and the incubation temperature ranged from 23.9°C to 25.3°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 8914 lux.

AE F130619 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.

**□ Results:**Validity Criteria:

The study met all validity criteria requested by the mentioned guideline. The frond number increased in the control by a factor of 21.5 within 7 days corresponding to a doubling time (Td) of about 1.6 days, respectively. Mean growth rate  $\mu$  [1/d] (0→7 d) in control was 0.438; under static conditions.

Analytical findings:

On day 0, between 84 and 110% (average 100%) of nominal were found. On day 7 there were analytical findings between 83 and 112% (average 101%) of nominal. Therefore all results are based on nominal concentrations of the metabolite.

**Table B.9.2.7.11.-1: Measured concentrations of AE F130619 in test solutions**

Nominal (µg/L)	measured day 0		measured day 7 (old)	
	µg a.i./L	% nominal	µg a.i./L	% nominal
control	<0.0106	--	<0.0106	--
solvent control	<0.0106	--	<0.0106	--
0.179	0.178	99	0.172	96
0.572	0.602	105	0.641	112
1.84	2.02	110	2.06	112
5.86	6.01	103	5.9	101
18.7	15.7	84	15.6	83

Growth rate:

Results for the effects of the static 7 day growth inhibition test are listed in the table below.

**Table B.9.2.7.11.-2: Survey of biological results and derived inhibition percentages according to growth rates**

nominal test concentration $\mu\text{g p.m./L}$	final frond no. (replicate means, day 7)	final total frond area of plants (replicate means) [mm <sup>2</sup> ]	% inhibition (compared to pooled control)	
			mean growth rate for frond no.	mean growth rate for total frond area of plants
control	257.7	1956.3	--	--
solvent control	254.7	1898.0	--	--
pooled control	256.2	1927.2	--	--
0.179	355.7	2125.7	-10.8	-4.6
0.572	78.7 *	439.7 *	38.6	50.2
1.84	39.3 *	248.7 *	61.2	70.6
5.86	24.7 *	166.7 *	76.5	82.9
18.7	25.0 *	155.3 *	76.1	85.8

\* Results which were significantly different (based on Williams Multiple sequential t-test Procedure) from pooled control

Observed visual effects:

On day 7 the fronds observed at 0.572  $\mu\text{g p m./L}$  were smaller than the control plants.

The results based on nominal concentrations of AE F130619 are shown in the table below.

**Table B.9.2.7.11.-3: Survey of 7-day endpoints for AE F130619**

end point (0-7 day)	effect on mean growth rate of frond no. [ $\mu\text{g p m./L}$ ]	effect on mean growth rate of total frond area of plants [ $\text{mg form./L}$ ]
ErC <sub>50</sub> (CI 95%)	1.50 (0.026 - 42.4)	0.889 (n.d. - n.d.)
ErC <sub>70</sub> (CI 95%)	0.272 (n.d. - 0.982)	0.244 (n.d. - n.d.)
ErC <sub>90</sub> (CI 95%)	0.111 (n.d. - 0.537)	0.124 (n.d. - n.d.)
LOErC	0.572	0.572
NOErC	0.179	0.179

**Conclusion:** The most sensitive response variable in this study was total frond area of plants resulting in a (0-7 day) - LC<sub>50</sub> of 0.889  $\mu\text{g AE F130619/L}$ . The lowest NOErC was 0.179  $\mu\text{g AE F130619/L}$  and was based on statistical data analysis of frond number and the total frond area of plants.

□ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

**B.9.2.7.12. Studies on the metabolite 4-Amino-N-methylbenzamide (BCS-CV29520) on *Lemna gibba* G3 (static)**

<b>Report:</b>	<u>KCA 8.2.7 /13; Bruns, E.;2013; M-464163-01</u>
<b>Title:</b>	<i>Lemna gibba</i> G3 - Growth inhibition test with BCS-CV29520 (metabolite of foramsulfuron) under static conditions
<b>Report No:</b>	EBFSN010
<b>Document No:</b>	<u>M-464163-01-1</u>
<b>Guidelines:</b>	<b>EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4400; none</b>
<b>GLP/GEP:</b>	yes

□ **Summary:**

The aim of this growth inhibition test was, to verify the assumption that the metabolite 4-amino-N-methylbenzamide (BCS-CV29520) will cause no adverse effects on the growth of *Lemna gibba* G3 at the limit test item concentration of 10 mg pure metabolite/L. 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<sub>50</sub>) was determined where possible. Based on the low difference compared to the controls, which are far below 10 % difference, this statistically significance can be judged as biologically non-relevant. The overall threshold level has been set as  $\geq 10$  mg/L.

□ **Materials and methods:**

<b>Test Material:</b>	BCS-CV29520
<b>Chemical name:</b>	4-amino-N-methylbenzamide
<b>Lot/Batch No:</b>	origin batch no.: GSE 61195-2-2, Batch ID: BCS-CV29520-PU-01
<b>Actual content of active ingredient:</b>	97.6%
<b>Description:</b>	white powder
<b>Stability of test compound:</b>	10 – 30 °C
<b>Expiry date:</b>	3 June 2014
<b>Density</b>	Not applicable
<b>Treatments</b>	
<b>Test concentrations:</b>	control; nominal concentrations of 10 mg p m./L
<b>Solvent:</b>	None
<b>Vehicle and/or positive control:</b>	None reported
<b>Analysis of test concentrations:</b>	Yes, analysis of BCS-CV29520 was conducted at the start and the end of testing using HPLC-UV analysis.
<b>Test organisms</b>	
<b>Species:</b>	<i>Lemna gibba</i> G3
<b>Source:</b>	Bayer CropScience AG Development, Environmental Safety, Testing 40789 Monheim, Germany, the plant material was transferred to the laboratory on June 27, 2002 and kept since then.
<b>Original supplier:</b>	Dr. Janet Slovin, Horticulture Crops Quality Laboratory, U.S. Department of Agriculture, Beltsville, MD 20705, U.S.A.
<b>Test vessels:</b>	Glass dishes with a diameter of 10 cm and a height of 6 cm (total volume of approx. 470 mL), covered with lids of glass to permit gas exchange and illumination
<b>Test medium:</b>	20X AAP- Medium
<b>Replication:</b>	Six the control and six vessels for test concentration
<b>Initial frond number:</b>	12 fronds per vessel
<b>Exposure regime:</b>	static
<b>Duration:</b>	7 days
<b>Environmental conditions</b>	
<b>Temperature:</b>	24.7 – 25.3°C
<b>pH:</b>	7.5 – 9.0
<b>Lighting:</b>	Continuous illumination, mean of 6920 lux (min. 6630; max. 7140)



### □ Principle of the test:

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 p.m./L in comparison to a water control. The pH values ranged from 7.5 to 9.0 in the control and the incubation temperature ranged from 24.7°C to 25.3°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6.92 klux (average of nine measurements). 4-amino-N-methylbenzamide (BCS-CV29520) 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.

### □ Results:

#### Validity Criteria:

The study met all validity criteria requested by the mentioned guideline. The frond number in the control increased by a factor of 17.7 within 7 days, corresponding to a doubling time (Td) of about 1.69 days. Growth rate  $\mu$  [1/d] (0→7 d) was 0.410; under static conditions.

#### Analytical findings:

The analytical finding of 4-amino-N-methylbenzamide (BCS-CV29520) found on day 0 was 109 % of nominal and 115 % of nominal on day 7. All reported results are based on nominal values of the test item. The static 7 day growth inhibition test provided the following tabulated effects:

**Table B.9.2.7.12.-1: Survey of biological results and derived inhibition percentages based on growth rates**

nominal concentration p.m./L]	test [mg]	final frond no. (replicate means, day 7)	final total frond area of plants (replicate means) [mm <sup>2</sup> ]	% inhibition	
				mean growth rate for frond no.	mean growth rate for total frond area of plants
control		212.3	1639.5	--	--
10.0		208.8	1574.8	0.6	2.5

#### 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 4-amino-N-methylbenzamide are shown in the table below.

**Table B.9.2.7.12.-2: Survey of 7-day endpoints for 4-amino-N-methylbenzamide**

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]
ErC <sub>50</sub>	>10.0	>10.0
LOErC	>10.0	10.0
NOErC	10.0	< 10.0*)

\*) The statistical evaluation yielded a statistical significant effect for the mean growth rate of total frond area after 7 days. The actual inhibition for this endpoint was obviously below 10 % compared to the controls.

### □ Conclusion:

4-Amino-N-methylbenzamide (BCS-CV29520) caused no adverse effects on the growth of *Lemna gibba* G3 up to a test item concentration of 10 mg pure metabolite/L.

For the endpoint mean growth rate of total frond area of plants a significant difference to the controls was statistically evaluated. The observed difference in comparison to the control was 2.5 %. Due to the low variability of the data a Minimum Detectable Difference of -2 % was evaluable by the student-t-test. After 2 and 4 days the endpoint related no statistically significant difference to the controls. Based

on the low difference compared to the controls, which are far below 10 % difference, this statistically significance can be judged as biologically non-relevant. Therefore the overall threshold level has been set by the study director as  $\geq 10$  mg/L.

- ❑ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

**B.9.2.7.13. Studies on the metabolite 4-Formamido-N-methylbenzamide (BCS-CW90756) on *Lemna gibba* G3 (static)**

<b>Report:</b>	<u>KCA 8.2.7 /14;Bruns, E.;2013;M-464321-01</u>
<b>Title:</b>	<i>Lemna gibba</i> G3 - Growth inhibition test with BCS-CW90756 (metabolite of foramsulfuron) under static conditions
<b>Report No:</b>	EBFSN011
<b>Document No:</b>	<u>M-464321-01-1</u>
<b>Guidelines:</b>	<b>EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4400; OECD Guideline 221 (March 23, 2006); A slight deviation concerning the inoculum of replicate D at the limit test concentration of 10 mg/L is explained and discussed within chapter 4 (Method)</b>
<b>GLP/GEP:</b>	yes

❑ **Summary:**

The objective of this growth inhibition test was to verify the assumption that the test item 4-formamido-N-methylbenzamide (BCS-CW90756) will cause no adverse effects on the growth of *Lemna gibba* G3 at the limit test item concentration of 10 mg pure metabolite/L. 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<sub>50</sub>) was determined where possible. The test item caused no adverse effects on the growth of *Lemna gibba* G3 up to the limit test item concentration of 10 mg pure metabolite/L.

❑ **Materials and methods:**

<b>Test Material:</b>	BCS-CW90756
<b>Chemical name:</b>	4-amino-2-[3-(4,6-dimethoxypyrimidin-2-yl)ureidosulfonyl]-N,N-dimethylbenzamide
<b>Lot/Batch No:</b>	origin batch no.: GSE 61182-2-1, Batch ID: BCS-CW90756-PU-01
<b>Actual content of active ingredient:</b>	99.0 %
<b>Description:</b>	white powder
<b>Stability of test compound:</b>	10 – 30 °C
<b>Expiry date:</b>	1 March 2014
<b>Density</b>	Not applicable
<b>Treatments</b>	
<b>Test concentrations:</b>	control; 10 mg p m./L
<b>Solvent:</b>	None
<b>Vehicle and/or positive control:</b>	None reported
<b>Analysis of test concentrations:</b>	Yes, analysis of BCS-CW90756 was conducted at the start and the end of testing from all tested concentration using HPLC-UV.
<b>Test organisms</b>	
<b>Species:</b>	<i>Lemna gibba</i> G3
<b>Source:</b>	Bayer CropScience AG Development-Environmental Safety - Testing 40789 Monheim, Germany, The plant material was transferred to the laboratory on June 27, 2002 and kept since then.



<b>Original supplier:</b>	Dr. Janet Slovin, Horticulture Crops Quality Laboratory, U.S. Department of Agriculture, Beltsville, MD 20705, U.S.A.
<b>Test design</b>	
<b>Test vessels:</b>	Glass dishes with a diameter of 10 cm and a height of 6 cm (total volume of approx. 470 mL), covered with lids of glass to permit gas exchange and illumination
<b>Test medium:</b>	20 X AAP-Medium
<b>Replication:</b>	6 replicate vessels per test level and 6 replicate vessels per control
<b>Initial frond number:</b>	2 fronds per vessel
<b>Exposure regime:</b>	static
<b>Duration:</b>	7 days
<b>Environmental conditions</b>	
<b>Temperature:</b>	24.7 – 25.3°C
<b>pH:</b>	7.5 – 9.0
<b>Lighting:</b>	Continuous illumination, mean of 6920 lux (min. 6630; max. 7140)

#### □ Principle of the test:

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 p.m./L in comparison to a water control. The pH values ranged from 7.5 to 9.0 in the control and the incubation temperature ranged from 24.7°C to 25.3°C (measured in an additional incubated glass vessel) over the whole period of testing at a continuous illumination of 6.92 klux (average of nine measurements). 4-formamido-N-methylbenzamide (BCS-CW90756) 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.

#### □ Results:

##### Validity Criteria:

The study met all validity criteria requested by the mentioned guideline. The frond number in the control increased by a factor of 17.7 within 7 days, corresponding to a doubling time (Td) of about 1.69 days. Growth rate  $\mu$  [1/d] (0→7 d) was 0.410; under static conditions.

##### Analytical findings:

The analytical finding of 4-formamido-N-methylbenzamide (BCS-CW90756) found on day 0 was 108% of nominal and 114 % of nominal on day 7. All reported results are based on nominal values of the test item.

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

**Table B.9.2.7.13.-1: Survey of biological results and derived inhibition percentages based on growth rates**

nominal test concentration [mg p.m./L]	final frond number (replicate means, day 7)	final total frond area of plants (replicate means) [mm <sup>2</sup> ]	% inhibition	
			mean growth rate for frond no.	mean growth rate for total frond area of plants
control	212.3	1639.5	--	--
10.0	219.2	1660.5	-0.6	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 4-formamido-N-methylbenzamide (BCS-CW90756) are shown in the table below.

**Table B.9.2.7.13.-2: Survey of 7-day endpoints for 4-formamido-N-methylbenzamide**

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 <sub>r</sub> C <sub>50</sub>	>10.0	>10.0
LOE <sub>r</sub> C	>10.0	>10.0
NOE <sub>r</sub> C	≥10.0	≥10.0

- **Conclusion:** 4-formamido-N-methylbenzamide (BCS-CW90756) caused no adverse effects on the growth of *Lemna gibba* G3 up to the limit test item concentration of 10 mg pure metabolite/L.
- **Comment (Co-RMS and RMS):** No comments study is acceptable.

#### **B.9.2.7.14. Studies on the metabolite Foramsulfuron-sulfamic acid (BCS-AW41401) on *Lemna gibba* G3 (static)**

<b>Report:</b>	<u>KCA 8.2.7 /15; Hoffmann, K.:2013;M-464386-01</u>
<b>Title:</b>	<i>Lemna gibba</i> G3 - Growth inhibition test with BCS-AW41401 under static conditions
<b>Report No:</b>	EBFSN012
<b>Document No:</b>	<u>M-464386-01-1</u>
<b>Guidelines:</b>	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4400; OECD Guideline 221 (March 23, 2006); none
<b>GLP/GEP:</b>	yes

□ **Summary:**

The objective of this growth inhibition test was, to verify the assumption that the metabolite foramsulfuron-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<sub>50</sub>) was determined where possible.

Foramsulfuron-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.

□ **Materials and methods:**

<b>Test Material:</b>	BCS-AW41401
<b>Chemical name:</b>	[(4, 6-dimethoxypyrimidin-2-yl) carbamoyl]sulfamic acid
<b>Lot/Batch No:</b>	origin batch no.: GSE 61222-2-3; Batch ID: BCS-AW41401-01-01
<b>Actual content of active ingredient:</b>	89.7 %
<b>Description:</b>	white powder
<b>Stability of test compound:</b>	10 – 30 °C
<b>Expiry date:</b>	17 June 2014
<b>Density</b>	Not applicable
<b>Treatments</b>	
<b>Test concentrations:</b>	control; 10 mg p m./L
<b>Solvent:</b>	None
<b>Vehicle and/or positive control:</b>	None reported

<b>Analysis of test concentrations:</b>	Yes, analysis of BCS-AW41401 was conducted at the start and the end of testing from all tested concentration using HPLC-UV.
<b>Test organisms</b>	
<b>Species:</b>	<i>Lemna gibba</i> G3
<b>Source:</b>	Bayer CropScience AG Development -Environmental Safety - Testing 40789 Monheim, Germany, The plant material was transferred to the laboratory on June 24, 2013 and kept since then.
<b>Original supplier:</b>	Eurofins Agrosience Services EcoChem GmbH Eutinger StraGe 24, 75223 Niefern-Oschelbronn, Germany
<b>Test design</b>	
<b>Test vessels:</b>	Glass dishes with a diameter of 10 cm and a height of 6 cm (total volume of approx. 470 mL), covered with lids of glass to permit gas exchange and illumination
<b>Test medium:</b>	20 X AAP-Medium
<b>Replication:</b>	6 replicate vessels per test level and 6 replicate vessels per control
<b>Initial frond number:</b>	12 fronds per vessel
<b>Exposure regime:</b>	static
<b>Duration:</b>	7 days
<b>Environmental conditions</b>	
<b>Temperature:</b>	24.4 – 24.9 °C
<b>pH:</b>	7.6 – 8.8
<b>Lighting:</b>	Continuous illumination, mean of 6700 lux (min. 6520; max. 6920)

#### □ Principle of the test:

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). Foramsulfuron-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.

#### □ Results:

##### Validity Criteria:

The study met all validity criteria requested by the mentioned guideline. The frond number in the control increased by a factor of 16.2 within 7 days, corresponding to a doubling time (Td) of about 1.74 days. Growth rate  $\mu$  [1/d] (0→7 d) was 0.397; under static conditions.

##### 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 foramsulfuron-sulfamic acid (BCS-AW41401).

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

**Table B.9.2.7.14.-1: Survey of biological results and derived inhibition percentages based on growth rates**

nominal test concentration [mg p.m./L]	final frond number (replicate means, day 7)	final total frond area of plants (replicate means) [mm <sup>2</sup> ]	% inhibition	
			mean growth rate for frond no.	mean growth rate for total frond area of plants
control	194.0	1400.8	--	--
10.0	207.8	1469.7	-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 foramsulfuron-sulfamic acid (BCS-AW41401) are shown in the table below.

**Table B.9.2.7.14.-2: Survey of 7-day endpoints for foramsulfuron-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]
ErC <sub>50</sub>	>10.0	>10.0
LOErC	>10.0	>10.0
NOErC	≥10.0	≥10.0

- ❑ **Conclusion:** Foramsulfuron-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.
- ❑ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

Studies on the product Equip OD 45**B.9.2.7.15. Effects of the product AE F130360 + AE F122006 flowable: AE F130360 01 1K05 on *Lemna gibba***

<b>Report:</b>	KCP 10.2.1 /05; Boeri, R.; Wyskiel, D.; Ward, T.; 2000; M-238581-01
<b>Title:</b>	Toxicity to the Duckweed, <i>Lemna gibba</i> : AE F130360 + AE F122006 flowable: AE F130360 01 1K05
<b>Report No:</b>	B002845
<b>Document No:</b>	M-238581-01
<b>Guidelines:</b>	USEPA (=EPA): 123-3; Deviations: not specified
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** Exposure of the duckweed, *Lemna gibba*, to AE F130360 + AEF122006 oil flowable resulted in a 7-day ErC<sub>50</sub> of 45 µg product/L (corresponds to 1.01 µg a.s./L) (95% confidence interval = 39 to 53 (µg/L) when calculated using the growth rate and a 7-day EbC<sub>50</sub> of 25 µg/L (95% confidence interval = 18 to 35 µg/L) when calculated using the area under the growth curve. The 7-day NOEC is 10 µg/ product/L (corresponds to 0.225 µg a.s./L) AE F130360 + AE F122006 oil flowable when calculated using both the growth rate and the area under the growth curve.
- ❑ **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

**B.9.2.7.16. Effects of the product AE F130360 + AE F122006 + AE F115008 water dispersible granule, 30 + 30 + 2% w/w including a methylated rapeseed oil surfactant on *Lemna gibba* G3 (static renewal test)**

<b>Report:</b>	KCP 10.2.1 /06; Madsen, T. J.; Bussard, J. B.; 2000; M-238567-01
<b>Title:</b>	Toxicity of AE F130360 + AE F122006 + AE F115008, water dispersible granule, 30 + 30 + 2% w/w including a methylated rapeseed oil surfactant to duckweed, <i>Lemna gibba</i> G3 determined under static renewal test conditions: AE F130360 02 WG62 A10
<b>Report No:</b>	B002838
<b>Document No:</b>	M-238567-01
<b>Guidelines:</b>	OECD Draft Test Guideline; Deviations: not specified
<b>GLP/GEP:</b>	yes

**□ Conclusion:**

Based on nominal formulation concentrations of AE F130360 + AE F122006 + AE F115008; water dispersible granule 30 + 30 + 2% w/w; Code: AE F130360 02WG62 A104 including a methylated rapeseed oil surfactant (adjuvant) in the test medium, the 3- and 7-day IC<sub>50</sub>, NOEC, and LOEC values were calculated as follows:

Area Under the Growth Curve:

3-day IaC<sub>50</sub> = 5.6 µg/L with 95% confidence limits of 3.8 and 7.4 µg/L

7-day IaC<sub>50</sub> = estimated to be between 1.0 and 2.0 µg/L

7-day NOEC = 1.0 µg/L

7-day LOEC = 2.0 µg/L

Growth Rate:

3-day IrC<sub>50</sub> = estimated to be approximately 8.0 µg/L

7-day IrC<sub>50</sub> = 2.8 µg/L with 95% confidence limits of 2.2 and 3.4 µg/L

7-day NOEC = 1.0 µg/L

7-day LOEC = 2.0 µg/L

Final Biomass (Dry Weight):

7-day IbC<sub>50</sub> = 5.8 µg/L with 95% confidence limits of 3.3 and 8.3 µg/L

7-day NOEC = 1.0 µg/L

7-day LOEC = 2.0 µg/L

**□ Comment (Co-RMS and RMS):**

Refer to original EU review. No new data or assessments of the study are provided.

**B.9.2.7.17. Effects of the product AE F130360 + AE F122006 + AE F115008, water dispersible granule, 30+ 30 + 2% w/w, Code: AE F130360 02 WG62 A104 on *Lemna gibba* G3 (static renewal test)**

<b>Report:</b>	KCP 10.2.1 /07; Madsen, T. J.; Bussard, J. B.; 2000; M-238536-01
<b>Title:</b>	Toxicity of AE F130360 + AE F122006 + AE F115008, water dispersible granule, 30 + 30 + 2% w/w including a methylated rapeseed oil surfactant to duckweed, <i>Lemna gibba</i> G3 determined under static renewal test conditions: AE F130360 02 WG62 A10
<b>Report No:</b>	B002810
<b>Document No:</b>	M-238536-01
<b>Guidelines:</b>	OECD: Draft test (12/99); Deviations: not specified
<b>GLP/GEP:</b>	yes

#### □ Conclusion:

Based on nominal formulated total product test concentrations of AE F130360 + AE F122006 + AE F115008; Water Dispersible Granule 30 + 30 + 2% w/w; Code: AE F13036002 WG62 A104 in test medium, the 4- and 7-day  $IC_{50}$ , NOEC, and LOEC values were calculated as follows:

##### Area Under the Growth Curve:

4-day  $I_aC_{50}$  = 3.8 µg/L with 95% confidence limits of 3.0 and 4.6 µg/L

7-day  $I_aC_{50}$  = 2.5 µg/L with 95% confidence limits of 2.1 and 3.0 µg/L

7-day NOEC = 1.0 µg/L

7-day LOEC = 2.0 µg/L

##### Growth Rate:

4-day  $I_rC_{50}$  = estimated to be between 4.0 and 8.0 µg/L

7-day  $I_rC_{50}$  = estimated to be between 4.0 and 8.0 µg/L

7-day NOEC = 1.0 µg/L

7-day LOEC = 2.0 µg/L

##### Final Biomass (Dry Weight):

7-day  $I_bC_{50}$  = estimated to be 8.0 µg/L

7-day NOEC = 1.0 µg/L

7-day LOEC = 2.0 µg/L

- **Comment (Co-RMS and RMS):** Refer to original EU review. No new data or assessments of the study are provided.

#### **B.9.2.7.18. Effects of the product Foramsulfuron Oil Flowable 22.5 g/L Formulation (AE F130360 01 1K05 A304) on Duckweed *Lemna gibba***

<b>Report:</b>	KCP 10.2.1 /08; Hoberg, J. R.; 2002; M-240877-01;
<b>Title:</b>	Foramsulfuron Oil Flowable 22.5 g/L Formulation (AE F130360 01 1K05 A304) - Toxicity To Duckweed, <i>Lemna gibba</i>
<b>Report No:</b>	B003893
<b>Document No:</b>	M-240877-01
<b>Guidelines:</b>	USEPA (=EPA): OPPTS 850.4400; Deviations: not specified
<b>GLP/GEP:</b>	yes

#### □ Conclusion:

Results (mg/L) related to measured a.s. concentrations

Effect (%)

Time    End point    NOEC    LOEC     $EC_{50}$

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7	d	Fronds	0.0002	0.00045	0.0007
7	d	Growth	0.0002	0.00045	0.0011

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The risk assessment presented in the monograph was based on an EC<sub>50</sub> of 0.00065 mg/L. The EC<sub>50</sub> value of 0.0007 mg/L established in the new study is nearly the same. Therefore, it is not warranted to change the risk assessment.

- ❑ **Comment (Co-RMS and RMS):** Refer to Addendum 3 to the Monograph of 01 April 2001. No new data or assessments of the study are provided.

#### B.9.2.7.19. Effects of EQUIP OD 45 (AE F130360 01 1K05 A9) on *Lemna gibba* G3 (semi-static)

<b>Report:</b>	<u>KCP 10.2.1 /09; Banman, C. S.; Hoffmann, J. M.; Lam, C. V.; 2008; M-296352-01-</u>
<b>Title:</b>	Toxicity of foramsulfuron + isoxadifen-ethyl OD 22.5+22.5 g/L (AE F130360 01 1K05 A9) to duckweed ( <i>Lemna gibba</i> G3) under static-renewal conditions
<b>Report No:</b>	EBFSX011
<b>Document No:</b>	M-296352-01
<b>Guidelines:</b>	FIFRA Guideline 123-2 (1982), OPPTS Guideline 850.4400 (2006 draft) OECD Guideline 221 (2006); Deviations: not specified
<b>GLP/GEP:</b>	yes

❑ **Summary:**

A 7-day static-renewal duckweed growth test was conducted to determine the growth effects of Foramsulfuron + Isoxadifen-Ethyl OD 22.5+22.5 g/L (AE F130360 01 1K05 A9) on *Lemna gibba* G3. The duckweed *Lemna gibba* G3 was exposed for 7 days under static-renewal (Day 3 and Day 5 renewal) conditions. Nominal (mean measured) concentrations were control (<0.01), 0.094 (0.10), 0.19 (0.19), 0.38 (0.41), 0.75 (0.77), 1.5 (1.53), and 3.0 (3.08) µg a.s./L. Concentrations were based on the amount of Foramsulfuron in the Foramsulfuron + Isoxadifen-Ethyl OD 22.5+22.5 g/L (AE F130360 01 1K05 A9). Growth was determined by frond counts on days 0, 3, 5 and 7 and frond dry weights from day 0 and day 7. Results are reported as mean measured recoveries of foramsulfuron as measured on Day 0, Day 3 (old and new solutions), Day 5 (old and new solutions) and Day 7.

The NOEC and LOEC for the endpoint of growth rate for frond dry weight were 0.77 µg a.s./L and 1.5 µg a.s./L, respectively. For all other endpoints the NOEC and LOEC was < 0.10 µg a.s./L and 0.10 µg a.s./L, respectively. The endpoint with the most sensitive EC<sub>10</sub> was day 7 frond dry weight yield with an E<sub>y</sub>C<sub>10</sub> value of 0.14 µg a.s./L. The endpoint with the most sensitive EC<sub>50</sub> value was day 7 frond yield with an E<sub>y</sub>C<sub>50</sub> value of 0.75 µg a.s./L.

❑ **Materials and methods:**

**Test Material:**

Foramsulfuron + Isoxadifen-Ethyl OD 22.5+22.5 g/L (AE F130360 01 1K05 A9)

**Chemical name:**

2-[[[(4,6-Dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]-4-(formylamino)-N,N-dimethylbenzamide; Ethyl 4,5 -dihydro-5,5-diphenyl-3 -isoxazolecarboxylate

**Lot/Batch No:**

EFKM001398

**Actual content of active ingredient:**

2.32 % Foramsulfuron, 2.40 % Isoxadifen-Ethyl.

**Description:**

Beige liquid

**Stability of test compound:**

Room temperature (20 °C)

**Expiry date:**

10 October 2009

**Density**

Not applicable

**Treatments**



<b>Test concentrations:</b>	control (<0.01), 0.094 (0.10), 0.19 (0.19), 0.38 (0.41), 0.75 (0.77), 1.5 (1.53), and 3.0 (3.08) µg a.s./L
<b>Solvent:</b>	None
<b>Vehicle and/or positive control:</b>	None reported
<b>Analysis of test concentrations:</b>	Day 0 (new), Day 3 (new and old), Day 5 (new and old) and Day 7 (old), LC/MS/MS.
<b>Test organisms</b>	
<b>Species:</b>	<i>Lemna gibba</i> G3
<b>Source:</b>	Bayer CropScience Ecotoxicology 17745 South Metcalf Avenue Stilwell, Kansas 66085-9104
<b>Original supplier:</b>	United States Department of Agriculture Fruit Laboratory, Beltsville Maryland (received on June 3, 2004)
<b>Test design</b>	
<b>Test vessels:</b>	Sterile, 650-ml borosilicate glass crystallization dishes covered with sterile glass Petri dishes.
<b>Test medium:</b>	20X AAP
<b>Replication:</b>	Three replicates
<b>Initial frond number:</b>	Three plants for a total of 12 fronds
<b>Exposure regime:</b>	Static-renewal
<b>Duration:</b>	7 days
<b>Environmental conditions</b>	
<b>Temperature:</b>	23.3 – 23.8 °C
<b>pH:</b>	7.7 – 8.89
<b>Lighting:</b>	Continuous illumination, min. 5040- max. 6450

#### ❑ Principle of the test:

The duckweed *Lemna gibba* G3 was exposed for 7 days under static-renewal (Day 3 and Day 5 renewal) conditions. Nominal (mean measured) concentrations were control (<0.01), 0.094 (0.10), 0.19 (0.19), 0.38 (0.41), 0.75 (0.77), 1.5 (1.53), and 3.0 (3.08) µg a.s./L. Concentrations were based on the amount of Foramsulfuron in the Foramsulfuron + Isoxadifen-Ethyl OD 22.5+22.5 g/L (AE F130360 01 1K05 A9). Growth was determined by frond counts on days 0, 3, 5 and 7 and frond dry weights from day 0 and day 7. Results are reported as mean measured recoveries of foramsulfuron as measured on Day 0, Day 3 (old and new solutions), Day 5 (old and new solutions) and Day 7.

The test system consisted of three replicates per level. Each replicate contained three plants and twelve fronds for a total of 9 plants per level. Conductivity and pH value were measured on days 0, 3 (old and new solutions), 5 (old and new solutions) and 7 from all test levels. Visual observations were conducted on day 0, 3, 5 and 7.

Growth rate for frond counts and cumulative biomass for frond counts (as area under the growth curve) were measured. The variable used to calculate these response parameters was frond number as determined by direct frond counts. Frond dry weights measured at study initiation from representative samples and dry weights measured at study termination from the test samples were used to calculate response parameters for 7 day frond dry weight yield and growth rate for frond dry weight. Temperature during the test ranged between 23.3 and 23.8°C (recorded hourly), pH was 7.7 to 8.89, the photoperiod was 24 hours light and the light intensity was 5040 to 6450 lux. All test dishes were placed in an environmentally controlled chamber.

#### ❑ Results:

##### Validity Criteria:

All biological validity criteria for this study were met.

##### Biological findings:

On study Day 7 small fronds were noted in the 0.77, and 1.53 µg a.s. /L test vessels. Also noted on Day 7 in the 3.08 µg a.s./L was the appearance of brown fronds. All other plants in all test concentrations and controls appeared normal throughout the study period.

**Table B.9.2.7.19.-1: Toxicity to *Lemna gibba* G3**

Test Substance	Foramsulfuron + Isoxadifen-Ethyl OD 22.5+22.5 g/L (AE F130360 01 1K05 A9)
Test Object	<i>Lemna gibba</i> G3
Exposure	7-Day, static-renewal
7-day $E_yC_{10}$ - frond count	0.26 µg foramsulfuron/L
7-day $E_yC_{50}$ - frond count	0.75 µg foramsulfuron/L
7-day $E_rC_{10}$ - growth rate for frond numbers	0.32 µg foramsulfuron/L
7-day $E_rC_{50}$ - growth rate for frond numbers	1.56 µg foramsulfuron/L
7-day $E_bC_{10}$ - cumulative biomass for frond numbers	0.22 µg foramsulfuron/L
7-day $E_bC_{50}$ - cumulative biomass for frond numbers	0.86 µg foramsulfuron/L
7-day $E_yC_{10}$ - frond dry weight	0.14 µg foramsulfuron/L
7-day $E_yC_{50}$ - frond dry weight	2.1 µg foramsulfuron/L
7-day NOEC - growth rate for frond dry weight	0.77 µg foramsulfuron/L
7-day $E_rC_{50}$ - growth rate for frond dry weight	>3.08 µg foramsulfuron/L (greater than highest test concentration)
Lowest Concentration With an Effect (LOEC)	0.10 µg foramsulfuron/L
Highest Concentration Without Toxic Effect (NOEC)	< 0.10 µg foramsulfuron/L
Toxic Threshold Effect Concentration, TEC (Geometric mean of NOEC and LOEC)	NA

- ❑ **Conclusion:** The NOEC and LOEC in the 7-day exposure of *Lemna gibba* G3 to Foramsulfuron + Isoxadifen-Ethyl OD 22.5+22.5 g/L (AE F130360 01 1K0p A9) for the endpoint of growth rate for frond dry weight were 0.77 µg a.s./L and 1.5 µg a.s./L, respectively. For all other endpoints the NOEC and LOEC was < 0.10 µg a.s./L and 0.10 µg a.s./L, respectively. Additionally, following the recommendations in OECD 221,  $EC_{10}$ s were calculated for all endpoints. The endpoint with the most sensitive  $EC_{10}$  was day 7 frond dry weight yield with an  $E_yC_{10}$  value of 0.14 µg a.s./L. The endpoint with the most sensitive  $EC_{50}$  value was day 7 frond yield with an  $E_yC_{50}$  value of 0.75 µg a.s./L.
- ❑ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.2.8. Further testing on aquatic organisms

Also the potential effects of foramsulfuron on *Palaemonetes pugio* and *Crassostrea virginica* were investigated and evaluated within the process of Annex I inclusion and were considered acceptable by the RMS Germany. The 96 hours acute toxicity study to the grass shrimp was performed according to the OECD test guideline 203: Fish, Acute Toxicity Test and the 96 hours toxicity study to eastern oyster according to the US EPA: FIFRA 72-3. There is no OECD test guideline for mollusc shell deposition test.

##### B.9.2.8.1. Acute toxicity of foramsulfuron on *Palaemonetes pugio* (grass shrimp) (static)

Report:	<a href="#">KCA 8.2.8 /02; Stachura, B. J.; Ruff, D. F.; 1998; M-143552-01</a>
Title:	96 hour acute toxicity to the Grass Shrimp, <i>Palaemonetes pugio</i> , in a static system AE F130360 technical 94.2% w/w Code: AE F130360 00 1C94 0001
Report No:	A59902
Document No:	<a href="#">M-143552-01-1</a>
Guidelines:	OECD: 203; USEPA (=EPA): E 72-3; Deviation not specified
GLP/GEP:	yes

- ❑ **Conclusion:** The 96 hour LC<sub>50</sub> of AE F130360 technical to grass shrimp could not be determined under the conditions of this study, and thus is greater than 100 mg/L. The no observed effect concentration (NOEC) was 100 mg/L. The lowest observed effect concentration (LOEC) could not be determined under the conditions of this study.
- ❑ **Comment (Co-RMS and RMS):** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final). EC<sub>50</sub> > 100 mg a.s./L.

#### B.9.2.8.2. Acute toxicity of foramsulfuron on *Crassostrea virginica* (eastern oyster) (flow-through)

<b>Report:</b>	<u>KCA 8.2.8 /01;Boeri, R. L.; Magazu, J. P.; Ward, T. J.;1998;M-181443-01</u>
<b>Title:</b>	Flow-through mollusc shell deposition test AE F130360
<b>Report No:</b>	C000906
<b>Document No:</b>	<u>M-181443-01-1</u>
<b>Guidelines:</b>	USEPA (=EPA): FIFRA 72-3, SEP 540/9-85-011;Deviation not specified
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** After 96 hours of AE F130360 exposure, water control oysters produced a pooled average, new shell growth of 3.4 mm. Exposure of eastern oysters resulted in a 96-hour EC<sub>50</sub> of 118 mg/L AE F130360, with a 95% confidence interval of 85.1 to 164 mg/L. The 96-hour no observed effect concentration is 13.4 mg/L AE F130360, based upon mean measured concentrations and new shell growth.
- ❑ **Comment (Co-RMS and RMS):** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final). EC<sub>50</sub> = 118 mg a.s./L.

### B.9.3. EFFECTS ON ARTHROPODS

#### B.9.3.1. Effects on bees

Potential acute and contact effects of foramsulfuron on bees were evaluated within the process of Annex I inclusion and were considered acceptable by the RMS Germany. This study was not re-evaluated since two new studies, one with the active substance and one with the product Equip OD 45 were submitted for the process of renewal. These studies were performed according to the OECD test guidelines 213 and 214 (1998): Honeybees, Acute Oral and Contact Toxicity Tests. Foramsulfuron has a low acute toxicity to honey bees, with LD<sub>50</sub> (oral and contact) above the highest tested dose level (oral: LD<sub>50</sub> > 110.1 µg a.s./bee, contact: LD<sub>50</sub> > 100 µg a.s./bee).

For purpose of renewal also a chronic adult feeding study, a larvae study, a honey bee brood feeding study and a semi-field honey bee brood study are submitted and evaluated in this dRAR. The chronic adult feeding study and the larvae study showed low toxicity of foramsulfuron with NOED values of > 120 mg a.s./kg and > 100 µg a.s./larva, respectively. The chronic adult feeding study, larvae study and honey bee brood feeding study were performed with formulation Foramsulfuron 50 WG. Representative formulation Equip OD 45 contains foramsulfuron technical (herbicide) and isoxadifen – ethyl (safener) in comparison to tested product Foramsulfuron WG 50 which contains foramsulfuron and cyprosulfamide (safener). Obtained results from these studies are considered valid, in spite of tested active substance was used in form of Foramsulfuron WG 50.

The conducted bee brood feeding study (Oomen et al., 1992) found a slightly, but statistically significantly increased termination rate of young and old larvae; as the observed slightly elevated termination rate of larvae was in absolute terms low; this observation - if at all test item related - was as such biologically not relevant. The bee brood feeding study further did not reveal adverse effects on the survival of adult bees and pupae, behaviour, colony strength, colony development as well as the condition of the colonies. Nonetheless, to clarify whether the observations in the honey bee brood feeding study are due to natural variability or test-item related, foramsulfuron was subjected to *in-vitro* larval testing. The limit test revealed no adverse effects on larval mortality with LD<sub>50</sub> > 120 µg a.s./larva. Based on the findings of the *in-vitro* larvae study, the observations in the honey bee brood feeding study are rather to be attributed to natural variability than being test-item related (intrinsic).

In parallel, foramsulfuron was subjected to confined semi-field testing by applying the maximum rate of Foramsulfuron + Isoxadifen-ethyl OD 45 (60 g a.s./ha) to full-flowering *Phacelia* during honey bees actively foraging on the crop. The results of this higher tier study confirmed all conclusions made above on the basis of the outcome of the lower-tiered studies, as no adverse direct or delayed effects on mortality of worker bees or pupae, foraging activity, behaviour, nectar- and pollen storage, queen survival, colony strength, colony development as well as the development of bee brood were observed.

Details of studies on foramsulfuron and two foramsulfuron formulations are provided briefly in the Table B.9.3.1-1. Summaries of the studies are provided thereafter.

**Table B.9.3.1-1: Honey bee toxicity of foramsulfuron (tech.) and formulated foramsulfuron to bees**

Test substance, Test species	Test system	Endpoints	Reference
<b>Foramsulfuron, tech.</b>			
Honey bee ( <i>Apis mellifera</i> )	oral 48/72 h	LD <sub>50</sub> > 163 µg a.s./bee	Waltersdorfer, 1998 <a href="#">M-143626-01-1</a> KCA 8.3.1.1.1 /01
Honey bee ( <i>Apis mellifera</i> )	contact 48/72 h	LD <sub>50</sub> > 1.9 µg a.s./bee	Waltersdorfer, 1997 <a href="#">M-143215-01-1</a> KCA 8.3.1.1.2 /01
Honey bee ( <i>Apis mellifera</i> )	oral 48 h contact 48 h	LD <sub>50</sub> > 110.1 µg a.s./bee LD <sub>50</sub> > 100 µg a.s./bee	Schmitzer & Sekine, 2012 <a href="#">M-444765-01-1</a> KCA 8.3.1.1.1/02 & KCA 8.3.1.1.2 /02

Test substance, Test species	Test system	Endpoints	Reference
<b>Foramsulfuron WG 50</b>			
Honey bee ( <i>Apis mellifera</i> )	10 d chronic adult feeding study	LC50 > 120 mg a.s./kg NOEC ≥ 120 mg a.s./kg	Kling, (2013) M-470639-01-1 KCA 8.3.1.2/01
Honey bee ( <i>Apis mellifera</i> )	In vitro honey bee larvae laboratory study, single exposure test design	LD50 > 100 µg a.s./larva NOED ≥ 100 µg a.s./larva	Przygoda & Nikolakis, (2013) M-470485-01-1 KCA 8.3.1.3/01
Honey bee ( <i>Apis mellifera</i> )	Honey bee brood feeding (Oomen et al., 1992)	Slightly, but statistically significantly increased termination rate of young and old larvae, which is not biologically relevant; no adverse effects on the survival of adult bees and pupae, behaviour, colony strength, condition of the colonies, brood index and brood compensation index by feeding honey bee colonies sugar syrup at a foramsulfuron concentration typically present in the spray tank (100 ppm)	Jeker, (2013) M-465326-01-1 KCA 8.3.1.3/02
<b>Foramsulfuron + Isoxadifen-ethyl OD 45 (22.5 + 22.5)</b>			
Honey bee ( <i>Apis mellifera</i> )	oral 72 h	LD <sub>50</sub> = 226.3 µg product/bee (5.8 µg a.s./bee)	Waltersdorfer, A; 1999; M-187295-01-1 KCP 10.3.1.1.1 /01
Honey bee ( <i>Apis mellifera</i> )	contact 72 h	LD <sub>50</sub> > 392.2 µg product/bee (10.1 µg a.s./bee)	Waltersdorfer, A; 1999; M-187293-01-1 KCP 10.3.1.1.1 /02
Honey bee ( <i>Apis mellifera</i> )	oral 48 h contact 48 h	LD <sub>50</sub> > 214.4 µg product/bee (> 5.0 µg a.s./bee) LD <sub>50</sub> > 200.0 µg product/bee (> 4.66 µg a.s./bee)	Sekine, T; 2013; 1999 M-465361-01-1 KCP 10.3.1.1.1 /03
Honey bee ( <i>Apis mellifera</i> )	Semi-field honey bee brood study (acc. to OECD 75; forced exposure conditions) in <i>Phacelia</i> ; application during full-bloom and bees actively foraging	No adverse effects on mortality, flight intensity, behaviour, brood development (brood termination rate, brood index, compensation index) as well as on colony vitality at maximum application rate (2.67 L product/ha)	Schmitzer (2013) M-468794-01-1 KCA 8.3.1.3/03

### B.9.3.1.1. Contact and oral acute toxicity (Annex IIA 8.3, Annex IIIA 10.4)

#### B.9.3.1.1.1. Acute oral toxicity of foramsulfuron tech. on bees

<b>Report:</b>	<u>KCA 8.3.1.1.1 /01;Waltersdorfer, A.;1998;M-143626-01</u>
<b>Title:</b>	Code: AE F130360 00 1C98 0001; substance, technical - Oral toxicity (LD 50) to honey bees ( <i>Apis mellifera</i> L.)
<b>Report No:</b>	A59983
<b>Document No:</b>	<u>M-143626-01-1</u>
<b>Guidelines:</b>	EPPO: 170;Deviation not specified
<b>GLP/GEP:</b>	yes

□ **Conclusion:** The 72 hours oral LD<sub>50</sub> under laboratory test conditions was > 163.09 µg a.s./bee.

- ❑ **Comment (Co-RMS and RMS):** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

#### B.9.3.1.1.2. Acute contact toxicity of foramsulfuron tech. on bees

<b>Report:</b>	<u>KCA 8.3.1.1.2 /01;Waltersdorfer, A.;1997;M-143215-01</u>
<b>Title:</b>	Code: AE F130360 00 1C98 0001 - Contact toxicity (LD50) to honey bees ( <i>Apis mellifera</i> L.)
<b>Report No:</b>	A59544
<b>Document No:</b>	<u>M-143215-01-1</u>
<b>Guidelines:</b>	<b>EPPO: 170; USEPA (=EPA): L 141-1;Deviation not specified</b>
<b>GLP/GEP:</b>	<b>yes</b>

- ❑ **Conclusion:** The 72 hours contact LD50 under laboratory test conditions was > 1.9 µg a.s./bee.
- ❑ **Comment (Co-RMS and RMS):** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

#### B.9.3.1.1.3. Acute toxicity of foramsulfuron tech. on bees (new study)

<b>Report:</b>	<u>KCA 8.3.1.1.2 /02;Schmitzer, S.; Sekine, T;2012;M-444765-01</u>
<b>Title:</b>	Effects of foramsulfuron tech. (acute contact and oral) on honey bees ( <i>Apis mellifera</i> L.) in the laboratory
<b>Report No:</b>	75201035
<b>Document No:</b>	<u>M-444765-01-1</u>
<b>Guidelines:</b>	<b>OECD 213 and 214 (1998);none</b>
<b>GLP/GEP:</b>	<b>yes</b>

- ❑ **Deviations:** A 5 µL droplet was chosen in deviation to the guideline recommendation (OECD 214) of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item; IBACON experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected; [presented as a poster on the ICPBR Bee Protection Group meeting in Bologna, 2002].

❑ **Materials and methods:**

<b>Test Material:</b>	Foramsulfuron technical
<b>Description:</b>	white / solid
<b>Lot/Batch No.:</b>	AE F130360-01-02
<b>Purity:</b>	foramsulfuron (AE F130360): 97.3 % w/w (analytical), according to certificate of analysis.
<b>Stability:</b>	The test item was assumed to be stable for the period of use in the study.
<b>Storage:</b>	At 10 to 30 °C, under dark and dry conditions.
<b>Control:</b>	Contact - Tap water with 0.5 % Adhäsit* (applied after anesthetization with CO <sub>2</sub> ), Oral - 50% (w/v) aqueous sucrose solution
<b>Treatment rates:</b>	Contact: 100.0 µg a.s. bee <sup>-1</sup> ; Oral limit: nominal dose: 100.0 µg a.s. bee <sup>-1</sup> ; actual consumption doses: 110.1 µg a.s. bee <sup>-1</sup> ; Oral dose response: nominal doses: 75, 50 and 25 µg a.s. bee <sup>-1</sup> ; actual consumption doses: 81.4, 54.2 and 27.9 µg a.s. bee <sup>-1</sup> .
<b>Reference item:</b>	Perfekthion (a.i. dimethoate)
<b>Test organisms</b>	
<b>Species:</b>	Honey bee <i>Apis mellifera</i> (Hymenoptera: <i>Apidae</i> )
<b>Source:</b>	Honey bee colonies, disease-free and queen-right, bred by IBACON, Germany
<b>Food:</b>	Commercial ready-to-use sugar syrup (Apiinvert; 30 % sucrose, 31 % glucose, 39 % fructose) <i>ad libitum</i> . This was done with syringes that



were inserted into the cages *via* an opening in the top of the test units and from which bees accessed the food directly. No replacement of the food was necessary during the experimental period (48 h).

#### Test design

<b>Application:</b>	Contact: application of 5 µL droplet of test solution to dorsal body surface (thorax) Oral: oral ingestion of sucrose solution
<b>Replication:</b>	test units (limit test) / 3 test units (oral dose response test) per test item dose level, control and reference item dose level, respectively
<b>No. of bees per rep:</b>	10 bees per test unit
<b>Environmental conditions</b>	
<b>Temperature:</b>	25.0 °C
<b>Humidity:</b>	Contact and oral limit test: 53 - 89 %; Oral dose response test: 51 - 75 %
<b>Photoperiod:</b>	Complete darkness
<b>Duration of test:</b>	48 hours

#### □ Summary

The aim of this study was to determine the acute contact and oral toxicity of foramsulfuron tech. to the honey bee (*A. mellifera* L.) under laboratory conditions. For this purpose female worker bees (*Apis mellifera*) were exposed for 48 hours to a single dose of 100.0 µg a.s./bee by topical application (contact limit test) and to a single dose of 110.1 µg a.s./bee for feeding (oral limit test, value based on the actual intake of the test item). In addition to the oral limit toxicity test, in another oral dose response test 30 female worker bees per dose were exposed for 48 hours to 81.4, 54.2 and 27.9 µg a.s./bee for feeding (values based on the actual intake of the test item). Mortality of the bees was used as the toxic endpoint. Sub-lethal effects, such as changes in behaviour, were also assessed.

The contact LD50 (48 h) was > 100.0 µg a.s./bee. The oral LD50 (48 h) was > 110.1 µg a.s./bee. The contact NOED was ≥ 100 µg a.s./bee. The oral NOED was estimated in an additional dose response toxicity test. The oral NOED was 81.4 µg a.s./bee.

#### □ Study Design and Methods:

10 bees were used per test unit. 5 test units for the limit tests and 3 test units for the oral dose response test were used per test item dose level, control and reference item dose level, respectively. 50 female worker bees (*Apis mellifera*) were exposed for 48 hours to a single dose of 100.0 µg a.s./bee by topical application (contact limit test) and 50 female worker bees (*Apis mellifera*) were exposed for 48 hours for feeding to a single dose of 110.1 µg a.s./bee (oral limit test, value based on the actual intake of the test item). In addition to the oral limit toxicity test, in another oral dose response test 30 female worker bees per dose were exposed for 48 hours to 81.4, 54.2 and 27.9 µg a.s./bee for feeding (values based on the actual intake of the test item).

For the contact test a single 5 µL droplet of foramsulfuron tech., dissolved in tap water with 0.5 % Adhäsit, was placed on the dorsal bee thorax, likewise for the toxic reference (dimethoate) and the control (tap water). For both oral tests aqueous stock solutions of the test item and reference item were prepared and mixed with ready-to-use sugar syrup (30 % sucrose, 31 % glucose, 39 % fructose) at a concentration of 50 % (w/w). For the control, tap water and sugar syrup was used at the same ratio 50% (w/w) tap water, 50% (w/w) ready-to-use sugar syrup. The treated food was offered in syringes, which were weighed before and after introduction into the cages. After a maximum of 1 hour 50 minutes (limit test) or 2 hours 15 minutes (dose response test) the uptake was complete and the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food. The number of dead bees was determined 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 25 °C; relative humidity was 53 - 89% for the contact and oral limit test and 51 - 75% for the oral dose response test. Bees were kept in darkness (except during observation).

#### □ Findings:

Control mortality during the 48 hour observation period in the oral and contact tests was 0.0%. Mortality data for the test item and reference item are summarised in the table below.



**Table B.9.3.1.1.3-1: Summary of observed mortality in the oral and contact limit test**

Treatment oral/contact	Mortality (%)					
	Oral test			Contact test		
	4h	24 h	48 h	4h	24 h	48 h
Control	0.0	0.0	0.0	0.0	0.0	4.0
<b>Test item foramsulfuron tech. (<math>\mu\text{g ai/bee}</math>)</b>						
110.1/100.0	0.0	0.0	10.0	0.0	0.0	0.0
<b>Reference item "Perfekthion" (<math>\mu\text{g dimethoate/bee}</math>)</b>						
0.33 / 0.30	12.0	76.0	80.0	2.0	92.0	94.0
0.16 / 0.20	2.0	60.0	70.0	0.0	68.0	76.0
0.08 / 0.15	0.0	26.0	42.0	0.0	30.0	38.0
0.05 / 0.10	0.0	8.0	10.0	0.0	10.0	20.0

The 24-hour oral limit and contact LD<sub>50</sub> values for the reference item were 0.16 and 0.17  $\mu\text{g dimethoate bee}^{-1}$ , respectively.

**Table B.3.1.1.3-2: Summary of observed mortality in the oral dose response test**

Treatment Oral / Contact	Mortality (%)		
	Oral dose response test		
	4h	24 h	48 h
Control	0.0	0.0	0.0
<b>Test item foramsulfuron tech. (<math>\mu\text{g ai/bee}</math>)</b>			
81.4	0.0	0.0	3.3
54.2	0.0	0.0	3.3
27.9	3.3	3.3	6.7
<b>Reference item "Perfekthion" (<math>\mu\text{g dimethoate/bee}</math>)</b>			
0.31	63.3	100.0	100.0
0.16	10.0	83.3	86.7
0.08	0.0	23.3	33.3
0.06	0.0	3.3	6.7

The 24-hour oral dose response LD<sub>50</sub> value for the reference item was 0.11  $\mu\text{g dimethoate bee}^{-1}$ . LD<sub>50</sub> value for the reference item should be between 0.10 – 0.35  $\mu\text{g dimethoate bee}^{-1}$  (according to OECD 213 and 214).

**Table B.9.3.1.1.3-3: Summary of toxicity of the test item foramsulfuron tech. and toxic standard (dimethoate) to the honeybee**

Treatment	Exposure		LD <sub>50</sub> values	95% confidence interval $\mu\text{g a.i./bee}$
	Route	Duration (hours)		
Test material	Contact	LD <sub>50</sub> (48)	> 100.0 $\mu\text{g a.s./bee}$	-
		NOED*	$\geq 100.0 \mu\text{g } \mu\text{g a.s./bee}$	-
	Oral	LD <sub>50</sub> (48)	> 110.1 $\mu\text{g a.s./bee}$	-
		NOED*	$\geq 81.4 \mu\text{g a.s./bee}$	-
Toxic standard	Oral limit	LD <sub>50</sub> (24)	0.16 $\mu\text{g dimethoate/bee}$	0.11 to 0.22
	Contact	LD <sub>50</sub> (24)	0.17 $\mu\text{g dimethoate/bee}$	0.15 to 0.20
	Oral dose response	LD <sub>50</sub> (24)	0.11 $\mu\text{g dimethoate/bee}$	0.10 to 0.13

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

- ❑ **Conclusion:** The contact LD<sub>50</sub> (48 h) was > 100.0 µg foramsulfuron tech./bee. The oral LD<sub>50</sub> (48 h) was > 110.1 µg foramsulfuron tech./bee. The contact NOED was ≥ 100 µg foramsulfuron tech./bee. The oral NOED was estimated in an additional dose response toxicity test. The oral NOED was 81.4 µg foramsulfuron tech./bee.
- ❑ **Comment (Co-RMS and RMS):** The choice of 5 µL droplet what is in deviation to the guideline recommendation of a 1 µL droplet (OECD 214) seems to be reasonable. No other comments, study is acceptable.

#### B.9.3.1.1.4. Acute oral toxicity of foramsulfuron + isoxadifen-ethyl (oil-flowable) 45 (22.5+22.5) on bees

<b>Report:</b>	KCP 10.3.1.1.1 /01; Waltersdorfer, A; 1999; M-187295-01-1
<b>Title:</b>	Oral toxicity (LD50) to honey bees ( <i>Apis mellifera</i> L.) AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L
<b>Report No:</b>	CW 98/110
<b>Document No:</b>	M-187295-01-1
<b>Guidelines:</b>	EPPO – guideline No. 170
<b>GLP/GEP:</b>	Yes

- ❑ **Conclusion:** The 72 hours oral LD<sub>50</sub> under laboratory test conditions was 226.3 µg product/bee (5.8 µg a.s./bee).
- ❑ **Comment (Co-RMS and RMS):** The endpoint from this study is referred in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final). No other comments, study is acceptable.

#### B.9.3.1.1.5. Acute contact toxicity of foramsulfuron + isoxadifen-ethyl (oil-flowable) 45 (22.5+22.5) on bees

<b>Report:</b>	KCP 10.3.1.1.1 /02; Waltersdorfer, A; 1999; M-187293-01-1
<b>Title:</b>	Contact toxicity (LD50) to honey bees ( <i>Apis mellifera</i> L.) AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L
<b>Report No:</b>	CW 98/109
<b>Document No:</b>	M-187293-01-1
<b>Guidelines:</b>	EPPO – guideline No. 170
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** The 72 hours oral LD50 under laboratory test conditions was > 392.2µg product/bee (10.1 µg a.s./bee).
- ❑ **Comment (Co-RMS and RMS):** The endpoint from this study is referred in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final). No other comments, study is acceptable.

#### B.9.3.1.1.6. Acute toxicity of foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G on bees (new study)

<b>Report:</b>	KCP 10.3.1.1.1 /03; Sekine, T; 2013; 1999; M-465361-01-1
<b>Title:</b>	Effects of foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G (acute contact and oral) on honey bees ( <i>Apis mellifera</i> L.) in the laboratory.
<b>Report No:</b>	EBFSN048
<b>Document No:</b>	M-465361-01-1
<b>Guidelines:</b>	<b>OECD (1998a) 213; OECD (1998b) 214; Deviations:</b> A 5 µL droplet was chosen in deviation to the guideline recommendation (OECD 214) of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item; IBACON experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected; [presented as a poster on the ICPBR Bee Protection Group meeting in Bologna, 2002].
<b>GLP/GEP:</b>	yes

## □ Materials and methods:

<b>Test Material:</b>	Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G
<b>Description:</b>	liquid
<b>Lot/Batch No.:</b>	EFKM002442
<b>Purity:</b>	1.) Foramsulfuron (AE F130360): 2.33 % w/w, 22.41 g/L, 2.) Isoxadifen-ethyl (AE F122006): 2.29 % w/w, 21.96 g/L, according to certificate of analysis
<b>Stability:</b>	The test item was assumed to be stable for the period of use in the study
<b>Storage:</b>	At +2 to +30 °C, under dark and dry conditions
<b>Control:</b>	Contact - Tap water with 0.5 % Adhäsit (applied after anesthetization with CO <sub>2</sub> ), Oral - 50 % (w/w) aqueous sugar syrup solution (50 % tap water, 50 % ready-to-use sugar syrup)
<b>Treatment rates:</b>	Contact limit: 200.0 µg product per bee <sup>-1</sup> ; Oral limit: actual consumption dose of 214.4 µg product per bee <sup>-1</sup> .
<b>Reference item:</b>	Perfekthion EC (BAS 15211 I; a.i. dimethoate)
<b>Test organisms</b>	
<b>Species:</b>	Honey bee <i>Apis mellifera carnica</i> L. (Hymenoptera: <i>Apidae</i> )
<b>Source:</b>	Honey bee colonies, disease-free and queen-right, bred by IBACON, Germany
<b>Food:</b>	Commercial ready-to-use sugar syrup (Apiinvert; 30 % sucrose, 31 % glucose, 39 % fructose) <i>ad libitum</i> . This was done with syringes that were inserted into the cages <i>via</i> an opening in the top of the test units and from which bees accessed the food directly. No replacement of the food was necessary during the experimental period (48 h).
<b>Test design</b>	
<b>Application:</b>	Contact: application of 5 µL droplet of test solution to dorsal body surface (thorax) Oral: oral ingestion of sucrose solution
<b>Replication:</b>	5 test units per test item dose level, control and reference item dose level, respectively
<b>No. of bees per rep:</b>	10 bees per test unit
<b>Environmental conditions</b>	
<b>Temperature:</b>	24.0 - 25.0 °C
<b>Humidity:</b>	51 - 77 %;
<b>Photoperiod:</b>	darkness
<b>Duration of test:</b>	48 hours

## □ Study Design and Methods:

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 female worker bees (*Apis mellifera*) were exposed for 48 hours to a single dose of 200.0 µg product/bee by topical application (contact limit test) and 50 female worker bees (*Apis mellifera*) were exposed for 48 hours to a single dose of 214.4 µg product/bee by feeding (oral limit test, value based on the actual intake of the test item).

For the contact test a single 5 µL droplet of foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G, dissolved in tap water with 0.5 % Adhäsit, was placed on the dorsal bee thorax, likewise for the toxic reference (dimethoate) and the control (tap water). For the oral test aqueous stock solutions of the test item and reference item were prepared and mixed with ready-to-use sugar syrup (30 % sucrose, 31 % glucose, 39 % fructose) at a concentration of 50 % (w/w). For the control, tap water and sugar syrup was used at the same ratio 50% (w/w) tap water, 50% (w/w) ready-to-use sugar syrup. The treated food was offered in syringes, which were weighed before and after introduction into the cages. After a maximum 55 minutes, the uptake was complete and the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food.

The number of dead bees was determined 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 ( $\pm 2$  h) hours. Temperature during the test was 24 - 25 °C; relative humidity was 51 - 77%. Bees were kept in darkness (except during observation).

- **Findings:** Control mortality during the 48 hour observation period in the oral and contact tests was 0.0%. Mortality data for the test item and reference item are summarised in the table below.

**Table B.9.3.1.1.6-1: Summary of observed mortality in the oral and contact test**

Treatment Oral / Contact	Mortality (%)					
	Oral test			Contact test		
	4h	24 h	48 h	4h	24 h	48 h
<b>Control</b>	0.0	0.0	0.0	0.0	0.0	0.0
<b>Test item foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G (<math>\mu\text{g ai/bee}</math>)</b>						
214.4 / 200.0	0.0	2.0	2.0	2.0	2.0	2.0
<b>Reference item "Perfekthion" (<math>\mu\text{g dimethoate/bee}</math>)</b>						
0.33 / 0.30	4.0	100.0	100.0	0.0	72.0	78.0
0.16 / 0.20	0.0	68.0	72.0	0.0	58.0	64.0
0.08 / 0.15	0.0	4.0	8.0	0.0	18.0	26.0
0.05 / 0.10	0.0	4.0	4.0	0.0	4.0	6.0

The 24-hour oral and contact LD<sub>50</sub> values for the reference item were 0.13 and 0.19  $\mu\text{g dimethoate bee}^{-1}$ , respectively.

**Table B.9.3.1.1.6-2: Summary of toxicity of the test item foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G to the honeybee**

Treatment	Exposure		LD <sub>50</sub> values	95% confidence interval
	Route	Duration (hours)		
Test material	Contact	LD <sub>50</sub> (24)	> 200.0 $\mu\text{g product/bee}$ > 4.66 $\mu\text{g a.s./bee}^{*1}$	-
		NOED(24)	$\geq 200.0 \mu\text{g product/bee}^{*}$ > 4.66 $\mu\text{g a.s./bee}^{*1}$	-
	Oral	LD <sub>50</sub> (24)	> 214.4 $\mu\text{g product/bee}$ > 5.0 $\mu\text{g a.s./bee}^{*1}$	-
		NOED(24)	$\geq 214.4 \mu\text{g product/bee}$ 5.0 $\mu\text{g a.s./bee}^{*1}$	-
Toxic standard	Oral	24	0.13 $\mu\text{g dimethoate/bee}$	0.08 to 0.16
	Contact	24	0.19 $\mu\text{g dimethoate/bee}$	0.15 to 0.30

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

\*<sup>1</sup> based on the analysed content of active ingredient of 2.33 % w/w, resp. 22.41 g/L

- **Conclusion:** The contact LD<sub>50</sub> (48 h) was > 200.0  $\mu\text{g product/bee}$  (> 4.66  $\mu\text{g a.s./bee}$ ). The oral LD<sub>50</sub> (48 h) was > 214.4  $\mu\text{g product/bee}$  (> 5.0  $\mu\text{g a.s./bee}$ ).
- **Comment (Co-RMS and RMS):** The choice of 5  $\mu\text{L}$  droplet what is in deviation to the guideline recommendation of a 1  $\mu\text{L}$  droplet (OECD 214) seems to be reasonable. No other comments, study is acceptable.

### B.9.3.1.2. Chronic toxicity on adult bees

#### B.9.3.1.2.1. Chronic toxicity of Foramsulfuron WG 50 W on bees

A 10 day chronic oral toxicity study was conducted with Foramsulfuron WG 50 as technical foramsulfuron was not well soluble in water. Representative formulation Equip OD 45 contains foramsulfuron technical (herbicide) and isoxadifen – ethyl (safener) in comparison to tested product Foramsulfuron WG 50 which contains a.s. foramsulfuron (TOX-No.: 09720-01; Batch ID: 2012-001517; Specification no.: 102000026995; content of a.s. (analysed): 49.4% w/w).

<b>Report:</b>	<u>KCA 8.3.1.2 /01;Kling, A.;2013;M-470639-01</u>
<b>Title:</b>	Foramsulfuron WG 50 W - Assessment of chronic effects to the honeybee, <i>Apis mellifera</i> L., in a 10 days continuous laboratory feeding limit test
<b>Report No:</b>	S13-00153
<b>Document No:</b>	<u>M-470639-01-1</u>
<b>Guidelines:</b>	<b>No OECD test available; not applicable</b>
<b>GLP/GEP:</b>	<b>yes</b>

❑ **Objective:**

To investigate the potential chronic effects of foramsulfuron on the honey bee, *Apis mellifera* L., in a 10 days continuous feeding test in the laboratory and to investigate whether the LC<sub>50</sub>-/NOEC- value is greater than the tested concentration.

❑ **Materials and methods:**

<b>Test Material:</b>	Foramsulfuron WG 50 W
<b>Description:</b>	solid
<b>Lot/Batch No.:</b>	2012-001517
<b>Purity:</b>	49.4 % w/w (analysed)
<b>Stability:</b>	The test item was assumed to be stable for the period of use in the study
<b>Storage:</b>	At +2 to +30 °C
<b>Control:</b>	oral – untreated 50% (w/v) aqueous sucrose solution
<b>Treatment rates:</b>	Oral, nominal dose: over a test period of 10 days, honey bees were fed continuously and <i>ad libitum</i> with a 50 % (w/v) aqueous sucrose solution, containing the test item Foramsulfuron WG 50 W at the nominal concentration level of 120 mg a.s./kg. The unit a.s. refers to the analysed content of the active substance foramsulfuron (49.4 % w/w).
<b>Reference item:</b>	none
<b>Test organisms</b>	
<b>Species:</b>	Honey bee <i>Apis mellifera</i> (Hymenoptera: <i>Apidae</i> ), young adult worker bees (newly hatched; 1 to 4 days old)
<b>Source:</b>	Honey bee colonies descended from a breeding line of a beekeeper in Rheinland-Pfalz, Germany (Mr. Gerald Wolters, Im Bannen 38-54, 56727 Mayen, Germany)
<b>Food:</b>	aqueous sucrose solution (50 w/v). The application (feeding) solutions were offered <i>ad libitum</i> to each cage of 10 bees in plastic syringes (Omnifix®, 5 mL, B. Braun, Melsungen, Germany). The tip of each syringe was removed so that the bees had access to the application (feeding) solution. Every morning the syringes of all test cages (i.e. test item and control) were replaced by new syringes, filled with freshly prepared application (feeding) solution over a period of 10 days. The weight of the syringes was determined before and after feeding on the next day in order to determine the mean food consumption of the bees per replicate.
<b>Test unit</b>	During the entire test period, the bees were kept in cages made of stainless steel (base: 8 cm x 4 cm; height: 6 cm). The front side of the cages were equipped with a transparent pane so that the bees could be observed. The bottom of the cages consisted of a perforated board,

which guaranteed sufficient air supply for the test animals. The test units were lined with filter paper.

The application (feeding) solutions were offered to the bees in syringes fitted into the test units.

#### Test design

**Application:** Ingestion in aqueous sucrose solution (50 w/v) over a test period of 10 days.

**Replication:** For each treatment group, 10 replicates (cages) each containing 10 bees were tested (100 bees in total)

#### Environmental conditions

**Temperature:** 31.7 – 33.3 °C

**Humidity:** 55.9 – 69.5 %

**Photoperiod:** Complete darkness

**Duration of test:** 10 days

#### □ Study Design and Methods:

Over a period of 10 days, honey bees were exposed to 50 % (w/v) aqueous sucrose application (feeding) solution, containing nominally 120 mg a.s./kg of the test item Foramsulfuron WG 50 W by continuous and *ad libitum* feeding. The control group was exposed for the same period of time under identical exposure conditions to untreated 50 % (w/v) aqueous sucrose application (feeding) solution. Mortality, sub-lethal effects and behavioural observations were assessed every day throughout the 10 days exposure period. Furthermore, the daily food uptake was determined.

#### □ Findings:

After 10 days of continuous exposure, mortality at the test item treatment level of 120 mg a.s./kg of Foramsulfuron WG 50 W was not statistically significantly different when compared to the control group. The cumulative control mortality was 3.0 %, as determined at the final assessment after 10 days (see table below). The cumulative mortality at the treatment level of 120 mg a.s./kg Foramsulfuron WG 50 W was 2.0 % at the final assessment. At 120 mg a.s./kg Foramsulfuron WG 50 W, no sub-lethal effects or behavioural abnormalities were observed throughout the entire observation period of 10 days.

**Table B.9.3.1.2.1-1: Cumulative mortality over 10 days of test period**

Treatment [mg a.s./kg]	Cumulative mortality [%]									
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10
Control	0.0	1.0	2.0	2.0	2.0	2.0	2.0	3.0	3.0	3.0
<b>Foramsulfuron WG 50 W: Actual and Corrected* Cumulative mortality [%]<sup>2</sup></b>										
120**	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	2.0	2.0
120*	0.0	-1.0	-2.0	-2.0	-1.0	-1.0	-1.0	-1.0	-1.0	-1.0

E = Assessment

<sup>1</sup> Application (feeding) solution: 50 % (w/v) aqueous sucrose solution

<sup>2</sup> Application (feeding) solution: 50 % (w/v) aqueous sucrose solution containing Foramsulfuron WG 50 W

\* Negative values indicate lower mortality in the test item group compared to the control group

\*\*Determined to be the NOEC based on mortality (not significantly different compared to the control; Fisher's Exact Test (one-sided,  $p \leq 0.05$ ))

After 10 days of continuous exposure, by considering the actual food consumption of the honey bees, the accumulated nominal intake of the test item Foramsulfuron WG 50 W at the treatment level of 120 mg a.s./kg was 52.44 µg a.s./bee, the corresponding average daily dose was therefore 5.2 µg a.s./bee (see table below).

**Table B.9.3.1.2.1-2: Mean nominal intake of active substance accumulated over test days**

Treatment [mg a.s./kg]	Mean accumulated nominal intake of active substance [µg a.s./bee]									
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
Control <sup>1</sup>	-	-	-	-	-	-	-	-	-	-
<b>Foramsulfuron WG 50 W<sup>2</sup></b>										
120	5.64	9.60	14.28	19.20	24.24	29.40	34.92	40.80	46.80	52.44

A = Application

<sup>1</sup> Application (feeding) solution: 50 % (w/v) aqueous sucrose solution

<sup>2</sup> Application (feeding) solution: 50 % (w/v) aqueous sucrose solution containing Foramsulfuron WG 50 W

The overall mean daily consumption of application (feeding) solution (i.e. the average value over 10 days) in the test item treatment group was not statistically significantly different (lower) when compared to the untreated control group (43.6 mg/bee at 120 mg a.s./kg, compared to 40.2 mg/bee in the control group). The mean daily consumption of the aqueous sucrose application (feeding) solution was not statistically significantly different (lower) between the control group and the test item treatment group throughout the entire testing period (day-by-day comparison).

- ❑ **Conclusion:** It can be concluded that the continuous ad libitum feeding of honey bees in the laboratory over a period of 10 consecutive days with the test item Foramsulfuron WG 50 W at the treatment level of 120 mg a.s./kg caused no adverse effect regarding mortality, sub-lethal effects and behaviour.

The overall mean daily consumption of application (feeding) solution (i.e. the average value over 10 days) in the test item treatment group was not statistically significantly different when compared to the untreated control group. Further, on every single day during the 10 day continuous exposure period the mean food consumption per bee was not statistically significantly different (lower) in the test item treatment group compared to the control group.

As the overall mean daily food uptake in the test item treatment group was not significantly lower compared to the control group, it can be concluded that there was no repellent effect of the test item at the treatment level of 120 mg a.s./kg.

The NOEC for mortality was determined at the end of the test period to be 120 mg a.s./kg (nominal). The LC50 was determined to be >120 mg a.s./kg (nominal).

- ❑ **Comment (Co-RMS and RMS):** The author states that a toxic standard was not included in this study, since a toxic reference substance had not been defined or validated for this type of study. This is acceptable.

No harmonised guideline is as yet available for this type of study, but recent description of this study (10-d oral feeding study on adult honeybees) in Appendix M of the EFSA GD on bees and Appendix O of the final version of the EFSA GD on bees (published July 2013) includes a control mortality of <15%. This is fulfilled, because the cumulative control mortality was 3.0 %, as determined at the final assessment after 10 days. The cumulative mortality at the treatment level of 120 mg a.s./kg Foramsulfuron WG 50 W was 2.0 % (corrected mortality: -1.0 %) at the final assessment. No sub-lethal effects or behavioural abnormalities were observed throughout the entire observation period of 10 days.

Temperature during the test was higher (31.7 – 33.3 °C) than those recommended in OECD 213 or 214 (25±2 °C). Technical comment (typo) – author of the study used wrong name of tested item in chapter 5.4.3. Preparation of application (feeding) solutions: Isoxaflutole WG 75W vs. Foramsulfuron WG 50. After 10 days of continuous exposure, by considering the actual food consumption of the honey bees, the accumulated nominal intake of the test item Foramsulfuron WG 50 W at the treatment level of 120 mg a.s./kg was 52.44 µg a.s./bee, the corresponding average daily dose was therefore 5.2 µg a.s./bee.

Obtained result is considered valid, in spite of tested active substance was used in form of Foramsulfuron WG 50. No other comment, study is acceptable.

### **B.9.3.1.3. Effects on honeybee development and other honeybee life stages (annex IIA 8.3.1.3)**

#### **B.9.3.1.3.1. An *in vitro* honey bee larvae laboratory study**

Study was conducted with Foramsulfuron WG 50 as technical foramsulfuron was not well soluble in water. Representative formulation Equip OD 45 contains foramsulfuron technical (herbicide) and isoxadifen – ethyl (safener) in comparison to tested product Foramsulfuron WG 50 which contains a.s. foramsulfuron (TOX-No.: 09720-01; Batch ID: 2012-001517; Specification no.: 102000026995; content of a.s. (analysed): 49.4% w/w).



<b>Report:</b>	<u>KCA 8.3.1.3 /01; Przygoda, D.; Nikolakis, A.:2013; M-470485-01</u>
<b>Title:</b>	Foramsulfuron WG 50 W: Effects of a single exposure to spiked diet on honey bee larvae ( <i>Apis mellifera carnica</i> ) in an in vitro laboratory testing design
<b>Report No:</b>	E3174533-6
<b>Document No:</b>	<u>M-470485-01-1</u>
<b>Guidelines:</b>	<b>EU Directive 91/414/EEC</b> <b>Regulation (EC) No. 1107/2009</b> <b>US EPA OCSPP 850.supp; not specified</b>
<b>GLP/GEP:</b>	<b>yes;</b> (certified laboratory). The rearing of honey bee larvae in their respective bee hives was not part of GLP. The preparation of saturated solutions of K <sub>2</sub> SO <sub>4</sub> and the preparation of solutions for the disinfection of grafting cells as well as for the wetting of dental rolls were not part of GLP. The procedure of the disinfection of grafting cells and the preparation of the test plates, were not part of the GLP.

□ **Materials and methods:**

<b>Test Material:</b>	Foramsulfuron WG 50 W
<b>Description:</b>	not stated
<b>Lot/Batch No.:</b>	2012-001517
<b>Purity:</b>	49.4 % w/w (analysed)
<b>Stability:</b>	The test item was assumed to be stable for the period of use in the study
<b>Storage:</b>	At -20 ± 5 °C
<b>Treatment rates:</b>	On day +4, the artificial diet was treated according to the respective test group. In the test item treatment group, foramsulfuron WG 50 W was incorporated into the artificial diet at the nominal test dose of 100 µg a.s./larva, corresponding to the nominal test concentration of 3030.3 mg a.s./kg diet.
<b>Control:</b>	water was incorporated into the artificial diet on day +4
<b>Reference item:</b>	Dimethoate was incorporated into the artificial diet at a nominal dose of 8.8 µg a.s./larva, corresponding to 266.7 mg a.s./kg diet.
<b>Administration:</b>	The larvae were fed with standardised amounts of artificial diet on day +1, +3, +4, +5 and +6.

**Test organisms**

<b>Species:</b>	Synchronised first instar larvae of <i>Apis mellifera carnica</i> (Hymenoptera: <i>Apidae</i> ), from three different honey bee colonies, each representing a replicate
<b>Source:</b>	Mr. Manfred Flosbach, An der Gerichtslinde 12, 42929 Wermelskirchen, North-Rhine Westphalia, Germany
<b>Food:</b>	The following three diet compositions (A, B, C) were used for feeding the bee larvae:

Diet A (day +1): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose.

Diet B (day +3): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose.

Diet C (from day +4 to day +6): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose.

Filtered, deionised water was used for the preparation of the diets (Milli-Q Academic system; 0.22 µm). For each diet, the sugar solution was filtered at 0.22 µm before being mixed to the royal jelly. De-frosted royal jelly (stored beforehand at about -20°C in a freezer) was used for the preparation of the respective diets. After preparation, the diets (A, B and C) were stored in a fridge at *ca.* +5°C until portioning of the untreated diets. Thereafter, the untreated portions of diets were stored in a freezer at *ca.* -20°C until use. The royal jelly was obtained from the apiary of Dr. Pia Aumeier, University of Bochum, Faculty of Biology, D-44801 Bochum, Germany.

**Test unit:**

During the test, larvae were maintained in 48-well culture plates with commercial grafting cells with an internal diameter of 9 mm. Before grafting, the grafting cells were disinfected for a minimum of 1.5 hours in a 1% aqueous Milton® sterilisation solution. The disinfected grafting cells were dried and distributed thereafter into the wells of a 48-well culture plate, which were previously half-filled with a piece of a dental roll (cellulose, wetted with a solution of 15% glycerol in 1% Milton® sterilisation solution). The 48-well culture plates were thereafter closed with a lid and stored at approximately +5°C in a refrigerator until use. During the biological phase of the test, no lid was used on the 48-well culture plates. The disinfection of grafting cells, the preparing of the solution for the wetting of dental rolls as well as the preparation of the test plates were not part of the GLP.

**Feeding and Maintenance of Honey Bee Larvae**

The feeding of the honey bee larvae took place once a day (except of day +2) according to the following schedule:

**Table B.9.3.1.3.1-1: Feeding schedule of the honey bee larvae**

Test group	Day	+1	+2	+3	+4	+5	+6
	Diet type	A	n.a.	B	C	C	C
Control	Volume of diet/larva [µL]	20 (untreated)	n.a.	20 (untreated)	30 (untreated)	40 (untreated)	50 (untreated)
Test item	Volume of diet/larva [µL]	20 (untreated)	n.a.	20 (untreated)	30 (treated) 100µg a.s./larva	40 (untreated)	50 (untreated)
reference Dimethoate	Volume of diet/larva [µL]	20 (untreated)	n.a.	20 (untreated)	30 (treated) 8.8µg a.s./larva	40 (untreated)	50 (untreated)

n.a. not applicable

The diets were placed in the incubator to attain a temperature of 30 - 35°C (handwarm) before use. Before feeding the larvae, dead larvae were discarded for sanitary reasons. Mortalities were recorded on day +5, day +6, day +7 (according to the study plan), and additionally on day +8.

**Test design**

**Application:** oral ingestion of artificial diet

**Replication:** For each treatment group 3 replicates from three different honey bee colonies.

**Environmental conditions**

From day +1 to day +8, the larvae were incubated in a hermetic container at about +35°C, containing a dish filled with a saturated solution of K<sub>2</sub>SO<sub>4</sub> in order to keep a relative humidity of on average 95 ± 5%. In order to avoid any potential contamination of the control larvae, the control group was stored in a separate incubator with the same test conditions as described above from day +1 to day +8. The desired test conditions were recorded with suitable and calibrated instruments. The preparation of saturated solutions of K<sub>2</sub>SO<sub>4</sub> and NaCl were not part of GLP.

**□ Study Design:**

At day +1 (Day 0 was the anticipated day of larval hatching), first instar bee larvae (*Apis mellifera carnica*) were transferred from their bee hive into an artificial in vitro testing system. The larvae were fed with standardised amounts of artificial diet on day +1, +3, +4, +5 and +6. On day +4, the artificial diet was treated according to the respective test group. In the test item treatment group, foramsulfuron WG 50 W was incorporated into the artificial diet at the nominal test dose of 100 µg a.s./larva, corresponding to the nominal test concentration of 3030.3 mg a.s./kg diet. In the reference item treatment group dimethoate was incorporated into the artificial diet at a nominal dose of 8 µg a.s./larva, corresponding to 266.7 mg a.s./kg diet. In the control group water was incorporated into the artificial diet. The actual concentration of foramsulfuron in the stock solution was determined according to Analytical Method 01340 for the determination of residues of foramsulfuron and its metabolite AE F153745 in/on plant matrix (sugar beet body and leaf) by HPLC-MS/MS.

During their development the honey bee larvae were incubated at about +35°C. The relative humidity inside the incubator was on average  $95 \pm 5\%$  from day +1 to +8. As the assessment endpoint mortality of the honey bee larvae was recorded on day +5, day +6, day +7 (according to the study plan), and additionally on day +8 (according to amendment no. 6). Dead test animals were discarded for sanitary reasons.

A first run of the study, conducted with the test item foramsulfuron tech. (TOX 09600-00), was stopped on 03 June 2013 (day +4 of the study) due to solubility problems of the technical material. It was therefore decided to conduct the study with the above-mentioned straight formulation.

#### □ Findings:

The validity criteria of the study were met (i.e. larval mortality in the control group from day +4 to day +7 was  $\leq 15\%$  and the larval mortality in the reference group was  $\geq 50\%$  from day +4 until day +7). In the control group, as well as in the test item treatment group, no larvae died until day +7. Until day +8, one single larva died in the control and in the test item treatment group, respectively.

**Table B.9.3.1.3.1-2: Control, test item and reference item performance and associated statistical evaluation**

Test object	Honey bee larvae ( <i>Apis mellifera carnica</i> )		
	Control (untreated diet)	Test Item (Foramsulfuron WG 50 spiked diet)	Reference Item (dimethoate, tech. spiked diet)
Test concentration (nominal) [mg a.s./kg diet]	---	3030.3	266.7
Feeding dose (nominal) [ $\mu\text{g}$ a.s./larva]	---	100	8.8
Total larval mortality until day +7 [%]	0.0	0.0	89.6
Abbott-corrected total mortality until day +7 [%]	0.0	0.0	89.6
* Statistical comparison to the control at day +7	---	n.s.	---
NOED at day +7 [ $\mu\text{g}$ a.s./larva]	---	$\geq 100$	---
LOED at day +7 [ $\mu\text{g}$ a.s./larva]	---	$> 100$	---
LD50 at day +7 [ $\mu\text{g}$ a.s./larva]	---	$> 100$	---
Total larval mortality until day +8 [%]	2.1	2.1	100
Abbott-corrected total mortality until day +8 [%]	---	0.0	100
*Statistical comparison to the control at day +8	---	n.s.	---
NOED at day +8 [ $\mu\text{g}$ a.s./larva]	---	$\geq 100$	---
LOED at day +8 [ $\mu\text{g}$ a.s./larva]	---	$> 100$	---
LD50 at day +8 [ $\mu\text{g}$ a.s./larva]	---	$> 100$	---

\* Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater,  $\alpha = 0.05$

n.s.: mean value is not statistically significantly different compared to the control

a.s.: active substance

The chemical analysis of the Foramsulfuron WG 50 stock solution, which was equivalent to the test item spiking solution used to treat the larval diet in the test item treatment group, revealed that the actual foramsulfuron concentration was well in line with the nominal foramsulfuron concentration (118% of nominal).

□ **Conclusion:** This *in vitro* honey bee larvae study, conducted with three replicates, complies with the validity criteria according to the OECD Draft Test Guideline on Honey Bee (*Apis mellifera*) Larval Toxicity Test, Single Exposure (Version of 21 February 2013) and the current draft version of the Post-WNT25 Approved Larval Honey Bee Test, dated April 2013.

The chemical analysis of the test item treated stock solution, which was equivalent to the test item spiking solution used to treat the larval diet in the test item treatment group, revealed that the actual foramsulfuron concentration was well in line with the nominal concentration.

The statistical processing of the data as obtained in the study, revealed that mortality of exposed honey bee larvae until day +8 (end of the test) did not differ significantly between the control and the test item treatment group of nominal 100 µg foramsulfuron a.s./larva, corresponding to nominal 3030.3 mg foramsulfuron a.s./kg diet (Fisher's Exact Binomial Test with Bonferroni Correction, one-sided greater,  $\alpha = 0.05$ ). Overall, it can be concluded that the No Observed Effect Dose (NOED) determined in this *in vitro* honey bee larvae study is  $\geq 100$  µg foramsulfuron a.s./larva (based on nominal) and the Lowest Observed Effect Dose (LOED) as well as the LD50 is  $> 100$  µg foramsulfuron a.s./larva (based on nominal).

- ❑ **Comment (Co-RMS and RMS):** Obtained result is considered valid, in spite of tested active substance was used in form of Foramsulfuron WG 50. No comment, study is acceptable.

#### B.9.3.1.3.2. Bee brood feeding test (Oomen *et al.*, 1992)

Representative formulation Equip OD 45 contains foramsulfuron technical (herbicide) and isoxadifen – ethyl (safener) in comparison to tested product Foramsulfuron WG 50 which contains foramsulfuron and cyprosulfamide (safener).

<b>Report:</b>	<u>KCA 8.3.1.3 /02;Jeker, L.;2013;M-465326-01</u>
<b>Title:</b>	Foramsulfuron WG 50 - A honeybee brood feeding study to evaluate potential effects on brood development and mortality of the honeybee, <i>Apis mellifera</i> L. (Hymenoptera: <i>Apidae</i> )
<b>Report No:</b>	20110170
<b>Document No:</b>	<u>M-465326-01-1</u>
<b>Guidelines:</b>	<b>Oomen, P. A., de Ruijter, A. and van der Stehen, J. (1992). Method for honeybee brood feeding tests with insect growth-regulating insecticides. EPPO Bulletin, 22, 613-616 [1].; not specified</b>
<b>GLP/GEP:</b>	yes

❑ **Summary:**

The purpose of this study was to evaluate potential effects of Foramsulfuron WG 50 administered together with the herbicide safener Cyprosulfamide SC 500 G on brood development and mortality of adult worker honey bees, *Apis mellifera* L. To assess the potential effects of Foramsulfuron WG 50 on honeybee brood development, the test item was administered in 1 L 50% (w/v) aqueous sucrose solution at a concentration of 0.198 g formulated test item/L (= 0.1 g foramsulfuron/L) + 0.101 mL formulated herbicide safener/L (0.05 g cyprosulfamide/L) per colony in summer 2012.

Mortality of worker bees, larvae and pupae and behavior around the hive were observed for a period of 21 days after application. Condition of the colonies and brood development were also assessed. The method of investigating the development of the honey bee brood is based on the method of Oomen *et al.* (1992). The administration of foramsulfuron WG 50 + the herbicide safener cyprosulfamide SC 500 G to honey colonies caused no adverse effects on the survival of adult bees and pupae, behaviour, colony strength, condition of the colonies, brood index and brood compensation index. In contrast, brood termination rate of young and old larvae was statistically significantly increased when compared to the control treatment. Despite of the slightly elevated termination rates in the test item treatment group, overall colony performance was normal and not impaired. Overall, due to the colony development progress during the course of the study, the observed effects in the test item group can be considered as biologically not relevant.

❑ **Materials and methods:**

<b>Test Material:</b>	Foramsulfuron WG 50
<b>Lot/Batch No.:</b>	2012-001517
<b>Purity:</b>	Nominal content of a.i.: 500 g/kg; Analysed content of a.i.: 506 g/kg
<b>Herbicide Safener:</b>	Cyprosulfamide SC 500 G
<b>Lot/Batch No.:</b>	2012-002411
<b>Purity:</b>	Nominal content of a.i.: 500 g/L; Analysed content of a.i.: 493.4 g/L

<b>Stability:</b>	The test item was assumed to be stable for the period of use in the study kept in the dark at ambient temperature and dry conditions
<b>Storage:</b>	
<b>Control:</b>	
<b>Treatment rates:</b>	The test item, herbicide safener and reference item were weighed and pipetted in the laboratory and set up with 50 % w/v aqueous sucrose solution. For the test item treatment, first the test item was dispersed in water and then the herbicide safener was added. After mixing the test item and the herbicide safener the appropriate amount of sugar and water was added to achieve the final volume. Test item: 0.198 g Foramsulfuron WG 50 / 1 L 50% (w/v) aqueous sucrose solution (= 0.1 g foramsulfuron/L) Safener: 0.101 mL Cyprosulfamide SC 500 G / 1 L 50% (w/v) aqueous sucrose solution (0.05 g cyprosulfamide/L). Calculation was based on the analysed active ingredient. All treatments were administered in 1 L 50% (w/v) aqueous sucrose solution per colony.
<b>Reference item:</b>	3 g Insegar® 25 WG in 1 L 50% (w/v) aqueous sucrose solution (= 0.75 g fenoxycarb/L). Calculation was based on the nominal active ingredient.
<b>Administration:</b>	Ingestion in aqueous sucrose solution (50 w/v) over a test period of 21 days. The treatment administration was conducted simultaneously to all hives in the afternoon at the time of low flight activity via commercial bee feeder as a single treatment. The feeder was placed beneath the hive roof over the hole on top of the crown board. The bee feeders were left at the colonies until total consumption of the feeding solution.
<b>Test organisms</b>	
<b>Species:</b>	Honey bee <i>Apis mellifera</i> (Hymenoptera: <i>Apidae</i> ), healthy bee colonies (sister queens) with one body (9 combs) containing between 10750 to 15850 bees, and 6 to 8 combs and 7 to 9 combs containing brood and stores, respectively.
<b>Source:</b>	Jacques Breiter, Wuhrweg, 35, 4450 Sissach, Switzerland
<b>Preparation of the Colonies:</b>	Healthy and well fed bee colonies were used for the test. The colonies were produced at the same time with sister queens in order to guarantee uniform bee material in all treatments. Colonies were free of symptoms of <i>Nosema</i> and <i>Varroa</i> , <i>Amoebiosis</i> , Chalkbrood, Sackbrood and American or European foulbrood at the beginning of the study.
<b>Settings of the Bees:</b>	Four days prior treatment application (DAT-4) the colonies including spares were delivered by the beekeeper and set up at the study site. For recording bee mortality, each colony was equipped with a dead-bee trap. The colonies were inspected by the beekeeper to ensure that they were suitable (e.g., healthy queen) to conduct the study. Thereafter (DAT -3 to DAT 0), natural bee mortality (remaining dead bees in the dead-bee trap), colony conditions and behaviour of the bees were assessed.
<b>Food:</b>	The sucrose feeding solutions were prepared 2 hours before administration to the honeybee colonies (1 L per colony). Due to rainy weather and low flight activity of the honeybees, the treatments were administered simultaneously to all hives in the afternoon via commercial bee feeder as a single treatment. The feeder was placed beneath the hive roof over the hole on top of the crown board, so bees had access to the contents through the central feed-hole of the feeder. The bee feeders were left at the colonies until total consumption of the feeding solution. The consumption progress of the treatment solution was recorded daily. Empty feeders were removed and the hole on top of the crown board was sealed.
<b>Test unit:</b>	The bees were free flying, with access to natural nectar and pollen sources. During the entire test period, the bees were kept in in Swiss format hives (Apibox CH-14) made out of polystyrene (38 cm width × 62 cm height × 62 cm depth). Swiss format frames were used with a comb area of 921 cm <sup>2</sup> .
<b>Location of the Field:</b>	IES Ltd facilities in 4108 Witterswil, Switzerland
<b>Replicates:</b>	For all treatments, three replicates (colonies) were selected and set up 3 days before treatment (DAT -3)



### □ Study Design:

To assess the potential effects of Foramsulfuron WG 50 on honeybee brood development, the test item was administered in 1 L 50% (w/v) aqueous sucrose solution at a concentration of 0.198 g formulated test item/L (= 0.1 g foramsulfuron/L) + 0.101 mL formulated herbicide safener/L (0.05 g cyprosulfamide/L) per colony in summer 2012. Mortality of worker bees, larvae and pupae and behavior around the hive were observed for a period of 21 days after application. Condition of the colonies and brood development (using a digital brood assessment method described by Jeker *et al.* 2012) were also assessed. The method of investigating the development of the honey bee brood is based on the method of Oomen *et al.* (1992).

### □ Findings:

**Validity:** The overall daily mean adult and pupae mortality of the reference item was significantly greater when compared to the control, indicating that sufficient exposure of the honeybees had taken place and thus the suitability of the test system to detect potential effects on the bee brood. The mortality of adult honeybees and brood stages in the control treatment during the course of the study remained low. In addition, the mean brood termination rate in the toxic reference treatment of all monitored brood stages on BFD 21 (eggs: 85.4%, young larvae: 43.9%, old larvae: 51.8%) was considerable increased and statistically significantly greater when compared to the control (eggs: 41.1%, young larvae: 7.7%, old larvae: 5%). Regarding the overall performance of the reference item and control treatment, the study validity criteria were fulfilled.

### Mortality (adult and young worker bees)

The overall daily mean bee mortality observed on the days before application was similar in all treatments (22.8 to 33.4 bees per colony per day) indicating well adapted colonies. The overall daily mean bee mortality after application of all treatments was 11.2, 18.9 and 23.6 in the control, test item and reference item treatment, respectively. Both test item and the reference item treatments were statistically significantly greater when compared to the control. Furthermore, the mortality was statistically significantly increased on DAT 2 (test item) and on DAT 5, 7 and 19 (reference item) when compared to the control treatment.

### Mortality (pupae)

The overall daily mean pupae mortality observed on the days before application was low and similar in all treatments (0.1 to 0.2 pupae per colony per day). The overall daily mean pupae mortality after application of all treatments was 0.5, 0.3 and 34.8 in the control, test item and reference item treatment, respectively. The reference item treatment was statistically significantly greater when compared to the control. Furthermore, statistically significant increased pupae mortality was observed in the reference item treatment at DAT 10 to 21 (6.7 to 105 mean pupae per colony). This indicated that honey bee brood was well exposed during the test and that the test system was sensitive to detect potential brood effect of plant protection products.

**Table B.9.3.1.3.2-1: Effects of Foramsulfuron WG 50 (+ Cyprosulfamide SC 500 G) on honeybee mortality and honeybee brood development**

Test item	Foramsulfuron WG 50 (+ Cyprosulfamide SC 500 G)		
Test object	Honeybee <i>Apis mellifera</i> L. (complete colonies)		
Exposure	Via treated 50 % (w/v) aqueous sucrose solution		
Assessment	Control n = 3	Test item n = 3	Reference Item n = 3
Mean mortality of worker bees - freshly emerged worker bees/colony			
Pre-application(DAT -3 to 0)	22.8 ± 6.5	33.4 ± 7.7	31.0 ± 16.9
Post-application(DAT 1 to 21)	11.2 ± 0.9	18.9 ± 6.3 <sup>a</sup>	23.6 ± 7.4 <sup>a</sup>
Mean mortality of pupae/colony			
Pre-application(DAT -3 to 0)	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.3
Post-application(DAT 1 to 21)	0.5 ± 0.2	0.3 ± 0.2	34.8 ± 17.9 <sup>a</sup>

Mean values of brood development (eggs)			
Brood termination rate (%) at BFD 21 (DAT 20)	41.1 ± 33.2	43.6 ± 33.3	85.4 ± 10.9 <sup>b</sup>
Brood index at BFD 21 (DAT 20)	2.9 ± 1.7	2.8 ± 1.7	0.7 ± 0.5
Compensation index at BFD 21 (DAT 20)	3.7 ± 0.7	3.5 ± 0.9	1.0 ± 0.8
Mean values of brood development (young larvae = 1-3 days old)			
Brood termination rate (%) at BFD 21 (DAT 20)	7.7 ± 4.5	27.0 ± 24.9 <sup>b</sup>	43.9 ± 35.6 <sup>b</sup>
Brood index at BFD 21 (DAT 20)	4.6 ± 0.2	3.6 ± 1.2	2.8 ± 1.7
Compensation index at BFD 21 (DAT 20)	4.8 ± 0.1	3.8 ± 1.2	2.9 ± 1.8
Mean values of brood development (old larvae = 4-6 days old)			
Brood termination rate (%) at BFD 21 (DAT 20)	5.0 ± 4.3	11.0 ± 14.1 <sup>b</sup>	51.8 ± 13.4 <sup>b</sup>
Brood index at BFD 21 (DAT 20)	4.7 ± 0.2	4.4 ± 0.7	2.4 ± 0.7 <sup>c</sup>
Compensation index at BFD 21 (DAT 20)	4.8 ± 0.2	4.5 ± 0.8	2.8 ± 0.3 <sup>c</sup>

Values are mean ± SD

<sup>a</sup> Statistically significantly greater when compared to the control (Mann-Whitney,  $\alpha=0.05$ , alternative one-sided smaller)

<sup>b</sup> Statistically significantly greater when compared to the control (Fisher's exact test,  $\alpha=0.05$ , alternative one-sided smaller)

<sup>c</sup> Statistically significantly smaller when compared to the control (t-test,  $\alpha=0.05$ , alternative one-sided greater)

DAT Days After Treatment

BFD Brood area Fixing Day

SD Standard Deviation

### Behaviour

In all treatments, no abnormal behavioural symptoms were observed during the whole study period.

### Colony strength

The mean colony strength before treatment administration was 13600, 13617 and 13267 bees/colony in the control, test item and reference item treatment, respectively, and was thus similar in all treatments. During the course of the study, the mean colony strength in the control, test item and reference item treatment displayed a relative increase of 22%, 15% and -27%, respectively, and was at study termination 16617, 15683 and 9700 bees per colony, respectively. No distinct differences between the control and test item treatment were observed.

### Brood nest (eggs/larvae/pupae)

At the 1st assessment a healthy queen was present and the brood nest was similar in all colonies indicating healthy colonies. During the course of the study the proportion of the brood nest in the control, test item and reference item displayed a relative decrease of 13%, 16% and 41%, respectively. The brood nest decrease in the test item treatment was similar to the control treatment, whereas the reference item showed a distinct decrease when compared to the control.

### Stores (pollen/nectar/honey)

At the 1st assessment (DAT -2) a sufficient amount of nectar, honey and pollen was available in all colonies. During the course of the study the proportion of stores in the control, test item and reference item displayed a relative decrease of 1%, 2% and 1%, respectively. Thus, stores remained similar in all treatments during the course of the study.

### Brood termination rate

#### Selected eggs at BFD 0:

The mean brood termination rate of the control, test item and reference item treatment at the last assessment (BFD 21) was 41.1%, 43.6% and 85.4%, respectively.

#### Selected young larvae (1-3 days old) at BFD 0:

The mean brood termination rate of the control, test item and reference item treatment at the last assessment (BFD 21) was 7.7%, 27% and 43.9%, respectively.

#### Selected old larvae (4 - 6 days old) at BFD 0:

The mean brood termination rate of the control, test item and reference item treatment at the last assessment (BFD 21) was 5%, 11% and 51.8%, respectively.



Overall, the mean brood termination of the test item was statistically significantly greater for young and old larvae, whereas the selected eggs at BFD 0 were not statistically significantly different when compared to the control. In the reference item treatment, brood termination rate was statistically significantly higher in all selected brood stages (eggs, young and old larvae) when compared to the control. This indicated that the test system was sensitive to detect potential brood effects of plant protection products.

#### **Brood index**

Brood indices generally correlate with the termination rates: the higher the termination rates the lower the brood indices and vice versa.

Selected eggs at BFD 0:

The mean brood index of the control, test item and reference item treatment at the last assessment (BFD 21) was 2.9, 2.8 and 0.7, respectively.

Selected young larvae at BFD 0:

The mean brood index of the control, test item and reference item treatment at the last assessment (BFD 21) was 4.6, 3.6 and 2.8, respectively.

Selected old larvae at BFD 0:

The mean brood index of the control, test item and reference item treatment at the last assessment (BFD 21) was 4.7, 4.4 and 2.4, respectively.

Overall, the brood indices of the control and test item displayed a continuous and comparable increase, indicating a successful development of the brood. In contrast, the mean brood indices of the reference item were distinctly lower when compared to the control.

#### **Brood compensation index**

Generally the brood compensation indices of all treatment groups were slightly higher than the corresponding brood-indices at all days indicating that cells with terminated brood were at least partially refilled with new eggs, which developed successfully.

Selected eggs at BFD 0:

The mean brood compensation index of the control, test item and reference item treatment at the last assessment (BFD 21) was 3.7, 3.5 and 1.0, respectively.

Selected young larvae at BFD 0:

The mean brood index of the control, test item and reference item treatment at the last assessment (BFD 21) was 4.8, 3.8 and 2.9, respectively.

Selected old larvae at BFD 0:

The mean brood index of the control, test item and reference item treatment at the last assessment (BFD 21) was 4.8, 4.5 and 2.8, respectively.

Overall, the brood compensation indices of the control and test item displayed a continuous and comparable increase, indicating a successful development of the brood. In contrast, the mean brood indices of the reference item were distinctly lower when compared to the control.

- ❑ **Conclusion:** The administration of Foramsulfuron WG 50 + the herbicide safener Cyprosulfamide SC 500 G in 1 L 50% (w/v) aqueous sucrose solution at a concentration of 0.198 g formulated test item/L (= 0.1 g foramsulfuron/L) + 0.101 mL formulated herbicide safener/L (0.05 g cyprosulfamide/L) per colony caused no adverse effects on the survival of adult bees and pupae, behaviour, colony strength, condition of the colonies, brood index and brood compensation index.

In contrast, brood termination rate of young and old larvae was statistically significantly increased when compared to the control treatment. Despite of the slightly elevated termination rates in the test item treatment group, overall colony performance was normal and not impaired. Overall, due to the colony development progress during the course of the study, the observed effects in the test item group can be considered as biologically not relevant.

#### ❑ **Comment (Co-RMS and RMS):**

The Applicant has performed this study according to Oomen *et al.* (1992), as Foramsulfuron WG 50 + the herbicide safener Cyprosulfamide SC 500 G were administered in the sucrose solution (1L) from the top feeder. Using this way of exposure is insufficient, because certain percentage of offered sucrose solution was stored and used later and it is impossible to set the exact level of exposure for larvae. That is why nowadays this method is no more considered not to be a standard test but only as a screening test

(Aupinell *et al.*, 2007<sup>1</sup>). However, statistically significant effect on mean mortality of worker bees + freshly emerged worker bees and pupae were observed in reference item treatment compared to control treatment. Also the mean values for brood development (eggs, young and old larvae) were statistically significantly different in reference treatment. These effects show that the test was able to detect harmful effects and can therefore be considered valid.

No adverse effect on egg stage was seen between the test item and control group in the test (all assessed parameters are not statistically significantly different than control treatment). Brood termination rate of young and old larvae was statistically significantly increased when compared to the control treatment, this means direct influence of tested item (positive) on larvae stage.

Mean brood indexes of the control (eggs, young and old larvae group) were not statistically significantly different from test item, in contrast with mean brood index of the reference item (old larvae) which is distinctly lower when compared to the control treatment.

Mean brood compensation index is an indicator for recovery of a colony, this parameter in the study was not statistically significantly different (control vs. test item treatment in all observed development stages) in contrast with mean brood index of the reference item (fenoxycarb) which is distinctly lower when compared to the control treatment in all observed development stages.

Obtained result is considered valid, in spite of tested active substance was used in form of Foramsulfuron WG 50. No comment, study is acceptable.

#### B.9.3.1.3.3. A honey bee brood test under semi-field conditions - Tunnel test study

<b>Report:</b>	<u>KCA 8.3.1.3 /03;Schmitzer, S.:2013;M-468794-01</u>
<b>Title:</b>	Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L): Effects on honey bee brood ( <i>Apis mellifera</i> L.) under semi-field conditions - Tunnel test
<b>Report No:</b>	79091033
<b>Document No:</b>	<u>M-468794-01-1</u>
<b>Guidelines:</b>	<b>GLP compliant study based on OEPP/EPPO guideline No. 170 (4) (OEPP/EPPO, 2010), OECD Number 75 (2007) and current recommendations of the AG Bienenschutz (2011);</b> The post-application exposure phase in the tunnel was reduced to 4 days due to the herbicide mode of action of the test item against the Phacelia-crop; at the end of the 4th day after application, the Phacelia-crop was no longer attractive to bees (faded) and did not longer support the confined colonies
<b>GLP/GEP:</b>	<b>yes</b>

#### □ Materials and methods:

<b>Test Material:</b>	Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G
<b>Description:</b>	liquid
<b>Lot/Batch No.:</b>	EFKM002442
<b>Purity:</b>	1.) Foramsulfuron (AE F130360): 2.33 % w/w, 22.41 g/L, 2.) Isoxadifen-ethyl (AE F122006): 2.29 % w/w, 21.96 g/L, according to certificate of analysis
<b>Stability:</b>	The test item was assumed to be stable for the period of use in the study
<b>Storage:</b>	At +2 to +30 °C, under dark and dry conditions
<b>Control:</b>	The crop and the bees in the control were treated with tap water at a rate equivalent to 400 L/ha, applied to the full-flowering <i>Phacelia</i> -crop during honey bees actively foraging on the crop.
<b>Target amount of test item:</b>	60 g foramsulfuron a.s./ha via 400 L spray solution/ha; according to Certificate of Analysis: 2.68 L (2575.5 g) product in 400 L tap water/ha (corresponding to 6.439 g product/L); applied to the full-flowering <i>Phacelia</i> -crop during honey bees actively foraging on the crop.

<sup>1</sup> Aupinel P, Fortini D, Michaud B, Marolleau F, Tasei JN and Odoux JF, Toxicity of dimethoate and fenoxycarb to honey bee brood (*Apis mellifera*), using a new in vitro standardized feeding method. *Pest Manag Sci* 63: 1090-1094 (2007).

<b>Reference item:</b>	Insegar (L 160112; fenoxycarb 250 g/kg)
<b>Target amount of reference item:</b>	Nominally 300 g fenoxycarb a.s. (1200 g product)/ha via 400 L spray solution/ha, prepared with tap water (corresponding to nominally 3.00 g product/L); applied to the full-flowering <i>Phacelia</i> -crop during honey bees actively foraging on the crop.
<b>Test organisms</b>	
<b>Species:</b>	Honey bee <i>Apis mellifera carnica</i> L. (Hymenoptera: <i>Apidae</i> )
<b>Source:</b>	Small honey bee colonies bred according to normal beekeeping practice, by the responsible beekeeper Mr. Marcus Amann, IBACON, Germany.
<b>Preparation of the Colonies:</b>	Healthy and well fed bee colonies were used for the test. The colonies were produced at the same time with sister queens (from 2012) in order to guarantee uniform bee material in all treatments. Colonies were free of obvious bee diseases. The colonies contained 11 combs with at least 5 brood combs containing all brood stages and an appropriate amount of nectar and pollen. The queen-right colonies contained about 5000 honey bees. No medical treatments were used in the hives prior to the experimental start. All hives were equipped with dead bee traps.
<b>Settings of the Bees:</b>	The bees were placed in the tunnels 3 days prior to application. At the end of the 4th day after application, due to the herbicide mode of action of the test item, the <i>Phacelia</i> -crop was no longer attractive to bees (faded) and did not longer support the confined colonies. Thus, all bee colonies were relocated after 4 complete days of confined exposure from their respective tunnels and placed in an area with no main flowering, bee attractive crops. Distance between tunnels and remote area: ca. 5.3 km
<b>Test Field, Plants and Tunnels</b>	
<b>Location of the Field:</b>	District authority: Darmstadt-Dieburg, Germany Municipality: 64354 Reinheim-Spachbrücken Flur 7, Flurstück 110. 111, Im Kreuzgrund
<b>Size of the Field:</b>	ca. 11300 m <sup>2</sup>
<b>Plants:</b>	<i>Phacelia tanacetifolia</i> , Type: "Balo" was used as test plants. Flowering <i>Phacelia</i> is a highly attractive plant for honey bees. The seeds were sown ca. 10 weeks before start of the experiment at a sowing rate of 10 kg seeds/ha. Plots of ca. 75 m <sup>2</sup> were prepared per replicate prior to the setting of the tunnels. At the time of application, the height of the plants was ca. 90 - 100 cm, at 90 - 100 % flowering and the coverage of the vegetation was 100 %.
<b>Size of the tunnels:</b>	20 m length x 5.5 m width x 2.5 m height, tunnels were semi-circular in cross-section and constructed out of a tubular steel frame, covered with synthetic gauze (mesh size ca. 2 mm). The tunnels were placed over the flowering plants a few days before experimental starting date with a distance of $\geq 2$ meters in between.
<b>Set up of the Plots:</b>	Each plot was subdivided in the middle by a cleared alleyway (ca. 50 cm), which served as a trail for carrying out the application. To facilitate the collection of the dead bees, in the middle of the tunnels, the plants were removed in order to prepare plane paths at ground level and covered by 50 cm wide gauze. Additionally at the front and head side of each tunnel, gauze was laid on the ground in order to collect the dead bees. A water supply for the bees was placed into each tunnel. Application was done during full flowering of the crop. The bees were exposed in the tunnels for a period of 4 days following the application. After 4 days the plants in the test item (herbicide) treated tunnels starts to dry up and the plants got loss of attractiveness to the bees. In order to avoid starvation to the bees and to avoid negative side-effect by this the bees were removed from all tunnels in the evening on day 4. The bees were then relocated to a field ("Witzel-Koppel/D-64380 Rossdorf: longitude: 49° 51' 16" north, latitude: 008° 44' 32" east) at a distance of ca. 5.3 km to the original

<p><b>Food and water:</b></p>	<p>experimental plot. At this place there were no main flowering crops in the surrounding.</p> <p>No additional feeding was offered to the colonies during the confined exposure period was necessary or offered. On day 15 following the application, 2 L commercial ready-to-use syrup (Apiinvert; 30 % sucrose, 31 % glucose, 39 % fructose) was supplied to each of the colonies. During the 4th colony assessment, it was observed that some of the colonies started to have an insufficient amount of nectar/honey stores. Therefore, in order to prevent artefacts from insufficient food supply/starvation, it was necessary to provide an exactly dosed, small amount of supplemental food to all colonies in order to avoid a decline of the colonies. This situation was caused by the very limited natural food resources available to the colonies at the open field location during the assessment period.</p> <p>Water was offered in each tunnel in a drinking trough (the trough was removed during application).</p>
<p><b>Course of the Test</b></p> <p><b>Replicates:</b></p> <p><b>Setting of the Bees:</b></p> <p><b>Experimental Time:</b></p>	<p>4 tunnels with 1 colony per treatment group</p> <p>In the evening 3 days before application</p> <p>As the test item acts as an herbicide, the bee colonies in all treatment groups (control, reference item and test item) were exposed in their respective tunnels following the applications until the crop in the test item treated tunnels was no longer attractive to bees (faded) and did not longer support the confined colonies. This was observed at the end of the 4th day after application, when the foraging activity in the test item treated tunnels decreased and the crop obviously did not longer act as a food supply for the test item treatment colonies. The bee colonies in all tunnels (control, reference item and test item) were relocated out of their tunnels after 4 complete days of confined exposure.</p> <p>The overall study duration was 4 weeks. The hives were placed inside their respective tunnels 2 days before and 4 days after the day of application. At the end of the 4th day after application, the hives were relocated from their tunnels and set up on an area without main flowering crops until day +27. In the tunnels, assessments on mortality, foraging activity, behaviour, brood development (general and of individually marked cells), colony condition and strength were carried out. Outside the tunnels, assessments of mortality, brooddevelopment (general and of individually marked cells), colony condition and strength were carried out.</p>

#### □ Study Design:

The test was conducted under forced/confined exposure conditions (tunnel), in order to assess potential effects of Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) 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 m<sup>2</sup> plot of *Phacelia tanacetifolia* (2 x 36 m<sup>2</sup>). Small bee colonies were introduced to the tunnels 3 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 4 days following the test item application. At the end of the 4th day after application, due to the herbicide mode of action of the test item, the *Phacelia*-crop was no longer attractive to bees (faded) 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 4 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 a brood comb and taking a digital picture of the brood comb. After saving the file on a computer, 220 - 270 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 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: 2 days before to 27 days after application (= end of the trial);
- Behavioural abnormalities: 2 days before to 27 days after application (= end of the trial);
- Foraging activity of the bees: 2 days before to 4 days after application;
- Condition of the colonies (food stores, brood status and colony strength): 1 day before and 5, 9, 15, 21 and 27 days after application;
- Bee brood development (eggs): 1 day before (= BFD0) and 5 (= BFD 6), 9 (= BFD 10), 15 (= BFD 16), 21 (= BFD 22) days after the application

Application Rates (during full flowering when honey bees were actively foraging on the crop):

Control: 400 L tap water/ha; Test Item: 60 g foramsulfuron a.s./ha; 2.68 L (2575.5 g) product in 400 L tap water/ha (corresponding to 6.439 g product/L); Reference Item: 300 g fenoxycarb a.s. (1200 g product)/ha in 400 L spray solution/ha (corresponding to nominally 3.00 g product/L),

#### Test Conditions:

Natural field conditions were observed during the experiment. On the application day, due to the warm and sunny weather, there was a very high honeybee foraging activity on the crop within the tunnels. Mean temperature during the whole experiment was between 12.9 and 29.1°C. First precipitation (28 mm) occurred in the night on day 2 (ca. 35 hours following the application). Thereafter rain occurred on days 6 (13 mm), 8 (2 mm), 9 (7 mm), 10 (6 mm) and 14 (6 mm).

#### Statistics:

Statistical evaluation was done for mortality, foraging activity, colony strength and the brood termination rate using Shapiro-Wilk's test (check for normal distribution), Levene's test (check for homogeneity of variance), Student or Welch t- test (pairwise comparison); (software: TOX Rat Professional, Version 2.10.05, ® ToxRat Solutions GmbH).

### ❑ Findings:

#### **Mortality of the adult bees (worker bees)**

##### Pre-application phase (day- 2 to day 0 before application):

Mortality of the pre-application phase in the control and the test item group was 24.8 and 17.6 dead bees/colony/day, respectively. The mortality in the reference item was 74.3 dead bees/colony/day. 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 4):

There was no sign of an acute effect on the mortality of the bees following the test item treatment. Average control mortality of adult bees during the exposition phase (day 0 to day 4 following the application) was 19.9 dead bees/colony/day. The average mortality in the test item group was slightly higher with 26.0 dead bees/colony/day, but not statistically significant to the control values (Student t-test, pairwise comparison, one-sided greater,  $\alpha = 0.05$ ). Reference Item mortality was 36.2 dead bees/colony/day (no statistical significant difference, Student t-test, pairwise comparison one-sided

greater,  $\alpha = 0.05$ ; *Nota bene*: The absence of acute effects of the Reference Item is in line to its mode of action).

Phase outside the tunnels (day 5 after application to day 27):

An overall comparison of the mean number of dead bees found in the traps and on the gauze after the application from day 5 to day 27 did also not show a statistical significant difference between the control and the Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) - treatment (Student t-test, pairwise comparison, one-sided greater,  $\alpha = 0.05$ ). A mean of 3.8 dead bees per day and tunnel was found for the period from day 5 to day 27 after treatment in the test item group, whereas a mean of 5.4 dead bees were found in the control group. There was no impact of the reference item to the adult bee mortality, which is not to be expected due to mode of action of the reference item.

### **Mortality of pupae**

Pre-application phase (day -2 to day 0 before application):

Mortality of pupae in the control, test item and reference item groups was 2.3, 0.1 and 3.4 dead pupae/colony/day, respectively. There was no statistically significant difference between the groups 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 4):

Mean pupae mortality during the exposure phase in the test item treated group was 0.6 dead pupae/day/colony and therefore lower compared to the mean value of the control group (0.8 dead pupae/day/colony). Accordingly, this was not statistically significantly different to the control group (Student t-test, pairwise comparison one-sided greater,  $\alpha = 0.05$ ). The application of the reference item resulted in a higher number of dead pupae following the application: 5.3 dead pupae/day/colony, which was statistically significantly different to the control group.

Phase outside the tunnels (day 5 after application to day 27):

The same as observed for the confinement period holds true for the phase outside the tunnels: the test item treated group showed a lower pupae mortality rate compared to the control group, whereas pupae mortality in the reference item group was increased and statistically significant different to the control group. Mean pupae mortality from day 5 to day 27 was 0.1 dead pupae/colony/day in the test item group and 0.4 dead pupae/colony/day in the control group. Reference item induced pupae mortality was 22.3 dead pupae/colony/day.

### **Foraging Activity**

Pre-application phase (day -2 to day 0 before application):

The mean foraging activity in the intended test item group and reference item groups was comparable to the control group, resulting in overall daily mean values of 15.4, 17.3 and 19.6 bees/m<sup>2</sup>/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.

Exposure phase in the tunnels (day 0 after application to day 4):

There was a slight decrease in foraging activity after application in the test item group. Mean foraging activity on each occasion was lower compared to the control values on these days. Nevertheless, these lower flight activities were not statistically significant different (Student t-test, pair-wise comparison to the control, one-sided smaller,  $\alpha = 0.05$ ). The overall daily mean foraging activity from day 0 to day 4 in the test item group was 11.4 bees/m<sup>2</sup>/day compared to 15.7 bees/m<sup>2</sup>/day the control group.

The reference item (Insegar) resulted in no reduction of the foraging activity on the day of application and on all following days.

### **Behavioural abnormalities**

After application of Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) no 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 brood stages (eggs, larvae and closed brood), as well as a sufficient amount of nectar and pollen storage, was found in all colonies as an indication of healthy colonies.

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. 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 very similar one day before application and did not differ statistically (mean of 4736 to 5018 per colony). The subsequent development of the colony strength among the colonies in the control and test item treatment groups followed the same pattern. There was a continuous increase of colony strength observable, which was stronger in the test item group compared to the control group. No statistical significant difference in the colony strength between the test item treated colonies and the control colonies occurred at any assessment date. Overall, no adverse effects of the test item on colony strength and population development have been observed throughout the study.

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

**Table B.9.3.1.3.3-1: Colony strength**

Treatment group	Day-1	Day+5	Day+9	Day+15	Day+21	Day 27
Control	100%	123%	144%	159%	161%	148%
Test item	100%	151%	161%	176%	175%	169%
Toxic reference item	100%	141%	152%	140%	137%	108%

### 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 40.3 %. Although the termination rate in the test item group was slightly higher compared to the control group (30.2 %), this difference 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 82.3 %. This decrease was statistically significantly different compared to the control group.



**Table B.9.3.1.3.3-2: Effects of Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) on honey bee brood under semi-field conditions (Tunnel Test)**

Parameter	Treatment group <sup>1)</sup>		
	Control	Test Item [2.68 L/ha]	Reference Item Insegar [300 g a.s./ha]
Mean mortality of worker bees/colony/day [%] during			
pre-application phase <sup>2)</sup>	24.8 ± 11.6	17.6 ± 8.7 (n.s.)	74.3 ± 50.1 (n.s.)
exposure phase in the tunnels <sup>2)</sup>	19.9 ± 17.6	26.0 ± 13.9 (n.s.)	36.2 ± 16.9 (n.s.)
phase outside the tunnels <sup>5)</sup>	5.4 ± 4.9	3.8 ± 4.9 (n.s.)	6.8 ± 8.7 (n.s.)
overall after application	8.0 ± 9.9	7.8 ± 11.1 (n.s.)	12.0 ± 15.3 (n.s.)
Mean mortality of larvae and pupae [n] during			
pre-application phase <sup>4)</sup>	2.3 ± 2.7	0.1 ± 0.1 (n.s.)	3.4 ± 2.1 (n.s.)
exposure phase in the tunnels <sup>4)</sup>	0.8 ± 0.8	0.6 ± 0.5 (n.s.)	5.3 ± 3.7 (*)
phase outside the tunnels <sup>5)</sup>	0.4 ± 0.7	0.1 ± 0.2 (n.s.)	22.3 ± 28.6 (*)
overall after application	0.5 ± 0.7	0.2 ± 0.3 (n.s.)	19.3 ± 26.7 (*)
Mean foraging activity/m <sup>2</sup> /colony/day[n] during			
pre-application phase	15.4 ± 6.7	17.3 ± 6.3 (n.s.)	19.6 ± 7.2 (n.s.)
exposure phase in the tunnels	15.7 ± 5.3	11.4 ± 6.2 (n.s.)	16.3 ± 6.6 (n.s.)
Mean brood termination rate [%] <sup>6)</sup>	30.2	40.3 (n.s.)	82.3 (*)

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; n.s. = not statistically significant compared to the control; \* = statistically significant compared to the control Statistic: Student or Welch t-test,  $\alpha=0.05$ , pairwise; before application: two-sided; after application one-sided greater (mortality and termination rate), one-sided smaller (foraging activity, colony strength)

- **Conclusion:** To assess the potential effects of Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) on honey bee colonies including brood development, 2.68 L product in 400 L tap water/ha (corresponding to 60 g foramsulfuron a.s./ha), a water treated control and a reference item were applied to a full-flowering and highly bee-attractive crop (i.e. *Phacelia tanacetifolia*) under semi-field (tunnel) condition during bee-flight. No adverse effects on mortality of worker or pupae, foraging activity, behaviour, nectar- and pollen storage as well as on queen survival were observed. No effects on colony development, colony strength or bee brood were observed.

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.

Based on the results of this study, it can be concluded that Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) does not adversely affect honey bees and honey bee brood when applied at a rate of 2.68 L product in 400 L tap water/ha (corresponding to 60 g foramsulfuron a.s./ha), during honey bees actively foraging on a bee-attractive, flowering crop.

- **Comment (Co-RMS and RMS):** There was a little deviation to the study plan, concretely bee inspection on -3D was postponed to -2d. This “inspection shift” has no effect on natural background

mortality and foraging activity of colony. The reduction of post-application exposure phase in the tunnel to 4 days (7 days according to OECD 75, 2007) is logic due to the herbicide mode of action of the test item against the *Phacelia*-crop, faded crop was no longer attractive for bees.

During the 4th colony assessment, some of the colonies were observed to start to have an insufficient amount of nectar/honey stores. On day 15 following the application, 2 L commercial ready-to-use syrup (Apiinvert; 30 % sucrose, 31 % glucose, 39 % fructose) was supplied to each of the colonies, what is in line with OECD 75, 2007.

No other comment, study is acceptable.

### B.9.3.2. Effects on non-target arthropods other than bees

Potential effects of FSN + IDF OD 45 on standard non-target arthropods, *Aphidius rhopalosiphii* and *Typhlodromus pyri* and on additional non-target arthropods, *Chrysoperla carnea*, *Aleochara bilineata*, *Poecilus cupreus* and *Pardosa sp.* were evaluated within the process of Annex I inclusion and were considered acceptable by the RMS Germany. These studies were not re-evaluated since the studies followed guidelines valid still today. Also new studies were submitted and were evaluated in this dRAR.

For the process of renewal a new study on the mortality of *Typhlodromus pyri* exposed to Equip OD 45 on glass plates was provided. The study was a dose-response study aiming for calculation of a LR<sub>50</sub> value and a LR<sub>50</sub> > 60 g a.s./ha was obtained. *Typhlodromus pyri* showed 20% corrected mortality and no effect on reproduction in an extended laboratory study on *Polygonum convolvulus* leaves at the highest test rate (90 g a.s./ha). Brief summaries of the old studies and a detailed summary of the new study are provided.

For the process of renewal a new study on the mortality of *Aphidius rhopalosiphii* exposed to Equip OD 45 on glass plates was provided. The study was a dose-response study aiming for calculation of a LR<sub>50</sub> value and a LR<sub>50</sub> > 60 g a.s./ha was obtained. *Aphidius rhopalosiphii* showed some mortality in a tier 1 glass plate study, no effects on mortality or reproduction were observed in a higher tier extended lab/aged residue study when exposed to freshly dried residues on maize leaves and residues aged for 3 and 7 days. Brief summaries of the old studies and a detailed summary of the new study are provided.

For *Chrysoperla carnea*, *Aleochara bilineata*, *Poecilus cupreus* and *Pardosa sp.* FSN + IDF OD 45 had no or only low effects on mortality, reproduction and feeding rate of these species. Brief summaries of the old studies are provided below.

#### B.9.3.2.1. Laboratory, extended laboratory and semi-field tests (annex IIA 8.3.2; annex IIIA 10.5.1)

##### Effects on *Typhlodromus pyri*

For the formulation FSN + IDF OD 45 toxicity studies on the predatory mite *Typhlodromus pyri* were performed. Details of all studies are provided briefly in the Table 9.3.2.1-1.

**Table 9.3.2.1-1: Toxicity data of foramsulfuron to *Typhlodromus pyri***

Test species	Tested Formulation, study type, exposure	Ecotoxicological Endpoint		Reference
<i>Typhlodromus pyri</i>	FSN + IDF OD 45			
	Laboratory, glass plate	Corr. Mortality [%]	Effect on Reproduction [%]	Waltersdorfer, 1999
	267 mL prod./ha	-8.5 <sup>A</sup>	-0.8 <sup>B</sup>	M-191384-01-1
	2667 mL prod./ha	53	33.9	KCA 8.3.2.2 /01 [CW99/003]

Test species	Tested Formulation, study type, exposure	Ecotoxicological Endpoint	Reference
<i>Typhlodromus pyri</i>	FSN + IDF OD 45 Extended lab., exposure on detached <i>Polygonum convolvulus</i> leaves 2000 mL prod./ha 4000 mL prod./ha	<b>LR<sub>50</sub> &gt; 3500 mL prod./ha</b>  Corr. Mortality [%]      Effect on Reproduction [%] -1.3 <sup>A</sup> 9.4 20.0                        -10.4 <sup>B</sup>	Waltersdorfer, 1999 <a href="#">M-192822-01-1</a> KCA 8.3.2.2 /02 [CW99/092]
<i>Typhlodromus pyri</i>	FSN + IDF OD 45 Laboratory, glass plate 267 mL prod./ha 475 mL prod./ha 844 mL prod./ha 1501 mL prod./ha 2670 mL prod./ha	<b>LR<sub>50</sub> &gt; 2670 mL prod./ha</b>  Corr. Mortality [%] 1.0 5.1 38.8 36.7 48.0	Roehlig, 2013 <a href="#">M-457360-01-1</a> KCA 8.3.2.2 /03 [13 10 48 031 A]

<sup>A</sup>: A negative value indicates a lower mortality in the treatment than in the control

<sup>B</sup>: A negative value indicates a higher reproduction rate in the treatment than in the control.

prod.: product

**Bold letters:** Values considered relevant for risk assessment

#### B.9.3.2.1.1. Toxicity of AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L on *Typhlodromus pyri* (laboratory test)

<b>Report:</b>	<a href="#">KCA 8.3.2.2 /01;Waltersdorfer, A.;1999;M-191384-01</a>
<b>Title:</b>	Toxicity to the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN (Acari, <i>Phytoseiidae</i> ) in the laboratory AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L Code: AE F130360 01 1K05 A301
<b>Report No:</b>	C005111
<b>Document No:</b>	<a href="#">M-191384-01-1</a>
<b>Guidelines:</b>	Deviation not specified
<b>GLP/GEP:</b>	yes

##### □ Conclusion:

Application rate	Mortality	Sublethal effects
0.233 L product/ha (6 g a.s./ha)	0%	0% (fertility)
2.33 L product/ha (60 g a.s./ha)	53%	33.9% (fertility)

- **Comment (Co-RMS and RMS):** Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final): 55 % mortality, 34 % fertility (60 g a.s./ha).

#### B.9.3.2.1.2. Toxicity of AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L on *Typhlodromus pyri* (extended laboratory test)

<b>Report:</b>	<a href="#">KCA 8.3.2.2 /02;Waltersdorfer, A.;1999;M-192822-01</a>
<b>Title:</b>	Toxicity to the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN (Acari, <i>Phytoseiidae</i> ) using an extended laboratory test AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L Code: AE F130360 01 1K05 A301
<b>Report No:</b>	C005863
<b>Document No:</b>	<a href="#">M-192822-01-1</a>
<b>Guidelines:</b>	Deviation not specified
<b>GLP/GEP:</b>	yes

##### □ Conclusion:



Application rate	Mortality	Sublethal effects
1.75 L product/ha (45 g a.s./ha)	0 %	9.0 % (fertility)
3.50 L product/ha (90 g a.s./ha)	20 %	0 % (fertility)

- **Comment (Co-RMS and RMS):** Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final): 20 % mortality, 0 % fertility (90 g a.s./ha).

#### B.9.3.2.1.3. Toxicity of Foramsulfuron + Isoxadifen-ethyl OD 45 (22.5 + 22.5 g/L) on *Typhlodromus pyri* (laboratory test) (new study)

<b>Report:</b>	<u>KCA 8.3.2.2 /03;Roehlig, U.;2013;M-457360-01</u>
<b>Title:</b>	Effects of foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) on the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN in a laboratory test
<b>Report No:</b>	13 10 48 031 A
<b>Document No:</b>	<u>M-457360-01-1</u>
<b>Guidelines:</b>	IOBC (BLUEMEL et al. 2000);none
<b>GLP/GEP:</b>	yes

#### □ Summary

The purpose of this study was to determine a rate-response relationship for mortality of the predatory mite *Typhlodromus pyri* SCHEUTEN in a worst-case laboratory test. Mites were exposed on glass plates to application rates of 267, 475, 844, 1501 and 2670 ml product/ha in 200 L deionised water/ha and effects on mortality were compared to those of deionised water treated controls (200 L/ha). Dimethoate (applied at 15 mL product/ha, nominally equivalent to 6 g a.s./ha, in 200 L deionised water/ha) was used as reference item. Survival of the predatory mites was used as test endpoint with the aim to calculate the LR<sub>50</sub> if possible. The test was performed according to the IOBC Guideline (Blümel et al. 2000) taking account of the recommendations given by Grimm et al. (2001), but without performance of a reproduction assessment. The LR<sub>50</sub> for *Typhlodromus pyri* was estimated to be > 2670 mL product/ha in 200 L water/ha, the highest rate tested. All validity criteria according to the guideline were met.

#### □ Materials and methods:

<b>Test Material:</b>	Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5)
<b>Description:</b>	beige suspension
<b>Lot/Batch No.:</b>	EFKM002442
<b>Purity:</b>	1.) Foramsulfuron (AE F130360): 2.33 % w/w, 22.41 g/L, 2.) Isoxadifen-ethyl (AE F122006): 2.29 % w/w, 21.96 g/L, according to certificate of analysis
<b>Density:</b>	0.961 g/mL
<b>Stability:</b>	stable under the given storage conditions
<b>Storage:</b>	25±5 °C, + 2 °C to + 30 °C are also acceptable (at BioChem agrar: at about 20 °C, dark and dry)
<b>Control:</b>	The control group was treated with deionised water only (200 L/ha).
<b>Toxic standard:</b>	Dimethoate (nominal - 400 g/L; analysed - 411.7g/L)
<b>Spray volume rate:</b>	200 L/ha
<b>Application method:</b>	Commercial sprayer (producer: Schachtner, 71640 Ludwigsburg, Germany) with one nozzle (Lechler ES 90-015) and operated at 3.4 bar pressure. Distance between the target area and the nozzle: 37cm.
<b>Test rates:</b>	0 (control) and 267 – 475 – 844 – 1501 – 2670 mL product/ha in 200 L/ha of deionised water
<b>Test organisms</b>	
<b>Species:</b>	<i>Typhlodromus pyri</i> (Acari: Phytoseiidae)
<b>Source:</b>	Katz Biotech AG, An der Birkenpfehlheide 10, 15837 Baruth, Germany (in the stage of eggs) on May 17, 2013

**Food:** pollen: pine (*Pinus nigra*) and birch (*Betula pendula*), 1:1  
**Age at test start:** Less than 24-h old protonymphs

#### Test design

**Test unit:** glass plates (cover glasses: 50 mm x 22 mm stuck together along their longitudinal sides) with a barrier of sticky material on moistened filter paper on a sponge placed in a plastic tray - Bellaplast (inside dimensions: 165 mm x 120 mm x 60 mm) filled with tap water up to a height of approx. 15 mm

**Replication:** 5

**No. of mites/ Test unit:** 20

#### Environmental test conditions

**Temperature:** 23-27°C

**Humidity:** 68 – 72%

**Photoperiod:** 16-h photoperiod (2020 lx)

**Duration of test:** 7 days

#### □ Study Design and Methods:

The test item was tested under laboratory conditions after contact exposure of protonymphs of the predatory mite *Typhlodromus pyri* SCHEUTEN to dried spray residues of the test item with rates of 267, 475, 844, 1501 and 2670 mL product/ha in 200 L deionised water/ha applied on glass plates. The control was treated with deionised water (200 L/ha). Dimethoate EC 400 (15 mL product/ha, nominally equivalent to 6 g a.s./ha, in 200 L deionised water/ha) was used as a toxic reference item. Protonymphs of the predatory mite *Typhlodromus pyri* SCHEUTEN were exposed in 5 replicates per treatment group and 20 mites per replicate to the residues of the test item, reference item and control treatments, respectively. During the assessments the mites were fed with a mix of pine (*Pinus nigra*) and birch (*Betula pendula*) pollen, 1:1. The number of surviving, dead, trapped and escaped predatory mites was recorded over a period of 7 days. From these data the endpoint mortality was calculated.

Toxic standard: (Dimethoate EC 400): 15 mL product/ha (nominally equivalent to 6 g a.s./ha) in 200 L/ha of deionised water; control: deionised water only (200 L/ha).

#### □ Findings:

Mortality is summarised in the table below:

**Table B.9.3.2.1.3-1: Effects on mortality of *Typhlodromus pyri***

Treatment <sup>1</sup> (kg Foramsulfuron + Isoxadifen-ethyl OD 45 /ha)	mortality <sup>2</sup> at 7 DAT (%)	Corrected % mortality <sup>3</sup> at 7 DAT (%)
Control	2.0	-
267 mL product/ha	3.0 (n.s.)	1.0
475 mL product/ha	7.0 (n.s.)	5.1
844 mL product/ha	40.0*	38.8
1501 mL product/ha	38.0*	36.7
2670 mL product/ha	49.0*	48.0
Toxic reference Dimethoate EC 400 15 mL product/ha	86.0*	85.7

<sup>1</sup> Application rate in 200 L water/ha

<sup>2</sup> Mortality after exposure to residues on treated glass plates. The results for mortality in individual treatments were compared to that in the control using Fisher's Exact Binomial test ( $\alpha = 0.05$ ).

<sup>3</sup> Corrected mortality according to Abbott (1925)

(n.s.) not statistically significantly different compared to the control: Fisher`s Exact Binomial test with Bonferroni correction ( $\alpha = 0.05$ )

\* statistically significantly different compared to the control: Fisher`s Exact Binomial test with Bonferroni correction ( $\alpha = 0.05$ ) for test item and Fisher`s Exact Binomial test ( $\alpha = 0.05$ ) for reference item

The results of the control group indicated that the test organisms were in a good condition (mortality: 2.0 %). The results of the reference item group indicated that the test system was sensitive to harmful substances (corrected mortality: 85.7 %). Concerning mortality in the control group and as well the susceptibility of the test organisms to the reference item the study is proved to be valid.

After 7 days, the mortality in the test item treatments ranged between 3.0 % and 49.0 % in comparison to 2.0 % in the control. Based on these results the corrected mortality for the different rates ranged between 1.0 % and 48.0 %.

- ❑ **Conclusion:** The 7-day LR50 for Foramsulfuron + Isoxadifen-ethyl OD 45 (22.5+22.5 g/L) was estimated to be > 2670 mL product/ha in 200 L water/ha, the highest rate tested (equivalent to > 62.2 g foramsulfuron/ha).
- ❑ **Comment (Co-RMS and RMS):** No comment. All validity criteria according to BLÜMEL *et al.* (2000) were met, study is acceptable.

### Effects on *Aphidius rhopalosiphi*

For the formulation FSN + IDF OD 45 toxicity studies on the parasitic wasp *Aphidius rhopalosiphi* were performed. Details of all studies are provided in the following table.

Table 9.3.2.1-2: Toxicity data of foramsulfuron to *Aphidius rhopalosiphi*

Test species	Tested Formulation, study type, exposure	Ecotoxicological Endpoint	Reference
<i>Aphidius rhopalosiphi</i>	FSN + IDF OD 45 Laboratory, glass plate 2 <sup>nd</sup> test run: 35 mL prod./ha 62 mL prod./ha 111 mL prod./ha 197 mL prod./ha 350 mL prod./ha 1 <sup>st</sup> test run: 267 mL prod./ha 475 mL prod./ha 844 mL prod./ha 1501 mL prod./ha 2670 mL prod./ha	<b>LR<sub>50</sub> 241 mL prod./ha</b> Corr. Mortality [%]  0 0 7.5 50.0 57.5  61.5 89.7 100 100 100	Roehlig, 2013 <u>M-461455-01-1</u> KCA 8.3.2.1 /03 [13 10 48 030 A]
<i>Aphidius rhopalosiphi</i>	FSN + IDF OD 45 Laboratory, glass plate 160 mL prod./ha 2000 mL prod./ha 4000 mL prod./ha	<b>Corr. Mortality [%] Effect on Reproduction [%]</b> 26 25.3 100 n.a. 100 n.a.	Kleiner, 1999 <u>M-191908-01-1</u> KCA 8.3.2.1 /01 [991048029]
<i>Aphidius rhopalosiphi</i>	FSN + IDF OD 45 Aged residues, spray deposits on potted maize plants 107 mL prod./ha Residues aged for 0 days: Residues aged for 3 days: Residues aged for 7 days: 2000 mL prod./ha Residues aged for 0 days: Residues aged for 3 days: Residues aged for 7 days: 2670 mL prod./ha Residues aged for 0 days: Residues aged for 3 days: Residues aged for 7 days:	<b>LR<sub>50</sub> &gt; 2670 mL prod./ha</b> Corr. Mortality [%] Effect on Repr. [%] Repellency (30 min) [%] Repellency (2h) [%] 0 -3 <sup>A</sup> 32 39 0 2 -7 <sup>B</sup> 6 0 -3 <sup>A</sup> 11 -24 <sup>B</sup> 0 5 6 70 0 0 13 6 0 -6 <sup>A</sup> 5 -13 <sup>B</sup> 0 3 5 21 5 6 -12 <sup>B</sup> 7 0 -2 <sup>A</sup> 5 -4 <sup>B</sup>	Barth, 2000 <u>M-198973-01-1</u> KCA 8.3.2.1 /02 [001048067]

<sup>A</sup>: A negative value indicates a higher reproduction rate in the treatment than in the control.

<sup>B</sup>: A negative value indicates a higher percentage of wasps found on plants in the treatment than in the control.

prod.: product

n.a.: not assessed

**Bold letters:** Values considered relevant for risk assessment

#### B.9.3.2.1.4. Toxicity of AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L on *Aphidius rhopalosiphi* (laboratory test)

Report:	KCA 8.3.2.1 /01;Kleiner, R.;1999;M-191908-01
Title:	Toxicity to the parasitoid <i>Aphidius rhopalosiphi</i> (Destefani-Perez) / adults under laboratory conditions according to IOBC Guidelines (Mead-Briggs 1992/1997) Code: AE F130360 01 1K05 A304
Report No:	C005357
Document No:	M-191908-01-1
Guidelines:	IOBC;;Deviation not specified
GLP/GEP:	yes

#### □ Conclusion:



Application rate	Mortality	Sublethal effects
0.16 L product/ha (3.6 g a.s./ha)	26 %	25.3 % (parasitism capacity)
2.0 L product/ha (45 g a.s./ha)	100 %	n.d.
4.0 L product/ha (2x45 g a.s./ha)	100 %	n.d.

- **Comment: (Co-RMS and RMS):** Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final): 100 % mortality, - fertility not determined (45 g a.s./ha).

#### B.9.3.2.1.5. Toxicity of AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L on *Aphidius rhopalosiphii* (extended laboratory test)

Report:	KCA 8.3.2.1 /02;Barth, M.;2000;M-198973-01
Title:	Toxicity of AE F130360 01 1K05 A304 to the cereal aphid parasitoid <i>Aphidius rhopalosiphii</i> (Destefani-Perez) (extended laboratory test/"aged residue test") Code: AE F130360 01 1K05 A304
Report No:	C010411
Document No:	M-198973-01-1
Guidelines:	ESCORT: Barrett et al. 1994; IOBC: Mead-Briggs & Longley 1997;Deviation not specified
GLP/GEP:	yes

#### □ Conclusion:

□ Application rate	Mortality [%] 0 DAT/ 3 DAT / 7 DAT	Sublethal effects (parasitism capacity) [%] 0 DAT/ 3 DAT / 7 DAT
0.107 L product/ha (2.4 g a.s./ha)	0/0/0	0/2/0
2.0 L product/ha (45 g a.s./ha)	0/0/0	5/0/0
2.67 L product/ha (60 g a.s./ha)	0/5/0	3/6/0

- **Comment (Co-RMS and RMS):** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

#### B.9.3.2.1.6. Toxicity of Foramsulfuron + Isoxadifen-ethyl OD 45 (22.5 + 22.5 g/L) on *Aphidius rhopalosiphii* (laboratory test) (new study)

Report:	KCA 8.3.2.1 /03;Roehlig, U.;2013;M-461455-01
Title:	Effects of foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) on the parasitic wasp <i>Aphidius rhopalosiphii</i> (DESTEFANI-PEREZ) in a laboratory test
Report No:	13 10 48 030 A
Document No:	M-461455-01-1
Guidelines:	IOBC (MEAD-BRIGGS et al. 2000);none
GLP/GEP:	yes

#### □ Summary:

The purpose of this study was to determine a rate-response relationship for mortality of the parasitic wasp *Aphidius rhopalosiphii* (DESTEFANI-PEREZ) in a laboratory test. Adult wasps (used within 48 hours after hatching, 4 x 7 females and 4 x 3 males for the control groups and the treatment groups) were exposed to

control (deionised water) and dried spray residues of the test item with rates of 267, 475, 844, 1501 and 2670 mL product/ha (1<sup>st</sup> test run) and 35, 62, 111, 197 and 350 mL product/ha (2<sup>nd</sup> test run) in 200 L deionised water/ha applied on glass plates. Dimethoate EC 400 (0.3 mL product/ha in 200 L deionised water/ha) was used as a toxic reference item. Survival of the parasitic wasps was used as test endpoint with the aim to calculate the LR<sub>50</sub>, if possible. The LR<sub>50</sub> for *Aphidius rhopalosiphi* was calculated to be 241 mL product/ha in 200 L water/ha based on the results of the 1<sup>st</sup> and 2<sup>nd</sup> test run.

The test was performed following the IOBC Guideline (MEAD-BRIGGS *et al.* 2000) taking account of the recommendations given by GRIMM *et al.* (2001), but without performance of a reproduction assessment.

#### □ Materials and methods:

##### Test Material: Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5)

<b>Description:</b>	beige suspension
<b>Lot/Batch No.:</b>	EFKM002442
<b>Purity:</b>	1.) Foramsulfuron (AE F130360): 2.33 % w/w, 22.41 g/L, 2.) Isoxadifen-ethyl (AE F122006): 2.29 % w/w, 21.96 g/L, according to certificate of analysis
<b>Density:</b>	0.961 g/mL
<b>Stability:</b>	stable under the given storage conditions
<b>Storage:</b>	25±5 °C, + 2 °C to + 30 °C are also acceptable (at BioChem agrar: at about 20 °C, dark and dry)
<b>Control:</b>	The control group was treated with deionised water only (200 L/ha).
<b>Toxic standard:</b>	Dimethoate (nominal - 400 g/L; analysed - 411.7g/L)
<b>Spray volume rate:</b>	00 L/ha
<b>Application method:</b>	Commercial sprayer (producer: Schachtner, 71640 Ludwigsburg, Germany) with one nozzle (Lechler ES 90-015) and operated at 3.4 bar pressure. Distance between the target area and the nozzle: 37cm.
<b>Test rates:</b>	1st test run: 0 (control) and 267 - 475 - 844 - 1501 - 2670 mL product/ha in 200 L/ha of deionised water 2nd test run: 0 (control) and 35 - 62 - 111 - 197 - 350 mL product/ha in 200 L/ha of deionised water
<b>Test organisms</b>	
<b>Species:</b>	parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) / adults (Hymenoptera: Braconidae)
<b>Source:</b>	Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany (in the stage of mummies) on May 31, 2013 (1st test run) and June 21, 2013 (2nd test run)
<b>Food:</b>	25 % w/w aqueous fructose solution
<b>Age at test start:</b>	Less then 48-h after hatching

##### Test design

<b>Test unit:</b>	Square glass plates (13 cm x 13 cm), held apart by an aluminium frame (13 cm x 13 cm x 1.4 cm) with gauze covered holes for forced air ventilation (blowing air; flow rate: 2.5 L/min) Both glass plates were fitted treated surface inwards onto a square aluminium frame as floor and ceiling. Three sides of the frame contained 6 ventilation holes each (1 cm diameter). The inside surface of the frame was coated with black tight cotton material to seal the ventilation holes. The fourth side of the frame contained an oval hole which was used for the introduction of the wasps and closed from the outside with black paper and adhesive tape.
<b>Replication:</b>	4
<b>No. of wasps/ test unit:</b>	7 females + 3 males

##### Environmental test conditions

<b>Temperature:</b>	1st test run: 19-21 °C 2nd test run: 19-21 °C
<b>Humidity:</b>	1st test run: 68-73 % 2nd test run: 68-72 %

**Photoperiod:** 16-h photoperiod, 1st test run: 1930lx and 2nd test run: 1950lx  
**Duration of test:** 48h

#### □ Study Design and Methods:

The test item was tested under laboratory conditions after contact exposure of adults of the parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ) to dried spray residues of the test item with rates of 267, 475, 844, 1501 and 2670 mL product/ha (1st test run) and 35, 62, 111, 197 and 350 mL product/ha (2nd test run) in 200 L deionised water/ha applied on glass plates. The control was treated with deionised water (200 L/ha). Dimethoate EC 400 (0.3 mL product/ha in 200 L deionised water/ha) was used as a toxic reference item. Adults of the parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ) were exposed in 4 replicates per treatment group and 7 females and 3 males per replicate to the residues of the test item, reference item and control treatments, respectively. During the exposure phase the adult wasps were fed with 25 % w/w aqueous fructose solution. The number of surviving, affected, moribund and dead wasps was recorded over a period of 48 hours. From these data the endpoint mortality was calculated.

#### □ Findings:

Mortality is summarised in the table below:

**Table B.9.3.2.1.6-1: Effects on mortality of *Aphidius rhopalosiphi* (DESTEFANI-PEREZ)**

Treatment <sup>1</sup> (kg Foramsulfuron + Isoxadifen-ethyl OD 45/ha)	dead wasps (number)	moribund wasps (number)	surviving wasps (number)	mortality <sup>2</sup> (%)	corrected mortality <sup>3</sup> (ABBOTT) (%)
<b>1<sup>st</sup> test run</b>					
Control	1	0	39	2.5	-
267 mL product/ha	25	0	15	62.5*	61.5
475 mL product/ha	36	0	4	90.0*	89.7
844 mL product/ha	40	0	0	100*	100
1501 mL product/ha	40	0	0	100*	100
2670 mL product/ha	40	0	0	100*	100
Toxic reference Dimethoate EC 400 0.3 mL product/ha	40	0	0	100*	100
<b>2<sup>nd</sup> test run</b>					
Control	0	0	40	0	-
267 mL product/ha	0	0	40	0 (n.s.)	0
475 mL product/ha	0	0	40	0 (n.s.)	0
844 mL product/ha	3	0	37	7.5 (n.s.)	7.5
1501 mL product/ha	20	0	20	50.0*	50.0
2670 mL product/ha	23	0	17	57.5*	57.5
Toxic reference Dimethoate EC 400 0.3 mL product/ha	40	0	0	100*	100

<sup>1</sup> Application rate in 200 L water/ha

<sup>2</sup> Mortality after exposure to residues on treated glass plates. The results for mortality in individual treatments were compared to that in the control using Fisher's Exact Binomial test ( $\alpha = 0.05$ ).

<sup>3</sup> Corrected mortality according to Abbott (1925)

(n.s.) not statistically significantly different compared to the control: Fisher's Exact Binomial test with Bonferroni correction ( $\alpha = 0.05$ )

\* statistically significantly different compared to the control: Fisher's Exact Binomial test with Bonferroni correction ( $\alpha = 0.05$ ) for test item and Fisher's Exact Binomial test ( $\alpha = 0.05$ ) for reference item

Validity of the test:

The results of the control group indicated that the test organisms were in a good condition (mortality: 2.5 % in the 1st test run and 0 % in the 2nd test run). The results of the reference item group indicated that the test system was sensitive to harmful substances (corrected mortality: 100 %, both test runs). Concerning mortality in the control group and as well the susceptibility of the test organisms to the reference item the study is proved to be valid.

#### Mortality:

##### 1st test run

After 48 hours, the mortality in the test item treatments ranged between 62.5 % and 100 % in the test item groups in comparison to 2.5 % in the control. Based on these results the corrected mortality for the different rates was between 61.5 % and 100 %.

##### 2nd test run

After 48 hours, the mortality in the test item treatments ranged between 0 % and 57.5 % in the test item groups in comparison to 0 % in the control. Based on these results the corrected mortality for the different rates was between 0 % and 57.5 %. No unusual observations were noted in the control and all test item treatment groups at any observation point during the test.

#### **□ Conclusion:**

The 48h LR<sub>50</sub> for Foramsulfuron + Isoxadifen-ethyl OD 45 (22.5+22.5 g/L) was estimated to be 241 mL product/ha in 200 L water/ha based on the results of the 1st and 2nd test run (equivalent to value of 5.6 g foramsulfuron/ha).

- Comment (Co-RMS and RMS):** No comment. All validity criteria according to MEAD-BRIGGS et al. (2000) were met, study is acceptable.

#### **Effects on other non-target arthropods**

Additional species *Chrysoperla carnea*, *Aleochara bilineata*, *Poecilus cupreus* and *Pardosa sp.* were tested. These studies showed that FSN + IDF OD 45 had no or only low effects on mortality, reproduction and feeding rate of these additional species, except for *Aleochara bilineata* for which 46 % mortality was observed already in 45 g a.s./ha. Details of the studies with these additional species are provided in the table below.

**Table 9.3.2.1-3: Toxicity data of foramsulfuron to non-target arthropods other than bees**

Test species	Tested Formulation, study type, exposure	Ecotoxicological Endpoint	Reference
<i>Chrysoperla carnea</i>	FSN + IDF OD 45 Laboratory, glass plate Control 160 mL prod./ha 2000 mL prod./ha 4000 mL prod./ha	LR <sub>50</sub> > 4000 mL prod./ha Corr. Mortality [%] Eggs/Female/Day Hatching [%] - 17.8 81.3 13 15.7 81.7 2 15.0 80.8 35 15.4 81.7	Kleiner, 2000 M-194627-01-1 KCA 8.3.2 /04 [991048098]
<i>Aleochara bilineata</i>	FSN + IDF OD 45 Laboratory, spray deposits on quartz sand 160 mL prod./ha 2000 mL prod./ha 4000 mL prod./ha	ER <sub>50</sub> > 4000 mL prod./ha Mortality Effect on Reproduction [%] 0 46 10 63 15	Kleiner, 1999 M-193482-01-1 KCA 8.3.2 /03 [991048095]
<i>Poecilus cupreus</i>	FSN + IDF OD 45 Laboratory, spray deposits on quartz sand 2667 mL prod./ha 5333 mL prod./ha	LR <sub>50</sub> > 4660 mL prod./ha Corr. Mortality [%] Effect on Feeding Rate [%] 0 -22.5 <sup>A</sup> 0 -12.4 <sup>A</sup>	Waltersdorfer, 1999 M-186968-01-1 KCA 8.3.2 /02 [CW98/112]

Test species	Tested Formulation, study type, exposure	Ecotoxicological Endpoint	Reference
<i>Pardosa</i> sp.	FSN + IDF OD 45 Laboratory, spray deposits on quartz sand 160 mL prod./ha 2000 mL prod./ha 4000 mL prod./ha	<b>LR<sub>50</sub> &gt; 4000 mL prod./ha</b>  Corr. Mortality [%]      Effect on Feeding Rate [%] 0                                  8 5                                  3 0                                  2	Kleiner, 1999 <u>M-188675-01-1</u> KCA 8.3.2 /01 [991048030]

<sup>A</sup>: A negative value indicates a higher feeding rate in the treatment than in the control. prod.: product

**Bold letters:** Values considered relevant for risk assessment

#### B.9.3.2.1.7. Toxicity of AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L on plant dwelling species *Chrysoperla carnea* (laboratory test)

Report:	<u>KCA 8.3.2 /04; Kleiner, R.:2000;M-194627-01</u>
Title:	Toxicity to the foliage dwelling predator <i>Chrysoperla carnea</i> STEPH. (laboratory) following the IOBC Guideline (BIGLER 1988), ringtest method (VOGT et al. 1997) and OECD Guideline proposal (VOGT et al. 1999) Code: AE F130360 01 1K05 A304
Report No:	C006791
Document No:	<u>M-194627-01-1</u>
Guidelines:	<b>IOBC: 1988; 1999; Deviation not specified</b>
GLP/GEP:	yes

##### □ Conclusion:

Application rate	Mortality	Sublethal effects
0.16 L product/ha (3.6 g a.s./ha)	13 %	12 % (fertility)
2.0 L product/ha (45 g a.s./ha)	2 %	17 % (fertility)
4.0 L product/ha (2x45 g a.s./ha)	35 %	13 % (fertility)

- **Comment (Co-RMS and RMS):** Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final): 35 % mortality, 13 % fertility (90 g a.s./ha).

#### B.9.3.2.1.8. Toxicity of AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L on soil dwelling species *Aleochara bilineata* (laboratory test)

Report:	<u>KCA 8.3.2 /03; Kleiner, R.:1999;M-193482-01</u>
Title:	Toxicity to the ground dwelling predator <i>Aleochara bilineata</i> Gyll. (laboratory) according to IOBC Guideline (Moreth & Naton 1992) Code: AE F130360 01 1K05 A304
Report No:	C006202
Document No:	<u>M-193482-01-1</u>
Guidelines:	<b>IOBC: Moreth &amp; Naton, 1992; Deviation not specified</b>
GLP/GEP:	yes

##### □ Conclusion:

Application rate	Mortality	Sublethal effects
0.16 L product/ha (3.6 g a.s./ha)	34 %	5 % (egg-hatch)
2.0 L product/ha (45 g a.s./ha)	46 %	14 % (egg-hatch)
4.0 L product/ha (2x45 g a.s./ha)	63 %	17 % (egg-hatch)



- ❑ **Comment (Co-RMS and RMS):** Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final): 46 % mortality, 14 % fertility (45 g a.s./ha).

**B.9.3.2.1.9. Toxicity of AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L on soil dwelling species *Poecilus cupreus* (laboratory test)**

<b>Report:</b>	<u>KCA 8.3.2 /02;Waltersdorfer, A.:1999;M-186968-01</u>
<b>Title:</b>	Toxicity to the ground dwelling predator <i>Poecilus cupreus</i> L. (Coleoptera, Carabidae) in the laboratory AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L Code: AE F130360 01 1K05 A301
<b>Report No:</b>	C003899
<b>Document No:</b>	<u>M-186968-01-1</u>
<b>Guidelines:</b>	BBA: VI 23-2.1.8;Deviation not specified
<b>GLP/GEP:</b>	yes

❑ **Conclusion:**

Application rate	Mortality	Sublethal effects
2.33 L product/ha (60 g a.s./ha)	0 %	+22 % (food uptake)
4.66 L product/ha (120 g a.s./ha)	0 %	+12 % (food uptake)

- ❑ **Comment (Co-RMS and RMS):** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final). The value should be included in the new list of end points.

**B.9.3.2.1.10. Toxicity of AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L on soil dwelling species *Pardosa spp.* (laboratory test)**

<b>Report:</b>	<u>KCA 8.3.2 /01;Kleiner, R.:1999;M-188675-01</u>
<b>Title:</b>	Toxicity to the ground dwelling predator <i>Pardosa</i> spp. (laboratory) according to IOBC Guideline (Wehling et al. 1998) Code: AE F130360 01 1K05 A304
<b>Report No:</b>	C004831
<b>Document No:</b>	<u>M-188675-01-1</u>
<b>Guidelines:</b>	IOBC: Wehling et al. 1998;Deviation not specified
<b>GLP/GEP:</b>	yes

❑ **Conclusion:**

Application rate	Mortality	Sublethal effects (reduction in feeding capacity)
0.16 L product/ha (3.6 g a.s./ha)	0 %	8 %
2.0 L product/ha (45 g a.s./ha)	5 %	3 %
4.0 L product/ha (2x45 g a.s./ha)	0 %	2 %

- ❑ **Comment (Co-RMS and RMS):** Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final): 5 % mortality, 8 % food consumption (120 g a.s./ha). Maximum tested concentration in a study was 90 g a.s./ha, not 120 g a.s./ha as stated in the Review Report for foramsulfuron.



**B.9.3.2.2. Field tests (annex IIIA 10.3.2.4)**

Field studies are not required as the risk assessment carried out with laboratory data indicate that use of EQUIP OD 45 poses an acceptable risk to non-target arthropods (see vol 3 CP Section 9.6).

#### B.9.4. EFFECTS ON NON-TARGET SOIL MESO- AND MACROFAUNA

In the new data requirements (EU Commission regulation No. 283/2013) the acute toxicity to earthworms needs not to be studied any longer. Two old acute studies are briefly summarised in this dRAR, one study with the active substance and one with the product Equip OD 45. These studies were submitted and reviewed during Annex I inclusion and were considered acceptable by the RMS Germany. These studies were not re-evaluated since they are performed according to the OECD 207: Acute Toxicity Test. The third study with the metabolite, AE F153745, as not previously evaluated at EU level, has been added in this dRAR for completeness, and has been evaluated. LC<sub>50</sub>s ranged from  $\geq 453$  mg product/kg dws to  $\geq 1000$  mg/kg dws for the active substance and metabolite AE F153745.

Details of all three studies are provided in the following Table 9.4-1.

**Table 9.4-1: Acute toxicity data of foramsulfuron, Equip OD 45 and metabolite AE F153745 to *Eisenia fetida***

Test substance	Test species, test design	Endpoint	Reference
Foramsulfuron	Acute toxicity to earthworms	LC <sub>50</sub> > 1000 mg a.s kg/kg dsw	<u>Heusel, R.:1998</u> <u>M-142934-01</u> <u>KCA 8.4 /01</u>
Equip OD 45	Acute toxicity to earthworms	LC <sub>50</sub> > 452.95 mg product/kg dsw	<u>Nienstedt, K.</u> <u>M.:1999;</u> <u>M-193746-01-1</u> <u>KCP 10.4.1 /01;</u>
AE F153745	Acute toxicity to earthworms	LC <sub>50</sub> > 1000 mg/kg dsw	<u>Sowig, P.; Gosch,</u> <u>H.:1999</u> <u>M-192813-01</u> <u>KCA 8.4 /02</u>

dws = dry weight soil

##### B.9.4.1. Earthworm – acute toxicity

##### B.9.4.1.1. AE F130360 substance, technical code: AE F130360 00 1C98 0002 Acute toxicity to earthworms (*Eisenia fetida*)

<b>Report:</b>	<u>KCA 8.4 /01;Heusel, R.:1998;M-142934-01</u>
<b>Title:</b>	Acute toxicity to earthworms ( <i>Eisenia fetida</i> ) AE F130360 substance, technical Code: AE F130360 00 1C98 0002
<b>Report No:</b>	A59245
<b>Document No:</b>	<u>M-142934-01-1</u>
<b>Guidelines:</b>	EU (=EEC): 92/69; OECD: 207; Deviation not specified
<b>GLP/GEP:</b>	yes

☐ **Findings:**

LC<sub>50</sub>: >1000 mg/kg  
 Lowest lethal conc.: >1000 mg/kg  
 NOEC: 1000 mg/kg

☐ **Conclusion:** In the test was used next concentration of technical foramsulfuron: 0/100/180/320/560/1000 mg/kg. LC<sub>50</sub> (endpoint) of technical foramsulfuron (purity 98,1 %) is >1000 mg/kg

☐ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.4.1.2. Acute toxicity to earthworms (*Eisenia fetida*) **AE F153745**; substance, technical (impurity of AE F130360)

<b>Report:</b>	KCA 8.4 /02;Sowig, P.; Gosch, H.;1999;M-192813-01
<b>Title:</b>	Acute toxicity to earthworms ( <i>Eisenia fetida</i> ) AE F153745 (impurity of AE F130360) substance, technical Code: AE F153745 00 1C98 0001
<b>Report No:</b>	C005859
<b>Document No:</b>	M-192813-01-1
<b>Guidelines:</b>	EU (=EEC): 92/69/EEG Part C; OECD: 207; Deviation not specified
<b>GLP/GEP:</b>	yes

##### □ Summary:

The acute toxicity of AE F153745 to earthworms of the species *Eisenia fetida* was examined in an artificial soil test according to OECD guideline 207. The following concentrations were tested: 100, 180, 320, 560 and 1000 mg test substance/kg artificial soil (dry weight) and an untreated control with 10 worms in each of the 4 replicates. Mortality and intoxication symptoms were determined 7 and 14 days after application. Weight of worms was determined at start and end of testing. Weight changes were compared with the untreated control. No mortality occurred and no intoxication symptoms were observed in any of the tested concentrations and in the untreated control. The LC<sub>50</sub> value after 7 and 14 days test duration was > 1000 mg test substance/kg dry soil. The NOEC regarding to mortality, weight loss and intoxication symptoms was > 1000 mg test substance/kg dry soil after 14 days test duration.

##### □ Materials and methods:

<b>Test Material:</b>	AE F153745; technical (metabolite of AE F130360); Code: AE F153745 00 1C98 0001
<b>Lot/Batch No:</b>	AZ 07716
<b>Actual content of a.s:</b>	foramsulfuron - analysed purity: 97.8 % w/w
<b>Description:</b>	substance
<b>Stability of test compound:</b>	At 25 ± 5 °C (+2°C to +30°C are also acceptable), under dark and dry conditions
<b>Reanalysis/Expiry date:</b>	January 25, 2000
<b>Treatments</b>	
<b>Test rates:</b>	Treatment group were exposed in an artificial soil (with 10% peat content) to the nominal test concentrations of 100, 180, 320, 560 and 1000 mg test substance/kg test substrate (dry weight)
<b>Control:</b>	Untreated (and moistened with deionised water)
<b>Toxic standard:</b>	Cloroacetamide – substance, technical (Code: AE F018608 00 1B99 0001)
<b>Test organisms</b>	
<b>Species:</b>	<i>Eisenia fetida</i> ( <i>Esenia fetida Andrei</i> , det. BOUCHÉ 1982)
<b>Age and weight range at test start:</b>	Adults, older than 2 months, with well developed clitellum, Weight of 10 worms – 5.04 and 5.71 g
<b>Source:</b>	Bred under standardised conditions in the animal maintenance room of the Ecobiology of AgrEvo in plastic boxes, stored at room temperature
<b>Feeding:</b>	None during test
<b>Test design</b>	
<b>Vessels:</b>	The test was conducted in 1.5 L glass jars , containing 750 g of test substrate. The jars had diameter of about 10 cm a height about 20 cm. The vessels were covered with glass lids
<b>Substrate:</b>	According to OECD 222: - 10% sphagnum-peat (residual moisture content of 13.61% taken into calculation) - 20% kaolin clay (particle analysis: 0.05 -0.2 mm > 50%)

- 70% industrial sand (quartz sand type 155-0, Gebr. Willersinn GmbH & Co. KG, Keisterbach, FRG)
- 72 g Calcium carbonate (CaCO<sub>3</sub>) was added to adjust pH to 6.3

**Replication**

<b>Test groups:</b>	4 replicates for the test item treatments; 6 treatment groups (5 test item concentrations + control)
<b>No. of worms/arena:</b>	10
<b>Duration of test:</b>	14 day

**Environmental test conditions**

<b>Temperature:</b>	20±2 °C
<b>pH of soil:</b>	test start: 6.1 – 6.4 test end: 5.8 – 6.4
<b>Water content of soil:</b>	at experimental start: 25.13% experimental end: 25.06% at
<b>Photoperiod: light:</b>	16 h light: 8 h dark

**□ Study design and methods:**

Principles of the testing procedure: Adult *Eisenia fetida* (older than 2 months and showing a clitellum), 4 x 10 animals for the control group and for each test concentration of the treatment group were exposed in an artificial soil (with 10% peat content) to the nominal test concentrations of 100, 180, 320, 560 and 1000 mg test substance/kg test substrate (dry weight). The test substance was mixed in industrial sand. Mortality and intoxication symptoms were determined 7 and 14 days after application. Weight of worms was determined at start and end of testing. Weight changes were compared with the untreated control.

**□ Findings:**Physical and chemical parameters:

At the start of testing the moisture content of the control substrate was 25.13% and 25.06% at experimental termination. At the beginning of the test the pH value of the test substrate ranged from 6.1 to 6.4. At the end of the test the pH value of the test substrate was between 5.8 and 6.4. The pH value of the basic substrate was 6.3.

Biological results:

No mortality occurred and no intoxication symptoms were observed in any of the tested concentrations and in the untreated control (see Table below).

**Table 9.4.1.2-1: % mortality and symptoms**

Nominal concentration in mg/kg (dry weight)	Jar No.	7 d test duration (mean values)		14 d test duration (mean values)	
		% mortality	symptoms	% mortality	symptoms
Control	1-4	0	-	0	-
100	5-8	0	-	0	-
180	9-12	0	-	0	-
320	13-16	0	-	0	-
560	17-20	0	-	0	-
1000	21-24	0	-	0	-

At the start of the test there was no significant difference of worm weights between the treatments and the control (see Table below).

At the end of the test the mean weight of surviving worms was reduced by 11.2 - 15.8% of initial weight in the treated groups and the control group. This weight loss increased with the dose of the test substance. There were no significant differences between the treatments and the control regarding the

absolute or the percent weight loss compared to controls.

The no observed effect concentration (NOEC) regarding to mortality, weight loss and intoxication symptoms was 1000 mg test substance/kg dry soil after 14 days test duration.

**Table 9.4.1.2-2: Weight of *Eisenia fetida* after treatment with AE F153745**

Nominal concentration in mg/kg (dry weight)	Mean start weight in g	Mean weight difference start - end in g	Mean percent weight loss <sup>1</sup>
Control	5.3650 A	0.071250 A B	13.340 A B
100	5.4075 A	0.071750 A B	13.277 A B
180	5.3975 A	0.070750 A B	13.187 A B
320	5.4375 A	0.061000 B	11.201 B
560	5.4925 A	0.076500 A B	13.922 A B
1000	5.4325 A	0.086250 A	15.824 A

<sup>1</sup> mean percent weight loss = mean weight difference (start - end) in percent of mean start weight  
Concentrations with the same letter within each column are not significantly different.

**Validity criteria:**

Validity criterion for the control group was met:

Adult mortality: ≤ 10% (being 0 % after 14 days).

Mean loss of control earthworm biomass < 20% (11.2 – 15.8%)

**□ Conclusion:**

In a 14-day Artificial Soil Test (CE99/141-1, method OECD / EU) to determine the effects of AE F153745 to *Eisenia fetida* (earthworm) the LC<sub>50</sub> value after 7 and 14 days test duration was >1000 mg/kg (dry weight) in comparison with the untreated control group.

The highest concentration tested without mortality, without intoxication symptoms and without a significantly higher weight loss compared to the control (NOEC) after 14 days test duration was > 1000 mg test substance / kg dry test substrate.

**□ Comment (Co-RMS and RMS):** No comments, study is acceptable.

**B.9.4.1.3. AE F130360 01 1K05 A304: A 14-day acute toxicity test with the earthworm (*Eisenia fetida*)**

Report:	KCP 10.4.1 /01; Nienstedt, K. M.:1999; M-193746-01-1
Title:	AE F130360 01 1K05 A304: A 14-day acute toxicity test with the earthworm ( <i>Eisenia fetida</i> )
Report No:	C006356
Document No:	M-193746-01-1
Guidelines:	OECD: 207; Deviation not specified
GLP/GEP:	yes

**Table B.9.4.1.3-1: Toxicity of formulation (2.42 % foramsulfuron and 2.44 % isoxadifen) on *Eisenia fetida***

Test material	Species	Test	NOEC	LC <sub>50</sub>
formulation (AE F130360 01 1K05 A304)	<i>Eisenia fetida</i>	acute	319 mg/kg	452.95 mg/kg

❑ **Conclusion:**

LC<sub>50</sub>: 452.95 mg/kg  
 Lowest lethal conc.: 565 mg/kg  
 NOEC: 319 mg/kg

❑ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.4.2. Earthworm – sub-lethal effects

One long-term toxicity study with the active substance was submitted and reviewed during Annex I inclusion and was considered acceptable by the RMS Germany. This study is not re-evaluated since it was performed according to BBA: VI, 2-2 which is in line with the OECD 222: Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia Andrei*).

For the process of renewal three new studies with metabolites, AE F092944, AE F130619 and AE F153745 were submitted. Studies have been evaluated and details of all four studies are provided in the Table 9.4.2-1. In all studies no mortality occurred. NOECs ranged from  $\geq 2.75$  mg/kg dws for the parent compound to  $\geq 100$  mg/kg dws for metabolite AE F153745.

Details of studies on foramsulfuron and metabolites are provided briefly in the Table B.9.4.2-1. Summaries of the studies are provided thereafter.

**Table 9.4.2-1: Reproductive toxicity data of foramsulfuron and metabolites to *Eisenia fetida***

Test substance	Test species, test design	Endpoint	Reference
Foramsulfuron	<i>Eisenia fetida</i> reproduction, 56 d (10% peat in test soil), test item sprayed on soil surface	NOEC $\geq 600$ g a.s./ha = $\geq 2.75$ mg a.s./kg dws <sup>1)</sup>	Sowig & Gosch, 2000 <u>M-193508-01-1</u> KCA 8.4.1 /01
AE F092944	<i>Eisenia fetida</i> reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC <b>10 mg/kg dws</b>	Kratz, 2013 <u>M-461051-01-1</u> KCA 8.4.1 /02
AE F130619	<i>Eisenia fetida</i> reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC <b>56 mg/kg dws</b>	Kratz, 2013 <u>M-461453-01-1</u> KCA 8.4.1 /03
AE F153745	<i>Eisenia fetida</i> reproduction, 56 d (10% peat in test soil), test item mixed into soil	NOEC $\geq 100$ mg/kg dws	Kratz, 2013 <u>M-459518-01-1</u> KCA 8.4.1 /04

<sup>1)</sup> Considering a jar surface area of 283.4 cm<sup>2</sup> and an amount of 618 g dry soil per jar – BCS calculation results in 2.75 mg a.s./kg dws.

dws = dry weight soil

**Bold letters:** Values considered relevant for risk assessment



#### B.9.4.2.1. Effects on growth and reproduction of earthworms (*Eisenia fetida*) AE F130360 substance, technical

<b>Report:</b>	<u>KCA 8.4.1 /01;Sowig, P.; Gosch, H.:2000;M-193508-01</u>
<b>Title:</b>	Effects on growth and reproduction of earthworms ( <i>Eisenia fetida</i> ) AE F130360 substance, technical Code: AE F130360 00 1C98 0002
<b>Report No:</b>	C006218
<b>Document No:</b>	<u>M-193508-01-1</u>
<b>Guidelines:</b>	BBA: VI, 2-2;Deviation not specified
<b>GLP/GEP:</b>	yes

##### □ Findings:

**Table B.9.4.2.1-1: Effects of technical foramsulfuron on reproduction and biomass of *Eisenia fetida***

Dose level (kg as/ha)	Adult mortality	Adult weight (% of initial weight)	Mean number of juveniles/test box
control	0	102.1	109
0.06	0	105.3	109
0.6	0	108.2	112

- **Conclusion:** Endpoint according to the Review Report for foramsulfuron (SANCO/10324/2002-Final): NOEC = 0.6 kg a.s./ha (corresponds to 3.24 mg a.s./kg)

\* Considering a jar surface area of 283.4 cm<sup>2</sup> and an amount of 618 g dry soil per jar – BCS calculation results in 2.75 mg a.s./kg dws.

- **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### Studies on the metabolites of foramsulfuron

#### B.9.4.2.2. AE F092944 (BCS-AA25052): Effects on survival, growth and reproduction of the earthworm *Eisenia fetida* tested in artificial soil

<b>Report:</b>	<u>KCA 8.4.1 /02;Kratz, M. A.:2013;M-461051-01</u>
<b>Title:</b>	AE F092944 (BCS-AA25052): Effects on survival, growth and reproduction of the earthworm <i>Eisenia fetida</i> tested in artificial soil
<b>Report No:</b>	kra/Rg-R-147/13
<b>Document No:</b>	<u>M-461051-01-1</u>
<b>Guidelines:</b>	OECD Guideline 222 (2004); deviation: none
<b>GLP/GEP:</b>	yes

##### □ Summary:

The purpose of this study was to assess the effect of AE F092944 on survival, growth and reproduction on the earthworm *Eisenia fetida* during an exposure in an artificial soil with one test concentration in the first run and five different test concentrations in the second run.

In the first run adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control group and 8 x 10 animals for the treatment group) were exposed in artificial soil (with 10 % peat content) to the nominal test concentration of 100 mg test item/ kg dry weight artificial soil. In the second run adult *Eisenia fetida* (approx. 5 months old, 8 x 10 animals for the control group and 4 x 10 animals per test concentration of the treatment group) were exposed in artificial soil (with 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. 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 observed on growth and reproduction, the overall NOEC was determined to be 10 mg test item/ kg dry weight artificial soil. The overall LOEC was determined to be 18 mg test item/kg dry weight artificial soil. The validity criteria of the test according to the guideline were fulfilled.

□ **Materials and methods:**

**Test Material:** AE F092944 (BCS-AA25052);  
**Lot/Batch No:** 23503LR  
**Actual content of active ingredient:** Purity: 99.8 % w/w  
**Description:** White powder  
**Preparation:** Technical substance  
**Stability of test compound:** Stable under standard conditions  
**Reanalysis/Expiry date:** 2015-12-18

**Treatments**

**Test rates:** 5.6, 10, 18, 32 and 56 mg test item/ kg dry weight artificial soil  
**Control:** Untreated soil  
**Toxic standard:** Carbendazim EC 360 G: 1.25 - 2.5 - 5.0 mg a.s./kg soil d.w.  
 (corresponds to 3.94 - 15.78 mg test item/ kg soil d.w.)

**Test organisms**

**Species:** *Eisenia fetida andrei*  
**Age and weight range attest start:** Approx. 5 to 6 months old and showing a clitellum  
**Source:** Forschungsanstalt, 38104 Braunschweig, Germany  
**Feeding:** Dried ground cow manure. Once per week during the test period with approximately 5 g food per vessel

**Test design:**

**Vessels:** Non-re-usable plastic boxes, containing approx. 500 g artificial soil  
**Substrate:**  
 - 10% sphagnum-peat (shredded)  
 - 20% kaolin clay (content of Kaolinite 30.2%)  
 - 69% industrial sand (Particle size 0.2–0.05 mm =91.35%)  
 - 1% food (dried ground cow manure)  
 - Calcium carbonate (CaCO<sub>3</sub>) was added to adjust pH to 6.0±0.5

**Replication**

**Test groups:** 1<sup>st</sup> run – 8  
 2<sup>nd</sup> run – 4  
 control group - 8  
**No. of worms/arena:** 1<sup>st</sup> run – 10  
 2<sup>nd</sup> run – 10  
 control group – 10  
**Duration of test:** 1<sup>st</sup> run – 4 weeks  
 2<sup>nd</sup> run – 4 weeks  
 total duration – 8 weeks

**Environmental test conditions**

**Temperature:** 20±2°C  
**pH of soil:** test start: 5.56 – 6.24  
 test end: 6.23 – 6.54  
**Water content of soil:** test start: from 32.23 to 34.10%  
 test end: from 32.45 to 34.88%  
**Photoperiod: light:** 16 h light: 8 h dark; intensity at light period between approximately 400 -800 Lux

□ **Study design and methods:**

In the first run adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control group and 8 x 10 animals for the treatment group) were exposed in artificial soil (with 10 % peat content) to the nominal test concentration of 100 mg test item/ kg dry weight artificial soil. In the second run adult *Eisenia fetida* (approx. 5 months old, 8 x 10 animals for the control group and 4 x 10 animals per test

concentration of the treatment group) were exposed in artificial soil (with 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.

□ **Findings:**

**Table 9.4.2.2-1: Effects 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	<i>Eisenia fetida</i>							
	1 <sup>st</sup> run		2 <sup>nd</sup> 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
							<b>Reproduction</b>	
EC <sub>10</sub> (mg test item/kg dry weight soil <sup>1)</sup> ) (95% confidence limits)							15.35 (n.d.)	
EC <sub>20</sub> (mg test item/kg dry weight soil <sup>1)</sup> ) (95% confidence limits)							54.06 (n.d.)	
EC <sub>50</sub> (mg test item/kg dry weight soil <sup>1)</sup> ) (95% confidence limits)							n. d.	

\* statistical significance compared to the control

(1<sup>st</sup> run: Student t-test; 2<sup>nd</sup> run: Williams Multiple Sequential t-test, two-sided,  $\alpha = 0.05$  )

\*\* statistical significance compared to the control

(1<sup>st</sup> run: Student t-test; 2<sup>nd</sup> run: Williams Multiple Sequential t-test, one-sided

smaller,  $\alpha = 0.05$ ) <sup>1)</sup> Probit analysis

n.d. - not determined due to mathematical reasons or inappropriate data

**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 (2<sup>nd</sup> 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 2<sup>nd</sup> 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

**Validity criteria**

The validity criteria for the control group were accomplished:

- adult mortality: ≤ 10 % (being 0 % after 8 weeks)
- number of juveniles per replicate ≥ 30  
     1<sup>st</sup> run – 391, 335, 260, 313, 330, 399, 371, 387  
     2<sup>nd</sup> run – 246, 350, 278, 228, 285, 232, 254, 287
- coefficient of variation for reproduction: ≤ 30 %  
     1<sup>st</sup> run – 13.7%  
     2<sup>nd</sup> run – 14.7%

- ❑ **Conclusion:** 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.
- ❑ **Comment (Co-RMS and RMS):** The NOEC for the mean number of offspring of > 100 mg/kg from the 1<sup>st</sup> run is much higher than the NOEC of 10 mg/kg from the 2<sup>nd</sup> run. In the 1st test run adult *Eisenia fetida* approx. 6 months old was used. In the 2nd test run adult *Eisenia fetida* approx. 5 months old was used under the same test conditions as in the 1st test run. Differences in values of NOEC could be caused by the age of earthworms and the date of experimental works -1st test run - July 2012 (summer-time) and 2nd - April 2013 (spring-time). It is possible to predict that younger earthworms are more sensitive to the exposure of the test substance than older one. Study is acceptable.

#### **B.9.4.2.3. AE F130619 (BCS-AU59648): Effects on survival, growth and reproduction of the earthworm *Eisenia fetida* tested in artificial soil**

<b>Report:</b>	<u>KCA 8.4.1 /02;Kratz, M. A.;2013;M-461051-01</u>
<b>Title:</b>	AE F092944 (BCS-AA25052): Effects on survival, growth and reproduction of the earthworm <i>Eisenia fetida</i> tested in artificial soil
<b>Report No:</b>	kra/Rg-R-147/13
<b>Document No:</b>	<u>M-461051-01-1</u>
<b>Guidelines:</b>	<b>EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP Not Applicable; none</b>
<b>GLP/GEP:</b>	yes

❑ **Summary:**

The purpose of this study was to assess the effect of AE F130619 on survival, growth and reproduction

on the earthworm *Eisenia fetida* during an exposure in an artificial soil with one test concentration in the first run and five different test concentrations in the second run.

In the first run adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control group and 8 x 10 animals for the treatment group) were exposed in artificial soil (with 10 % peat content) to the test concentration of 100 mg pure metabolite/ kg dry weight artificial soil (corresponding to 106 mg test item/ kg dry weight artificial soil). In the second run adult *Eisenia fetida* (approx. 10 months old, 8 x 10 animals for the control group and 4 x 10 animals per test concentration of the treatment group) were exposed in an artificial soil (with 10 % peat content) to the test concentrations of 5.6, 10, 18, 32 and 56 mg pure metabolite/ kg dry weight artificial soil (corresponding to 6, 10.7, 19.0, 33.7 and 59.9 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 observed on growth and reproduction, the overall NOEC was determined to be 56 mg pure metabolite/ kg dry weight artificial soil. The overall LOEC was determined to be 100 mg pure metabolite/kg dry weight artificial soil. The validity criteria of the test according to the guideline were fulfilled.

□ **Materials and methods:**

<b>Test Material:</b>	AE F130619 (BCS-AU59648)
<b>Lot/Batch No:</b>	SES 10641-3-3
<b>Actual content of a.s:</b>	Purity: 94 % w/w
<b>Description:</b>	Off white powder
<b>Preparation:</b>	Technical substance
<b>Stability of test compound:</b>	Stable under standard conditions
<b>Reanalysis/Expiry date:</b>	2012-11-28
<b>Treatments</b>	
<b>Test rates:</b>	5.6, 10, 18, 32 and 56 mg pure metabolite/ kg dry weight artificial soil (corresponding to 6, 10.7, 19.0, 33.7 and 59.9 mg test item/ kg dry weight artificial soil).
<b>Control:</b>	Untreated soil
<b>Toxic standard:</b>	Carbendazim EC 360 G: 1.25 - 2.5 - 5.0 mg a.s./kg soil d.w. (corresponds to 3.94 - 15.78 mg test item/ kg soil d.w.)
<b>Test organisms</b>	
<b>Species:</b>	<i>Eisenia fetida andrei</i>
<b>Age and weight range at test start:</b>	Approx. 6 months old and showing a clitellum, body weight at start test is 0.30 to 0.50 g per worm
<b>Source:</b>	Forschungsanstalt, 38104 Braunschweig, Germany
<b>Feeding:</b>	Dried ground cow manure. Once per week during the test period with approximately 5 g food per vessel
<b>Test design</b>	
<b>Vessels:</b>	Non-re-usable plastic boxes, containing approx. 500 g artificial soil
<b>Substrate:</b>	- 10% sphagnum-peat (shredded) - 20% kaolin clay (content of Kaolinite 30.2%) - 69% industrial sand (Particle size 0.2–0.05 mm =91.35%) - 1% food (dried ground cow manure) - Calcium carbonate (CaCO <sub>3</sub> ) was added to adjust pH to 6.0±0.5
<b>Replication:</b>	
<b>Test groups:</b>	1 <sup>st</sup> run – 8 2 <sup>nd</sup> run – 4 control group - 8
<b>No. of worms/arena:</b>	1 <sup>st</sup> run – 10 2 <sup>nd</sup> run – 10 control group – 10

<b>Duration of test:</b>	1 <sup>st</sup> run – 4 weeks 2 <sup>nd</sup> run – 4 weeks total duration – 8 weeks
<b>Environmental test conditions</b>	
<b>Temperature:</b>	20±2°C
<b>pH of soil:</b>	test start: 5.69 – 6.24 test end: 6.36 – 6.54
<b>Water content of soil:</b>	test start: from 32.80 to 36.74% test end: from 34.34 to 36.82%
<b>Photoperiod: light:</b>	16 h light : 8 h dark; intensity at light period between approximately 400 -800 Lux

□ **Study design and methods:**

In the first run adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control group and 8 x 10 animals for the treatment group) were exposed in an artificial soil (with 10 % peat content) to the test concentration of 100 mg pure metabolite/ kg dry weight artificial soil (corresponding to 106 mg test item/ kg dry weight artificial soil). In the second run adult *Eisenia fetida* (approx. 10 months old, 8 x 10 animals for the control group and 1 x 10 animals per test concentration of the treatment group) were exposed in an artificial soil (with 10 % peat content) to the test concentrations of 5.6, 10, 18, 32 and 56 mg pure metabolite/ kg dry weight artificial soil (corresponding to 6, 10.7, 19.0, 33.7 and 59.9 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 - 15.78 mg test item/ kg soil d.w.); control: quartz sand, solvent control: none.

□ **Findings:**

**Table 9.4.2.3-1: Effects 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	<i>Eisenia fetida</i>							
	1st run		2nd run					
	Control	AE F130619	Control	AE F130619				
mg pure metabolite/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	38.29*	34.35	33.7	42.06	38.10	37.90	40.15
Standard Deviation	4.05	6.83	4.53	3.42	2.19.0	1.91	6.80	6.44
Mean number of offspring per test vessel after 56 days	348.3	363.0	290.5	317.8	279.8	291.0	322.8	280.3
**								
Standard Deviation	47.8	65.4	26.6	57.5	39.1	22.3	32.1	65.2
Coefficient of variance (%)	13.7	18.1	9.1	18.1	14.0	7.6	10.0	23.3



Test object	<i>Eisenia fetida</i>							
	1st run		2nd run					
Test item	Control	AE F130619	Control	AE F130619				
% of control	...	104.2	...	109.4	96.3	100.2	111.1	96.5
							<b>Reproduction</b>	
EC <sub>10</sub> (mg pure metabolite/kg dry weight soil <sup>1)</sup> ) (95% confidence limits)							n.d.	
EC <sub>20</sub> (mg pure metabolite/kg dry weight soil <sup>1)</sup> ) (95% confidence limits)							n.d.	
EC <sub>50</sub> (mg pure metabolite/kg dry weight soil <sup>1)</sup> ) (95% confidence limits)							n.d.	

- \* statistical significance compared to the control  
(1<sup>st</sup> run: Student t-test; 2<sup>nd</sup> run: Williams Multiple Sequential t-test, two-sided,  $\alpha = 0.05$  )
- \*\* statistical significance compared to the control  
(1<sup>st</sup> run: Student t-test; 2<sup>nd</sup> run: Williams Multiple Sequential t-test, one-sided smaller,  $\alpha = 0.05$ )
- <sup>1)</sup> Probit analysis
- n.d. - not determined due to mathematical reasons or inappropriate data

#### 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 1<sup>st</sup> run. Therefore, based on biological and statistical significance (for both test runs):

NOEC related to growth: 56 mg pure metabolite/kg dry weight artificial soil  
LOEC related to growth: 100 mg pure metabolite /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 all tested concentrations the first and second run. Therefore, based on biological and statistical significance (for both test runs):

NOEC related to reproduction: 100 mg pure metabolite /kg dry weight artificial soil  
LOEC related to reproduction: 100 mg pure metabolite /kg dry weight artificial soil

#### **Validity criteria**

The validity criteria for the control group were accomplished:

- adult mortality:  $\leq 10$  % (being 0 % after 8 weeks)
- number of juveniles per replicate  $\geq 30$   
1<sup>st</sup> run – 448, 354, 314, 269, 374, 299, 424, 422  
2<sup>nd</sup> run – 280, 292, 286, 268, 316, 316, 245, 321
- coefficient of variation for reproduction:  $\leq 30$  %  
1<sup>st</sup> run – 13.7%  
2<sup>nd</sup> run – 9.1%

#### **Conclusion:**

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 56 mg pure metabolite/kg dry weight artificial soil. Thus, the overall LOEC is determined to be 100 mg pure metabolite/kg dry weight artificial soil.

#### **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.4.2.4. Foramsulfuron AE F153745 (BCS-AU80017): Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil

<b>Report:</b>	KCA 8.4.1 /04;Kratz, M. A.;2013;M-459518-01
<b>Title:</b>	Foramsulfuron-AE F153745 (BCS-AU80017): Effects on survival, growth and reproduction on the earthworm <i>Eisenia fetida</i> tested in artificial soil
<b>Report No:</b>	kra/Rg-R-140/13
<b>Document No:</b>	M-459518-01-1
<b>Guidelines:</b>	ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004; US EPA OCSP: None; none
<b>GLP/GEP:</b>	yes

##### □ Summary:

The purpose of this study was to assess the effect of AE F153745 on survival, growth and reproduction on the earthworm *Eisenia fetida* during an exposure in an artificial soil with one test concentration (limit test). Adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control group and 8 x 10 animals for the treatment group) were exposed in artificial soil (with 10 % peat content) to the nominal test concentration of 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. The test was performed as a limit test according to the guideline ISO 11268-2 (1998) and the OECD Guideline 222 (2004). The overall NOEC related to reproduction was determined to be 100 mg test item/kg soil dry weight. The LOEC related to reproduction was determined to be > 100 mg test item/kg soil dry weight. The validity criteria of the test according to the guideline were fulfilled.

##### □ Materials and methods:

<b>Test Material:</b>	Foramsulfuron-AE F153745 (BCS-AU80017)
<b>Lot/Batch No:</b>	ZER0234
<b>Actual content of a.s:</b>	Purity: 98.2 % w/w
<b>Description:</b>	Beige powder
<b>Preparation:</b>	Technical substance
<b>Stability of test compound:</b>	Stable under standard conditions
<b>Reanalysis/Expiry date:</b>	2016-10-14
<b>Treatments</b>	
<b>Test rates:</b>	The nominal test concentration of 100 mg test item/ kg dry weight artificial soil
<b>Control:</b>	Untreated soil
<b>Toxic standard:</b>	Carbendazim EC 360 G: 1.25 - 2.5 - 5.0 mg a.s./kg soil d.w. (corresponds to 3.94 - 15.78 mg test item/ kg soil d.w.)
<b>Test organisms</b>	
<b>Species:</b>	<i>Eisenia fetida andrei</i>
<b>Age and weight range at test start:</b>	Approx. 6 months old and showing a clitellum, body weight at start test is 0.30 to 0.50 g per worm
<b>Source:</b>	Forschungsanstalt, 38104 Braunschweig, Germany
<b>Feeding:</b>	Dried ground cow manure. Once per week during the test period with approximately 5 g food per vessel
<b>Test design</b>	
<b>Vessels:</b>	Non-re-usable plastic boxes, containing approx. 500 g artificial soil
<b>Substrate:</b>	- 10% sphagnum-peat (shredded) - 20% kaolin clay (content of Kaolinite 30.2%) - 68.7% industrial sand (Particle size 0.2–0.05mm =91.35%) - 1% food (dried ground cow manure)

	- 0.3% Calcium carbonate (CaCO <sub>3</sub> ) was added to adjust pH to 6.0±0.5
<b>Replication:</b>	
<b>Test groups:</b>	8 - treatment group 8 – control group
<b>No. of worms/arena:</b>	10
<b>Duration of test:</b>	8 weeks
<b>Environmental test conditions</b>	
<b>Temperature:</b>	20±2°C
<b>pH of soil:</b>	test start: 6.14 – 6.24 test end: 6.43 – 6.54
<b>Water content of soil:</b>	test start: from 34.10 to 34.37% test end: from 34.34 to 35.23%
<b>Photoperiod: light:</b>	16 h light : 8 h dark; intensity at light period between approximately 400 -800 Lux

□ **Study design and methods:**

Adult *Eisenia fetida* (approx. 6 months old, 8 x 10 animals for the control group and 8 x 10 animals for the treatment group) were exposed in artificial soil (with 10 % peat content) to the nominal test concentration of 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. 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.

□ **Findings:**

**Table B.9.4.2.4-1: Effects 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**

Test object	<i>Eisenia fetida</i>	
	Control	AE F153745
mg test item/kg dry weight artificial soil	-	100
Mortality of adult earthworms [%] after 28 days	0	0
Mean change of body weight of the adults from day 0 to day 28 [%] *	31.75	35.91
Standard Deviation	4.05	6.84
Mean number of offspring per test vessel after 56 days	348.3	342.1
Standard Deviation	47.8	32.3
Coefficient of variance (%)	13.7	9.5
% of control	-	98.2

**Mortality:**

After 28 days of exposure no worms died in the control group and no mortality was observed at the tested concentration of 100 mg test item/kg dry weight soil.

Effects on growth:

No statistically significant different value for the growth, relative to the control, was observed at the tested concentration of 100 mg test item/kg dry weight soil. 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 value for the number of juveniles per test vessel relative to the control was observed at the tested concentration of 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

**Validity criteria**

The validity criteria for the control group were accomplished:

- adult mortality: ≤ 10 % (0 % after 8 weeks)
- number of juveniles per replicate ≥ 30 (348.3)
- coefficient of variation for reproduction: ≤ 30 % (13.7%)

- ❑ **Conclusion:** 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 > 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.
- ❑ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### **B.9.4.2.5. Effects of Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G on Reproduction and Growth of Earthworms *Eisenia fetida* in Artificial Soil**

<b>Report:</b>	KCP 10.4.1.1 /01; Witte, B.;2013;M-464888-01-1
<b>Title:</b>	Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G: Effects on reproduction and growth of earthworms <i>Eisenia fetida</i> in artificial soil
<b>Report No:</b>	83352022
<b>Document No:</b>	M-464888-01-1
<b>Guidelines:</b>	<b>OECD, Guideline for the testing of chemicals No. 222, Earthworm, Reproduction Test; Deviations: None</b>
<b>GLP/GEP:</b>	yes

- ❑ **Summary:**

The purpose of this study was to investigate the effects of foramsulfuron + isoxadifen-ethyl OD45 (22.5 + 22.5) G on the mortality, body weight, feeding activity and reproduction of adult *Eisenia fetida*. Adult *Eisenia fetida* (with clitellum and weight range 300 to 600 mg, 9 to 10 months old, 8 x 10 animals for the control group and 4 x 10 animals for each treatment group) were exposed in artificial soil (with 10 % peat content) to an untreated control and to the nominal concentrations of 13, 21, 34, 55, 88, 142, 229 and 370 mg test item/ kg dry weight artificial soil. The test item was incorporated into the soil.

After 28 days exposure of adult worms the mortality, behavioural effects and biomass development was carried out. After additional 28 days the reproduction rate (number of offspring) was assessed. The NOEC for mortality, growth and feeding activity of the earthworm *Eisenia fetida* was determined to be ≥370 mg test item/kg soil, i.e. the highest concentration tested. The NOEC for reproduction was determined to be the concentration of 229 mg test item/kg soil. The LOEC was determined to be 370 mg test item/kg soil and the EC10, EC20 and EC50 values were determined to be 273.9 mg test item/kg soil, 305.8 mg test item/kg soil and 369.2 mg test item/kg soil.

**❑ Materials and methods:**

<b>Test Material:</b>	Foramsulfuron + isoxadifen-ethyl OD 45 (22.5 + 22.5) G (BCS-AH47626 + BCS- AI19578)
<b>Lot/Batch No:</b>	Batch ID: EFKM002442; Material No.: 06321801; Specification No.: 102000011304-06
<b>Actual content of a.s.:</b>	foramsulfuron (AE F130360): 2.33% w/w, 22.41 g/L, isoxadifen-ethyl (AE F122006): 2.29% w/w, 21.96 g/L
<b>Description:</b>	T0X10129-00, beige, liquid
<b>Stability of test compound:</b>	At $25 \pm 5$ °C (+2°C to +30°C are also acceptable), under dark and dry conditions
<b>Preparation:</b>	OD (Oil dispersion)
<b>Reanalysis/Expiry date:</b>	April 25, 2015
<b>Treatments:</b>	
<b>Test rates:</b>	13, 21, 34, 55, 88, 142, 229 and 370 mg test item/ kg dry weight artificial soil
<b>Control:</b>	Untreated (and moistened with deionised water)
<b>Toxic standard:</b>	Luxan Carbendazim 500 FC): 0.57 - 0.87 - 1.30 - 1.96 - 2.91 mg a.s./kg soil d.w. (corresponds to 1.3 - 2.0 - 3.0 - 4.5 - 6.7 mg test item/ kg soil d.w.) The EC <sub>50</sub> for reproduction was calculated to be 1.7 mg carbendazim/kg soil dry weight. Statistically significant effects on reproduction were observed at concentrations of 1.3 to 2.91 mg carbendazim/kg artificial soil (dry weight), indicating that the sensitivity of the worms was consistent with the level proposed by the OECD 222 guideline (significant effects between 1 and 5 mg carbendazim/kg soil dry weight).
<b>Test organisms:</b>	
<b>Species:</b>	<i>Eisenia fetida</i> (Savigny 1826)
<b>Age and weight range at test start:</b>	Adults, 9 to 10 months, with well developed clitellum, age range between test individuals not differing by more than 4 weeks, body weight: 300-600 mg.
<b>Source:</b>	Bred under standardised conditions in IBACON laboratories in a breeding medium of cattle manure, peat, sand, calcium carbonate and straw, fed with cattle manure, stored at room temperature.
<b>Feeding:</b>	Finely ground cattle manure was added as food. 10 g/kg dry soil was mixed into the artificial soil 1 day before the start of the study; 5 g/container was scattered on the soil surface at day 1 after application and was moistened with 5 g deionised water; 5 g/container (moistened with 2 - 3 g deionised water) was added each week for the first 4 weeks of the experiment, when the food of the previous week had almost been consumed. If the food was not quite fully consumed, the added amount of food was adjusted to replace the visually estimated consumption. Four weeks after application, the food was mixed into the substrate following removal of the adult worms. Cattle manure was supplied by H. Lautz, Ober Ramstadt, Germany and prepared at IBACON.
<b>Test design:</b>	
<b>Vessels:</b>	Plastic boxes (18.3 cm x 13.6 cm x 6 cm, tapered towards the bottom, with a soil surface of approximately 16.5 cm x 11.5 cm = 189.75 cm <sup>2</sup> ) with perforated transparent lids to enable exchange of air, to minimise evaporation from the artificial soil, and to prevent the worms from escaping. Each container was filled with 640.3 g of the prepared soil (500 g dry weight plus 135.3 g deionised water plus 5 g food). The height of the soil layer in the containers was approximately 4 - 5 cm.
<b>Substrate:</b>	According to OECD 222:

	-10% Sphagnum-peat, air-dried and finely ground (2 mm with no visible plant remains); (Floragard, Vertriebs GmbH für Gartenbau, 26138 Oldenburg, Germany)
	-20% kaolin clay (Kaolinite content >30%) (Erbsloh, 65558 Lohrheim, Germany)
	-69.6% fine quartz-sand (F34) containing more than 50% by mass of particle size 0.05 mm to 0.2 mm; (Quarzwirke Frechen, Postfach 1780, 50207 Frechen, Germany)
	-0.4% Calcium carbonate (CaCO <sub>3</sub> ) was added to adjust pH to 6.0 ± 0.5 (Merck, 64293 Darmstadt, Germany).
	The artificial soil was moistened to approximately half of the final water content 1 day before the application. The additional water required to achieve the final water content was added when applying the test item.
<b>Replication:</b>	4 replicates for the test item treatments and 8 replicates for the control.
<b>Test groups:</b>	9 treatment groups (8 test item concentrations, control)
<b>No. of worms/arena:</b>	10
<b>Duration of test:</b>	8 weeks
<b>Environmental test conditions:</b>	
<b>Temperature:</b>	18 - 22 °C
<b>pH of soil:</b>	test start: 5.9 – 6.1 test end: 5.9 – 6.0
<b>Water content of soil:</b>	Water content was determined at the start and end of the experiment for each test concentration, according to ISO 11465. At experimental start: 25.8% to 29.3% (49.6% to 56.4% of the maximum water holding capacity, <i>i.e.</i> within the recommended range 40-60 % of the total water holding capacity). At experimental end: 30.2% to 34.2% (58.1% to 65.8% of the maximum water holding capacity)
<b>Photoperiod: light:</b>	16 h light : 8 h dark, light intensity: 400 lux to 800 lux.

#### ❑ Study design and methods:

Adult *Eisenia fetida* (with clitellum and weight range 300 to 600 mg, 9 to 10 months old, source: from an in-house culture, 8 x 10 animals for the control group and 4 x 10 animals for each treatment group) were exposed in artificial soil (with 10 % peat content) to an untreated control and to the nominal concentrations of 13, 21, 34, 55, 88, 142, 229 and 370 mg test item/ kg dry weight artificial soil. The test item was incorporated into the soil.

After 28 days exposure of adult worms in treated artificial soil the mortality, behavioural effects and biomass development was carried out. After additional 28 days the reproduction rate (number of offspring) was assessed (assessed 56 days after application).

#### ❑ Findings:

##### Mortality:

No mortality was observed in any treatment group.

##### Weight change:

The body weight changes of the earthworms after 4 weeks exposure to foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G were not statistically significantly different compared to the control up to and including the highest test concentration of 370 mg test item/kg soil (Williams t-test,  $\alpha = 0.05$ , two sided).

##### Reproduction:

The reproduction rates were not significantly different compared to the control up to and including the test concentration of 229 mg test item/kg soil (Williams t-test,  $\alpha = 0.05$ , one-sided smaller). At the highest test concentration of 370 mg test item/kg soil a statistically significantly reduced reproduction was observed.



No behavioral abnormalities were observed in any of the treatment groups. The feeding activity in all the treated groups was comparable to the control (see Table below).

**Table B.9.4.2.5-1: Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G: Effect on earthworms (*Eisenia fetida*) in a 56-day reproduction study**

Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G [mg/kg soil dry weight]	Control	13	21	34	55	88	142	229	370
Mortality (day 28) [%]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Significance	-	-	-	-	-	-	-	-	-
Weight change (day 28) [%]	26.3	24.1	23.6	25.0	24.4	20.2	21.5	25.4	31.6
Significance <sup>1</sup>	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mean No. of juveniles (day 56)	237	239	236	251	266	275	278	231	118
Significance <sup>1</sup>	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
Reproduction in [%] of control (day 56)	-	100.8	99.5	105.6	112.2	115.6	117.2	97.1	49.6
Food consumption [g]	24.4	24.8	25.0	24.3	24.5	24.8	25.0	24.5	24.3
<b>Endpoints [mg/kg soil dry weight]</b>									
NOEC (day 28 mortality and weight)	≥ 370								
NOEC (day 56 reproduction)	229								
LOEC (day 56 reproduction)	370								
EC Values (reproduction) <sup>2)</sup>	EC <sub>10</sub>			EC <sub>20</sub>			EC <sub>50</sub>		
	273.9 (269.3 to 278.1)			305.8 (302.5 to 308.8)			369.2 (368.2 to		

- = not applicable

n.s. = not significantly different compared to the control \* = significantly different compared to the control Williams t-test,  $\alpha = 0.05$ , two-sided for weight changes and one-sided smaller for reproduction <sup>2)</sup> Logit Analysis

#### Validity criteria:

Validity criteria	Recommended	Obtained
Mortality of adults in the control	≤ 10 %	0 %
Reproduction of control (number of juvenile worms per replicate)	≥ 30	146 - 382
Coefficient of variation of reproduction in the control	≤ 30 %	29.1 %

All study validity criteria were met.

In the most recent GLP conducted experiment with the reference item Luxan Carbendazim 500 FC (performed under IBACON Project No. 46645022 from August 2012 to October 2012), there were statistically significant effects on reproduction at a concentration of 1.30 mg carbendazim/ kg soil and higher. The EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> (reproduction) were calculated to be 1.2, 1.4 or 1.7 mg a.s./kg artificial soil dry weight.

- **Conclusion:** In an earthworm reproduction and growth study with foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G the NOEC for mortality, growth and feeding activity of the earthworm *Eisenia fetida* was determined to be ≥370 mg test item/kg soil, i.e the highest concentration tested.

The NOEC for reproduction was determined to concentration of 229 mg test item/kg soil. The LOEC was determined to be 370 mg test item/kg soil and the EC<sub>10</sub>, EC<sub>20</sub> and EC<sub>50</sub> values were determined to be 273.9 mg test item/kg soil (95% confidence limits of 269.3 to 278.1 mg test item/kg soil), 305.8 mg

test item/kg soil (95% confidence limits of 302.5 to 308.8 mg test item/kg soil) and 369.2 mg test item/kg soil (95% confidence limits of 368.2 to 370.3 mg test item/kg soil).

□ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.4.3. Effects on non-target soil meso- and macrofauna (other than earthworms)

For the purpose of renewal many new toxicity studies on reproduction of *Hypoaspis aculeifer* and *Folsomia candida* were performed for foramsulfuron and its metabolites AE F092944, AE F153745, AE F130619 and for the formulation Equip OD 45. All these studies are evaluated in this dRAR and details of the studies are provided briefly in the Table B.9.4.3-1. All the studies showed low long-term toxicity with NOEC values > 100 mg/kg dw (see Table below).

**Table B.9.4.3-1: Reproductive toxicity data of foramsulfuron and metabolites to other non-target macro-organisms**

Test substance	Test species	Endpoint	Reference
Foramsulfuron	<i>Hypoaspis aculeifer</i>	NOEC ≥ 1000 mg a.s./kg dws	Kratz, 2012 <u>M-443308-01-1</u> KCA 8.4.2.1/01
	<i>Folsomia candida</i>	NOEC 178 mg a.s./kg dws	Frommholz, 2012 <u>M-443369-01-1</u> KCA 8.4.2.1/02
AE F092944	<i>Hypoaspis aculeifer</i>	NOEC ≥ 100 mg/kg dws	Schulz, 2013 <u>M-454043-01-1</u> KCA 8.4.2.1/03
	<i>Folsomia candida</i>	NOEC ≥ 100 mg/kg dws	Friedrich, 2013 <u>M-451142-01-1</u> KCA 8.4.2.1/04
AE F130619	<i>Hypoaspis aculeifer</i>	NOEC ≥ 100 mg/kg dws	Schulz, 2013 <u>M-454051-01-1</u> KCA 8.4.2.1/05
	<i>Folsomia candida</i>	NOEC ≥ 100 mg/kg dws	Friedrich, 2013 <u>M-450824-01-1</u> KCA 8.4.2.1/06
AE F153745	<i>Hypoaspis aculeifer</i>	NOEC ≥ 100 mg/kg dws	Schulz, 2013 <u>M-447606-01-1</u> KCA 8.4.2.1/07
	<i>Folsomia candida</i>	NOEC ≥ 100 mg/kg dws	Friedrich, 2013 <u>M-450830-01-1</u> KCA 8.4.2.1/08
Equip OD 45	<i>Hypoaspis aculeifer</i>	NOEC ≥ 370 mg/kg dws	Witte, B.;2013 M-462827-01-1 KCP 10.4.2.1 /01
	<i>Folsomia candida</i>	NOEC 142 mg/kg dws	Witte, B.;2013 M-462835-01-1 KCP 10.4.2.1 /02

dws = dry weight soil

**Bold letters:** Values considered relevant for risk assessment

##### **B.9.4.3.1. Foramsulfuron (AE F130360) a.s.: Influence on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested in artificial soil**

<b>Report:</b>	<u>KCA 8.4.2.1 /01;Kratz, M. A.;2012;M-443308-01</u>
<b>Title:</b>	Foramsulfuron (AE F130360) a.s.: Influence on mortality and reproduction on the soil mite species <i>Hypoaspis aculeifer</i> tested in artificial soil
<b>Report No:</b>	KRA-HR-78/12
<b>Document No:</b>	<u>M-443308-01-1</u>
<b>Guidelines:</b>	<b>OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (<i>Hypoaspis</i> (Geolaelaps) <i>aculeifer</i>) reproduction test in soil; not applicable</b>
<b>GLP/GEP:</b>	yes

❑ **Summary:**

The purpose of this study was to assess the effects of foramsulfuron (AE F130360) a.s. on mortality and reproduction on the soil mite species *Hypoaspis aculeifer* tested during an exposure of 14 days in artificial soil comparing control and treatment. 10 adult, fertilized, female *Hypoaspis aculeifer* per replicate (8 control replicates and 4 replicates for each test item concentration) were exposed to control and treatments. Concentrations of 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil were tested. After a period of 14 days, the surviving adults and living juveniles were extracted and counted under a binocular.

The LC<sub>50</sub> could not be calculated and it is considered to be > 1000 mg test item/kg dry weight artificial soil. The No-Observed-Effect-Concentration (NOEC) for reproduction was > 1000 mg test item/kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction was >1000 mg test item/kg dry weight artificial soil. The EC<sub>50</sub>-value could not be calculated and it was considered to be > 1000 mg test item/kg dry weight artificial soil. All validity criteria (for the untreated controls) according to the guideline were met.

❑ **Materials and methods:**

<b>Test Material:</b>	Foramsulfuron (AE F130360) a.s. (BCS-AH47626)
<b>Lot/Batch No:</b>	ELIR004294
<b>Actual content of a.s.:</b>	97.3 % w/w
<b>Description:</b>	white powder, herbicide
<b>Stability of test compound:</b>	10 - 30°C (stable under standard conditions)
<b>Preparation:</b>	technical product
<b>Reanalysis/Expiry date:</b>	December 01, 2013

**Treatments**

<b>Test rates:</b>	control, 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil
<b>Control:</b>	artificial soil with deionised water
<b>Toxic standard:</b>	Dimethoate EC 400: 1.0 - 1.8 - 3.2 - 5.6 - 10.00 mg a.s./kg dry weight artificial soil

**Test organisms**

<b>Species:</b>	<i>Hypoaspis aculeifer</i> (Acari: Laelapidae)
<b>Age at test start:</b>	28 days after start of egg-laying
<b>Source:</b>	ECT Oekotoxikologie GmbH, 65439 Flörsheim, Germany.
<b>Feeding:</b>	cheese mites ( <i>Tyrophagus putrescentiae</i> ), 57 – 121 ng food per test vessel

**Test design**

<b>Vessels:</b>	Glass vessel, volume 140 ML, diameter 5 cm at the bottom, height 7 cm.
<b>Substrate:</b>	Artificial soil containing 75 % fine quartz sand, 20 % kaolin clay, 5 % sphagnum peat, air dried and finely ground, and CaCO <sub>3</sub> for the adjustment to pH to 6.0 ± 0.5.
<b>Replication:</b>	8 control replicates and 4 replicates for each test item concentration
<b>Test groups:</b>	6 treatment groups (5 test item concentrations, control)
<b>Individuals:</b>	10 per unit
<b>Duration of test:</b>	14 days
<b>Environmental test conditions</b>	
<b>Temperature:</b>	20 ± 2 °C

<b>pH of soil:</b>	test start: 5.72 – 5.82 test end: 5.85 – 6.42
<b>Water content of soil:</b>	test start: 19.88 – 20.31 % test end: 19.32 – 20.66 %
<b>Photoperiod:</b>	light : dark = 16 h : 8 h
<b>Light intensity:</b>	400 Lux to 800 Lux.

#### □ Study design and methods:

Ten adult, fertilized, female *Hypoaspis aculeifer* were exposed to control and to concentrations of 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil. In each test vessel 20 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 *Tyrophagus putrescentiae* (cheese mites) which were bred on brewer's yeast.

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.

#### □ Findings:

**Table 9.4.3.1-1: Effects on mortality and reproduction of *Hypoaspis aculeifer***

Test item Test object Exposure		Foramsulfuron (AE F130360) a.s. <i>Hypoaspis aculeifer</i> 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	5.0	356.0 ± 30.2	100	
100	5.0	363.5 ± 55.4	102.1	
178	12.5	340.5 ± 23.9	95.6	
316	2.5	363.8 ± 32.8	102.2	-
562	0.0	397.8 ± 19.8	111.7	-
1000	2.5	402.8 ± 38.6	113.1	-
NOEC <sub>reproduction</sub> (mg test item/kg dry weight artificial soil)			≥1000	
LOEC <sub>reproduction</sub> (mg test item/kg dry weight artificial soil)			>1000	

(\*)= Williams-t.-test one sided smaller;  $\alpha=0.05$

#### Mortality

In the control group 5 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤20 % mortality. The LC<sub>50</sub> could not be calculated and is considered to be > 1000 mg test item/kg dry weight artificial soil.

#### Reproduction

Concerning the number of juveniles statistical analysis (William's t-test one-sided smaller,  $\alpha = 0.05$ ) revealed no significant difference between control and any treatment group. Therefore the NOEC for reproduction is > 1000 mg test item/kg dry weight artificial soil. The LOEC for reproduction is > 1000 mg test item/kg dry weight artificial soil. The EC<sub>50</sub>-value could not be calculated and is considered to be >1000 mg test item/kg dry weight artificial soil.

#### **Validity criteria:**

Validity criteria	Recommended	Obtained
Control mortality (mean)	≤20 %	5 %
Control reproduction (mean number of juveniles per replicate)	≥ 100	423 - 555
Coefficient of variation of the control reproduction	≤30 %	10.7 %

All validity criteria were met. Therefore this study is valid.

- ❑ **Conclusion:** The NOEC for reproduction was determined to be  $\geq 1000$  mg test item/kg dry weight artificial soil, and the LOEC for reproduction was determined to be  $> 1000$  mg test item/ kg dry weight artificial soil.
- ❑ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

**B.9.4.3.2. Foramsulfuron (AE F130360) a.s.: Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil**

<b>Report:</b>	<u>KCA 8.4.2.1 /02;Frommholz, U.;2012;M-443369-01</u>
<b>Title:</b>	Foramsulfuron (AE F130360) a.s.: Influence on the reproduction of the collembolan species <i>Folsomia candida</i> tested in artificial soil
<b>Report No:</b>	FRM-Coll-147/12
<b>Document No:</b>	<u>M-443369-01-1</u>
<b>Guidelines:</b>	<b>OECD 232 adopted, September 07, 2009: Collembolan Reproduction Test in Soil</b> <b>US EPA OCSPP Deviations:</b> None; not specified
<b>GLP/GEP:</b>	yes

❑ **Summary:**

The purpose of this study was to assess the effects of Foramsulfuron (AE F130360) a.s. 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 4 replicates for each treatment group) were exposed to control (water treated), 100, 178, 316, 562 and 1000 mg test item/kg artificial soil dry weight.

After a period of 28 days, mortality and reproduction were determined. The NOEC for reproduction is 178 mg test item/kg dry weight artificial soil. The LOEC for reproduction is 316 mg test item/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:**

<b>Test Material:</b>	Foramsulfuron (AE F130360) a.s. (BCS-AH47626)
<b>Lot/Batch No:</b>	ELIR004294
<b>Actual content of a.s:</b>	97.3 % w/w
<b>Description:</b>	white powder
<b>Stability of test compound:</b>	10 - 30°C (stable under standard conditions)
<b>Preparation:</b>	a.s., technical product
<b>Reanalysis/Expiry date:</b>	December 01, 2013
<b>Treatments</b>	
<b>Test rates:</b>	control, 100, 178, 316, 562 and 1000 mg test item/kg artificial soil dry weight
<b>Control:</b>	artificial soil with deionised water
<b>Toxic standard:</b>	44 - 67 - 100 - 150 - 225 mg boric acid/kg soil d.w.
<b>Test organisms</b>	
<b>Species:</b>	<i>Folsomia Candida</i> (Collembolan, Isotmidae)
<b>Age at test start:</b>	10-12 days old, from cultures held at the laboratory
<b>Source:</b>	Ibacon, Institute for Analytic and Consulting, GmbH, 64380 Rossdorf, Germany
<b>Feeding:</b>	granulated dry yeast
<b>Test design</b>	
<b>Vessels:</b>	Plastic vessels (9.5 cm diameter).
<b>Substrate:</b>	According to OECD 232:

	75 % fine quartz-sand (Particle size 0.2–0.05mm =91.35%)
	20% kaolin clay
	10% sphagnum-peat (air dried and finely ground)
	calcium carbonate (CaCO <sub>3</sub> ) was added to adjust pH to 6.0 ± 0.5
<b>Replication:</b>	8 replicates for the control group and 4 replicates for each treatment group
<b>Test groups:</b>	6 treatment groups (5 test item concentrations, control)
<b>Individuals:</b>	10 per unit
<b>Duration of test:</b>	28 days
<b>Environmental test conditions</b>	
<b>Temperature:</b>	22±2 °C
<b>pH of soil:</b>	test start: 5.72 – 5.82 test end: 5.46 – 5.55
<b>Water content of soil:</b>	test start: 19.88 – 20.31 % test end: 19.78 – 20.80 %
<b>Photoperiod:</b>	16 h light: 8 h dark
<b>Light intensity:</b>	at test start: 648 Lux after 14 days: 613 Lux at the end of the test (ay 28): 630 Lux

□ **Study design and methods:**

The purpose of this study was to determine potential effects of foramsulfuron on the reproductive output of the as *Folsomia candida* a representative of soil micro-arthropods during a test period of 28 days. 10 collembolans (10 - 12 days old) were exposed to untreated control and to concentrations of 100, 178, 316, 562 and 1000 mg test item/kg dry weight artificial soil. Each test vessel of the 8 control and the 4 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

□ **Findings:**

**Table B.9.4.3.2-1: Effects on mortality and reproduction of *Folsomia candida***

Test item Test object Exposure	Foramsulfuron (AE F130360) a.s. <i>Folsomia candida</i> Artificial soil		
	mg test item/kg soil dry weight nominal concentration	Adult mortality (%)	Mean number of juveniles ± SD
	Control	10.0	1395.4 ± 244.5
	100	7.5	1343.0 ± 150.3
	178	10.0	1172.5 ± 368.7
	316	17.5	1044.3 ± 147.5
	562	15.0	1175.0 ± 187.4
	1000	7.5	1111.3 ± 199.9
	NOEC <sub>reproduction</sub> (mg test item/kg soil dry weight)		178
	LOEC <sub>reproduction</sub> (mg test item/kg soil dry weight)		316

The calculations were performed with un-rounded values

SD = Standard deviation

\* = statistically significant (William's-t test one-sided-smaller,  $\alpha = 0.05$ )

n.s. = statistically not significant (William's-t test one-sided-smaller,  $\alpha = 0.05$ )

**Mortality**

In the control group 10.0 % of the adult *Folsomia candida* died which is below the allowed maximum of < 20 % mortality. The LC<sub>10</sub>, LC<sub>20</sub> and the LC<sub>50</sub> values could not be determined and are considered to be > 1000 mg test item/kg artificial soil dry weight.



**Reproduction:**

Concerning the number of juveniles, statistical analysis (William's-t test, one-sided smaller,  $\alpha = 0.05$ ) has revealed a significant difference between control and the treatment groups with 316, 562 and 1000 mg test item/kg artificial soil dry weight. Therefore the NOEC for reproduction is 178 mg test item/kg artificial soil dry weight. The LOEC for reproduction is 316 mg test item/kg artificial soil dry weight. The EC10, EC20 and LC50 values could not be determined since no clear dose response relation was observed

**Validity criteria:**

Validity criteria	Recommended	Obtained
Control mortality (mean)	$\leq 20 \%$	10 %
Mean number of juveniles per replicate (with 10 collembolans)	$\geq 100$	423 - 555
Coefficient of variation of the control reproduction	$\leq 30 \%$	17.5 %

All validity criteria were met. Therefore this study is valid.

- ☐ **Conclusion:** The NOEC for reproduction is 178 mg test item/kg dry weight artificial soil, and the LOEC for reproduction is 316 mg test item/kg dry weight artificial soil.
- ☐ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

**B.9.4.3.3. AE F092944 (BCS-AA25052): Effects on the reproduction of the predatory mite *Hypoaspis aculeifer***

<b>Report:</b>	<u>KCA 8.4.2.1 /03;Schulz, L.;2013;M-454043-01</u>
<b>Title:</b>	AE F092944 (BCS-AA25052): Effects on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i>
<b>Report No:</b>	13 10 48 044 S
<b>Document No:</b>	<u>M-454043-01-1</u>
<b>Guidelines:</b>	OECD 226 (2008); none
<b>GLP/GEP:</b>	yes

☐ **Summary:**

The purpose of this study was to determine potential effects of 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 overall NOEC was determined to be  $> 100$  mg test item/kg soil dry weight. The LOEC was determined to be  $> 100$  mg test item/kg soil dry weight. The validity criteria for the control group of the study were accomplished.

☐ **Materials and methods:**

**Test Material:** AE F092944 (BCS-AA25052)  
**Lot/Batch No:** 23503LR  
**Actual content of a.s.:** 99.8 % w/w  
**Description:** white powder  
**Stability of test compound:** 10 - 30°C (stable under standard conditions)  
**Preparation:** technical product  
**Reanalysis/Expiry date:** December 18, 2015

**Treatments**

**Test rates:** control, 100 mg test item/kg soil dry weight  
**Control:** artificial soil with deionised water

<b>Toxic standard:</b>	Dimethoate EC 400: .10 - 5.12 - 6.40 - 8.00 - 10.00 mg a.s./kg soil d.w
<b>Test organisms</b>	
<b>Species:</b>	<i>Hypoaspis aculeifer</i> (Canestrini)
<b>Age at test start:</b>	adults from a synchronised culture with an age difference of 2 days
<b>Source:</b>	Katz Biotech AG, Birkenpfehlheide 10, 15837 Baruth, Germany.
<b>Feeding:</b>	every 2 days - <i>Tyrophagus putrescentiae</i> (Schränk)
<b>Test design</b>	
<b>Vessels:</b>	100 ml SCHOTT-bottle with screw cap (4 cm diameter, 11 cm high)
<b>Substrate:</b>	Artificial soil containing 74.7 % fine quartz sand, 20 % kaolin clay, 5 % sphagnum peat, air dried and finely ground, and CaCO <sub>3</sub> for the adjustment to pH to 6.0 ± 0.5
<b>Replication:</b>	8 replicates for the control group and 8 replicates for each treatment group
<b>Test groups:</b>	8 treatment groups
<b>Individuals:</b>	10 per unit
<b>Duration of test:</b>	14 days
<b>Environmental test conditions</b>	
<b>Temperature:</b>	20 ± 2 °C
<b>pH of soil:</b>	test start: 6.4 test end: 6.5 - 6.6
<b>Water content of soil:</b>	test start: 18.64 – 18.89 % test end: 17.99 – 18.01 %
<b>Photoperiod:</b>	light : dark = 16 h : 8 h
<b>Light intensity:</b>	400 Lux to 800 Lux.; Measured: 580 Lux

□ **Study design and methods:**

The purpose of this study was to determine potential effects of AE F092944 on the reproductive output of the *Hypoaspis aculeifer* as a representative of soil micro-arthropods during a test period of 14 days. Per test vessel 10 adult soil mites (females) were exposed to untreated control and to 100 mg test item/kg dry weight of soil 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.

□ **Findings:**

**Table B.9.4.3.3-1: Effects of AE F092944 on mortality and reproduction of *Hypoaspis aculeifer***

Test item Test object Exposure	AE F092944 <i>Hypoaspis aculeifer</i> Artificial soil	
	Adult mortality	Reproduction
	(mg test item/kg soil d.w.)	
NOEC	≥ 100	≥ 100
LOEC	> 100	> 100
EC <sub>10</sub>	-	-
EC <sub>20</sub>	-	-
LC <sub>50</sub> /EC <sub>50</sub>	> 100	> 100
95 % confidence limit	-	-
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 (Chi<sup>2</sup> 2x2 Test for mortality,  $\alpha = 0.05$ ; Student t-test for reproduction;  $\alpha = 0.05$ )

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 (Chi<sup>2</sup> 2x2 Test,  $\alpha = 0.05$ , one-sided greater) and reproduction (Student t-test,  $\alpha = 0.05$ , one-sided smaller) of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg test item/kg soil dry weight.

#### Validity criteria:

Validity criteria	Recommended	Obtained
Mean mortality of adult females	$\leq 20 \%$	7.5 %
Mean number of juveniles per replicate	$\geq 50$	263.9
Coefficient of variation calculated for the number of juveniles per replicate	$\leq 30 \%$	16.4 %

All validity criteria were met. Therefore this study is valid.

- **Conclusion:** 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.

**Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.4.3.4. AE F092944 (BCS-AA25052): Effects on the reproduction of the collembolan *Folsomia candida*

<b>Report:</b>	KCA 8.4.2.1 /04:Friedrich, S.;2013:M-451142-01
<b>Title:</b>	AE F092944 (BCS-AA25052): Effects on the reproduction of the collembolan <i>Folsomia candida</i>
<b>Report No:</b>	13 10 48 045 S
<b>Document No:</b>	M-451142-01-1
<b>Guidelines:</b>	OECD 232 (2009), ISO 11267 (1999); 'none
<b>GLP/GEP:</b>	yes

#### □ Summary:

The purpose of this study was to determine potential effects of the test item 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 overall NOEC was determined to be > 100 mg test item/kg soil dry weight. The LOEC was determined to be > 100 mg test item/kg soil dry weight. The validity criteria for the control group of the study were accomplished.

#### □ Materials and methods:

<b>Test Material:</b>	AE F092944 (BCS-AA25052)
<b>Lot/Batch No:</b>	23503LR
<b>Actual content of a.s.:</b>	99.8 % w/w
<b>Description:</b>	white powder
<b>Stability of test compound:</b>	10 - 30°C (stable under standard conditions)
<b>Preparation:</b>	metabolite, technical product
<b>Reanalysis/Expiry date:</b>	December 18, 2015
<b>Treatments</b>	
<b>Test rates:</b>	control, 100 mg test item/kg soil dry weight
<b>Control:</b>	artificial soil with deionised water
<b>Toxic standard:</b>	44 - 67 - 100 - 150 - 225 mg boric acid/kg soil d.w.
<b>Test organisms</b>	
<b>Species:</b>	<i>Folsomia Candida</i> (Willem)
<b>Age at test start:</b>	10-12 days old
<b>Source:</b>	Biologische Bundesanstalt (BBA), Berlin-Dahlem, Germany
<b>Feeding:</b>	granulated dry yeast
<b>Test design</b>	
<b>Vessels:</b>	Glass container (approximately 150 ml) covered with a glass lid; surface area of soil 18.9 cm <sup>2</sup>
<b>Substrate:</b>	According to OECD 232: 74.7% industrial quartz-sand (Particle size 0.2–0.05mm =91.35%) 20% kaolin clay 5% sphagnum-peat (air dried and finely ground) 0.3 % calcium carbonate (CaCO <sub>3</sub> ) was added to adjust pH to 6.0 ± 0.5
<b>Replication:</b>	8 replicates for the control group and 8 replicates for each treatment group
<b>Test groups:</b>	8 treatment groups
<b>Individuals:</b>	10 per unit
<b>Duration of test:</b>	28 days
<b>Environmental test conditions</b>	
<b>Temperature:</b>	22±2 °C
<b>pH of soil:</b>	test start: 6.22 – 6.25 test end: 6.07 – 6.09
<b>Water content of soil:</b>	test start: 25.0 % test end: 24.5 %
<b>Photoperiod:</b>	16 h light : 8 h dark
<b>Light intensity:</b>	580 Lux

#### ❑ Study design and methods:

The purpose of this study was to determine potential effects of AE F092944 on the reproductive output of the *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. 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 and were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

#### ❑ Findings:

**Table B.9.4.3.4-1: Effects on mortality and reproduction of *Folsomia candida***

Test item Test object Exposure	AE F092944 <i>Folsomia candida</i> Artificial soil	
	Adult mortality	Reproduction
	(mg test item/kg soil d.w.)	
LOEC	> 100	> 100
NOEC	≥ 100	≥ 100
LC <sub>50</sub> /EC <sub>50</sub>	> 100	> 100
95 % confidence limit	-	-
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,  $\alpha = 0.05$ , one-sided greater) and reproduction (Student-t-test,  $\alpha = 0.05$ , one-sided smaller)

CV: coefficient of variation, d.w.: dry weight (of artificial soil)

Calculations were done using unrounded values

Percent reproduction:  $(R_t / R_c) * 100 \%$

$R_t$  = mean number of juveniles observed in the treated groups

$R_c$  = 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 observed in the control. No statistically significant effect (Fisher's Exact Binomial Test,  $\alpha = 0.05$ , 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,  $\alpha = 0.05$ , 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 NOEC was determined to be > 100 mg test item/kg dry weight.

#### Validity criteria:

Validity criteria	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 were met. Therefore this study is valid.

- ❑ **Conclusion:** 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.
- ❑ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.4.3.5. **AE F130619 (BCS-AU59648): Effects on the reproduction of the predatory mite *Hypoaspis aculeifer***

<b>Report:</b>	KCA 8.4.2.1 /05;Schulz, L.;2013;M-454051-01
<b>Title:</b>	Foramsulfuron-AE F130619 (BCS-AU59648): Effects on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i>
<b>Report No:</b>	13 10 48 046 S
<b>Document No:</b>	M-454051-01-1
<b>Guidelines:</b>	OECD 226 (2008); <b>Deviations: none</b>
<b>GLP/GEP:</b>	yes

##### □ **Summary:**

The purpose of this study was to determine potential effects of AE F130619 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 control replicates and 8 replicates for the test item concentration) were exposed to 100 mg metabolite/kg soil dry weight (corresponding to 106 mg test item/kg soil dry weight). After 2 weeks the number of juveniles and surviving parental mites was determined. The test was performed as a limit test.

The NOEC was determined to be > 100 mg metabolite/kg soil d.w.. The LOEC was determined to be > 100 mg metabolite/kg soil d.w.. All validity criteria (for the control group) according to the guideline were accomplished.

##### □ **Materials and methods:**

**Test Material:** Foramsulfuron-AE F130619 (BCS-AU59648)

**Lot/Batch No:** SES 10641-3-3

**Actual content of a.s.:** 94 % w/w

**Description:** off white powder

**Stability of test compound:** stable under standard conditions

**Preparation:** metabolite, technical product

**Reanalysis/Expiry date:** January 16, 2016

##### **Treatments**

**Test rates:** control, 100 mg test item/kg soil dry weight (corresponding to 106 mg test item/kg soil dry weight)

**Control:** artificial soil with deionised water

**Toxic standard:** Dimethoate EC 400: 4.10 - 5.12 - 6.40 - 8.00 - 10.00 mg a.s./kg soil d.w

##### **Test organisms**

**Species:** *Hypoaspis aculeifer* (Canestrini)

**Age at test start:** adults from a synchronised culture with an age difference of 2 days

**Source:** Katz Biotech AG, Birkenpfehlheide 10, 15837 Baruth, Germany.

**Feeding:** every 2 days - *Tyrophagus putrescentiae* (Schränk)

##### **Test design**

**Vessels:** 100 ml SCHOTT-bottle with screw cap (4 cm diameter, 11 cm high)

**Substrate:** Artificial soil containing 74.7 % fine quartz sand, 20 % kaolin clay, 5 % sphagnum peat, air dried and finely ground, and CaCO<sub>3</sub> for the adjustment to pH to 6.0 ± 0.5

**Replication:** 8 replicates for the control group and 8 replicates for each treatment group

**Test groups:** 8 treatment groups

**Individuals:** 10 per unit

**Duration of test:** 14 days

##### **Environmental test conditions**

**Temperature:** 20 ± 2 °C



<b>pH of soil:</b>	measured: 19.5 – 21,2 °C test start: 6.4 - 6.5 test end: 6.8
<b>Water content of soil:</b>	test start: 20.40 – 20.57 % test end: 20.25 – 20.89 %
<b>Photoperiod:</b>	light : dark = 16 h : 8 h
<b>Light intensity:</b>	400 Lux to 800 Lux. measured: 540 Lux

#### □ Study design and methods:

The purpose of this study was to determine potential effects of AE F130619 on the reproductive output of the *Hypoaspis aculeifer* as a representative of soil micro-arthropods during a test period of 14 days. 10 adult soil mites (females) were exposed to 100 mg metabolite/kg soil and were fed every 2 days with *Tyrophagus putrescentiae* (SCHRANK). Mortality and reproduction were determined after 14 days of exposure.

#### □ Findings:

**Table B.9.4.3.5-1: Effects of AE F130619 on mortality and reproduction of *Hypoaspis aculeifer***

Test item Test object Exposure	AE F130619 <i>Hypoaspis aculeifer</i> Artificial soil	
	Adult mortality	Reproduction
	(mg test item/kg soil d.w.)	
NOEC	≥ 100	≥ 100
LOEC	> 100	> 100
EC <sub>10</sub>	-	-
EC <sub>20</sub>	-	-
LC <sub>50</sub> /EC <sub>50</sub>	> 100	> 100
95 % confidence limit	-	-
Endpoint	AE F130619 (mg metabolite/kg soil d.w.)	
	control	100
Mortality of collembolans after 14 days (%)	1.3	0.0
Mean number of juveniles after 14 days	268.4	256.1
CV %	8.0	11.8
Reproduction (% to control)	100	95

No statistically significant differences compared to the control were calculated (Fisher's Exact Binomial Test for mortality,  $\alpha = 0.05$ ; Student-t-test for reproduction;  $\alpha = 0.05$ )

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, 1.3 % parental mortality could be observed at the end of the 14-day exposure period. In the test item treatment group no parental mortality could be observed at the end of the test. Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 268.4 in the control and 256.1 in the test item treatment group. The test item caused no statistically significantly adverse effects on adult mortality (Fisher's Exact Binomial Test,  $\alpha = 0.05$ , one-sided greater) and reproduction (Student t-test,  $\alpha = 0.05$ , one-sided smaller) of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg metabolite/kg soil dry weight.

#### Validity criteria:

Validity criteria	Recommended	Obtained
Mean mortality of adult females	≤20 %	1.3 %
Mean number of juveniles per replicate	≥ 50	268.4
Coefficient of variation calculated for the number of juveniles per replicate	≤30 %	8.0 %

All validity criteria were met. Therefore this study is valid.

- ❑ **Conclusion:** The test item AE F130619 showed no statistically significantly adverse effects on adult mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg metabolite/kg soil dry weight. Therefore, the overall NOEC was determined to be > 100 mg metabolite/kg soil dry weight, and the LOEC was determined to be > 100 mg metabolite/kg soil dry weight.
- ❑ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.4.3.6. AE F130619 (BCS-AU59648): Effects on the reproduction of the collembolan *Folsomia candida*

<b>Report:</b>	<u>KCA 8.4.2.1 /06:Friedrich, S.:2013:M-450824-01</u>
<b>Title:</b>	Foramsulfuron-AE F130619 (BCS-AU59648): Effects on the reproduction of the collembolan <i>Folsomia candida</i>
<b>Report No:</b>	13 10 48 047 S
<b>Document No:</b>	<u>M-450824-01-1</u>
<b>Guidelines:</b>	OECD 232 (2009), ISO 11267 (1999); <b>Deviations: none</b>
<b>GLP/GEP:</b>	yes

❑ **Summary:**

The purpose of this study was to determine potential effects of AE F130619 on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. 10 collembolans (9-12 days old) per replicate (8 control replicates and 8 replicates for the test item concentration) were exposed to 100 mg metabolite/kg soil dry weight (corresponding to 106 mg test item/kg soil dry weight). After 4 weeks the number of offspring (juveniles) and surviving parental collembolans was counted. The NOEC was determined to be > 100 mg metabolite/kg soil d.w.. The LOEC was determined to be > 100 mg metabolite/kg soil d.w.. All validity criteria (for the control group) according to the guideline were fulfilled.

❑ **Materials and methods:**

<b>Test Material:</b>	Foramsulfuron-AE F130619 (BCS-AU59648)
<b>Lot/Batch No:</b>	SES 10641-3-3
<b>Actual content of a.s.:</b>	99.4 % w/w
<b>Description:</b>	off white powder
<b>Stability of test compound:</b>	stable under standard conditions
<b>Preparation:</b>	metabolite, technical product
<b>Reanalysis/Expiry date:</b>	January 22, 2016

**Treatments**

<b>Test rates:</b>	control, 100 mg metabolite/kg soil dry weight (corresponding to 106 mg test item/kg soil dry weight)
<b>Control:</b>	artificial soil with deionised water
<b>Toxic standard:</b>	44 - 67 - 100 - 150 - 225 mg boric acid/kg soil d.w.

**Test organisms**

<b>Species:</b>	<i>Folsomia Candida</i> (Willem)
<b>Age at test start:</b>	10-12 days old
<b>Source:</b>	Biologische Bundesanstalt (BBA), Berlin-Dahlem, Germany
<b>Feeding:</b>	granulated dry yeast

**Test design**

<b>Vessels:</b>	Glass container (approximately 150 ml) covered with a glass lid; surface area of soil 18.9 cm <sup>2</sup>
<b>Substrate:</b>	According to OECD 232: 74.7% industrial quartz-sand (Particle size 0.2–0.05mm =91.35%) 20% kaolin clay 5% sphagnum-peat (air dried and finely ground) 0.3 % calcium carbonate (CaCO <sub>3</sub> ) was added to adjust pH to 6.0 ± 0.5
<b>Replication:</b>	8 replicates for the control group and 8 replicates for each treatment group
<b>Test groups:</b>	8 treatment groups
<b>Individuals:</b>	10 per unit
<b>Duration of test:</b>	28 days
<b>Environmental test conditions</b>	
<b>Temperature:</b>	18.3 – 21.0 °C
<b>pH of soil:</b>	test start: 6.16 – 6.19 test end: 5.90 – 5.97
<b>Water content of soil:</b>	test start: 24.9 – 25.0 % test end: 24.5 %
<b>Photoperiod:</b>	16 h light : 8 h dark
<b>Light intensity:</b>	650 Lux

□ **Study design and methods:**

The purpose of this study was to determine potential effects of AE F130619 on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. 10 Collembola (9-12 days old) were exposed to 100 mg metabolite/kg soil dry weight containing 74.7% quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % CaCO<sub>3</sub>, 18.3 - 21.0 °C and a photoperiod: light : dark = 16 h : 8 h (650 lx) and were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

□ **Findings:**

**Table B.9.4.3.6-1: Effects of AE F130619 on mortality and reproduction of *Folsomia candida***

Test item Test object Exposure	AE F130619 <i>Folsomia candida</i> Artificial soil	
	Adult mortality	Reproduction
	(mg test item/kg soil d.w.)	
LOEC	> 100	> 100
NOEC	≥100	≥ 100
LC <sub>50</sub> /EC <sub>50</sub>	> 100	> 100
95 % confidence limit	-	-
Endpoint	AE F130619 (mg test item/kg soil d.w.)	
	control	100
Mortality of parental collembolans after 4 weeks (%)	6.3	3.8
Mean number of juveniles after 4 weeks	778	768
CV %	14.4	14.2
Reproduction (% to control)	100	99

No statistically significant differences compared to the control were calculated for mortality (Fisher's Exact Binomial Test,  $\alpha = 0.05$ , one-sided greater) and reproduction (Student-t-test,  $\alpha = 0.05$ , one-sided smaller)

CV: coefficient of variation, d.w.: dry weight (of artificial soil)

Calculations were done using unrounded values

Percent reproduction:  $(R_t / R_c) * 100 \%$

$R_t$  = mean number of juveniles observed in the treated groups

$R_c$  = mean number of juveniles observed in the control group

The test item caused 3.8 % parental mortality at a concentration of 100 mg metabolite/kg soil d.w. 6.3 % parental mortality was observed in the control. No statistically significant effect (Fisher's Exact Binomial Test,  $\alpha = 0.05$ , 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 778 in the control and 768 at 100 mg metabolite/kg soil d.w. No statistically significant effects (Student-t-test,  $\alpha = 0.05$ , one-sided smaller) on the number of juveniles compared to the control group were found at 100 mg metabolite/kg soil d.w.. The NOEC was determined to be  $\geq 100$  mg metabolite/kg soil d.w.

#### Validity criteria:

Validity criteria	Recommended	Obtained
Mean adult mortality	$\leq 20 \%$	6.3 %
Mean number of juveniles per replicate	$\geq 100$	778
Coefficient of variation (mean number of juveniles per replicate)	$\leq 30 \%$	14.4 %

All validity criteria were met. Therefore this study is valid.

□ **Conclusion:** The test item AE F130619 showed no statistically significant adverse effects on adult mortality and reproduction of the collembolan *Folsomia candida* in artificial soil at 100 mg metabolite/kg soil d.w. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be  $> 100$  mg metabolite/kg soil d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be 100 mg metabolite/kg soil d.w.

□ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.4.3.7. AE F153745 (BCS-AU80017): Effects on the reproduction of the predatory mite *Hypoaspis aculeifer*

<b>Report:</b>	<u>KCA 8.4.2.1 /07;Schulz, L.;2013;M-447606-01</u>
<b>Title:</b>	Foramsulfuron-AE F153745 (BCS-AU80017): Effects on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i>
<b>Report No:</b>	13 10 48 048 S
<b>Document No:</b>	<u>M-447606-01-1</u>
<b>Guidelines:</b>	<b>OECD 226 (2008);Deviations: not specified</b>
<b>GLP/GEP:</b>	yes

#### □ **Summary:**

The purpose of this study was to determine potential effects of AE F153745 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. Ten 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 overall NOEC was determined to be  $> 100$  mg test item/kg soil dry weight. The LOEC was determined to be  $> 100$  mg test item/kg soil dry weight. The validity criteria for the control group of the study were accomplished.

#### □ **Materials and methods:**

<b>Test Material:</b>	Foramsulfuron-AE F153745 (BCS-AU80017)
<b>Lot/Batch No:</b>	ZER0234
<b>Actual content of a.s.:</b>	98.2 % w/w
<b>Description:</b>	beige powder
<b>Stability of test compound:</b>	stable under standard conditions
<b>Preparation:</b>	metabolite, technical product
<b>Reanalysis/Expiry date:</b>	October 14, 2016
<b>Treatments</b>	
<b>Test rates:</b>	control, 100 mg test item/kg soil dry weight
<b>Control:</b>	artificial soil with deionised water
<b>Toxic standard:</b>	Dimethoate EC 400: 4.10 - 5.12 - 6.40 - 8.00 - 10.00 mg a.s./kg soil d.w
<b>Test organisms</b>	
<b>Species:</b>	<i>Hypoaspis aculeifer</i> (Canestrini)
<b>Age at test start:</b>	adults from a synchronised culture with an age difference of 2 days
<b>Source:</b>	Katz Biotech AG, Birkenpfehlheide 10, 15837 Baruth, Germany.
<b>Feeding:</b>	every 2 days - <i>Tyrophagus putrescentiae</i> (Schrack)
<b>Test design</b>	
<b>Vessels:</b>	100 ml SCHOTT-bottle with screw cap (4 cm diameter, 11 cm high)
<b>Substrate:</b>	Artificial soil containing 74.7 % fine quartz sand, 20 % kaolin clay, 5 % sphagnum peat, air dried and finely ground, and CaCO <sub>3</sub> for the adjustment to pH to 6.0 ± 0.5
<b>Replication:</b>	8 replicates for the control group and 8 replicates for each treatment group
<b>Test groups:</b>	8 treatment groups
<b>Individuals:</b>	10 per unit
<b>Duration of test:</b>	14 days
<b>Environmental test conditions</b>	
<b>Temperature:</b>	20 ± 2 °C measured: 19.5 – 21.5 °C
<b>pH of soil:</b>	test start: 6.4 test end: 6.7
<b>Water content of soil:</b>	test start: 19.36 – 19.49 % test end: 18.26 – 18.32 %
<b>Photoperiod:</b>	light : dark = 16 h : 8 h
<b>Light intensity:</b>	400 Lux to 800 Lux. measured: 457 Lux

❑ **Study design and methods:**

The purpose of this study was to determine potential effects of AE F153745 on the mortality and the reproductive output of the soil mite species *Hypoaspis aculeifer* as a representative of soil microarthropods during a test period of 14 days. Per test vessel 10 adult soil mites (females) were exposed to untreated control and to 100 mg test item/kg dry weight of soil and were fed every 2 days with *Tyrophagus putrescentiae*. Mortality and reproduction were determined after 14 days of exposure.

❑ **Findings:**



**Table B.9.4.3.7-1: Effects of AE F153745 on mortality and reproduction of *Hypoaspis aculeifer***

Test item Test object Exposure	AE F153745 <i>Hypoaspis aculeifer</i> Artificial soil	
	Adult mortality	Reproduction
	(mg test item/kg soil d.w.)	
NOEC	≥ 100	≥ 100
LOEC	> 100	> 100
EC <sub>10</sub>	-	-
EC <sub>20</sub>	-	-
LC <sub>50</sub> /EC <sub>50</sub>	> 100	> 100
95 % confidence limit	-	-
Endpoint	AE F153745 (mg metabolite/kg soil d.w.)	
	control	100
Mortality of collembolans after 14 days (%)	1.3	3.8
Mean number of juveniles after 14 days	242.3	246.5
CV %	19.9	13.1
Reproduction (% to control)	100	102

No statistically significant differences compared to the control were calculated (Fisher's Exact Binomial Test for mortality,  $\alpha = 0.05$ ; Student-t-test for reproduction;  $\alpha = 0.05$ )

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 1.3 % and 3.8 %, 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 242.3 in the control and 246.5 in the test item treatment group. The test item caused no statistically significantly adverse effects on adult mortality (Fisher's Exact Binomial Test,  $\alpha = 0.05$ , one-sided greater) and reproduction (Student t-test,  $\alpha = 0.05$ , one-sided smaller) of the predatory mite *Hypoaspis aculeifer* in artificial soil at 100 mg test item/kg soil dry weight.

#### Validity criteria:

Validity criteria	Recommended	Obtained
Mean mortality of adult females	≤20 %	1.3 %
Mean number of juveniles per replicate	≥ 50	242.3
Coefficient of variation calculated for the number of juveniles per replicate	≤30 %	19.9 %

All validity criteria were met. Therefore this study is valid.

#### □ Conclusion:

The test item AE F153745 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 NOEC was determined to be ≥100 mg test item/kg soil dry weight, and the LOEC was determined to be > 100 mg test item/kg soil dry weight.

#### □ Comment (Co-RMS and RMS):

No comments, study is acceptable.



#### B.9.4.3.8. AE F153745 (BCS-AU80017): Effects on the reproduction of the collembolan *Folsomia candida*

<b>Report:</b>	KCA 8.4.2.1 /08;Friedrich, S.;2013;M-450830-01
<b>Title:</b>	Foramsulfuron-AE F153745 (BCS-AU80017): Effects on the reproduction of the collembolan <i>Folsomia candida</i>
<b>Report No:</b>	13 10 48 049 S
<b>Document No:</b>	M-450830-01-1
<b>Guidelines:</b>	OECD 232 (2009), ISO 11267 (1999);deviations: none
<b>GLP/GEP:</b>	yes

##### □ Summary:

The purpose of this study was to determine potential effects of AE F153475 on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. Ten 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 overall NOEC was determined to be > 100 mg test item/kg soil dry weight. The LOEC was determined to be > 100 mg test item/kg soil dry weight. The validity criteria for the control group of the study were accomplished.

##### □ Materials and methods:

<b>Test Material:</b>	Foramsulfuron-AE F153745 (BCS-AU80017)
<b>Lot/Batch No:</b>	ZER0234
<b>Actual content of a.s.:</b>	98.2 % w/w
<b>Description:</b>	beige powder
<b>Stability of test compound:</b>	stable under standard conditions
<b>Preparation:</b>	metabolite, technical product
<b>Reanalysis/Expiry date:</b>	October 14, 2016

##### Treatments

<b>Test rates:</b>	control, 100 mg metabolite/kg soil dry weight (corresponding to 106 mg test item/kg soil dry weight)
<b>Control:</b>	artificial soil with deionised water
<b>Toxic standard:</b>	44 - 67 - 100 - 150 - 225 mg boric acid/kg soil d.w.

##### Test organisms

<b>Species:</b>	<i>Folsomia Candida</i> (Willem)
<b>Age at test start:</b>	10-12 days old
<b>Source:</b>	Biologische Bundesanstalt (BBA), Berlin-Dahlem, Germany
<b>Feeding:</b>	granulated dry yeast

##### Test design

<b>Vessels:</b>	Glass container (approximately 150 ml) covered with a glass lid; surface area of soil 18.9 cm <sup>2</sup>
<b>Substrate:</b>	According to OECD 232: 74.7% industrial quartz-sand (Particle size 0.2–0.05mm =91.35%) 20% kaolin clay 5% sphagnum-peat (air dried and finely ground) 0.3 % calcium carbonate (CaCO <sub>3</sub> ) was added to adjust pH to 6.0 ± 0.5
<b>Replication:</b>	8 replicates for the control group and 8 replicates for each treatment group
<b>Test groups:</b>	8 treatment groups
<b>Individuals:</b>	10 per unit
<b>Duration of test:</b>	28 days
<b>Environmental test conditions</b>	
<b>Temperature:</b>	19.1 – 20.7 °C
<b>pH of soil:</b>	test start: 6.22 – 6.26 test end: 6.13 – 6.14
<b>Water content of soil:</b>	test start: 25.0 % test end: 24.5 – 24.6 %

**Photoperiod:** 16 h light : 8 h dark  
**Light intensity:** 580 Lux

□ **Study design and methods:**

The purpose of this study was to determine potential effects of AE F153475 on the reproductive output of the collembolan *Folsomia candida* as a representative of soil micro-arthropods during a test period of 28 days. Ten 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 and were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.

□ **Findings:**

**Table B.9.4.3.8-1: Effects of AE F153745 on mortality and reproduction of *Folsomia candida***

Test item Test object Exposure	AE F153745 <i>Folsomia candida</i> Artificial soil	
	Adult mortality	Reproduction
	(mg test item/kg soil d.w.)	
LOEC	> 100	> 100
NOEC	≥100	≥ 100
LC <sub>50</sub> /EC <sub>50</sub>	> 100	> 100
95 % confidence limit	-	-
Endpoint	AE F153745 (mg test item/kg soil d.w.)	
	control	100
Mortality of parental collembolans after 4 weeks (%)	3.8	2.5
Mean number of juveniles after 4 weeks	639	646
CV %	13.5	12.6
Reproduction (% to control)	100	101

No statistically significant differences compared to the control were calculated for mortality (Fisher's Exact Binomial Test,  $\alpha = 0.05$ , one-sided greater) and reproduction (Student-t-test,  $\alpha = 0.05$ , one-sided smaller)

CV: coefficient of variation, d.w.: dry weight (of artificial soil)

Calculations were done using unrounded values

Percent reproduction:  $(R_t / R_c) * 100 \%$

$R_t$  = mean number of juveniles observed in the treated groups

$R_c$  = 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. 3.8% parental mortality was observed in the control. No statistically significant effect (Fisher's Exact Binomial Test,  $\alpha = 0.05$ , 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 639 in the control and 646 at 100 mg test item/kg soil d.w. No statistically significant effects (Student-t-test,  $\alpha = 0.05$ , 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 NOEC was determined to be > 100 mg test item/kg dry weight.

**Validity criteria:**

Validity criteria	Recommended	Obtained
Mean adult mortality	≤20 %	3.8 %
Mean number of juveniles per replicate	≥ 100	639
Coefficient of variation (mean number of juveniles per replicate)	≤30 %	13.5 %

All validity criteria were met. Therefore this study is valid.

- ❑ **Conclusion:** The test item AE F153745 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.

- ❑ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.4.3.9. Effects of Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G on Reproduction and Growth of the collembola *Folsomia candida* in Artificial Soil

<b>Report:</b>	KCP 10.4.2.1 /01; Witte, B.:2013; M-462827-01-1
<b>Title:</b>	Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G: (FSN+IDF OD 45 (22.5+22.5) G): Effects on reproduction of the collembola <i>Folsomia candida</i> in artificial soil
<b>Report No:</b>	83353016
<b>Document No:</b>	M-462827-01-1
<b>Guidelines:</b>	OECD 232 (2009), ISO 11267 (1999); deviations: none
<b>GLP/GEP:</b>	yes

##### ❑ Summary:

The purpose of the study was to determine the effects of foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G (FSN+IDF OD 45 (22.5+22.5) G) on mortality and reproduction of the Collembola *Folsomia candida* in artificial soil. 10 collembolans (10-12 days old) per replicate (8 replicates for the control group, 4 replicates for each treatment group) were exposed to control (water treated), 13, 21, 34, 55, 88, 142, 229 and 370 mg test item/kg soil dry weight. After a period of 28 days, mortality, behavioural effects and reproduction were determined. The overall NOEC was determined to be 142 mg test item/kg soil dry weight. The overall LOEC was determined to be 229 mg test item/kg soil dry weight. All validity criteria for the untreated control of the study according to the OECD Guideline 232 have been fulfilled.

##### ❑ Materials and methods:

<b>Test Material:</b>	Foramsulfuron + isoxadifen-ethyl OD 45 (22.5 + 22.5) G (FSN+IDF OD 45 (22.5+22.5) G)
<b>Lot/Batch No:</b>	Batch ID: EFKM002442; Material No.: 06321801; Specification No.: 102000011304-06
<b>Actual content of a.s:</b>	foramsulfuron (AE F130360): 2.33% w/w, 22.41 g/L, isoxadifen-ethyl (AE F122006): 2.29% w/w, 21.96 g/L
<b>Description:</b>	T0X10129-00, beige, liquid
<b>Stability of test compound:</b>	At 25 ± 5 °C (+2°C to +30°C are also acceptable), under dark and dry conditions
<b>Preparation:</b>	OD (Oil dispersion)
<b>Reanalysis/Expiry date:</b>	April 25, 2015
<b>Treatments</b>	
<b>Test rates:</b>	control, 13, 21, 34, 55, 88, 142, 229 and 370 mg test item/ kg dry weight artificial soil
<b>Control:</b>	artificial soil with deionised water
<b>Toxic standard:</b>	33.6 - 53.7 - 85.9 - 137.5 - 220.0 mg boric acid/kg soil d.w.

**Test organisms**

<b>Species:</b>	<i>Folsomia candida</i> (Willem 1902)
<b>Age at test start:</b>	10-12 days old, from cultures held at the laboratory
<b>Source:</b>	The synchronised individuals were bred at IBACON and were fed with granulated dry yeast and kept under breeding conditions until test start.
<b>Feeding:</b>	After the introduction of the test organisms (day 0), and after 14 days, approximately 2 mg (half of a small spatula) of granulated dried yeast was spread over the soil surface.

**Test design**

<b>Vessels:</b>	Glass containers (volume: 100 mL; diameter: 5 cm), closed tightly to avoid water evaporation, filled with 30 g $\pm$ 1.0 g artificial soil fresh weight.
<b>Substrate:</b>	According to OECD 232: 5% Sphagnum-peat, air-dried and finely ground (2 mm); (Floragard, Vertriebs GmbH für Gartenbau, 26138 Oldenburg, Germany) 20% kaolin clay (Erbsloh, 65558 Lohrheim, Germany) 74.8% fine quartz-sand (F34) containing more than 50% by mass of particle size 0.05 mm to 0.2 mm; (Quarzwirke Frechen, Postfach 1780, 50207 Frechen, Germany) 0.2% calcium carbonate (CaCO <sub>3</sub> ) was added to adjust pH to 6.0 $\pm$ 0.5 (Merck, 64293 Darmstadt, Germany). The artificial soil was moistened to approximately half of the final water content 2 days before the application. The additional water required to achieve the final water content was added when applying the test item.
<b>Replication:</b>	4 replicates for the test item treatments and 8 replicates for the control. 1 additional container per treatment to check the pH and water content of the test substrate after 28 days
<b>Test groups:</b>	9 treatment groups (8 test item concentrations, control)
<b>Individuals:</b>	10 per unit
<b>Duration of test:</b>	28 days

**Environmental test conditions**

<b>Temperature:</b>	18 - 22 °C
<b>pH of soil:</b>	test start: 5.7 – 5.8 test end: 5.7
<b>Water content of soil:</b>	At test start: 21.2% to 21.7% (55.8% to 57.1% of the maximum water holding capacity, i.e. within the recommended range 40-60 % of the total water holding capacity) At test end: 20.0% to 21.9% (52.7% to 57.6% of the maximum water holding capacity)
<b>Photoperiod:</b>	16 h light : 8 h dark
<b>Light intensity:</b>	400 lux to 800 lux.

**□ Study design and methods:**

10 Collembola per replicate (8 replicates for the control group, 4 replicates for each treatment group) were exposed 28 days in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil before the Collembola were introduced on top of the soil; 8 concentrations (13, 21, 34, 55, 88, 142, 229 and 370 mg test item/kg soil) and one control (untreated) were tested. The collembolans were fed with approximately 2 mg dry yeast for each test vessel at the beginning of the test and on day 14. The assessments of adult mortality, behavioural effects and reproduction were performed after 28 d.

**□ Findings:****Mortality:**

A mortality of 58% was observed in the highest test item treated group of 370 mg test item/kg soil dry weight, which was statistically significantly different compared to the control, where 5% of the

Collembola died (Fisher's Exact test,  $\alpha = 0.05$ , one-sided greater). At the lower test concentrations no significant increased mortality was observed, except at the concentration of 21 mg test item/kg soil dry weight, which is not considered to be treatment related since at the higher concentrations up to and including 229 mg test item/kg soil dry weight no effects were observed.

#### Reproduction:

The reproduction of the Collembola exposed to foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G (FSN+IDF OD 45 (22.5+22.5) G) was not statistically significantly different compared to the control up to and including the test concentration of 142 mg test item/kg soil dry weight (Bonferroni- Welch t-test,  $\alpha = 0.05$ , one-sided smaller).

No behavioural abnormalities were observed in any of the treatment groups.

**Table B.9.4.3.9-1: Effect of foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G (FSN+IDF OD 45 (22.5+22.5) G) on Collembola (*Folsomia candida*) in a 28-day reproduction study**

Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G [mg/kg soil dry weight]	Control	13	21	34	55	88	142	229	370
Mortality (day 28) [%]	5	13	23	15	15	20	13	15	58
Statistical significance <sup>1)</sup>	-	n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.	*
No. of juveniles (day 28)	487	532	386	467	532	432	512	321	53
Reproduction in [%] of control (day 28)	-	109	79	96	109	89	105	66	11
Statistical significance <sup>2)</sup>	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*
<b>Endpoints [mg/kg soil]</b>									
NOEC(mortality)	229								
NOEC (reproduction)	142								
LOEC (reproduction)	229								
EC Values (reproduction) <sup>3)</sup>	EC <sub>10</sub>			EC <sub>20</sub>			EC <sub>50</sub>		
	179.05			203.10			258.48		

n.s. = not significantly different compared to the control \* = significantly different compared to the control

<sup>1)</sup> Fisher's Exact Test,  $\alpha = 0.05$ , one-sided greater <sup>2)</sup> Bonferroni-Welch t-test,  $\alpha = 0.05$ , one-sided smaller

<sup>3)</sup> Probit analysis

#### Validity criteria:

Validity criteria	Recommended	Obtained
Control mortality (mean)	≤20 %	5 %
Control reproduction (mean number of juveniles per replicate)	≥ 100	423 - 555
Coefficient of variation of the control reproduction	≤30 %	10.7 %

All validity criteria were met. Therefore this study is valid.

In a separate GLP conducted study (study code 61403016) the reference item Boric acid showed statistically significant effects on mortality and reproduction at concentrations of  $\geq 53.7$  mg/kg soil dry weight; the EC<sub>50</sub> for reproduction was calculated to be 59.9 mg/kg soil dry weight.

- **Conclusion:** Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G (FSN+IDF OD 45 (22.5+22.5) G) caused no significant effects on mortality of *Folsomia candida* up to and including the concentration of 229 mg test item/kg soil dry weight and no effects on reproduction up to and including the concentration of 142 mg test item/kg soil dry weight. Therefore, the overall NOEC was determined to be 142 mg test item/kg soil dry weight. The overall LOEC was determined to be 229 mg test item/kg soil dry weight. The EC<sub>50</sub> was determined to be 258.48 mg test item/kg soil dry weight.
- **Comment (Co-RMS and RMS):** No comments, study is acceptable.

#### B.9.4.3.10. Effects of Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G on Reproduction of the predatory mite *Hypoaspis aculeifer* in Artificial Soil

<b>Report:</b>	KCP 10.4.2.1 /02; Witte, B.;2013; M-462835-01-1
<b>Title:</b>	Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G: (FSN+IDF OD 45 (22.5+22.5) G): Effects on reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil
<b>Report No:</b>	83351089
<b>Document No:</b>	M-462835-01-1
<b>Guidelines:</b>	OECD 232 (2009), ISO 11267 (1999); deviations: none
<b>GLP/GEP:</b>	yes

##### □ Summary:

The purpose of the study was to determine the effects of foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G (FSN+IDF OD 45 (22.5+22.5) G) on mortality and reproduction of the Predatory Mite *Hypoaspis aculeifer*. 10 adult soil mites (females) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated), 13, 21, 34, 55, 88, 142, 229 and 370 mg test item/kg soil dry weight. Two weeks after start of exposure, the number of juveniles and surviving adult female. The overall NOEC was determined to be  $\geq 370$  mg test item/kg soil dry weight. The overall LOEC and the EC<sub>50</sub> were estimated to be greater than 370 mg test item/kg soil dry weight. The validity criteria for the control group of the study were accomplished.

##### Materials and methods:

<b>Test Material:</b>	Foramsulfuron + isoxadifen-ethyl OD 45 (22.5 + 22.5) G (FSN+IDF OD 45 (22.5+22.5) G)
<b>Lot/Batch No:</b> Batch ID:	EFKM002442; Material No.: 06321801; Specification No.: 102000011304-06
<b>Actual content of a.s:</b>	foramsulfuron (AE F130360): 2.33% w/w, 22.41 g/L, isoxadifen-ethyl (AE F122006): 2.29% w/w, 21.96 g/L
<b>Description:</b>	T0X10129-00, beige, liquid
<b>Stability of test compound:</b>	At 25 ± 5 °C (+2°C to +30°C are also acceptable), under dark and dry conditions
<b>Preparation:</b>	OD (Oil dispersion)
<b>Reanalysis/Expiry date:</b>	April 25, 2015
<b>Treatments</b>	
<b>Test rates:</b>	Control, 13, 21, 34, 55, 88, 142, 229 and 370 mg test item/ kg dry weight artificial soil
<b>Control:</b>	Artificial soil with deionised water
<b>Toxic standard:</b>	1.0 - 1.7 - 2.7 - 4.3 -6.8 mg dimethoate (BAS 152 I) /kg soil d.w. The reference item test (dose response) is performed at least once a year at the test facility as a mean of ensuring that the laboratory test conditions are adequate and to verify that the response of the test organisms does not change significantly over time. The GLP conducted experiment was performed in May/June 2012 and is archived under the IBACON Project No. 74661089.
<b>Test organisms</b>	
<b>Species:</b>	<i>Hypoaspis aculeifer</i> (Canestrini 1883)
<b>Age at test start:</b>	Adults, approximately 9 days after reaching the adult stage (30 days after placing adult females in clean rearing vessels over a period of 3 days)
<b>Source:</b>	Cultured by IBACON
<b>Feeding:</b>	One spatula of cheese mites ( <i>Tyrophagus putrescentiae</i> cultured by IBACON) at experimental start and on day 2, 5, 7, 9 and 12
<b>Test design</b>	
<b>Vessels:</b>	Glass containers (volume: 100 mL; diameter: 5 cm), tight screw top closure to avoid water evaporation, filled with approximately 20 g ± 1.0 g artificial soil dry weight.
<b>Substrate:</b>	According to OECD 226:



	5% Sphagnum-peat, air-dried and finely ground (< 2 mm); (Floragard, Vertriebs GmbH für Gartenbau, 26138 Oldenburg, Germany)
	20% kaolin clay (Erbsloh, 65558 Lohrheim, Germany)
	74.8% fine quartz-sand (F34) containing more than 50% by mass of particle size 0.05 mm to 0.2 mm; (Quarzwirke Frechen, Postfach 1780, 50207 Frechen, Germany)
	0.2% Calcium carbonate (CaCO <sub>3</sub> ) extra pure (Merck, 64293 Darmstadt, Germany) to adjust pH to 6.0 ± 0.5.
	The artificial soil was moistened to approximately half of the final water content 2 days before the application. The additional water required to achieve the final water content was added when applying the test item.
<b>Replication:</b>	4 replicates for the test item treatments and 8 replicates for the control. 1 additional container per treatment to check the pH and water content of the test substrate after 14 days
<b>Test groups:</b>	9 treatment groups (8 test item concentrations, control)
<b>Individuals:</b>	10 adult female mites per unit
<b>Duration of test:</b>	14 days
<b>Environmental test conditions</b>	
<b>Temperature:</b>	18 - 22 °C
<b>pH of soil:</b>	test start: 5.7 – 5.8 test end: 5.8 – 5.9
<b>Water content of soil:</b>	At test start: 21.2% to 21.7% (55.8% to 57.1% of the maximum water holding capacity, i.e. within the recommended range 40-60 % of the total water holding capacity) At test end: 20.3% to 21.8% (53.5% to 57.5% of the maximum water holding capacity)
<b>Photoperiod:</b>	16 h light : 8 h dark
<b>Light intensity:</b>	400 lux to 800 lux.

❑ **Study design and methods:**

10 predatory mites (adult females, approximately 9 days after reaching the adult stage) per replicate (8 replicates for the control group, 4 replicates for each treatment group) were exposed 14 days in treated artificial soil. Different concentrations of the test item were mixed homogeneously into the soil before the predatory mites were introduced on top of the soil; 8 concentrations (13, 21, 34, 55, 88, 142, 229 and 370 mg test item/kg soil dry weight) and one control (untreated) were tested. The predatory mites were fed with cheese mite (*Tyrophagus putrescentiae*) *ad libitum* at test start and on day 2, 5, 7, 9 and 12. The assessments of adult mortality, morphological differences and reproduction were performed after 14 d.

❑ **Findings:**

Mortality:

Mortality of *Hypoaspis aculeifer* in the test item treated group ranged from 3% to 13%. The values were not significantly different compared to the control where 4% of the soil mites died (Fisher's Exact Test,  $\alpha = 0.05$ , one-sided greater). No differences in morphology of the mites between the test item treated groups and the control were observed.

Reproduction:

There were no statistically significant effects on reproduction of *Hypoaspis aculeifer* up to and including the highest test concentration of 370 mg test item/kg soil (Williams t-test,  $\alpha = 0.05$ , one-sided smaller). The EC<sub>50</sub> for reproduction was 4.0 mg dimethoate/kg soil.

**Table B.9.4.3.10-1: Effect of foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G (FSN+IDF OD 45 (22.5+22.5) G) on the Predatory Mite *Hypoaspis aculeifer* in a 14-day reproduction study**

Foramsulfuron + isoxadifen- ethyl OD 45 (22.5+22.5) G (FSN+IDF OD 45 (22.5+22.5) G) [mg/kg soil dry weight]	Control	13	21	34	55	88	142	229	370
<b>Mortality (day 14) [%]</b>	4	8	8	8	13	8	8	3	8
Statistical significance <sup>1)</sup>	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<b>No. of juveniles (day 14)</b>	207	213	264	213	233	209	207	223	193
<b>Reproduction in [%] of control (day 14)</b>	-	103	127	103	112	101	100	108	93
Statistical significance <sup>2)</sup>	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
<b>Endpoints [mg/kg soil ]</b>									
NOEC(mortality)	> 370								
LC50 (mortality) <sup>3)</sup>	>370								
NOEC (reproduction)	≥370								
LOEC (reproduction)	>370								
EC <sub>50</sub> (reproduction) <sup>3)</sup>	>370								

n.s. = not significantly different compared to the control - not applicable

<sup>1)</sup> Fisher's Exact Test,  $\alpha = 0.05$ , one-sided greater

<sup>2)</sup> Williams t-test,  $\alpha = 0.05$ , one-sided smaller

<sup>3)</sup> estimated value

#### Validity criteria:

Validity criteria	Recommended	Obtained
Control Mortality (mean mortality of the adult female animals)	≤20 %	4 %
Control Reproduction (number of juvenile mites per replicate)	≥ 50	190 - 226
Coefficient of variation of the Control Reproduction	≤30 %	5.3 %

All validity criteria were met. Therefore this study is valid.

In a separate GLP conducted study (study code 74661089, performed in May/June 2012) the reference item dimethoate showed statistically significant effects on reproduction at a concentration of 1.7 mg dimethoate/kg soil dry weight and above. The EC<sub>50</sub> for reproduction was 4.0 mg dimethoate/kg soil dry weight.

- ❑ **Conclusion:** Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G (FSN+IDF OD 45 (22.5+22.5) G) caused no significant effects on mortality and reproduction of *Hypoaspis aculeifer* up to and including the highest test concentration of 370 mg test item/kg soil. Therefore, the overall NOEC was determined to be ≥370 mg test item/kg soil. The overall LOEC and the EC<sub>50</sub> were estimated to be greater than 370 mg test item/kg soil.

- ❑ **Comment (Co-RMS and RMS):** No comments, study is acceptable.

## B.9.5. EFFECTS ON SOIL NITROGEN TRANSFORMATION

### B.9.5.1. Laboratory testing

Toxicity studies on the effects of foramsulfuron, foramsulfuron and bound residues and product Equip OD 45 on nitrogen transformation and soil respiration were submitted and reviewed during Annex I inclusion and were considered acceptable by the RMS Germany. These studies were not re-evaluated since studies were performed either according to BBA: VI, 1-1 which is in line with the OECD: 216 (draft): Nitrogen Transformation Test and OECD 217 (draft): Carbon Transformation Test or according to the OECD guidelines. Only brief summaries of these tests are presented in this dRAR.

For the purpose of renewal three new studies on the effects of soil major metabolites, AE F092944, AE F130619 and AE F153745 on nitrogen transformation in soil were submitted. Studies have been evaluated in this dRAR and detailed summaries are provided. In none of the studies unacceptable effects were found at the highest tested dose level which ranged from 0.137 mg/kg dws to 0.735 mg/kg dws. Brief details of all studies are provided in the Table B.9.5.1-1.

**Table B.9.5.1-1: Toxicity data of foramsulfuron and its metabolites to soil non-target micro-organisms**

Test item	Test design	Ecotoxicological endpoint		Reference
N-transformation				
Foramsulfuron	28 d	no unacceptable effects	≥0.3 mg a.s./kg dws	Heusel, 1997 <u>M-142972-01-1</u> KCA 8.5/01
Foramsulfuron + bound residues	28 d	no unacceptable effects	≥0.735 mg a.s./kg dws	Sowig & Gildemeister, 2000 <u>M-193916-01-1</u> KCA 8.5/02
AE F153745	28 d	no unacceptable effects	≥0.240 mg/kg dws	Schulz, 2013 <u>M-453508-01-1</u> KCA 8.5/07
AE F130619	28 d	no unacceptable effects	≥0.375 mg/kg dws	Schulz, 2013 <u>M-453568-01-1</u> KCA 8.5/06
AE F092944	28 d	no unacceptable effects	≥0.137 mg/kg dws	Schulz, 2013 <u>M-453511-01-1</u> KCA 8.5/05
Soil respiration				
Foramsulfuron	28 d	no unacceptable effects	≥0.3 mg a.s./kg dws	Heusel R., 1998 <u>M-142971-01-1</u> KCA 8.5/03
Foramsulfuron + bound residues	28 d	no unacceptable effects	≥0.3 mg a.s./kg dws	Sowig & Gildemeister, 2000 <u>M-142971-01-1</u> KCA 8.5/04
N-transformation and soil respiration				
Equip OD 45	56 d	no unacceptable effects	≥0.135 mg a.s./kg dws	van der Kolk J, 1999 <u>M-193742-01</u> KCP 10.5 /01

dws = dry weight soil

**B.9.5.1.1. Toxicity of foramsulfuron on soil nitrification**

<b>Report:</b>	<u>KCA 8.5 /01;Heusel, R.;1997;M-142972-01</u>
<b>Title:</b>	AE F130360; substance, technical; Code: AE F130360 00 1C98 0002 - Effects on soil microbial activity (nitrogen turn-over)
<b>Report No:</b>	A59288
<b>Document No:</b>	<u>M-142972-01-1</u>
<b>Guidelines:</b>	<b>BBA: VI 1-1;Deviation not specified</b>
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** AE F130360 when applied at rate 0.06 mg test item/kg soil dry weight (45 g a.s./ha) and 5 times higher rate 0.3 mg test item/kg soil dry weight (225 g a.s./ha) had a negligible effect on soil microbial nitrogen turn-over ( $< \pm 15$  % deviation of the control treatment) in the loamy sand and loamy silt soil after 28 days.
- ❑ **Comment (Co-RMS and RMS):** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final), but should be included in the new list of end points. No comment, study is acceptable.

<b>Report:</b>	<u>KCA 8.5 /02;Sowig, P.; Gildemeister, H.;2000;M-193916-01</u>
<b>Title:</b>	Effects on soil microbial activity (nitrogen turn-over) bound residues of AE F130360 substance, technical Code: AE F130360 00 1C98 0002
<b>Report No:</b>	C006438
<b>Document No:</b>	<u>M-193916-01-1</u>
<b>Guidelines:</b>	<b>OECD: 216 (draft);Deviation not specified</b>
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** AE F130360 00 1C98 0002 when applied at the rate 0.147 mg AE F130360 / kg soil dry weight (60 g a.s./ha) and 5 times rate 0.735 mg AE F130360 / kg soil dry weight (300 g a.s./ha) had a tolerable effect on soil microbial nitrogen turn-over in the loamy sand between 77 and 105 days.
- ❑ **Comment (Co-RMS and RMS):** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final), but should be included in the new list of end points.. No comment, study is acceptable.

**B.9.5.1.2. Toxicity of foramsulfuron on soil respiration**

Studies below are carbon transformation studies submitted in the original European dossier. This study is no longer required under Regulation 1107/2009 but has been included here for completeness.

<b>Report:</b>	<u>KCA 8.5 /03;Heusel, R.;1998;M-142971-01</u>
<b>Title:</b>	AE F130360; substance, technical; Code: AE F130360 00 1C98 0002 - Effects on soil microbial activity (short-term respiration)
<b>Report No:</b>	A59287
<b>Document No:</b>	<u>M-142971-01-1</u>
<b>Guidelines:</b>	<b>BBA: VI, 1-1;Deviation not specified</b>
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** AE F130360 00 1C98 0002 when applied at the field rate (45 g a.s./ha) and 5 times field rate (225 g a.s./ha) had a negligible effect on soil microbial respiration in the loamy sand after 28 days.
- ❑ **Comment (Co-RMS and RMS):** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final), but should be included in the new list of end points.. No comment, study is acceptable.

<b>Report:</b>	<u>KCA 8.5 /04;Sowig, P.; Gildemeister, H.;2000;M-193914-01</u>
<b>Title:</b>	Effects on soil microbial activity (short-term respiration) bound residues of AE F130360 substance, technical Code: AE F130360 00 1C98 0002
<b>Report No:</b>	C006437
<b>Document No:</b>	<u>M-193914-01-1</u>
<b>Guidelines:</b>	<b>OECD: Draft 217, JAN 1999;Deviation not specified</b>
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** AE F130360 00 1C98 0002 when applied at the field rate (45 g a.s./ha) and 5 times field rate (225 g a.s./ha) had a tolerable effect on soil microbial respiration in the loamy sand between 77 and 105 days.
- ❑ **Comment (Co-RMS and RMS):** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final), but should be included in the new list of end points.. No comment, study is acceptable.

### Studies on metabolites

#### **B.9.5.1.3. Toxicity of AE F092944 on soil nitrification**

<b>Report:</b>	<u>KCA 8.5 /05;Schulz, L.;2013;M-453511-01</u>
<b>Title:</b>	AE F092944 (BCS-AA25052): Effects on the activity of soil microflora (Nitrogen transformation test)
<b>Report No:</b>	13 10 48 018 N
<b>Document No:</b>	<u>M-453511-01-1</u>
<b>Guidelines:</b>	<b>OECD 216 adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation; not applicable</b>
<b>GLP/GEP:</b>	yes

#### ❑ **Materials and methods:**

<b>Test Material:</b>	AE F092944 (BCS-AA25052)
<b>Description:</b>	white powder
<b>Lot/Batch No.:</b>	AE F092944 00 1B99 0002
<b>Purity:</b>	analysed – 99.8% w/w
<b>Density:</b>	n.a.
<b>Stability:</b>	Stable under normal use and storage conditions
<b>Rates used:</b>	0.021 kg test item/ha (0.028 mg test item / kg soil dry weight) and 0.103 kg test item/ha (0.137 mg test item / kg soil dry weight)
<b>Control:</b>	The control was prepared with quartz sand only.
<b>Toxic Standard:</b>	Dinoterb

#### **Test Design**

<b>Soil:</b>	Agricultural soil
<b>Test units:</b>	500 mL wide mouth glass flasks with airpermeable caps
<b>Replication:</b>	3
<b>Sampling intervals:</b>	0, 7, 14 and 28 days

#### **Environmental conditions**

<b>Temperature:</b>	18.7 - 21.1 °C
<b>Photoperiod:</b>	Continuous dark
<b>Soil moisture content:</b>	45.02 - 47.44 % of maximum water holding capacity
<b>Soil pH:</b>	6.5 – 6.6
<b>Duration of test:</b>	28 days

#### ❑ **Study Design and Methods:**

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<sub>3</sub>-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5 %). NH<sub>4</sub>-nitrogen, NO<sub>3</sub>- and NO<sub>2</sub>-nitrogen were determined using the Autoanalyser (BRAN+LUEBBE) at different sampling intervals (0, 7, 14 and 28 days after treatment).

#### □ Findings:

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.

The result for nitrogen transformation are summarised below:

**Table B.9.5.1.3-1: 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 <sup>1)</sup>			Nitrate-N <sup>1)</sup>		% difference to control		Nitrate-N <sup>1)</sup>		% 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

<sup>1)</sup> 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,  $p \leq 0.05$ )

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).

- **Conclusion:** AE F092944 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO<sub>3</sub>-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.
- **Comment (Co-RMS and RMS):** No comment. Validity criteria according to OECD 216 were met, study is acceptable.



**B.9.5.1.4. Toxicity of AE F130619 on soil nitrification**

<b>Report:</b>	<u>KCA 8.5 /06;Schulz, L.;2013;M-453568-01</u>
<b>Title:</b>	Foramsulfuron-AE F130619 (BCS-AU59648): Effects on the activity of soil microflora (nitrogen transformation test)
<b>Report No:</b>	13 10 48 019 N
<b>Document No:</b>	<u>M-453568-01-1</u>
<b>Guidelines:</b>	<b>OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation; none</b>
<b>GLP/GEP:</b>	<b>yes</b>

**❑ Materials and methods:**

<b>Test Material:</b>	Foramsulfuron-AE F130619
<b>Description:</b>	off white powder
<b>Lot/Batch No.:</b>	AE F130619-01-01
<b>Purity:</b>	analysed – 94% w/w
<b>Density:</b>	n.a.
<b>Stability:</b>	Stable under normal use and storage conditions
<b>Rates used:</b>	0.056 kg test item/ha (0.075 mg test item / kg soil dry weight) and 0.281 kg test item/ha (0.375 mg test item / kg soil dry weight)
<b>Control:</b>	The control was prepared with quartz sand only.
<b>Toxic Standard:</b>	Dinoterb

**Test Design**

<b>Soil:</b>	agricultural soil
<b>Test units:</b>	500 mL wide mouth glass flasks with airpermeable caps
<b>Replication:</b>	3
<b>Sampling intervals:</b>	0, 7, 14 and 28 days

**Environmental conditions**

<b>Temperature:</b>	18.7 - 20.6 °C
<b>Photoperiod:</b>	continuous dark
<b>Soil moisture content:</b>	45.7 - 48.15 % of maximum water holding capacity
<b>Soil pH:</b>	6.3 – 6.5
<b>Duration of test:</b>	28 days

**❑ Study Design and Methods:**

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.075 and 0.375 mg test item/kg soil dry weight. Application rates were equivalent to 0.056 and 0.281 kg test item/ha. Determination of the nitrogen transformation (NO<sub>3</sub>-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5 %). NH<sub>4</sub>-nitrogen, NO<sub>3</sub>- and NO<sub>2</sub>-nitrogen were determined using the Autoanalyser (BRAN+LUEBBE) at different sampling intervals (0, 7, 14 and 28 days after treatment).

**❑ Findings:**

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.

The result for nitrogen transformation are summarised below:

**Table B.9.5.1.4-1: Effects on nitrogen transformation in soil after treatment with AE F130619**

Time Interval (days)	Control			0.075 mg test item/kg soil dry weight equivalent to 0.056 kg test item/ha			0.375 mg test item/kg soil dry weight equivalent to 0.281 kg test item/ha				
	Nitrate-N <sup>1)</sup>			Nitrate-N <sup>1)</sup>			% difference to control	Nitrate-N <sup>1)</sup>		% difference to control	
0-7	3.18	±	0.11	3.67	±	0.37	+15.6 n.s.	3.81	±	0.40	+19.9 n.s.
7-14	1.87	±	0.09	1.39	±	0.17	-26.0 n.s.	1.15	±	0.24	-38.4 n.s.
14-28	0.91	±	0.08	0.97	±	0.01	+6.6 n.s.	1.10	±	0.15	+21.3 n.s.

The calculations were performed with unrounded values

<sup>1)</sup> 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,  $p \leq 0.05$ )

No adverse effects of Foramsulfuron-AE F130619 (BCS-AU59648) 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 +6.6 % (test concentration 0.075 mg/kg dry soil) and +21.3 % (test concentration 0.375 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

- **Conclusion:** AE F130619 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO<sub>3</sub>-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.375 mg test item/kg soil dry weight, which are equivalent to application rates up to 0.281 kg test item/ha.
- **Comment (Co-RMS and RMS):** No comment. Validity criteria according to OECD 216 (2000) were met, study is acceptable.

#### **B.9.5.1.5. Toxicity of AE F153745 on soil nitrification**

<b>Report:</b>	<u>KCA 8.5 /07;Schulz, L.:2013;M-453508-01</u>
<b>Title:</b>	Foramsulfuron-AE F153745 (BCS-AU80017): Effects on the activity of soil microflora (Nitrogen transformation test)
<b>Report No:</b>	1321048020N
<b>Document No:</b>	<u>M-453508-01-1</u>
<b>Guidelines:</b>	<b>OECD 216 adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation; not applicable</b>
<b>GLP/GEP:</b>	<b>no</b>

#### □ **Materials and methods:**

<b>Test Material:</b>	Foramsulfuron-AE F153745
<b>Description:</b>	beige powder
<b>Lot/Batch No.: A</b>	E F153745 00 1B98 0001
<b>Purity:</b>	analysed – 98.2% w/w
<b>Density:</b>	n.a.
<b>Stability:</b>	Stable under normal use and storage conditions
<b>Rates used:</b>	0.036 kg test item/ha (0.048 mg test item / kg soil dry weight) and 0.180 kg test item/ha (0.240 mg test item / kg soil dry weight)
<b>Control:</b>	The control was prepared with quartz sand only.
<b>Toxic Standard:</b>	Dinoterb
<b>Test Design</b>	
<b>Soil:</b>	agricultural soil
<b>Test units:</b>	500 mL wide mouth glass flasks
<b>Replication:</b>	3
<b>Sampling intervals:</b>	0, 7, 14 and 28 days
<b>Environmental conditions</b>	
<b>Temperature:</b>	18.7 - 21.1 °C in a climatic room
<b>Photoperiod:</b>	continuous dark
<b>Soil moisture content:</b>	44.78 - 47.44 % of maximum water holding capacity

**Soil pH:** 6.5 – 6.6  
**Duration of test:** 28 days

❑ **Study Design and Methods:**

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.048 and 0.240 mg test item/kg soil dry weight. Application rates were equivalent to 0.036 and 0.180 kg test item/ha. Determination of the nitrogen transformation (NO<sub>3</sub>-nitrogen production) in soil enriched with lucerne meal (concentration in soil 0.5 %). NH<sub>4</sub>-nitrogen, NO<sub>3</sub>- and NO<sub>2</sub>-nitrogen were determined using the Autoanalyser (BRAN+LUEBBE) at different sampling intervals (0, 7, 14 and 28 days after treatment).

❑ **Findings:**

In a separate study (dated 04.01. - 01.02.2013), 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.

The result for nitrogen transformation are summarised below:

**Table B.9.5.1.5-1: Effects on nitrogen transformation in soil after treatment with AE F153745**

Time Interval (days)	Control			0.048 mg test item/kg soil dry weight equivalent to 0.036 kg test item/ha			0.240 mg test item/kg soil dry weight equivalent to 0.180 kg test item/ha				
	Nitrate-N <sup>1)</sup>			Nitrate-N <sup>1)</sup>			% difference to control				
0-7	3.73	±	0.39	3.28	±	0.21	-12.0 n.s.	3.56	±	0.32	-4.5 n.s.
7-14	1.22	±	0.42	1.64	±	0.13	+34.8 n.s.	1.43	±	0.26	+ 17.2 n.s.
14-28	0.94	±	0.12	0.89	±	0.07	-5.3 n.s.	1.04	±	0.10	+10.9 n.s.

The calculations were performed with unrounded values

<sup>1)</sup> 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,  $p \leq 0.05$ )

The test item AE F153745 (BCS-AU80017) caused a temporary stimulation of the daily nitrate rate at the tested concentration of 0.048 mg/kg dry soil at time interval 7-14 days after application. However, no adverse effects of AE F153745 (BCS-AU80017) on nitrogen transformation in soil could be observed at both tested concentrations (0.048 mg and 0.240 mg test item/kg dry soil) at the end of the test, 28 days after application (time interval 14-28). Differences from the control of -5.3 % (test concentration 0.048 mg/kg dry soil) and +10.9 % (test concentration 0.240 mg/kg dry soil) were measured at the end of the 28-day incubation period (time interval 14-28).

- ❑ **Conclusion:** AE F153745 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO<sub>3</sub>-N production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.240 mg test item/kg soil dry weight, which are equivalent to application rates up to 0.180 kg test item/ha.
- ❑ **Comment (Co-RMS and RMS):** No comment. Validity criteria according to OECD 216 were met, study is acceptable.

#### B.9.5.1.6. Toxicity of AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L on soil nitrification and respiration

<b>Report:</b>	KCP 10.5 /01; van der Kolk J, 1999;M-193742-01
<b>Title:</b>	The effects on the respiration and nitrification of soil microflora Code: AE F130360 01 1K05 A304
<b>Report No:</b>	
<b>Document No:</b>	M-193742-01
<b>Guidelines:</b>	BBA: VI, 1-1, 1990; Deviations: not specified
<b>GLP/GEP:</b>	yes

- ❑ **Conclusion:** The results showed that at both test concentrations 0.06 mg a.i./kg soil dry weight and 0.6 mg a.i./kg soil dry weight (field rate of 45 g a.s./ha and 10 x exaggerated field rate of 450 g a.s./ha) the deviations were below the guideline's trigger value of 15% deviation to control after 28 days of exposure, for respiration and nitrification part of the study for the loamy sand soil and for the respiration part of the study for the sandy loam soil. Deviations larger than 15% were found for the nitrification part of the study for the sandy loam soil, but these effects were transient and were below 15% after 56 days of exposure. These results show that AE 130360 01 1K05A304 had no lasting effects on the respiration and nitrification processes in the soils at the application rates tested.
- ❑ **Comment (Co-RMS and RMS):** The endpoints from this study are reported in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final). No other comment, study is acceptable.

#### B.9.5.2. Additional testing (annex IIIA 10.7.2)

Neither foramsulfuron solo formulation nor its metabolites had any significant effects on nitrification of soil micro-organisms in soil at the highest concentration tested. It is therefore not necessary to investigate the rates of recovery following treatment.

### B.9.6. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS

#### B.9.6.1. Testing on non-target plants

Five toxicity studies on non-target plants were submitted. For foramsulfuron, a screening study on higher plant species was performed. As expected for a sulfonyl urea herbicide the compound showed significant herbicidal activity to several plants. Test results of studies on non-target plants are, by nature, related to the tested formulation. Three tests have been performed with the representative formulation: foramsulfuron + isoxadifen-ethyl OD45. For seedlings emergence, a tier 1 and a tier 2 study has been performed with the representative formulation. For vegetative vigour, only a tier 2 study was performed, as a tier 1 study was considered unprofitable due to the proven herbicidal activity of the compound.

In tier 2 seedling emergence test the most sensitive species was lettuce with an EC<sub>50</sub> = 38.8 g sum of a.i./ha. In tier 2 vegetative vigour test the most sensitive species was radish with an EC<sub>50</sub> = 1.88 g sum of a.i./ha. Brief details of the studies are provided in the Table B.9.6.1-1. Short summaries of the studies are provided thereafter.

**Table B.9.6.1-1: Toxicity of foramsulfuron and FSN + IDF OD 45 to non-target plants**

Terrestrial Non-Target Plants			
Number of species tested (species)	Test method Test substance Application rate	Effects	Reference
Crop plants (8 species) Broadleaf plants (17 species) Grass plants (11 species)	Greenhouse, seedling emergence and growth, 28 d <u>Foramsulfuron</u>	Post-emergence application: grass plants more susceptible than broadleaf plants Pre-emergence application: some broadleaf plants are quite susceptible to dosage range of $\geq 20$ g a.s./ha	Bieringer, 1999 M-191762-01-1 KCA 8.6.1 /01
Dicotyledoneae:6 (bean, cabbage, radish, tomato,soybean, lettuce) Monocotyledoneae:4 (rye grass, corn, wheat, onion)	Seedling emergence <u>FSN + IDF OD 45</u> 0 (control) and 60 g prod./ha with observations of emergence on Days 10, 14 and 21, with observations of height and condition on Day 21 and measurement of dry weight on Day 21	Reduction > 25 % (emergence) in onion and rye grass; reductions > 25 % (height and weight of seedlings, signs of phytotoxicity) in cabbage, lettuce, onion, radish, rye grass, tomato and wheat seedlings	Porch, Kendall& Krueger, 1999; B002673 <u>M-238408-01-2</u> KCP 10.6.2/01
Dicotyledoneae: 6 (bean, cabbage, radish, tomato, soybean, lettuce) Monocotyledoneae: 4 (rye grass, corn, wheat, onion)	Tier 2 vegetative vigour <u>FSN + IDF OD 45</u> 0 (control), 0.25, 0.74, 2.2, 6.7, 20 and 60 g prod./ha with height and condition observations on Days -1 or 0 (prior to application), 7, 14 and 21, dry weight measurements on Day 21	most sensitive species: radish; <b>lowest EC<sub>50</sub> = 1.88 g sum of a.i./ha<sup>1)</sup></b>	Porch, Kendall& Krueger, 1999; B002710 <u>M-238444-01-2</u> KCP 10.6.2/02
Dicotyledoneae: 4 (cabbage, radish, tomato, lettuce) Monocotyledoneae: 3 (rye grass, wheat, onion)	Tier 2 seedling emergence <u>FSN + IDF OD 45</u> 0 (control), 0.25, 0.74, 2.2, 6.7, 20 and 60 g prod./ha with observations of emergence on Days 10 and 14, with observations of height and condition on Day 14 and measurement of dry weight on Day 14	most sensitive species: lettuce; <b>lowest EC<sub>50</sub> = 38.8 g sum of a.i./ha<sup>1)</sup></b>	Porch, Kendall& Krueger, 2000; B002819 <u>M-238550-01-1</u> KCP 10.6.2/03

<sup>1)</sup> In all studies endpoints are given in g a.i./ha. Descriptions of the experimental design in the seedling emergence and vegetative vigour studies indicate that the endpoints are given as g (FSN + IDF) per hectare.



### B.9.6.1.1. Effects of Foramsulfuron solo formulation A8714C on non-target plants (terrestrial and aquatic)

#### B.9.6.1.1.1. Effects of foramsulfuron on seedling emergence

<b>Report:</b>	<u>KCA 8.6.1 /01; Bieringer, H.; 1999; M-191762-01</u>
<b>Title:</b>	Effectivity of the herbicide AE F130360 on higher plant species as applied under greenhouse conditions
<b>Report No:</b>	C005291
<b>Document No:</b>	<u>M-191762-01-1</u>
<b>Guidelines:</b>	None
<b>GLP/GEP:</b>	no

- ❑ **Conclusion:** Post-emergence application: grass plants are more susceptible than broadleaf plants Pre-emergence application: some broadleaf plants are quite susceptible to dosage range of > 20 g a.s./ha. The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).
- ❑ **Comment (Co-RMS and RMS):** No comments; study brings only supportive information.

#### B.9.6.1.1.2. Effects of AE F130360 + AE F122006, oil flowable, 22.5+22.5 g/L on seedling emergence

<b>Report:</b>	<u>KCP 10.6.2 /01; Porch, J. R.; Kendall, T. Z.; Krueger, H. O.; 1999; M-238408-01-2</u>
<b>Title:</b>	AE F130360 + AE F122006; oil flowable; 22.5 + 22.5 g/l. CODE: AE F130360 01 1K05 A304: A toxicity test to determine the effects of the test substance on seedling emergence of ten species of plants
<b>Report No:</b>	B002673
<b>Document No:</b>	<u>M-238408-01-2</u>
<b>Guidelines:</b>	U.S. EPA, Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.4100 (1); <b>Deviation: not specified changes in study protocol regarding the identification, study Monitor and analytical method.</b>
<b>GLP/GEP:</b>	yes

❑ **Conclusion:**

Treatment-related effects resulting from a single spray application of AE F130360 + AE F122006 at 60 g a.i./ha to planted seeds were observed in seven of ten test species. No apparent effects were observed on bean, corn, or soybean seedling emergence, growth, or condition. Reductions of 25% or more in emergence were observed in onion and rye grass. Reductions greater than 25% in height or weight of seedlings and apparent treatment-related signs of phytotoxicity were observed in cabbage, lettuce, onion, radish, rye grass, tomato, and wheat seedlings.

Number of species tested (species)	Test method Test substance Application rate	Effects
Dicotyledoneae: 6 (bean, cabbage, radish, tomato, soybean, lettuce) Monocotyledoneae: 4 (rye grass, corn, wheat, onion)	Seedling emergence FSN + IDF OD 45 0 (control) and 60 g prod./ha with observations of emergence on Days 10, 14 and 21, with observations of height and condition on Day 21 and measurement of dry weight on Day 21	Reduction > 25 % (emergence) in onion and rye grass; reductions > 25 % (height and weight of seedlings, signs of phytotoxicity) in cabbage, lettuce, onion, radish, rye grass, tomato and wheat seedlings

- ❑ **Comment (Co-RMS and RMS):** No comments, study is acceptable and brings supportive information.



### B.9.6.1.1.3. Effects of AE F130360 + AE F122006, oil flowable, 22.5+22.5 g/L on seedling emergence

Report:	<u>KCP 10.6.2 /03; Porch, J. R.; Kendall, T. Z.; Krueger, H. O.:2000; M-238550-01</u>
Title:	A tier II toxicity test to determine the effects of the test substance on seedling emergence of seven species of plants: AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L: AE F130360 01 1K05 A304
Report No:	B002819
Document No:	<u>M-238550-01</u>
Guidelines:	MAFF: 59 NohSan Notification No. 3850; OECD: ENV/MC/CHEM(98)17; USEPA (=EPA): 160; U.S. EPA Series 850 - Ecological Effects, Test Guidelines OPPTS Number 850.4225; Deviation: not specified
GLP/GEP:	yes

#### □ Conclusion:

Species	M/D	Seedling Emergence Test EC <sub>50</sub> [g as/ha], 14 days	
		Shoot Length	Shoot Dry Weight
<i>Brassica oleracea</i>	D	> 5	56.4
<i>Lactuca sativa</i>	D	> 5.8	38.8
<i>Allium cepa</i>	M	> 31.7	> 13.5
<i>Raphanus sativus</i>	D	54.9	39.9
<i>Lolium perenne</i>	M	> 52.0	> 25.5
<i>Lycopersicon esculentum</i>	D	> 60	> 60
<i>Triticum aestivum</i>	M	> 60	> 27.8

Seedling Emergence Test, endpoint emergence: EC<sub>50</sub> > 60 g as/ha for all species.

M = Monocotyledoneous, D = Dicotyledoneous

Treatment-related effects resulting from a single spray application of AE F130360 + AE F122006 to planted seeds were observed in six of seven test species. Of the species which showed effects, the most sensitive were cabbage and lettuce, which both had a NOEC of 0.74 g a.i./ha. Radish and onion had NOECs of 2.2 and 6.7 g a.i./ha, respectively, while perennial ryegrass and wheat had NOECs of 20 g a.i./ha. The calculated EC<sub>25</sub> estimate for lettuce height was 5.79 g a.i./ha, and the calculated EC<sub>25</sub> and EC<sub>50</sub> for lettuce weight were 1.88 and 38.8 g a.i./ha, respectively. No apparent effects were observed on tomato seedling emergence, growth, or condition.

□ Comment (Co-RMS and RMS): No comments study is acceptable.

### B.9.6.1.1.4. Effects of AE F130360 + AE F122006, oil flowable, 22.5+22.5 g/L on vegetative vigour

Report:	<u>KCP 10.6.2 /02; Porch, J. R.; Kendall, T. Z.; Krueger, H. O.:1999; M-238444-01-2</u>
Title:	A Tier II toxicity test to determine the effects of the test substance on vegetative vigor of ten species of plants: AE F130360 + AE F122006, oil flowable, 22.5+22.5 g/L
Report No:	B002710
Document No:	<u>M-238444-01-2</u>
Guidelines:	MAFF: 3850; OECD: (98)17; U.S. EPA Series 850 - Ecological Effects, Test Guidelines; OPPTS Number 850.4150 and 850.4250; Deviation: not specified
GLP/GEP:	yes

#### □ Conclusion:

Species	M/D	Vegetative Vigour Test EC <sub>50</sub> [g as/ha], 21 days	
		Shoot Length	Shoot Dry Weight
<i>Brassica oleracea</i>	D	6.2	2.43
<i>Lactuca sativa</i>	D	5.6	2.12
<i>Allium cepa</i>	M	60.0	38.5
<i>Raphanus sativus</i>	D	> 0.66	1.88
<i>Lolium perenne</i>	M	> 3.5	2.38
<i>Lycopersicon esculentum</i>	D	3.4	1.97
<i>Triticum aestivum</i>	M	19.9	4.10
<i>Zea mays</i>	M	> 60	> 60
<i>Phaseolus vulgaris</i>	D	5.4	5.21
<i>Glycine max</i>	D	12.6	15.2

M = Monocotyledoneous, D = Dicotyledoneous

The application of AE F130360 + AE F122006; OIL FLOWABLE; 22.5 + 22.5 g/L caused effects on plant condition and growth in all ten test species. Commonly observed effects included necrosis, chlorosis, formative and morphological abnormalities, height and weight reduction, and plant mortality. The most sensitive species was radish, which exhibited slight effects at 0.25 g a.i./ha, the lowest concentration tested. The EC<sub>25</sub> estimate (and 95% confidence limits) for plant height at Day 21 was 0.66 g a.i./ha (0.59 - 0.82 g a.i./ha). The EC<sub>50</sub> value could not be calculated but was predicted to be > 0.66 g a.i./ha. The EC<sub>25</sub> and EC<sub>50</sub> estimates (and 95% confidence limits) for plant dry weight at Day 21 were 0.57 g a.i./ha (0.43 - 0.79 g a.i./ha) and 1.88 g a.i./ha (1.40 - 2.88 g a.i./ha), respectively. Corn was the least sensitive species tested. At test termination, the reduction in all test endpoints for corn was less than 25%, therefore the EC<sub>25</sub> for corn height and weight at Day 21 were considered to be >60 g a.i./ha.

□ **Comment (Co-RMS and RMS):** No comments study is acceptable.

#### B.9.6.1.1.5. Literature search

The literature search revealed a paper by Roux et al. (2005) which presented effects of 22 ALS- inhibitors, one of which was foramsulfuron, on different mutants of *Arabidopsis thaliana*. Although the paper as a whole can be regarded as reliable, the endpoints presented in this paper are not considered in the risk assessment for foramsulfuron for the following reasons:

1. The test was conducted with strains which were susceptible to ALS-inhibitors and not to naturally occurring phenotypes of *A. thaliana*.
2. As far as described in the paper the test method used does not fully apply to OECD 2017. Especially the plant density (40 plants in a 1 L pot) was exceptionally high.

For sake of completeness and as supplementary information a summary of this paper is presented here:

<b>Report:</b>	<u>KCA 8.6.2 /01;Roux, F.; Matejicek, A.; Reboud, X.;2005;M-458576-01</u>
<b>Title:</b>	Response of <i>Arabidopsis thaliana</i> to 22 ALS inhibitors: Baseline toxicity and cross-resistance of <i>csr1-1</i> and <i>csr1-2</i> resistant mutants.
<b>Report No:</b>	<u>M-458576-01-1</u>
<b>Document No:</b>	<u>M-458576-01-1</u>
<b>Guidelines:</b>	<b>not applicable; not applicable</b>
<b>GLP/GEP:</b>	<b>no</b>

#### □ **Summary:**

Acetolactate synthase (ALS) is the target site of the herbicide family known as ALS inhibitors. The intensive use of the ALS inhibitors, together with an apparently high weed mutation rate and/or a wide range of resistance, have resulted in an increased occurrence of weed population resistance.

The aim was to study the relationships among 22 ALS-inhibiting herbicides using two *Arabidopsis thaliana* susceptible lines and to assess the cross-resistance pattern of chlorsulfuron- and imazapyr- resistant lines to these 22 ALS-inhibiting herbicides. Two susceptible (S) and two resistant (R) lines of *A. thaliana*: Columbia (Col) and Landsberg (Ler) inbred lines were chosen as the susceptible references. ED<sub>50</sub> values for the Col and Ler susceptible lines of *A. thaliana* were 333 mg/ha and 506 mg/ha, respectively.

## □ Material and methods:

### A. Materials

#### 1. Test material

<b>Test item:</b>	Foramsulfuron was obtained directly from the marketing company who provided a formulation containing the ALS inhibitor as the single herbicide active ingredient.
<b>Active substance(s):</b>	Foramsulfuron
<b>Adjuvant / Surfactant:</b>	Not given
<b>Source of test item:</b>	Bayer CropSciences (Lyon, France)
<b>Lot/Batch number:</b>	Not given
<b>Purity:</b>	22.5% a.i. (wt/wt)
<b>Stability of test item:</b>	Not given
<b>Water solubility:</b>	Not given

#### 2. Test organism(s)

<b>Species:</b>	Two susceptible (S) and two resistant (R) lines of <i>A. thaliana</i> : Columbia (Col) and Landsberg (Ler) inbred lines were chosen as the susceptible references. The <i>A. thaliana</i> chlorsulfuron- resistant (csr1-1 or GH50) and imazapyr-resistant (csr1-2 or GH90) mutants isolated by Haughn and Somerville (1986,1990) <sup>5</sup> from ethylmethane- sulfonate (EMS) mutagenized populations of the wild-type susceptible Col line were used. The csr1-1 mutant is resistant due to a point mutation resulting in a Pro to Ser substitution at the 197th amino acid, while the csr1-2 mutant is resistant due to a point mutation resulting in a Ser to Asn substitution at the 653rd amino acid (Haughn et al., 1988; Sathasivan et al., 1990, 1991) <sup>6</sup> .
<b>Cultivar:</b>	Not given
<b>Source of test species:</b>	All <i>A. thaliana</i> lines were provided by the Nottingham Stock Centre (Nottingham, UK).
<b>Crop growth stage at treatment:</b>	Post-emergence

### B. Study design and methods

#### 1. Test procedure

<b>Test system (study type):</b>	Laboratory assays
<b>Guideline/method:</b>	Not specified
<b>Duration of study:</b>	From seedlings to 20 days after 4 to 5 leaf stage
<b>Conduction:</b>	Seeds of <i>A. thaliana</i> were sown in 1-L plastic pots filled with a commercial

<sup>5</sup> Haughn GW & Somerville CR (1986) Sulfonylurea-resistant mutants of *Arabidopsis thaliana*. *Molecular and General Genetics* 204, 430–434.

Haughn GW & Somerville CR (1990) A mutation causing imidazolinone resistance maps to the *csr1* locus of *Arabidopsis thaliana*. *Plant Physiology* 92, 1081–1085.

<sup>6</sup> Haughn GW, Smith J, Mazur B & Somerville C (1988) Transformation with a mutant *Arabidopsis* acetolactate synthase gene renders tobacco resistant to sulfonylurea herbicides. *Molecular & General Genetics* 211, 266–271.

Sathasivan K, Haughn GW & Murai N (1990) Nucleotide sequence of a mutant acetolactate synthase gene from an imidazolinone-resistant *Arabidopsis thaliana* var. Columbia. *Nucleic Acids Research* 18, 2188.

Sathasivan K, Haughn GW & Murai N (1991) Molecular basis of imidazolinone herbicide resistance in *Arabidopsis thaliana* var. Columbia. *Plant Physiology* 97, 1044–1050.

	soil (Terreau Semis Bouturage Repiquage; Composana, Roche-les-Beaupré, France). They were grown in the greenhouse at 20/25°C (night/ day) under natural light supplemented by artificial sodium light to provide a 16-h photoperiod. The pots were regularly rotated during the growing period. The plants were watered twice a week with a standard nutrient solution.
<b>Application rates:</b>	Applied post-emergence at rates: 0.034, 0.103, 0.309, 0.926, 2.778, 8.33 and 25 g a.i./ha.
<b>Number of replicates:</b>	3 (randomized)
<b>Plot size:</b>	Before spraying, plants were thinned to 40 per pot.
<b>Application / device / nozzles:</b>	Laboratory track sprayer delivering 1 spray solution with a 110-04 nozzle operated at 400 kPa
<b>Water volume:</b>	300 L ha
<b>Verification of dispersion:</b>	Not specified

## **2. Test conditions**

<b>Soil type at study site:</b>	Commercial soil (Terreau Semis Bouturage Repiquage; Composana, Roche-les-Beaupré, France)
<b>pH:</b>	Not specified
<b>Organic matter (Corg):</b>	Not specified
<b>Others:</b>	Not specified

## **2. Observations and measurements:**

<b>Treatment at end of test:</b>	Two weeks after treatment plants were cut off at soil level and shoots were oven-dried at 70°C for 48 h.
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### **Biological parameters measured:**

<b>Statistical analyses:</b>	An observation corresponded to the dry shoot biomass of 40 plants per pot. Data were expressed as percentages of their untreated respective controls to standardise comparisons between Col and Ler lines. For each line a non-linear regression was used to describe the response of lines to ALS inhibiting herbicides. Following Kudsk and Streibig (1993) <sup>7</sup> , we used the equation given below and fitted the dose-response curve using SYSTAT <sup>8</sup> . An F-test (P = 0.05) was used to test significant differences of the regression parameters. Bonferroni's correction was applied to adjust the observed significance level for the fact that multiple comparisons were made (Scherrer, 1984) <sup>9</sup> . Comparisons of ED50 values among herbicides were carried out by examining the overlap between the 95% Wald's confidence limits. Wilcoxon's signed-rank test was then performed to test the effect of the Col or Ler genetic background of the S line on the ED50 (Scherrer, 1984).
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## **Results:**

### **1 Biological findings:**

**Baseline toxicity:** For each susceptible line the herbicide application rates were sufficient to establish the dose-response curve. ED<sub>50</sub> was used to characterize the baseline toxicity of the ALS-inhibiting herbicides studied for *A. thaliana*. Results for foramsulfuron are shown in Table B.9.6.1.1.5-1.

<sup>7</sup> Kudsk P & Streibig JC (1993) Formulations and adjuvants. In: Herbicide Bioassays (eds JC Streibig & P Kudsk), 99–116. CRC Press, Boca Raton, FL, USA.

<sup>8</sup> SYSTAT 10 (2000) SYSTAT, Release 10 for Windows. SPSS, Chicago, IL, USA.

<sup>9</sup> Scherrer B (1984) Biostatistiques (ed. B Scherrer), 593–596. Chicoutimi: Gae'tan Morin Editeur, Quebec, Canada.

**Table B.9.6.1.1.5-1: ED50 for the Col and Ler susceptible lines and resistance ratios (R:S) for the chlorsulfuron-resistant csr1-1 and imazapyr-resistant csr1-2 lines of *Arabidopsis thaliana* treated with 22 ALS-inhibiting herbicides - results for foramsulfuron**

Herbicide	Arabidopsis thaliana				csr1-1 R:S	csr1-2 R:S
	Col		Ler			
	ED50 [mg/ha]	CL* [mg/ha]	ED50 [mg/ha]	CL* [mg/ha]		
Foramsulfuron	333	95-570	506	291-722	2	1

\*CL: 95% Wald confidence limits R = resistant; S = susceptible

Data from 14 species were considered to be suitable for the study of the relationships between ED<sub>50</sub> for *A. thaliana* and other weed species. Foramsulfuron was not included in the comparison.

**Cross-resistance:** A cross-resistance pattern could be directly assessed by the inhibition of ALS enzyme activity. Here, the cross-resistance pattern on the 22 ALS-inhibiting herbicides, including foramsulfuron, used in the study was assessed for the homozygous chlorsulfuron- and imazapyr- resistant lines by recording plant dry matter. The resistance ratios for the csr1-1 and csr1-2 lines are indicated in Table B.9.6.1.1.5-1. The csr1-2 imazapyr-resistant line conferred little or no resistance to some sulfonylurea herbicides, including foramsulfuron (R:S ratio < 5). The same was observed for the csr1-1 chlorsulfuron-resistant line.

- **Conclusion:** ED50 values (dry shoot biomass) for the Col and Ler susceptible lines of *Arabidopsis thaliana* were 333 mg/ha and 506 mg/ha, respectively.

**Comment (Co-RMS and RMS):** No comments, study brings supplementary information but the results cannot be used in the risk assessment because of: 1).The test was conducted with strains which were susceptible to ALS-inhibitors and not to naturally occurring phenotypes of *A. thaliana*. 2).As far as described in the paper the test method used does not fully apply to OECD 2017. Especially the plant density (40 plants in a 1 L pot) was exceptionally high.

**B.9.7. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)**

For foramsulfuron a screening study on entomology species has been performed. Details of the study are provided in the Table B.9.7.2-1.

**Effectivity of the herbicide AE F130360 on entomology screening species**

<b>Report:</b>	<u>KCA 8.7/01:Thoenessen, M. T.:2000;M-194770-01</u>
<b>Title:</b>	Effectivity of the herbicide AE F130360 on entomology screening species
<b>Report No:</b>	C006863
<b>Document No:</b>	<u>M-194770-01-1</u>
<b>Guidelines:</b>	Deviation not specified
<b>GLP/GEP:</b>	no

- ❑ **Conclusion:** The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).

**Table B.9.7.2-1: Effect data of a straight foramsulfuron WG50 to entomology screening species**

Test design	Test species	Ecotoxicological endpoint	Reference (see IIA, Point 8)
<b>Foramsulfuron, formulated as WG 50</b>			
Root systemicity test, different treated stages (eggs, larvae, all stages), 6 d	<i>Spodoptera littoralis</i> , <i>Heliothis virescens</i> , <i>Apis fabae</i> , <i>Nilaparvata lugens</i> , <i>Diabrotica undecimpunctata</i> , <i>Meloidogyne incognita</i> , <i>Tetranychus urticae</i> , <i>Aphis fabae</i> (root systemic activity)	The test item is not effective on any tested species.  Most sensitive species: <i>Meoidogyne incognita</i> (larvae)	Thoenessen, 2000 <u>M-194770-01-1</u> KCA 8.7 /01

- ❑ **Comment (Co-RMS and RMS):** No comments, study brings supplementary information.



### B.9.8. EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT

#### B.9.8.1. Effects on biological methods for sewage treatment

For foramsulfuron, one study with activated sludge has been conducted. This study was evaluated within the process of Annex I inclusion and was considered acceptable by the RMS Germany. This study was not re-evaluated since the study was performed according to the OECD test guideline No. 209: Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation). Details of the study are provided in the Table B.9.8.1-1 briefly.

<b>Report:</b>	KCA 8.8 /01;Reinhardt, J.;1997;M-142587-01
<b>Title:</b>	Testing the respiration inhibition of activated sludge: Bacteria toxicity. Test substance: AE F130360, substance technical
<b>Report No:</b>	A58873
<b>Document No:</b>	M-142587-01-1
<b>Guidelines:</b>	EU (=EEC): 88/302 part C; ISO: 8192; OECD: 209; Deviation: not specified
<b>GLP/GEP:</b>	yes

- **Results:** Details of the results are provided in the following table.

**Table B.9.8.1-1: Effect data of foramsulfuron to activated sludge presented in this chapter**

Test species	Test design	Ecotoxicological endpoint	Reference
Foramsulfuron			
Activated sludge	Respiration inhibition, 3 h, static (OECD 209)	Activated sludge, inhibition of respiratory activity : EC <sub>20</sub> > 625.0 mg/L EC <sub>50</sub> > 625.0 mg/L EC <sub>80</sub> > 625.0 mg/L	Reinhardt, 1997 M-142587-01-1 KCA 8.8. /01

- **Conclusion:** Activated sludge, inhibition of respiratory activity: EC<sub>20</sub> > 625.0 mg/L, EC<sub>50</sub> > 625.0 mg/L, EC<sub>80</sub> > 625.0 mg/L. The endpoint from this study was not mentioned in the Review Report for foramsulfuron (SANCO/ 10324/2002-Final).
- **Comment (Co-RMS and RMS):** No comments, study is acceptable.

### B.9.9. MONITORING DATA

Monitoring data concerning adverse effects of the active substance to non-target organisms are not available.

### B.9.10. BIOLOGICAL ACTIVITY OF METABOLITES POTENTIALLY OCCURRING IN GROUNDWATER

PEC<sub>GW</sub> values of foramsulfuron and its major soil metabolites AE F130619, AE F153745 and AE F092944 were below the groundwater threshold value of 0.1µg/L (see Vol 3, B.8 for PPP: Equip OD 45). It can be concluded that the use of foramsulfuron in accordance with the use patterns is not likely to pose an unacceptable risk to groundwater. Hence, the biological activity of the metabolites needs not to be assessed.

**B.9.11. REFERENCES RELIED ON****Literature search:**

The literature search carried out by the applicant was summarised in Document MCA section 9. The RMS considers the literature search provided as acceptable.

Databases: STN, a scientific information platform hosted by CAS, itself a division of the American Chemical Society, was selected as the preferred provider. Following data bases were used for the literature search: Agricola, Biosis, CABA, Chemical Abstracts, Derwent Drug File (DRUGU), EMBASE, Esbiobase, IPA, Medline, Pascal, PQSciTech, Registry, Scisearch, Toxcenter, Ulidat and FSTA.

Time window: January 1<sup>st</sup> 2004 – August 2<sup>nd</sup> 2013 for the parent compound and metabolites. Input parameters: IUPAC name, CAS number, common name, code and abbreviation, molecular structure, molecular formula, molar mass and/or other names/codes, as far as available.

Results: A total of 430 identified and evaluated for potential relevance for foramsulfuron and its metabolites. Of these, 384 summary records were excluded after a rapid assessment of relevance, and 46 full-text documents were assessed in detail.

As a summary 45 studies were excluded from the risk assessment because the publications did not meet the relevance criteria for the detailed assessment. Moreover, one study was unclear of relevance and only one study from the whole literature search was revealed for further examination (KCA 8.6.2).

A reference list containing these 46 documents were included in Doc MCA section 9.

One reference was identified as relevant for the environment and non-target species (as defined in the EFSA Guidance Document). RMS agrees that other references should not be identified as relevant for the assessment of ecotoxicological effects of foramsulfuron in the environment.

<b>Report:</b>	<a href="#">KCA 8.6.2 /01;Roux, F.; Matejcek, A.; Reboud, X.:2005;M-458576-01</a>
<b>Title:</b>	Response of Arabidopsis thaliana to 22 ALS inhibitors: Baseline toxicity and cross-resistance of csr1-1 and csr1-2 resistant mutants.
<b>Report No:</b>	<a href="#">M-458576-01-1</a>
<b>Document No:</b>	<a href="#">M-458576-01-1</a>
<b>Guidelines:</b>	<b>not applicable; not applicable</b>
<b>GLP/GEP:</b>	<b>no</b>

This study has been evaluated in Volume 3 CA, Point B.9.6.1.1.5. This study was, however, not considered in the risk assessment for the following reasons:

1. The test was conducted with strains which were susceptible to ALS-inhibitors and not to naturally occurring phenotypes of *A. thaliana*.
2. As far as described in the paper the test method used does not fully apply to OECD 2017. Especially the plant density (40 plants in a 1 L pot) was exceptionally high.

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 8.1.1.1 /01	[REDACTED]	1998	Code: Hoe 130360 00 ZC98 0001 - Bobwhite quail acute oral toxicity study [REDACTED] Report No.: A59886, Report includes Trial Nos.: 96.0762 TOX96116 Edition Number: M-143541-01-1 EPA MRID No.: 45109733 Date: 1998-01-05 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y
KCA 8.1.1.1 /02	[REDACTED]	1997	Hoe 130360 (AE F130360); Code: Hoe 130360 00 ZC98 0001 - Mallard duck acute oral toxicity study [REDACTED] [REDACTED] eport No.: A59045, Report includes Trial Nos.: 96.0763 TOX96280 Edition Number: M-142752-01-1 EPA MRID No.: 45109734 Date: 1997-06-25 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y
KCP 10.1.1.1 /01	[REDACTED]	2000	Bobwhite quail acute oral toxicity (LD50) AE F130360 + AE F122006 flowable oil 22.5 + 22.5 g/l Code: AE F130360 01 1K05 A3 [REDACTED] Report No.: C005783, Report includes Trial Nos.: Tox99230 Edition Number: M-192635-01-1 Date: 2000-01-17 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 8.1.1.2 /01	[REDACTED]	1998	Bobwhite quail dietary LC50 study Code: AE F130360 00 1C98 0001 [REDACTED] Report No.: A67441, Report includes Trial Nos.: 96.0781 Tox96117 Edition Number: M-147825-01-1 EPA MRID No.: 45109735 Date: 1998-04-17 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y
KCA 8.1.1.2 /02	[REDACTED]	1998	Mallard duck dietary LC50 study Code: AE F130360 00 1C98 0001 [REDACTED] Report No.: A67442, Report includes Trial Nos.: 96.0780 Tox96118 Edition Number: M-147826-01-1 EPA MRID No.: 45109736 Date: 1998-03-09 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y
KCA 8.1.1.3 /01	[REDACTED]	1999	Northern Bobwhite quail dietary reproduction study AE F130360 Code: AE F130360 00 1C97 0002 [REDACTED] Report No.: C006593, Report includes Trial Nos.: TOX96125 Edition Number: M- 194248-01-1 EPA MRID No.: 45109901 Date: 1999-12-16 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 8.1.1.3 /02	[REDACTED]	1999	Mallard duck dietary reproduction study AE F130360 Code: AE F130360 00 1C97 0002 [REDACTED] Report No.: C006594, Report includes Trial Nos.: TOX96127 Edition Number: M- 194250-01-1 EPA MRID No.: 45109902 Date: 1999-12-16 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y
KCA 5.2.1 /01	[REDACTED]	1997	Hoe 130360 (AE F130360); Code: Hoe 130360 00 ZC98 0001 - Rat acute oral toxicity [REDACTED] Bayer CropScience, Report No.: A58267, Report includes Trial Nos.: TOX96110 Edition Number: M-141959-01-1 EPA MRID No.: 45109433 Date: 1997-01-24 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y
KCP 7.1.1 /01	[REDACTED]	1999	Rat acute oral toxicity AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/l Code: AE F130360 01 1K05 A3 [REDACTED] Report No.: C005915, Report includes Trial Nos.: TOX95126 Edition Number: M-192928-01-1 Date: 1999-11-19 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 5.6.1 /01	[REDACTED]	1999	Rat dietary two-generation reproductive toxicity study AE F130360 Code: AE F130360 00 1C99 0002 [REDACTED] Report No.: C004338, Report includes Trial Nos.: TOX96123 Edition Number: M-187748-01-1 EPA MRID No.: 45109616 Date: 1999-10-22 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y
KCA 5.6.2 /02	[REDACTED]	1997	Rat oral development toxicity (teratogenicity) study Code: Hoe 130360 00 ZC98 0001 [REDACTED] Report No.: A67035, Report includes Trial Nos.: TOX95390 Edition Number: M-147435-01 Date: 1997-12-10 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y



Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 5.6.2 /01	[REDACTED]	1997	Hoe 130360 (AE F130360); Code: Hoe 130360 00 ZC98 0001 - Rabbit oral developmental toxicity (teratogenicity) range finding study [REDACTED] Report No.: A 59486, Report includes Trial Nos.: TOX95391 Edition Number: M-143157-01 Date: 1997-09-30 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y
KCA 5.6.2 /03	[REDACTED]	1997	Code: Hoe 130360 00 ZC98 0001 - Rabbit oral developmental toxicity (teratogenicity) study [REDACTED] Report No.: A 67041, Report includes Trial Nos.: TOX95392 Edition Number: M-147441-01 Date: 1997-12-17 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 5.6.2 /04	[REDACTED]	2000	1st Addendum to Report number TOX/98/262-25 Rabbit oral developmental toxicity (teratogenicity) study: Provision of historical control body weight data as requested by the EU Code: Hoe 130360 00 ZC98 0001 [REDACTED] Report No.: C 010603, Edition Number: M-199311-01	Y	N	Not relevant	Bayer CropScience	Y
KCA 8.2.1 /01	[REDACTED]	1997	96 hour acute toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , in a static renewal system AE F130360 technical 98.6 % w/w Code: AE F130360 00 1C98 0001 [REDACTED] Report No.: A57725, Edition Number: M-141405-02-1 Date: 1997-05-13 ...Amended: 1997-06-05 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y
KCA 8.2.1 /02	[REDACTED]	1997	AE F130360; technical 98.6 percent w/w; Code: AE F130360 00 1C98 0001 - 96 hour acute toxicity to the bluegill sunfish, <i>Lepomis macrochirus</i> , in a static renewal system [REDACTED] Report No.: A57726, Edition Number: M-141406-02-1 Date: 1997-05-13 ...Amended: 1997-06-05 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 8.2.1 /03	[REDACTED]	1998	96 hour acute toxicity to the Sheepshead minnow ( <i>Cyprinodon variegatus</i> ) in a static system AE F130360 technical 94.2% w/w Code: AE F130360 00 1C94 0001 [REDACTED] Report No.: A59901, Edition Number: M-143551-01-1 EPA MRID No.: 45109927 Date: 1998-05-14 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y
KCA 8.2.1 /04	[REDACTED]	1993	Hoe 092944 - substance, technical (Hoe 092944 00 ZD99 0001) Effect to <i>Oncorhynchus mykiss</i> (Rainbow trout) in a Static-Acute Toxicity Test (method OECD) [REDACTED] Bayer CropScience, Report No.: A50396, Edition Number: M-131422-01-1 Date: 1993-04-13 GLP/GEP: yes, unpublished	Y	Y	New data requirement according to Regulations 91/414 and 1107/2009 – address aquatic toxicity of soil metabolite	Bayer CropScience	N
KCA 8.2.2.1 /01	[REDACTED]	1999	Prolonged toxicity to the rainbow trout, <i>Oncorhynchus mykiss</i> , in a flow through system AE F130360 technical 95.8 % w/w Code: AE F130360 00 1C96 0002 [REDACTED] Report No.: C004117, Edition Number: M-187354-01-1 EPA MRID No.: 45109905 Date: 1999-08-16 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 8.2.2.1 /02	[REDACTED]	2004	Early Life Stage Toxicity of Foramsulfuron (AE F130360) Technical to the Fathead Minnow ( <i>Pimephales promelas</i> ) Under Flow- Through Conditions [REDACTED] Report No.: B004606, Report includes Trial Nos.: EBFSX001 (A3841201) Edition Number: M-241508-01-1 Date: 2004-03-17 GLP/GEP: yes, unpublished	Y	Y	New data requirement according to Regulation 1107/2009	Bayer CropScience	N
KCA 8.2.4.1 /01	Stachura, B. J.; Ruff, D. F.	1997	AE F130360; technical 98.4 percent w/w; Code: AE F130360 00 1C98 0001 - The 48 hour acute toxicity to <i>Daphnia magna</i> , in a static renewal system AgrEvo USA Company, Pikeville, NC, USA Report No.: A57724, Edition Number: M-141404-02-1 Date: 1997-05-09 ...Amended: 1997-06-05 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y
KCA 8.2.4.1 /02	Heusel, R.	1993	Hoe 092944 - substance, technical (Hoe 092944 00 ZD99 0001) Effect to <i>Daphnia magna</i> (waterflea) in a Static -Acute Toxicity Test (method OECD) Hoechst AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: A50353, Edition Number: M-131382-01-1 Date: 1993-04-13 GLP/GEP: yes, unpublished	N	Y	New data requirement according to Regulations 91/414 and 1107/2009 – address aquatic toxicity of soil metabolite	Bayer CropScience	N

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 8.2.5.1 /01	Young, B. M.; Ruff, D. F.	1999	Effects on life-cycle of the water flea ( <i>Daphnia magna</i> ) in a static renewal system AE F130360 technical 95.8% w/w AgrEvo USA Company, Ecotoxicology, Pikeville, NC, USA Report No.: B002180, Report includes Trial Nos.: CF99W537 Edition Number: M-237962-01-2 Date: 1999-07-21 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y
KCA 8.2.6.1 /01	Christ, M. T.; Ruff, D. F.	1998	Effect to <i>Pseudokirchneriella subcapitata</i> (green alga) in a growth inhibition test AE F130360 technical 94.2% w/w AgrEvo USA Company, Ecotoxicology, Pikeville, NC, USA Report No.: A59926, Edition Number: M-143574-01-1 EPA MRID No.: 45109908 Date: 1998-06-26 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y
KCA 8.2.6.1 /02	Heusel, R.	1993	Hoe 092944 - substance, technical (Hoe 092944 00 ZD99 0001) Effect to <i>Scenedesmus subspicatus</i> (Green alga) in a Growth Inhibition Test (method OECD) Hoechst AG, Frankfurt am Main, Germany Bayer CropScience, Report No.: A50395, Edition Number: M-131421-01-1 Date: 1993-04-13 GLP/GEP: yes, unpublished	N	Y	New data requirement according to Regulations 91/414 and 1107/2009 – address aquatic toxicity of soil metabolite	Bayer CropScience	N

Data Point	Author(s)	Year	Title Compagny Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCA 8.2.6.1 /03	Dorgerloh, M.	2005	<i>Pseudokirchneriella subcapitata</i> - growth inhibition test with AE F099095 00 1B99 0001 Bayer CropScience, Report No.: EBMMX092, Edition Number: M-254084-01-1 Date: 2005-07-08 GLP/GEP: yes, unpublished	N	Y	To address aquatic toxicity of a new aquatic metabolite	Bayer CropScience	N
KCA 8.2.6.2 /01	Young, B. M.; Ruff, D. F.	1999	Effect to <i>Navicula pelliculosa</i> (freshwater diatom) in a growth inhibition test AE F130360 technical 94.6% w/w Code: AE F130360 00 1C94 0001 AgrEvo USA Company, Ecotoxicology, Pikeville, NC, USA Report No.: C002422, Edition Number: M-184469-01-1 EPA MRID No.: 45109910 Date: 1999-06-07 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y
KCA 8.2.6.2 /02	Christ, M. T.; Ruff, D. F.	1999	Effect to <i>Anabaena flos-aquae</i> (blue-green alga) in a growth inhibition test technical 94.6% w/w Code: AE F130360 00 1C94 0001 AgrEvo USA Company, Ecotoxicology, Pikeville, NC, USA Report No.: C003699, Edition Number: M-186627-01-1 EPA MRID No.: 45109909 Date: 1999-07-01 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y



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KCA 8.2.6.2 /03	Young, B. M.; Ruff, D. F.	1999	Effect to <i>Skeletonema costatum</i> (Marine Diatom) in a growth inhibition test AE F130360 technical 94.6 % w/w Code: AE F130360 00 1C94 0001 AgrEvo USA Company, Ecotoxicology, Pikeville, NC, USA Report No.: C002436, Edition Number: M-184494-01-1 Date: 1999-06-25 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y
KCA 8.2.7 /01	Christ, M. T.; Ruff, D. F.	1998	Effect to <i>Lemna gibba</i> (duckweed), in a growth inhibition test AE F130360 technical 96.1% w/w Code: AE F130360 00 1C96 0002 AgrEvo USA Company, Ecotoxicology, Pikeville, NC, USA Report No.: A67514, Report includes Trial Nos.: CF98W507 Edition Number: M-147891-02-1 EPA MRID No.: 45109911 Date: 1998-08-14 ...Amended: 1999-04-20 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y
KCA 8.2.7 /02	Christ, M. T.; Ruff, D. F.	2000	Effect to <i>Lemna gibba</i> (duckweed) in a growth inhibition test: AE F153745 technical 97.8% w/w Aventis CropScience USA LP, Ecotoxicology, Pikeville, NC, USA Report No.: B002765, Report includes Trial Nos.: CF99W565 Edition Number: M-240924-01-2 Date: 2000-02-11 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y

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KCA 8.2.7 /03	Christ, M. T.; Ruff, D. F.	2000	Effect to <i>Lemna gibba</i> (duckweed) in a growth inhibition test: AE 0338795 technical 90.2 percent w/w: AE 0338795 00 1C90 0001 Aventis CropScience USA LP, Ecotoxicology, Pikeville, NC, USA Report No.: B002774, Report includes Trial Nos.: CF99W566 Edition Number: M-238498-01-2 Date: 2000-02-23 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y
KCA 8.2.7 /04	Sowig, P.; Gildemeister, H.	2000	Effects on growth of rooted aquatic macrophytes ( <i>Valisneria</i> spec.) bound residues of AE F130360 substance, technical Code: AE F130360 00 1C98 0002 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Report No.: C006439, Edition Number: M-193919-01-1 EPA MRID No.: 45109939 Date: 2000-02-17 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y
KCA 8.2.7 /05	Dorgerloh, M.	2005	<i>Lemna gibba</i> G3 Exposure and recovery test with Foramsulfuron (tech.) (code: AE F130360 00 1D97 0001) BCS, Report No.: EBFSX010, Edition Number: M-250268-01-1 Date: 2005-04-26 GLP/GEP: yes, unpublished	N	Y	Study performed to refine risk assessment for aquatic plants ( <i>Lemna</i> )	Bayer CropScience	N

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KCA 8.2.7 /06	Bruns, E.	2013	<i>Lemna gibba</i> G3 - Growth inhibition test with foramsulfuron (tech) (AE F 130360) under peak exposure conditions Bayer CropScience, Report No.: EBFSN003, Edition Number: M-462569-01-1 Date: 2013-08-13 GLP/GEP: yes, unpublished	N	Y	Study performed to refine risk assessment for aquatic plants ( <i>Lemna</i> )	Bayer CropScience	N
KCA 8.2.7 /07	Kirkwood, A.	2012	Outdoor growth inhibition and recovery of aquatic plants exposed to foramsulfuron WG 50 percent Smithers Viscient, Wareham, MA, USA Bayer CropScience, Report No.: EBFSL012, Edition Number: M-429538-01-1 EPA MRID No.: 48869701 Date: 2012-04-13 GLP/GEP: yes, unpublished	N	Y	Study performed to refine risk assessment for aquatic plants ( <i>Lemna</i> )	Bayer CropScience	N
KCA 8.2.7 /08	Bruns, E.	2013	<i>Lemna gibba</i> G3 - Prolonged growth inhibition test with foramsulfuron (AE F130360) with stepwise decreasing concentrations over an 6 week test duration Bayer CropScience, Report No.: EBFSL014, Edition Number: M-464150-01-1 Date: 2013-09-10 GLP/GEP: yes, unpublished	N	Y	Study performed to refine risk assessment for aquatic plants ( <i>Lemna</i> )	Bayer CropScience	N

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KCA 8.2.7 /09	Banman, C. S. Alexander, T. M.; Lam, C. V.	2012	Toxicity of foramsulfuron technical to the aquatic macrophyte, <i>Myriophyllum spicatum</i> Bayer CropScience LP, Stilwell, KS, USA Bayer CropScience, Report No.: EBFSL004, Edition Number: M-431270-01-1 Date: 2012-05-17 GLP/GEP: yes, unpublished	Y	Y	New data requirement according to Regulation 1107/2009	Bayer CropScience	N
KCA 8.2.7 /10	Sowig, P.; Weller, O.	2000	Duckweed ( <i>Lemna gibba</i> G3) growth inhibition test AE F092944 (metabolite of ethoxysulfuron and amidosulfuron) substance technical Code: AE F092944 00 1C99 0001 Aventis CropScience GmbH, Frankfurt am Main, Germany Bayer CropScience, Report No.: C003865, Edition Number: M-186916-01-1 Date: 2000-11-03 GLP/GEP: yes, unpublished	N	Y	New data requirement according to Regulation 1107/2009 – address aquatic toxicity of soil metabolite	Bayer CropScience	N
KCA 8.2.7 /11	Dorgerloh, M.	2005	<i>Lemna gibba</i> G3 - growth inhibition test with AE F099095 under static conditions (Code: AE F099095 00 1B99 0001) BCS, Report No.: EBMMX091, Edition Number: M-254496-01-1 Date: 2005-07-14 GLP/GEP: yes, unpublished	N	Y	To address aquatic toxicity of a new aquatic metabolite	BCS	N

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KCA 8.2.7 /12	Bruns, E.	2013	<i>Lemna gibba</i> G3 - Growth inhibition test with with AE F130619 (metabolite of foramsulfuron) under static conditions Bayer CropScience, Report No.: EBFSL011, Edition Number: M-452669-01-1 Date: 2013-04-15 GLP/GEP: yes, unpublished	N	Y	New data requirement according to Regulations 91/414 and 1107/2009 – address aquatic toxicity of soil metabolite	Bayer CropScience	N
KCA 8.2.7 /13	Bruns, E.	2013	<i>Lemna gibba</i> G3 - Growth inhibition test with BCS-CV29520 (metabolite of foramsulfuron) under static conditions Bayer CropScience, Report No.: EBFSN010, Edition Number: M-464163-01-1 Date: 2013-08-29 GLP/GEP: yes, unpublished	N	Y	To address aquatic toxicity of a new aquatic metabolite	Bayer CropScience	N
KCA 8.2.7 /14	Bruns, E.	2013	<i>Lemna gibba</i> G3 - Growth inhibition test with BCS-CW90756 (metabolite of foramsulfuron) under static conditions Bayer CropScience, Report No.: EBFSN011, Edition Number: M-464321-01-1 Date: 2013-08-29 GLP/GEP: yes, unpublished	N	Y	To address aquatic toxicity of a new aquatic metabolite	Bayer CropScience	N
KCA 8.2.7 /15	Hoffmann, K.	2013	<i>Lemna gibba</i> G3 - Growth inhibition test with BCS-AW41401 under static conditions Bayer CropScience, Report No.: EBFSN012, Edition Number: M-464386-01-1 Date: 2013-08-29 GLP/GEP: yes, unpublished	N	Y	To address aquatic toxicity of a new aquatic metabolite	Bayer CropScience	N

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KCA 8.2.8 /01	Boeri, R. L.; Magazu, J. P.; Ward, T. J.	1998	Flow-through mollusc shell deposition test AE F130360 Wilbury Laboratories, Inc., Marblehead, MA, USA Report No.: C000906, Edition Number: M-181443-01-1 EPA MRID No.: 45109929 Date: 1998-11-24 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y
KCA 8.2.8 /02	Stachura, B. J.; Ruff, D. F.	1998	96 hour acute toxicity to the Grass Shrimp, <i>Palaemonetes pugio</i> , in a static system AE F130360 technical 94.2% w/w Code: AE F130360 00 1C94 0001 AgrEvo USA Company, Ecotoxicology, Pikeville, NC, USA Report No.: A59902, Edition Number: M-143552-01-1 EPA MRID No.: 45109928 Date: 1998-05-08 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y
AIIIA- 10.2.1	Hoberg, J.R.	2002	Foramsulfuron Oil Flowable 22,5 g/L Formulation (AE F130360 01 1K05 A304) - Toxicity to Duckweed, <i>Lemna gibba</i> Berichts-Nr.: B003893 GLP: yes unpublished WAT2002-392	N	Y		AVD	Y



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KCP 10.2.1 /01	[REDACTED]	2000	Static renewal toxicity with the rainbow trout, <i>Oncorhynchus mykiss</i> : AE F130360 + AE F122006: AE F130360 01 1K05 A304 [REDACTED] Report No.: B002796, Report includes Trial Nos.: 1889-AG CF00W543 Edition Number: M-238518-01-2 Date: 2000-03-09 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y
KCP 10.2.1 /02	[REDACTED]	2000	Static renewal toxicity with the bluegill sunfish, <i>Lepomis macrochirus</i> : AE F130360 + AE F122006: AE F130360 01 1K05 A304 [REDACTED] Report No.: B002795, Report includes Trial Nos.: 1888-AG CF99W542 Edition Number: M-238517-01-2 Date: 2000-03-09 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y

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KCP 10.2.1 /03	Boeri, R. L.; Ward, T. J.	2000	Growth and reproduction toxicity test with the freshwater alga, <i>Selenastrum capricornutum</i> : AE F130360 + AE F122006: AE F130360 01 1K05 A304 Wilbury Laboratories, Inc., Marblehead, MA, USA Report No.: B002798, Report includes Trial Nos.: 1891-AG CF00W545 Edition Number: M-238520-01-2 Date: 2000-03-06 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y
KCP 10.2.1 /05	Boeri, R.; Wyskiel, D.; Ward, T.	2000	Toxicity to the Duckweed, <i>Lemna gibba</i> : AE F130360 + AE F122006 flowable: AE F130360 01 1K05 Wilbury Laboratories, Inc., Marblehead, MA, USA Bayer CropScience, Report No.: B002845, Report includes Trial Nos.: 1928-AG CF99W571 Edition Number: M-238581-01-1 Date: 2000-06-09 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y

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KCP 10.2.1 /06	Madsen, T. J.; Bussard, J. B.	2000	Toxicity of AE F130360 + AE F122006 + AE F115008, water dispersible granule, 30 + 30 + 2 percent w/w including a methylated rapeseed oil surfactant to duckweed, <i>Lemna gibba</i> G3 determined under static renewal test conditions: AE F130360 02 WG62 A10 ABC Laboratories, Inc., Columbia, MO, USA Bayer CropScience, Report No.: B002838, Report includes Trial Nos.: 45737 Edition Number: M-238567-01-1 Date: 2000-03-30 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y
KCP 10.2.1 /07	Madsen, T. J.; Bussard, J. B.	2000	Toxicity of AE F130360 + AE F122006 + AE F115008, water dispersible granule, 30+ 30 + 2 percent w/w, Code: AE F130360 02 WG62 A104 to duckweed, <i>Lemna gibba</i> G3, determined under static renewal test conditions: AE F130360 02 WG62 A104 Aventis CropScience USA LP, Ecotoxicology, Pikeville, NC, USA Bayer CropScience, Report No.: B002810, Report includes Trial Nos.: CF99W572 Edition Number: M-238536-01-1 Date: 2000-03-10 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y

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KCP 10.2.1 /08	Hoberg, J. R.	2002	Foramsulfuron Oil Flowable 22.5 g/L Formulation (AE F130360 01 1K05 A304) - Toxicity To Duckweed, <i>Lemna gibba</i> Springborn Smithers Laboratories, Snow Camp, NC, USA Report No.: B003893, Report includes Trial Nos.: 13726.6166 Edition Number: M-240877-01-1 Date: 2002-05-21 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y
KCP 10.2.1 /09	Banman, C. S.; Hoffmann, J. M.; Lam, C. V.	2008	Toxicity of foramsulfuron + isoxadifen-ethyl OD 22.5+22.5 g/L (AE F130360 01 1K05 A9) to duckweed ( <i>Lemna gibba</i> G3) under static- renewal conditions Bayer CropScience LP, Stilwell, KS, USA BCS, Report No.: EBFSX011, Edition Number: M-296352-01-1 Date: 2008-01-14 GLP/GEP: yes, unpublished	N	Y	Test on sensitive species	BCS	N
KCP 10.2.2 /01	[REDACTED]	2000	Prolonged toxicity to the rainbow trout , <i>Oncorhynchus mykiss</i> , in a flow through system: AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L: AE F130360 01 1K05 A304 [REDACTED] Report No.: B002764, Report includes Trial Nos.: CF99W541 Edition Number: M-238492-01-2 Date: 2000-02-16 GLP/GEP: yes, unpublished	Y	N	Not relevant	Bayer CropScience	Y

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KCP 10.2.2 /02	Young, B. M.; Ruff, D. F.	2000	Effects on life-cycle of the water flea ( <i>Daphnia magna</i> ) in a static renewal system: AE F130360 + AE F122006, oil flowable 22.5 + 22.5 g/L : AE F130360 01 1K05 A304 Aventis CropScience USA LP, Ecotoxicology, Pikeville, NC, USA Report No.: B002760, Report includes Trial Nos.: CF99W540 Edition Number: M-238488-01-2 Date: 2000-02-16 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y
KCP 10.2 /01	Vrbka, L.	2013	Foramsulfuron (FSN) and metabolite: PECsw,sed FOCUS EUR (graphical outputs) - Use in maize in Europe Bayer CropScience, Report No.: EnSa-13-0880, Edition Number: M-468841-02-1 Date: 2013-11-05 ...Amended: 2013-11-18 GLP/GEP: no, unpublished	N	N	Not relevant	Bayer CropScience	N
KCA 8.3.1.1.1 /01	Waltersdorfer A.	1998	AE130360 00 1C98 0001 Sustance technical Oral toxicity (LD 50) to honey bees ( <i>Apis mellifera</i> L.). Hoechst Schering AgrEvo GmbH, Umweltforschung Oekobiologie, D-65926 Frankfurt am Main, Germany Report No. CW96/031 GLP, unpublished Bayer file No. M-143626-01-1	N	N	Not relevant	Bayer Crop Science	Y

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KCA 8.3.1.1.2 /01	Waltersdorfer A.	1997	AE130360 00 1C98 0001 Sustance technical Contact toxicity (LD 50) to honey bees ( <i>Apis mellifera</i> L.). Hoechst Schering AgrEvo GmbH, Umweltforschung Oekobiologie, D-65926 Frankfurt am Main, Germany Report No. CW96/130 GLP, unpublished Bayer file No. M-143215-01-1	N	N	Not relevant	Bayer Crop Science	Y
KCA 8.3.1.1.2 /02	Schmitzer S.; Sekine T.	2012	Effects of foramsulfuron tech. (acute contact and oral) on honey bees ( <i>Apis mellifera</i> L.) in the laboratory IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany Report No. EBFSN009 GLP, unpublished Bayer File No: M-444765-01-1	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009 and EFSA draft guidance document on the risk assessment of plant protection products on bees	Bayer Crop Science	N
KCP 10.3.1.1.1 /01	Waltersdorfer A.	1999	Oral toxicity (LD50) to honey bees ( <i>Apis mellifera</i> L.) AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L Hoechst Schering AgrEvo GmbH, Umweltforschung Oekobiologie, D-65926 Frankfurt am Main, Germany Report No. CW98/111 GLP, unpublished Bayer file No.: M-187295-01-1	N	N	Not relevant	Bayer Crop Science AG	Y



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KCP 10.3.1.1.2 /01	Waltersdorfer A.	1999	Contact toxicity (LD50) to honey bees ( <i>Apis mellifera</i> L.) AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L Hoechst Schering AgrEvo GmbH, Umweltforschung Oekobiologie, D-65926 Frankfurt am Main, Germany Report No. CW98/109 GLP, unpublished Bayer file No.: M-187293-01-1	N	N	Not relevant	Bayer Crop Science AG	Y
KCP 10.3.1.1.1 /02	Sekine T.	2013	Effects of foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G (acute contact and oral) on honey bees ( <i>Apis mellifera</i> L.) in the laboratory. IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany Report No. EBFSN048 GLP, unpublished Bayer File No: M-465361-01-1	N	Y	New data requirement	Bayer Crop Science AG	N
KCA 8.3.1.2 /01	Kling A.	2013	Foramsulfuron WG 50 W - Assessment of chronic effects to the honeybee, <i>Apis mellifera</i> L., in a 10 days continuous laboratory feeding limit test EurofinsAgroscience Services, EcoChem GmbH, Eutinger Straße 24, 75223 Niefern- Öschelbronn, Germany Report No. EBFSN022 GLP, unpublished Bayer File No: M-470639-01-1	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009 and EFSA draft guidance document on the risk assessment of plant protection products on bees	Bayer Crop Science	N

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KCA 8.3.1.3 /01	Przygoda D.; Nikolakis A.	2013	Foramsulfuron WG 50 W: Effects of a single exposure to spiked diet on honey bee larvae ( <i>Apis mellifera carnica</i> ) in an in vitro laboratory testing design Bayer CropScience AG, BCS-AG-D-EnSa-Testing, 40789 Monheim, Germany Report No. EBFSN044 GLP, unpublished Bayer File No: M-470485-01-1	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009 and EFSA draft guidance document on the risk assessment of plant protection products on bees	Bayer Crop Science	N
KCA 8.3.1.3/02	Jeker L.	2013	Foramsulfuron WG 50 W - honeybee brood feeding study to evaluate potential effects on brood development and mortality of the honeybee, <i>Apis mellifera</i> L. (Hymenoptera: Apidae) Innovative Environmental Services (IES) Ltd, Benkenstrasse 260, 4108 Witterswil, Switzerland Report No. EBFSL013 GLP, unpublished Bayer File No: M-465326-01-1	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009 and EFSA draft guidance document on the risk assessment of plant protection products on bees	Bayer Crop Science	N
KCA 8.3.1.3 /03	Schmitzer S.	2013	Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L): Effects on honey bee brood ( <i>Apis mellifera</i> L.) under semi-field conditions - Tunnel test – IBACON GmbH, Arheilger Weg 17, 64380 Rossdorf, Germany Report No. EBFSN034 GLP, unpublished Bayer File No: M-468794-01-1	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009 and EFSA draft guidance document on the risk assessment of plant protection products on bees	Bayer Crop Science	N

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KCP 10.3.2.1./02	Waltersdorfer A.	1999	Toxicity to the predatory mite <i>Typhlodromus pyri</i> Scheuten (Acari, Phytoseiidae) in the laboratory AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L AE F130360 01 1K05 A301 Hoechst Schering AgrEvo GmbH, Umweltforschung Oekobiologie, D-65926 Frankfurt am Main, Germany Report No. CW99/003 GLP, unpublished Bayer file No.: M-191384-01-1	N	N	Not relevant	Bayer Crop Science	Y
KCP 10.3.2.2./01	Waltersdorfer A.	1999	Toxicity to the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN (Acari, Phytoseiidae) using an extended laboratory test AEF130360+ AEF122006 Oil flowable 22.5 + 22.5 g/L Hoechst Schering AgrEvo GmbH, Umweltforschung Oekobiologie, D-65926 Frankfurt am Main, Germany Report No. CW99/092 GLP, unpublished Bayer file No.: M-192822-01-1	N	N	Not relevant	Bayer Crop Science	Y
KCA 8.3.2.2 /03	Roehlig U.	2013	Effects of foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) on the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN in a laboratory test BioChem agrar, Labor für biologische und chemische Analytik GmbH Kupferstraße 604827 Gerichshain, Germany Report No. 13 10 48 031 A GLP, unpublished Bayer file No.: M-457360-01-1	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer Crop Science	N

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KCP 10.3.2.1./01	Kleiner R.	1999	Toxicity to the <i>parasitoid</i> <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) / adults under laboratory conditions according to IOBC Guidelines (MEAD-BRIGGS 1992/1997) AE F130360 01 1K05 A304 using an extended laboratory test AEF130360+ AEF122006 Oil flowable 22.5 + 22.5 g/L BioChem agrar, Labor für biologische und chemische Analytik GmbH Kupferstraße 604827 Gerichshain, Germany Report No. 991048029 GLP, unpublished Bayer file No.: M-191908-01-1	N	N	Not relevant	Bayer Crop Science	Y
KCP 10.3.2.2./02	Barth M.	2000	Toxicity of AE F130360 01 1K05 A304 to the cereal aphid parasitoid <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) (extended laboratory test/"aged residue test") to the <i>parasitoid</i> <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) / adults under laboratory conditions according to IOBC Guidelines (MEAD-BRIGGS 1992/1997) AE F130360 01 1K05 A304 using an extended laboratory test AEF130360+ AEF122006 Oil flowable 22.5 + 22.5 g/ BioChem agrar, Labor für biologische und chemische Analytik GmbH Kupferstraße 604827 Gerichshain, Germany Report No. 1048067 GLP, unpublished Bayer file No.: M-198973-01-1	N	N	Not relevant	Bayer Crop Science	Y

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KCA 8.3.2.1./03	Roehlig U.	2013	Effects of foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5 g/L) on the parasitic wasp <i>Aphidius rhopalosiphi</i> (DESTEFANI-PEREZ) in a laboratory BioChem agrar, Labor für biologische und chemische Analytik GmbH Kupferstraße 604827 Gerichshain, Germany Report No. 131048030A GLP, unpublished Bayer file No.: M-461455-01- 1	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer Crop Science	N
KCA 8.3.2./04	Kleiner R.	2000	Toxicity to the foliage dwelling predator <i>Chrysoperla carnea</i> STEPH. (laboratory) following the IOBC Guideline (BiGLER 1988), ringtest method (VOGT <i>et al.</i> 1997) and OECD Guideline proposal (VOGT <i>et al.</i> 1999) AE F130360 01 1K05 A304 BioChem agrar, Labor für biologische und chemische Analytik GmbH Kupferstraße 604827 Gerichshain, Germany Report No. 991048098 GLP, unpublished Bayer file No.: M-194627-01- 1	N	N	Not relevant	Bayer Crop Science	Y
KCA 8.3.2.1./04	Waltersdorfer A.	1999	Toxicity to the ground dwelling predator <i>Poecilus cupreus</i> L. (Coleoptera, Carabidae) in the laboratory AEF130360+ EF122006 Oil flowable 22.5 + 22.5 g/L Hoechst Schering AgrEvo GmbH, Umweltforschung Oekobiologie, D-65926 Frankfurt am Main, Germany Report No. CW98/112 GLP, unpublished Bayer file No.: M-186968-01-1	N	N	Not relevant	Bayer Crop Science	Y

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KCA 8.3.2./04	Kleiner R.	2000	Toxicity to the ground dwelling predator <i>Aleochara bilineata</i> GYLL. (laboratory) according to IOBC Guideline (MORETH & NATON 1992) AE F130360 01 1K05 A304 BioChem agrar, Labor für biologische und chemische Analytik GmbH Kupferstraße 604827 Gerichshain, Germany Report No. 991048095 GLP, unpublished Bayer file No.: M-193482-01- 1	N	N	Not relevant	Bayer Crop Science	Y
KCA 8.3.2./01	Kleiner R.	1999	Toxicity to the ground dwelling predator <i>Pardosa</i> spp. (laboratory) according to IOBC Guideline (WEHLING <i>et al.</i> 1998) AE F130360 01 1K05 A304 BioChem agrar, Labor für biologische und chemische Analytik GmbH Kupferstraße 604827 Gerichshain, Germany Report No. 991048030 GLP, unpublished Bayer file No.: M-188675-01- 1	N	N	Not relevant	Bayer Crop Science	Y
KCA 8.4 /01	Heusel, R.	1998	Acute toxicity to earthworms ( <i>Eisenia fetida</i> ) AE F130360 substance, technical Code: AE F130360 00 1C98 0002 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Report No.: A59245, Edition Number: M-142934-01-1 EPA MRID No.: 45109923 Date: 1998-04-03 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y



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KCA 8.4 /02	Sowig, P.; Gosch, H.	1999	Acute toxicity to earthworms ( <i>Eisenia fetida</i> ) AE F153745 (impurity of AE F130360) substance, technical Code: AE F153745 00 1C98 0001 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Report No.: C005859, Edition Number: M-192813-01-1 EPA MRID No.: 45109924 Date: 1999-12-17 GLP/GEP: yes, unpublished	N	Y	Study submitted to provide complete view on available data set	Bayer CropScience	Y
KCP 10.4.1 /01	Nienstedt, K. M.	1999	A 14-day acute toxicity test with the earthworm ( <i>Eisenia fetida</i> ) Code: AE F130360 01 1K05 A304 Springborn Laboratories (Europe) AG, Horn, Switzerland Report No.: C006356, Edition Number: M-193746-01-1 Date: 1999-12-07 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y
KCA 8.4.1 /01	Sowig, P.; Gosch, H.	2000	Effects on growth and reproduction of earthworms ( <i>Eisenia fetida</i> ) AE F130360 substance, technical Code: AE F130360 00 1C98 0002 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Report No.: C006218, Edition Number: M-193508-01-1 Date: 2000-01-28 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer CropScience	Y
KCA 8.4.1 /02	Kratz, M. A.	2013	AE F092944 (BCS-AA25052): Effects on survival, growth and reproduction of the earthworm <i>Eisenia fetida</i> tested in artificial soil Bayer CropScience, Report No.: kra/Rg-R-147/13, Edition Number: M-461051-01-1 Date: 2013-07-31 GLP/GEP: yes, unpublished	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer CropScience	N

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KCA 8.4.1 /03	Kratz, M. A.	2013	AE F130619 (BCS-AU59648): Effects on survival, growth and reproduction of the earthworm <i>Eisenia fetida</i> tested in artificial soil Bayer CropScience, Report No.: kra/Rg-R-138/13, Edition Number: M-461453-01-1 Date: 2013-08-14 GLP/GEP: yes, unpublished	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer CropScience	N
KCA 8.4.1 /04	Kratz, M. A.	2013	Foramsulfuron-AE F153745 (BCS-AU80017): Effects on survival, growth and reproduction on the earthworm <i>Eisenia fetida</i> tested in artificial soil Bayer CropScience, Report No.: kra/Rg-R-140/13, Edition Number: M-459518-01-1 Date: 2013-07-17 GLP/GEP: yes, unpublished	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer CropScience	N
KCP 10.4.1.1 /01	Witte, B.	2013	Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G: Effects on reproduction and growth of earthworms <i>Eisenia fetida</i> in artificial soil IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 83352022, Edition Number: M-464888-01-1 Date: 2013-08-21 GLP/GEP: yes, unpublished	N	Y	New data requirement	Bayer CropScience	N
KCA 8.4.2.1 /01	Kratz, M. A.	2012	Foramsulfuron (AE F130360) a.s.: Influence on mortality and reproduction on the soil mite species <i>Hypoaspis aculeifer</i> tested in artificial soil Bayer CropScience, Report No.: KRA-HR-78/12, Edition Number: M-443308-01-1 Date: 2012-12-10 GLP/GEP: yes, unpublished	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer CropScience	<u>N</u>

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KCA 8.4.2.1 /02	Frommholz, U.	2012	Foramsulfuron (AE F130360) a.s.: Influence on the reproduction of the collembolan species <i>Folsomia candida</i> tested in artificial soil Bayer CropScience, Report No.: FRM-Coll-147/12, Edition Number: M-443369-01-1 Date: 2012-12-12 GLP/GEP: yes, unpublished	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer CropScience	N
KCA 8.4.2.1 /03	Schulz, L.	2013	AE F092944 (BCS-AA25052): Effects on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> BioChem agrar, Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Bayer CropScience, Report No.: 13 10 48 044 S, Edition Number: M-454043-01-1 Date: 2013-05-02 GLP/GEP: yes, unpublished	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer CropScience	N
KCA 8.4.2.1 /04	Friedrich, S.	2013	AE F092944 (BCS-AA25052): Effects on the reproduction of the collembolan <i>Folsomia candida</i> BioChem agrar, Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Bayer CropScience, Report No.: 13 10 48 045 S, Edition Number: M-451142-01-1 Date: 2013-03-28 GLP/GEP: yes, unpublished	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer CropScience	N

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KCA 8.4.2.1 /05	Schulz, L.	2013	Foramsulfuron-AE F130619 (BCS-AU59648): Effects on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> BioChem agrar, Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Bayer CropScience, Report No.: 13 10 48 046 S, Edition Number: M-454051-01-1 Date: 2013-05-02 GLP/GEP: yes, unpublished	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer CropScience	N
KCA 8.4.2.1 /06	Friedrich, S.	2013	Foramsulfuron-AE F130619 (BCS-AU59648): Effects on the reproduction of the collembolan <i>Folsomia candida</i> BioChem agrar, Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Bayer CropScience, Report No.: 13 10 48 047 S, Edition Number: M-450824-01-1 Date: 2013-03-28 GLP/GEP: yes, unpublished	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer CropScience	N
KCA 8.4.2.1 /07	Schulz, L.	2013	Foramsulfuron-AE F153745 (BCS-AU80017): Effects on the reproduction of the predatory mite <i>Hypoaspis aculeifer</i> BioChem agrar GmbH, Gerichshain, Germany Bayer CropScience, Report No.: 13 10 48 048 S, Edition Number: M-447606-01-1 Date: 2013-02-22 GLP/GEP: yes, unpublished	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer CropScience	N

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KCA 8.4.2.1 /08	Friedrich, S.	2013	Foramsulfuron-AE F153745 (BCS-AU80017): Effects on the reproduction of the collembolan <i>Folsomia candida</i> BioChem agrar, Labor fuer biologische und chemische Analytik GmbH, Gerichshain, Germany Bayer CropScience, Report No.: 13 10 48 049 S, Edition Number: M-450830-01-1 Date: 2013-03-28 GLP/GEP: yes, unpublished	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer CropScience	N
KCP 10.4.2.1 /01	Witte, B.	2013	Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G (FSN+IDF OD 45 (22.5+22.5) G): Effects on reproduction of the collembola <i>Folsomia candida</i> in artificial soil IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 83353016, Edition Number: M-462827-01-1 Date: 2013-07-23 GLP/GEP: yes, unpublished	N	Y	New data requirement	Bayer CropScience	N
KCP 10.4.2.1 /02	Witte, B.	2013	Foramsulfuron + isoxadifen-ethyl OD 45 (22.5+22.5) G (FSN+IDF OD 45 (22.5+22.5) G): Effects on reproduction of the predatory mite <i>Hypoaspis aculeifer</i> in artificial soil IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 83351089, Edition Number: <b>M-462835-01-1</b> Date: 2013-07-23 GLP/GEP: yes, unpublished	N	Y	New data requirement	Bayer CropScience	N

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KCA 8.5 /01	Heusel, R.	1997	AE F130360; substance, technical; Code: AE F130360 00 1C98 0002 - Effects on soil microbial activity (nitrogen turn-over) Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Report No.: CE97/006 GLP/GEP: yes, unpublished Bayer file No.: M-142972-01-1	N	N	Not relevant	Bayer Crop Science	Y
KCA 8.5 /02	Sowig, P.; Gildemeister, H.	2000	Effects on soil microbial activity (nitrogen turn-over) bound residues of AE F130360 substance, technical Code: AE F130360 00 1C98 0002 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Report No.: CE99/102 GLP/GEP: yes, unpublished Bayer file No.: M-193916-01-1	N	N	Not relevant	Bayer Crop Science	Y
KCA 8.5 /03	Heusel, R.	1998	AE F130360; substance, technical; Code: AE F130360 00 1C98 0002 - Effects on soil microbial activity (short-term respiration) Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Report No.: CE97/005 GLP/GEP: yes, unpublished Bayer file No.: M-142971-01-1	N	N	Not relevant	Bayer Crop Science	Y
KCA 8.5 /04	Sowig, P.; Gildemeister, H.	2000	Effects on soil microbial activity (short-term respiration) bound residues of AE F130360 substance, technical Code: AE F130360 00 1C98 0002 Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Report No.: CE99/101 GLP/GEP: yes, unpublished Bayer file No.: M-193914-01-1	N	N	Not relevant	Bayer Crop Science	Y



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KCA 8.5 /05	Schulz, L.	2013	AE F092944 (BCS-AA25052): Effects on the activity of soil microflora (Nitrogen transformation test) BioChem Agrar GmbH, Gerichshain, Germany Bayer CropScience, Report No.: 13 10 48 018 N, GLP/GEP: yes, unpublished Bayer file No.: M-453511-01-1	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer Crop Science	N
KCA 8.5 /06	Schulz, L.	2013	Foramsulfuron-AE F130619 (BCS-AU59648): Effects on the activity of soil microflora (nitrogen transformation test) BioChem Agrar GmbH, Gerichshain, Germany Bayer CropScience, Report No.: 13 10 48 019 N GLP/GEP: yes, unpublished Bayer file No.: M-453568-01-1	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer Crop Science	N
KCA 8.5 /07	Schulz, L.	2013	Foramsulfuron-AE F153745 (BCS-AU80017): Effects on the activity of soil microflora (Nitrogen transformation test) BioChem Agrar GmbH, Gerichshain, Germany Bayer CropScience, Report No.: 1321048020N GLP/GEP: yes, unpublished Bayer file No.: M-453508-01-1	N	Y	Study conducted to fulfil new data requirement acc. 1107/2009	Bayer Crop Science	N

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KCP 10.5 /01	van der Kolk, J.	1999	The effects on the respiration and nitrification of soil microflora Code: AE F130360 01 1K05 A304 Springborn Laboratories (Europe) AG, Horn, Switzerland Report No.: C006355 GLP/GEP: yes, unpublished Bayer file No.: : M-193742-01-1	N	N	Not relevant	Bayer Crop Science	Y
KCA 8.6.1 /01	Bieringer, H	1999	Effectivity of the herbicide AE F130360 on higher plant species as applied under greenhouse conditions Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Report No.: C005291, Edition Number: M-191762-01-1 EPA MRID No.: 45109401 GLP/GEP: no, unpublished ...also filed: KCA 3.3 /01	N	N	Not relevant	Bayer Crop Science	Y
KCP 10.6.2 /01	Porch, J. R.; Kendall, T. Z.; Krueger, H. O	1999	AE F130360 + AE F122006; oil flowable; 22.5 + 22.5 g/l. CODE: AE F130360 01 1K05 A304: A toxicity test to determine the effects of the test substance on seedling emergence of ten species of plants Wildlife International Limited, 8598 Commerce Drive, Easton, Maryland 21601, USA, Report No: B002673 Edition Number: M-238408-01-2, Date: 1999-11-22 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer Crop Science	Y

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KCP 10.6.2 /01	Porch, J. R.; Kendall, T. Z.; Krueger, H. O.	1999	A Tier II toxicity test to determine the effects of the test substance on vegetative vigor of ten species of plants: AE F130360 + AE F122006, oil flowable, 22.5+22.5 g/L Wildlife International, Ltd., Easton, MD, USA Report No.: B002710, Report includes Trial Nos.: 312-122 Edition Number: M-238444-01-2 Date: 1999-12-08 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer Crop Science	Y
KCP 10.6.2 /02	Porch, J. R.; Kendall, T. Z.; Krueger, H. O.	2000	A tier II toxicity test to determine the effects of the test substance on seedling emergence of seven species of plants: AE F130360 + AE F122006 oil flowable 22.5 + 22.5 g/L: AE F130360 01 1K05 A304 Wildlife International, Ltd., Easton, MD, USA Report No.: B002819, Report includes Trial Nos.: 312-123 CF00E582 Edition Number: M-238550-01-1 EPA MRID No.: 45109933 Date: 2000-03-08 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer Crop Science	Y

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KCA 8.6.2 /01	Roux, F.; Matejicek, A.; Reboud, X.	2005	Response of Arabidopsis thaliana to 22 ALS inhibitors: Baseline toxicity and cross-resistance of csr1-1 and csr1-2 resistant mutants. Journal: Weed Res., Volume:45, Issue:3, Pages:220-227, Year:2005, Report No.: M-458576-01-1, Edition Number: M-458576-01-1 Date: 2005-12-31 GLP/GEP: no, published	N	N	Not relevant	Public	N
KCA 8.7 /01 KCP 10.3.2.1 /07	Thoenessen, M. T.	2000	Effectivity of the herbicide AE F130360 on entomology screening species Hoechst Schering AgrEvo GmbH, Frankfurt am Main, Germany Report No.: C006863, Edition Number: M-194770-01-1 EPA MRID No.: 45109935 GLP/GEP: no, unpublished	N	N	Not relevant	Bayer Crop Science	N
KCA 8.8 /01	Reinhardt, J.	1997	Testing the respiration inhibition of activated sludge: Bacteria toxicity. Test substance: AE F130360, substance technical Hoechst AG, Frankfurt am Main, Germany Report No.: A58873, Edition Number: M-142587-01-1 EPA MRID No.: 45109936 Date: 1997-05-07 GLP/GEP: yes, unpublished	N	N	Not relevant	Bayer Crop Science	N