

## **ANNEX B**

### **Florasulam**

#### **B.8 Ecotoxicology**



### **B.8.1 Effects on birds (Annex IIA 8.1; Annex IIIA 10.1)**

#### **B.8.1.1 Acute oral toxicity (Annex IIA 8.1.1)**

##### **XDE-570 An acute oral toxicity study with the Japanese quail (Campbell, et al., 1994)**

###### Guidelines :

**FIFRA Guideline No. 71-1, as recommended in SETAC**

###### GLP :

Yes

###### Material and Methods :

*Test Substance:* florasulam, purity : 92.2%

*Test species :* Japanese quail (*Coturnix coturnix japonica*)

*Sex, weight, age :* 5 males and 5 females /test group, \_ : 154-188 g ; \_ : 150-178 g, 8 week old

*Applied concentrations :* untreated control, 175, 292, 486, 810, 1350, 2250 mg a.s. /kg body weight;

*Type of application :* by gavage in corn oil

*Time of exposure :* one single application, monitoring during 14 days

###### Findings :

*Mortality :* at doses  $\geq$  810 mg/kg

*Body weight :* no body weight change

*Clinical signs :* signs of toxicity at doses 295 to 2250 mg/kg.

*Feed consumption :* slight reduction at 1350 mg/kg

###### Conclusions :

LD<sub>50</sub> = 1046 mg a.s./kg bw

NOEL = 175 mg a.s./kg bw

#### **B.8.1.2 Avian dietary toxicity (5 day) (Annex IIA 8.1.2)**

##### **XDE-570 herbicide: 8-day acute dietary LC<sub>50</sub> study in mallard ducklings (Helsten, et al.,1994a)**

###### Guidelines :

FIFRA Guideline No. 71-2  $\equiv$  OECD Guideline No. 205

###### GLP :

Yes

###### Material and Methods :

*Test Substance:* florasulam, purity : 92.2%

*Test species :* Mallard (*Anas platyrhynchos*)

*Sex, weight, age :* 10 birds/test group, sex unspecified, mean weight 115-118 g, 11 days old

*Applied concentrations :* untreated control, 312, 625, 1250, 2500, 5000 mg/kg feed;

*Type of application :* a.s. mixed in the feed; stability of the a.s. in diet at ambient temperature over 7 days (99.0-107.7% of the nominal concentration at day 1) was determined.

*Time of exposure :* Short-term feeding test (5 days with exposition by the feed + 3 days observation)

###### Findings :

*Mortality :* none

*Body weight - Feed consumption :* No treatment related change

*Clinical signs - gross pathology :* none

###### Conclusions :

LC<sub>50</sub> (5d) > 5000 mg a.s./kg feed

NOEC (5d) = 5000 mg a.s./kg feed

##### **XDE-570 herbicide: 8-day acute dietary LC<sub>50</sub> study in Japanese quail (Helsten,1994b)**

Guidelines :

FIFRA Guideline No. 71-2 = OECD Guideline No. 205

GLP :

Yes

Material and Methods :

*Test Substance:* florasulam, purity : 92.2%

*Test species :* Japanese quail (*Coturnix coturnix japonica*)

*Sex, weight, age :* 10 birds/test group, sex unspecified, mean weight 55.29-58.79 g, 11 days old

*Applied concentrations :* untreated control, 312, 625, 1250, 2500, 5000 mg/kg feed;

*Type of application :* a.s. mixed in the feed; stability of the a.s. in diet at ambient temperature over 7 days (99.0-107.7% of the nominal concentration at day 1) was determined.

*Time of exposure :* Short-term feeding test (5 days with exposition by the feed + 3 days observation)

Findings :

*Mortality :* none

*Body weight - Feed consumption :* No treatment related change

*Clinical signs - gross pathology :* none

Conclusions :

LC<sub>50</sub> (5d) > 5000 mg a.s./kg feed

NOEC (5d) = 5000 mg a.s./kg feed

### B.8.1.3 Subchronic and reproductive toxicity (Annex IIA 8.1.3)

**XDE-570: a reproduction study with the northern bobwhite (*Colinus virginianus*) (Gallagher, et al., 1995a)**

Guidelines :

**FIFRA Guideline No. 71-4 (a) ≡ OECD Guideline No. 206**

GLP :

Yes

Material and Methods :

*Test substance* : florasulam, purity : 92.2%

*Test species* : Northern bobwhite (*Colinus virginianus*)

*Sex, weight, age* : 16 pens of 1 male and 1 female per test group; \_ mean weight : 195-200 g; \_ mean weight : 187-195g; 20 week old at test initiation

*Applied concentrations* : untreated control, 240, 600, 1500 mg/kg in the feed

*Type of application* : a.s. mixed in the feed; homogeneity (95.0-96.7%) and stability of the a.s. in diet at ambient temperature over 7 days (114-136% of the nominal concentration at day 1) were determined.

*Time of exposure* :

10 weeks : pre-egg production period

10 weeks : egg production period

1 week : post-egg laying period

Findings :

**Table B.8.1.3-1 : Major effects of florasulam observed during the reproduction study of Northern bobwhite**

Endpoints	Doses (mg/kg feed)			
	0	240	600	1500
Adults				
Mortality	No treatment related effects			
Clinical symptoms	No treatment related effects			
Body weight	No treatment related effects			
Food consumption	No treatment related effects			
Reproduction Parameters				
Total Eggs Laid	778	587	750	627
Eggs Laid/Maximum Laid (%)	71	57	73	61
Eggs Cracked/Eggs Laid (%)	2	2	1	1*
Egg shell thickness (mm)	0.208	0.204	0.210	0.209
Viable Embryos/Eggs Set (%)	95	93	91	91
Live 3-Week Embryos/Viable Embryos (%)	98	99	98	98
Hatchlings/Live 3-Week Embryos (%)	81	92*	88	87
14-Day Old Survivors/Hatchlings (%)	87	91	84	84
Hatchlings/Eggs Set (%)	75	84	79	78
14-Day Old Survivors/Eggs Set (%)	66	77	66	66
Hatchlings/Maximum Set (%)	51	46	56	45
14-Day Old Survivors/Maximum Set (%)	45	41	47	38

Conclusions :

NOEC (Northern bobwhite, 21 weeks) = 1500 mg a.s./kg feed.

**XDE-570: a reproduction study with the mallard (*Anas platyrhynchos*) (Gallagher, et al., 1995b)**

Guidelines :

**FIFRA Guideline No. 71-4 (a) = OECD Guideline No. 206**

GLP :

Yes

Material and Methods :

*Test substance* : florasulam, purity : 92.2%

*Test species* : Mallard (*Anas platyrhynchos*)

*Sex, weight, age* : 16 pens of 1 male and 1 female per test group; \_ mean weight : 1122-1150 g; \_ mean weight : 1011-1029 g; 28 week old at test initiation

*Applied concentrations* : untreated control, 240, 600, 1500 mg/kg in the feed

*Type of application* : a.s. mixed in the feed; homogeneity (95.0-96.7%) and stability of the a.s. in diet at ambient temperature over 7 days (94.3-113%% of the nominal concentration at day 1) were determined.

*Time of exposure :*

9 weeks : pre-egg production period

13 weeks : egg production period

1 week : post-egg laying period

Findings :

**Table B.8.1.3-2 : Major effects of florasulam observed during the reproduction study of mallard duck**

Endpoints	Doses (mg/kg feed)			
	0	240	600	1500
Adults				
Mortality	No treatment related effects			
Clinical symptoms	No treatment related effects			
Body weight	No treatment related effects			
Food consumption	No treatment related effects			
Reproduction Parameters				
Total Eggs Laid	873	1007	938	924
Eggs Laid/Maximum Laid (%)	63	72	72	66
Eggs Cracked/Eggs Laid (%)	2	1	1	1
Egg shell thickness (mm)	0.353	0.359	0.355	0.351
Viable Embryos/Eggs Set (%)	85	73	87	88
Live 3-Week Embryos/Viable Embryos (%)	98	97	97	98
Hatchlings/Live 3-Week Embryos (%)	39	44	36	44
14-Day Old Survivors/Hatchlings (%)	94	94	95	96
Hatchlings/Eggs Set (%)	32	29	31	37
14-Day Old Survivors/Eggs Set (%)	30	27	30	36
Hatchlings/Maximum Set (%)	18	21	20	22
14-Day Old Survivors/Maximum Set (%)	17	20	19	20

Conclusions :

NOEC (Mallard, 22 weeks) = 1500 mg a.s./kg feed.

2 reproduction pilot studies were submitted. These studies confirm the results of both main reproduction studies.

XDE-570 herbicide: a pilot reproduction study with the mallard (Beavers, et al., 1995)

The effects of florasulam on the reproduction of the mallard were assessed in a 6-week pilot studies. Effects on health, weight gain, feed consumption, egg production and egg shell thickness were evaluated. Due to effects on body weight at 2000 mg/kg, the NOEC was considered to be 800 mg a.s./kg food.

XDE-570 herbicide: a pilot reproduction study with the northern bobwhite (Beavers, et al., 1995)

The effects of florasulam on the reproduction of the Northern bobwhite quail were assessed in a 6-week pilot studies. Effects on health, weight gain, feed consumption, egg production and egg shell thickness were evaluated. The NOEC was considered to be 2000 mg a.s./kg food, the highest dietary concentration tested.

**B.8.1.4 Acute oral toxicity of the preparations (Annex IIIA 10.1.1)**

**B.8.1.5 Supervised cage or field trials (Annex IIIA 10.1.2)**

**B.8.1.6 Acceptance of bait, granules or treated seeds by birds (palatability test) (Annex IIIA 10.1.3)**

**B.8.1.7 Effects of secondary poisoning (Annex IIIA 10.1.4)**

These studies are not required.

### B.8.1.8 Summary of effects to birds - exposure and risk assessment for birds (Annex IIIA 10.1)

Table B.8.1.8-1 : Summary of effects of florasulam to birds.

Test species	Test System	Duration of exposure	Results	References
<i>Coturnix coturnix japonica</i>	acute	single appl.	LD <sub>50</sub> = 1046 mg a.s./kg bw	Campbell, et al., 1994
<i>Anas platyrhynchos</i>	short-term	5 days	LC <sub>50</sub> > 5000 mg a.s./kg food	Helsten, et al., 1994a
<i>Coturnix coturnix japonica</i>	short-term	5 days	LC <sub>50</sub> > 5000 mg a.s./kg food	Helsten, et al., 1994a
<i>Colinus virginianus</i>	reproduction	21 weeks	NOEC = 1500 mg a.s./kg food	Gallagher, et al., 1995a
<i>Anas platyrhynchos</i>	reproduction	22 weeks	NOEC = 1500 mg a.s./kg food	Gallagher, et al., 1995ab

The risk assessment for birds is based on the following assumptions :

- Food consumption of 30% bw for small birds
- The initial residue is estimated according to Hoerger and Kenaga (1972)
- the maximum application rate is g a.s./ha

The TER reveal that the acute, short-term and long-term risk to birds is negligible.

Table B.8.1.8-2 : Estimated initial concentration of florasulam in potential feed of birds

Target crop	Application rate (kg a.s./ha)	Estimated initial residues (mg a.s./kg food)	
		Small insects	Grass
Cereals	0.0075	0.2175	0.615

Table B.8.1.8-3 : Estimated oral uptake of florasulam by birds

Target crop	Bird type	food consumed	food consumption (% bw)	Max. daily intake (mg a.s. bw / day)
cereals	small bird (<100 g)	small insects	30	0.065
		grass	30	0.184

Table B.8.1.8-4 : Toxicity exposure ratios for birds exposed to florasulam - worst cases

Application rate (kg a.s./ha)	Crop	Organism	Time-scale	TER	Annex VI trigger
florasulam					
0.0075	Cereals	small insectivorous bird	acute	5669	10
		small grass-eating bird	acute	16031	10
		small insectivorous bird	short-term	8130	10
		small grass-eating bird	short-term	22989	10



		small insectivorous bird	long-term	2439	5
		small grass-eating bird	long-term	6897	5

## **B.8.2 Effects on aquatic organisms (fish, aquatic invertebrates, algae) (Annex IIA 8.2; Annex IIIA 10.2)**

### **B.8.2.1 Acute toxicity of the active substance and metabolites, degradation or reactions products to fish (Annex IIA 8.2.1)**

#### **Evaluation of the acute toxicity of XDE-570 herbicide to the rainbow trout, *Oncorhynchus mykiss* Walbaum (Kirk, et al., 1995a)**

##### Guidelines :

**EC Method C1, Directive 92/69 = OECD Guideline No. 203**

##### GLP :

Yes

##### Material and Methods :

*Test substance* : florasulam, purity : 92.2%

*Test species* : rainbow trout (*Oncorhynchus mykiss*)

*Number of organisms, weight, length, loading* : 20 fish/dose; 787.1 mg (548-1400 mg); 37.2 mm (34-43 mm); 0.787 g fish/l

*Type of test* : 96-hour static limit test

*Nominal and measured concentrations* :

nominal : water control, DMF control, 100 mg a.s./l

measured concentrations ranging from 94.1 to 99.6% of the nominal concentrations

*Test conditions* :

temperature : 12.2-12.6 °C,

pH : 6.9-7.9

oxygen content : 9.3-10.3 mg/l, >88.6% saturation

total hardness (as CaCO<sub>3</sub>): 72 mg/l

Photoperiod : 16 h light/8 h dark

*Analytical methods* : HPLC/UV

##### Findings :

*Mortality* : none

*Behavioral observations* : none

##### Conclusions :

LC<sub>50</sub> (96h) > 100 mg a.s./l

NOEC (96h) = 100 mg a.s./l

#### **Evaluation of the acute toxicity of XDE-570 herbicide to the bluegill, *Lepomis macrochirus* Rafinesque (Kirk, et al., 1995a)**

##### Guidelines :

**EC Method C1, Directive 92/69 = OECD Guideline No. 203**

##### GLP :

Yes

##### Material and Methods :

*Test substance* : florasulam, purity : 92.2%

*Test species* : bluegill, (*Lepomis macrochirus*)

*Number of organisms, weight, length, loading* : 20 fish/dose; 354.8 mg (273-761 mg); 25.4 mm (23-33 mm); 0.355 g fish/l

*Type of test* : 96-hour static limit test

*Nominal and measured concentrations* :

nominal : water control, DMF control, 100 mg a.s./l

measured concentrations ranging from 94.7 to 102% of the nominal concentrations

*Test conditions* :

temperature : 22.5-22.7 °C,

pH : 7.0-8.1

oxygen content : 7.9-8.5 mg/l, >94% saturation

total hardness (as CaCO<sub>3</sub>): 72 mg/l

Photoperiod : 16 h light/8 h dark

*Analytical methods* : HPLC/UV

**Findings :**

***Mortality : none***

***Behavioral observations : none***

**Conclusions :**

**LC<sub>50</sub> (96h) > 100 mg a.s./l**

**NOEC (96h) = 100 mg a.s./l**

**XDE-570: acute toxicity to the silverside, *Menidia beryllina* (Ward, et al, 1995)**

**Guidelines :**

**FIFRA Guideline No. 72-3 (a) ≡ OECD Guideline No. 203**

**GLP :**

**Yes**

**Material and Methods :**

***Test substance : florasulam, purity : 99.2%***

***Test species : silverside, *Menidia beryllina****

***Number of organisms, weight, length, loading :30 fish/dose; 130 mg; 23 mm; 0.09 g fish/l***

***Type of test : 96-hour static limit test***

***Nominal and measured concentrations :***

**nominal : water control, 122 mg a.s./l**

**Measured concentrations were 94% of the nominal concentrations**

***Test conditions :***

**temperature : 22.0-22.5°C,**

**pH : 7.6-8.0**

**oxygen content : 6.8-8.1 mg/l**

**salinity : 17 g/l**

**Photoperiod : 16 h light/8 h dark**

***Analytical methods : HPLC/UV***

**Findings :**

***Mortality : none***

***Behavioral observations : none***

**Conclusions :**

**LC<sub>50</sub> (96h) > 122 mg a.s./l**

**NOEC (96h) = 122 mg a.s./l**

**Evaluation of the acute toxicity of 5-hydroxy XDE-570 to the rainbow trout, *Oncorhynchus mykiss* Walbaum (Kirk, et al., 1996)**

**Guidelines :**

**OECD Guideline No. 203 ≡ EC Method C1, Directive 92/69 ≡ FIFRA Guideline No. 72-1 (c)**

**GLP :**

**Yes**

**Material and Methods :**

***Test substance : metabolite 5-hydroxy DE-570, purity : 97.0%***

***Test species : rainbow trout, (*Oncorhynchus mykiss*)***

***Number of organisms, weight, length, loading :30 fish/dose; 480.6mg; 34.5 mm; 0.481 g fish/l***

***Type of test : 96-hour static limit test***

***Nominal and measured concentrations :***

**nominal : water control, 91 mg/l**

**measured concentrations ranging from 76.3-99.3% of the nominal concentrations**

***Test conditions :***

**temperature : 12.2-12.5°C,**

**pH : 6.6-7.7**

**oxygen content : 8.7-10.0 mg/l, > 80% saturation**

**total hardness (as CaCO<sub>3</sub>): 67 mg/l**

**Photoperiod : 16 h light/8 h dark**

**Analytical methods : HPLC/UV**

**Findings :**

***Mortality : none***

***Behavioral observations : none***

**Conclusions :**

**LC<sub>50</sub> (96h, 5-hydroxy DE-570) > 91 mg/l**

**NOEC (96h, 5-hydroxy DE-570) = 91 mg/l**

**XDE-570: acute toxicity to the silverside, *Menidia beryllina* (Ward, et al, 1995c)**

**Guidelines :**

**FIFRA Guideline No. 72-3 (a) ≡ OECD Guideline No. 203**

**GLP :**

**Yes**

**Material and Methods :**

***Test substance : florasulam, purity : 99.2%***

***Test species : silverside, *Menidia beryllina****

***Number of organisms, weight, length, loading :30 fish/dose; 130 mg; 23 mm; 0.09 g fish/l***

***Type of test : 96-hour static limit test***

***Nominal and measured concentrations :***

**nominal : water control, 122 mg a.s./l**

**Measured concentrations were 94% of the nominal concentrations**

***Test conditions :***

**temperature : 22.0-22.5°C,**

**pH : 7.6-8.0**

**oxygen content : 6.8-8.1 mg/l**

**salinity : 17 g/l**

**Photoperiod : 16 h light/8 h dark**

**Analytical methods : HPLC/UV**

**Findings :**

***Mortality : none***

***Behavioral observations : none***

**Conclusions :**

**LC<sub>50</sub> (96h) > 122 mg a.s./l**

**NOEC (96h) = 122 mg a.s./l**

#### **B.8.2.2 Chronic toxicity to fish (Annex IIA 8.2.2)**

**Evaluation of the prolonged (28-day) toxicity of XDE-570 herbicide to the rainbow trout, *Oncorhynchus mykiss* Walbaum (Landre, et al., 1996)**

**Guidelines :**

**OECD Guideline No. 204**

**GLP :**

**Yes**

**Material and Methods :**

***Test substance : florasulam, purity : 99.2%***

***Test species : rainbow trout (*Oncorhynchus mykiss*)***

***Number of organisms, weight, length:20 fish/dose; 808.7 mg; 37.9 mm***

***Type of test : prolonged (28 day) toxicity test***

***Nominal and measured concentrations :***

**Measured concentrations : control, 9.6, 14.8, 25.9, 43.8, 70.9, 119 mg a.s./l**

**Measured concentrations were 91.4-98.8% of the nominal concentrations**

*Test conditions :*

temperature : 12.4-13.3°C

pH : 7.4-7.8

oxygen content : 8.7-11.8 mg/l; > 80% saturation

total hardness (as CaCO<sub>3</sub>): 70-81 mg/l

**Photoperiod : 16 h light/8 h dark**

**Analytical methods : HPLC/UV**

**Findings :**

**Mortality : none**

**Behavioral observations : none**

**Conclusions :**

LC<sub>50</sub> (28d) > 119 mg a.s./l

**NOEC (28 d) = 119 mg a.s./l**

**B.8.2.3 Bioaccumulation potential in fish (Annex IIA 8.2.3)**

The Bioconcentration of XDE-570 by the Rainbow Trout, *Oncorhynchus mykiss* Walbaum (Rick, et al, 1997)

**Guidelines :**

OPPTS Draft Guideline 850-1730 ≡ OECD Guideline No. 305E

**GLP :**

Yes

**Material and Methods :**

*Test substance* : florasulam, purity : 99.2%; <sup>14</sup>C phenyl labelled florasulam, radiochemical purity : 98.5%

*Test species* : rainbow trout, *Oncorhynchus mykiss*

*Number of organisms, weight, loading :*

Low concentration : 80 fish, 0.4074 ± 0.133 g, 0.1 g fish/l/d

High concentration : 220 fish, 0.6130 ± 0.2065 g, 0.5 g fish/l/d

*Type of test* : Continuous flow-through system ( 28-32 days exposure period and 28 days depuration period)

*Nominal and measured concentrations* : 5.1 and 50 µg/l water control.

A.s. concentrations were measured daily :

low concentration : 4.7-5.3 µg/l during the exposure period

high concentration : 48-51-(64) µg/l during the exposure period

*Test conditions* : Flow rate : 255 l per tank per day

Water quality data (temperature, flow rate, dissolved oxygen, pH) were monitored daily .

low concentration : temperature 11.7-14.8; pH : 5.9-6.8; oxygen content : 67-107% saturation; total hardness (as CaCO<sub>3</sub>): 65-74 mg/l

high concentration : temperature 12.4-15.5; pH : 6.4-7.5; oxygen content : 69-92% saturation; total hardness (as CaCO<sub>3</sub>): 47-64 mg/l

**Photoperiod : 16 h light/8 h dark**

**Analytical methods** : HPLC radioassay of parent/metabolites in water and fish

**Findings :**

Table B.8.2.3-1 : Major endpoints of the bioaccumulation study in rainbow trout.

	Remainder (*)	Muscle	Whole fish
BCF	2.2	1.4	0.8-2.2
T <sub>ss80%</sub> , time to achieve 80% steady state concentration (days)	41	47	19-34
T <sub>ss95%</sub> , time to achieve 95% steady state concentration (days)	100	98	45-85
Depuration half-life (days)	6.4	16	6.4-5.9

(\*) : head, skin, viscera, skeleton

*Identification of the radioactivity* : No radiolabelled peaks were detectable above background level in the solvent-

extracted whole fish.

Conclusion :

Florasulam is characterized by a low bioconcentration potential: BCF = 0.8-2.2

**B.8.2.4 Acute toxicity to invertebrates (Annex IIA 8.2.4)**

Evaluation of the acute toxicity of XDE-570 herbicide to the daphnid, *Daphnia magna* Straus (Kirk, et al., 1995)

Guidelines :

EC Method C2, Directive 92/69 ≡ OECD Guideline No. 202, Part 1

GLP :

Yes

Material and Methods :

*Test substance* : florasulam, purity : 99.2%

*Test species* : daphnid, *Daphnia magna*

*Number of organisms, age* : 20 organisms/ test group, < 24 h old

*Type of test* : 48 h static test

*Nominal and measured concentrations* :

Measured concentrations : control, 38.0, 63.0, 104, 174, 292 mg a.s./l

Measured concentrations ranging from 96.3 to 97.7 % of the nominal concentrations

*Test conditions* :

temperature : 19.5-20.2 °C,

pH : 6.3-7.8

oxygen content : 8.1-9.0 mg/l; > 91% saturation

total hardness (as CaCO<sub>3</sub>) : 166 mg/l

**Photoperiod : 16 h light/8 h dark**

*Analytical methods* : HPLC

Findings and conclusions :

LC<sub>50</sub> (48h) > 292 mg a.s./l

NOEC (48h) = 174 mg a.s./l

XDE-570: acute toxicity to the grass shrimp, *Palaemonetes pugio* (Ward, et al., 1995a)

Guidelines :

FIFRA Guideline No. 72-3 (c)

GLP :

Yes

Material and Methods :

*Test substance* : florasulam, purity : 99.2%

*Test species* : grass shrimp, *Palaemonetes pugio*

*Number of organisms, weight, length, loading* : 30 animals/test group, 0.16 g, 27 mm, 0.11 g/l

*Type of test* : 96 h static test

*Nominal and measured concentrations* :

Measured concentrations : control, 120 mg a.s./l

Measured concentrations were 92% of the nominal concentrations

*Test conditions* :

temperature : 22.1-22.6 °C

pH : 7.5-7.9

oxygen content : 6.0-8.1 mg/l

salinity : 17 g/l

**Photoperiod : 16 h light/8 h dark**

*Analytical methods* : HPLC with UV detection

Findings and conclusions :

LC<sub>50</sub> (96h) > 120 mg a.s./l

NOEC (96h) = 120 mg a.s./l

XDE-570: acute flow-through mollusc shell deposition test (Ward, et al., 1995b)

Guidelines :

FIFRA Guideline No. 72-3 (c)

GLP :

Yes

Material and Methods :

*Test substance* : florasulam, purity : 99.2%

*Test species* : Eastern oyster, *Crassostrea virginica*

*Number of organisms, length*,: 30 animals/test group, 29-44 mm at initiation of the test

*Type of test* : 96 h static test

*Nominal and measured concentrations* :

Measured concentrations : control, 125 mg a.s./l

Measured concentrations were 96 % of the nominal concentrations

*Test conditions* :

temperature : 20.2-21.0 °C

pH : 7.7-7.9

oxygen content : 6.3-7.2 mg/l

salinity : 32 g/l

Photoperiod : **16 h light/8 h dark**

*Analytical methods* : HPLC with UV detection

Findings and conclusions :

EC<sub>50</sub> (96h, shell deposition) > 125 mg a.s./l

NOEC (96h, shell deposition ) = 125 mg a.s./l

Evaluation of the acute toxicity of 5-hydroxy XDE-570 to the daphnid, *Daphnia magna* Straus (Kirk, et al, 1996)

Guidelines :

OECD Guideline No. 202 ≡ EC Method C2, Directive 92/69 ≡ FIFRA Guideline No. 72-2 (a)

GLP :

Yes

Material and Methods :

*Test substance* :metabolite 5-hydroxy DE-570, purity : 97.0%

*Test species* : daphnid, *Daphnia magna*

*Number of organisms, age* : 30 organisms/ test group, < 24 h old

*Type of test* : 48 h static test

*Nominal and measured concentrations* :

Measured concentrations : control, 96.7 mg/l

Measured concentrations were 96.7% of the nominal concentrations

*Test conditions* :

temperature : 19.0-21.0 °C,

pH : 6.3-7.6

oxygen content : 8.6-8.8 mg/l; > 93 % saturation

total hardness (as CaCO<sub>3</sub>) : 164 mg/l

Photoperiod : 16 h light/8 h dark

*Analytical methods* : HPLC with UV detection

Findings and conclusions :

EC<sub>50</sub> (48h) > 96.7 mg/l

NOEC (48h) =96.7 mg/l

### B.8.2.5 Chronic toxicity to aquatic invertebrates (Annex IIA 8.2.5)

Evaluation of the chronic toxicity of XDE-570 herbicide to the daphnid, *Daphnia magna* Straus (Kirk, et al., 1996)

Guidelines :

OECD Guideline No. 202, Part 2  $\equiv$  FIFRA Guideline No. 72-4

GLP :

Yes

Material and Methods :

Test substance : florasulam, purity : 99.2%

Test species : daphnid, *Daphnia magna*

Number of organisms, age : 10 organisms/ test group, < 24 h old

Type of test : 21 d semi- static test with renewal every 3 days

Nominal and measured concentrations :

Nominal concentrations : control, 14.0, 23.4, 38.9, 64.8, 108, 180 mg a.s./l

Measured concentrations ranging from 97.8 to 100 % of the nominal concentrations

Test conditions :

temperature : 19.3-20.9 °C,

pH : 6.5-8.0

oxygen content : 7.3 mg/l; > 82 % saturation

total hardness (as CaCO<sub>3</sub>) : 120-161mg/l

Photoperiod : 16 h light/8 h dark

Analytical methods : HPLC with UV detection

Table 8.2.5-1 : Effects on daphnids exposed to florasulam in a semi-static system over 21 days

	Concentration (mg a.s./l) (nominal)						
	0	14.0	23.4	38.9	64.8	108	180
% survival	90	100	100	70	80	80	0
Reproduction rate (offspring/adult)	138.3	160.1	163.5	154.1	118.6	39.8*	-
Mean day to first eggs	7.2	7.2	7.0	7.8	8.3	7.2	-
Mean day to first broods	9.6	10.2	9.6	10.4	10.5	13.4	-
Broods/adult	4.2	4.4	4.7	3.5	3.2	2.2*	-
Instars/brood	33	37	35	44	31	18	-
Weight (mg)	0.96	0.76*	0.91	0.84	0.66*	0.49*	-
Length (mm)	3.92	3.58*	3.83	3.78	3.72*	3.42*	-

Conclusions :

EC<sub>50</sub> (adult mortality, 21 d) = 169.2 mg a.s./l

NOEC (adult mortality, 21 d) = 23.4 mg a.s./l

NOEC (offspring/adult, 21 d) = 64.8 mg a.s./l

NOEC (broods/adult, 21 d) = 64.8 mg a.s./l

NOEC (weight, 21 d) = 38.9 mg a.s./l

NOEC (length, 21 d) = 38.9 mg a.s./l



#### **B.8.2.6 Effects on algal growth (Annex IIA 8.2.6)**

XDE-570 herbicide: the toxicity to the green alga, *Selenastrum capricornutum* Printz (Milazzo, et al. , 1995)

Guidelines :

EC Method C3, Directive 92/69  $\equiv$  OECD Guideline No. 201

GLP :

Yes

Material and Methods :

*Test substance* : florasulam, purity : 99.2%

*Test species* : green alga, *Selenastrum capricornutum*

*Number of organisms number of replicates* : 3000 cells/ml, 3 replicates per test concentration

*Type of test* : 72 h static test

*Nominal and measured concentrations* :

Measured concentrations : control, 0.788, 1.48, 3.02, 6.09, 11.9, 24.3, 49.3  $\mu\text{g a.s./l}$

Measured concentrations ranging from 95.5 to 102 % of the nominal concentrations

*Test conditions* :

temperature : 24-25  $^{\circ}\text{C}$ ,

pH : 7.0-7.2

oxygen content : -

total hardness : -

Photoperiod : continuous, 4300 lux

*Analytical methods* : HPLC with UV detection

Findings and conclusions :

$\text{EbC}_{50}$  (72 h) = 9.42  $\mu\text{g a.s./l}$  (confidence limits : 5.79-15.31)

$\text{ErC}_{50}$  (72 h) = 8.94  $\mu\text{g a.s./l}$  (confidence limits : 5.62-14.22)

NOEC (72 h) < 0.788  $\mu\text{g a.s./l}$

XDE-570 herbicide: the toxicity to the freshwater diatom, *Navicula pelliculosa* (Milazzo, et al. , 1996)

Guidelines :

FIFRA Guideline No. 123-2  $\equiv$  EC Method C3, Directive 92/69

GLP :

Yes

Material and Methods :

*Test substance* : florasulam, purity : 99.2%

*Test species* : freshwater diatom, *Navicula pelliculosa*

*Number of organisms number of replicates* : 10000 cells/ml, 4 replicates per test concentration

*Type of test* : 120 h static test

*Nominal and measured concentrations* :

Nominal concentrations :water control, DMF control, 0.0141, 0.0492, 0.162, 0.541, 1.77, 5.90 mg a.s./l

Measured concentrations ranging from 94.3 to 104 % of the nominal concentrations

*Test conditions* :

temperature : 23.9-24.3  $^{\circ}\text{C}$ ,

pH : 7.5-7.7

oxygen content : -

total hardness : -

Photoperiod : continuous, 3200-5400 lux

*Analytical methods* : HPLC with UV detection

Findings and conclusions :

$\text{EbC}_{50}$  (120 h) = 1.38 mg a.s./l (confidence limits : 0.03-71.01)

NOEC (120 h) < 0.0493 mg a.s./l

The toxicity of XDE-570 to *Skeletonema costatum* (Hughes, et al., 1995)

Guidelines :

FIFRA Guideline No. 123-2  $\equiv$  EC Method C3, Directive 92/69

GLP :

Yes

Material and Methods :

*Test substance* : florasulam, purity : 99.2%

*Test species* : marine diatom *Skeletonema costatum*

*Number of organisms number of replicates* : 10000 cells/ml, 3 replicates per test concentration

*Type of test* : 120 h static test

*Nominal and measured concentrations* :

Measured concentrations : control, 12.1, 22.8, 42.9, 89.8, 173 mg a.s./l

Measured concentrations ranging from 85.9 to 96.6 % of the nominal concentrations after 5 days

*Test conditions* :

temperature : 19.84-21.52 °C,

pH : 7.21-8.72

oxygen content : -

total hardness : -

Photoperiod : 14 hours light, 10 hours dark, 4306 lux

*Analytical methods* : HPLC with UV detection

Findings and conclusions :

EbC<sub>50</sub> (120 h) = 43.1 mg a.s./l (confidence limits : 32.1-57.8)

ErC<sub>50</sub> (120 h) = 47.6 mg a.s./l (confidence limits : 41.4-54.6)

NOEC (48 h) < 12.1 mg a.s./l

**XDE-570 herbicide: the toxicity to the blue-green alga, *Anabaena flos-aquae*** (Milazzo, et al., 1995)

Guidelines :

FIFRA Guideline No. 123-2  $\equiv$  EC Method C3, Directive 92/69

GLP :

Yes

Material and Methods :

*Test substance* : florasulam, purity : 99.2%

*Test species* : **blue-green alga, *Anabaena flos-aquae***

*Number of organisms number of replicates* : 10000 cells/ml, 3 replicates per test concentration

*Type of test* : 120 h static test

*Nominal and measured concentrations* :

Measured concentrations : water control, DMF control, 29, 58.3, 116, 235, 478 µg a.s./l

Measured concentrations ranging from 94.4 to 97.5 % of the nominal concentrations

*Test conditions* :

temperature : 24.8-24.9 °C,

pH : 7.3-7.4

oxygen content : -

total hardness : -

Photoperiod : continuous , 2000 lux

*Analytical methods* : HPLC with UV detection

Findings and conclusions :

EC<sub>50</sub> (96 h) = 363 µg a.s./l (confidence limits : 214-940)

NOEC (96 h) = 235 µg a.s./l

XDE-570 5-hydroxy: the toxicity to the freshwater green alga, *Selenastrum capricornutum* Printz (Milazzo, et al., 1996)

Guidelines :

OECD Guideline No. 201 = EC Method C3, Directive 92/69 = FIFRA Guideline No. 123-2

GLP :

Yes

Material and Methods :

*Test substance* : metabolite **5-hydroxy DE-570**, purity : 100 %

*Test species* : freshwater green alga, *Selenastrum capricornutum*

*Number of organisms number of replicates* : 10000 cells/ml, 3 replicates per test concentration

*Type of test* : 96 h static test

*Nominal and measured concentrations* :

Measured concentrations : water control, 3.48, 6.64, 13.0, 25.4, 49.5, 96.1 mg/l

Measured concentrations ranging from 96.1 to 111 % of the nominal concentrations

*Test conditions* :

temperature : 24.3-24.8 °C,

pH : 4.7-7.6 (pH decreasing in function of the test material concentration)

oxygen content : -

total hardness : -

Photoperiod : continuous , 4305 lux

*Analytical methods* : HPLC with UV detection

Findings and conclusions :

EbC<sub>50</sub> (96 h) = 21.32 mg/l (confidence limits : 12.61-36.07)

ErC<sub>50</sub> (96 h) = 21.57 mg/l (confidence limits : 13.36-34.84)

NOEC ( 96 h) = 6.64 mg/l

Preparation of Soil Extracts for Determination of the Algal Toxicity of XDE-570 Metabolites (Hastings, 1997)

Two aqueous soil extracts containing XDE-570 metabolites: Growth Inhibition of *Selenastrum capricornutum* (Preliminary Toxicity Screen) (Jenkins, 1997)

Guidelines :

OECD Guideline No. 201 = EC Method C3, Directive 92/69

GLP :

Yes

Material and methods :

Florasulam was applied to samples of 2 European soils at a rate of 50 µg/kg (equivalent to 50 g a.s./ha). The soils were incubated in the dark at 20°C, in a closed aerobic system for up to 112 days. Soils metabolites (**5-hydroxy DE-570**, M4, M6, ...) were extracted with water on day 112 in order to determine their toxicity to algae (the most sensitive aquatic organism). *Selenastrum capricornutum* were exposed in a 72 h to the 2 soil extracts, to spiked water (8.3 µg a.s./l) and to spiked soil extracts

Findings :

Table B.8.2.6-1 : Concentrations of the soil extracts (µg/l)

Soil type	florasulam	<b>5-hydroxy DE-570</b>	M-4	M-6
Speyer 2:2	not detected	0.4	0.5	1.1
Marcham SCL	not detected	not detected	1.5	1.1

Table B.8.2.6-2 : Effects of soil extracts to *Selenastrum capricornutum*

Sample identification	algal growth (% inhibition at 72 h)	biomass (% inhibition at 72 h)
spiked water (8.3 µg/l)	7*	24*
Speyer 2.2 (spiked soil)	8*	26*
Speyer 2.2 (soil extract)	1	5
Marcham SCL (spiked soil)	9*	30*
Marcham SCL (soil extract)	0	1

(\*) statistically significant

Conclusions :

Treated soil extracts did not inhibit the algal growth. These results are in accordance with the results of the study investigating the herbicidal activity of the florasulam metabolites (Ehr, et al., 1997).

**B.8.2.7 Effects on sediment dwelling organisms (Annex IIA 8.2.7)**

XDE-570: Toxicity to the Sediment Dwelling Phase of the Midge, *Chironomus riparius* (Kelly, 1997)

Guidelines :

BBA (1995), Streloke, M, Kopp, H - Long term toxicity test with *Chironomus riparius*: Development and validation of a new test system

GLP :

Yes

Material and Methods :

*Test substance* : florasulam, chemical purity : 99%; radiolabelled florasulam, radiochemical purity > 97%

*Test species* : midge, *Chironomus riparius*, first instar less than 24 h old

*Number of organisms* : 3 replicates each with 20 larvae per concentration

*Type of test* : 28 day long term toxicity test

*Nominal and measured concentrations* :

nominal : solvent control, water control, 0.46, 1.0, 2.2, 4.6, 10 mg a.s./l

Concentrations in overlying water decreased from 96-115% on day 0 to 89-95% of nominal on day 28.

Samples of the pore water collected after the 28 day period account for 0.24 to 0.57% of the total material added.

Samples of the dry sediment on day 28 contained 14 to 16% of the total test material added.

Total amount accounted for in all of the fractions on day 28 ranged from 104 to 110% of the test material added.

*Test conditions* :

temperature : 18-20 °C,

pH : 6.5-7.7

oxygen content : 6.8-10.5 mg/l; % saturation

Photoperiod : 16 h light/8 h dark

*Analytical methods* : LSC

Findings :

Table B.8.2.7-1 : Effects of florasulam to midges emergence

Dose applied (mg a.s./l)	Development and emergence	
	Mean development rate	Mean % emergence
sand control	0.0564	77.5
control	0.0590	85.0
solvent control	0.0673	88.3
0.46	0.0630	95.0
1.0	0.0654	85.0
2.2	0.0642	78.3
4.6	0.0654	83.3
10	0.0633 *	85.0

Conclusions :

EC<sub>50</sub> (emergence) > 10 mg a.s./l

NOEC (emergence) = 10 mg a.s./l

### B.8.2.8 Effects on aquatic plants (Annex IIA 8.2.8)

The toxicity of XDE-570 herbicide to the aquatic plant, duckweed, *Lemna gibba* L. G-3 (Milazzo, et al., 1995)

Guidelines :

FIFRA Guideline No. 123-2

GLP :

Yes

Material and Methods :

Test substance : florasulam, purity : 99.2%

Test species : duckweed, *Lemna gibba*

Number of organisms number of replicates : 16 fronds (4 plants, 4 fronds/plant)/ replicate, 3 replicates per test concentration

Type of test : 14 days static test

Nominal and measured concentrations :

Nominal concentrations : water control, DMF control, 0.157, 0.314, 0.628, 1.26, 2.50, 4.99 µg a.s./l

Test solutions at 0.314 and 0.628 µg/l were quantitated after 14 days. The measured concentrations were 95.8 and 98.5 % of the nominal concentrations. Other solutions could not be quantitated because there were too many interferences.

Test conditions :

temperature : 23.2-24.2 °C,

pH : 8.5

oxygen content : -

total hardness : -

Photoperiod : continuous , 5830 lux

Analytical methods : HPLC with UV detection

Findings and conclusions :

EC<sub>50</sub> (14 d) = 1.18 µg a.s./l (confidence limits : 0.39-3.53)

NOEC (14 d) = 0.62 µg a.s./l

The activity of DE-570 and soil metabolites of DE-570 on acetolactate synthase, *Lemna minor* and *Agrostis palustris* (Ehr, et al., 1997)

Guidelines :

-

GLP :

No

Material and Methods :

Test substances :

florasulam	> 99%
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metabolite 5-hydroxy-DE-570	> 99%
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M4 soil metabolite (5-(aminosulfonyl)-1H-1,2,4-triazole-3-carboxylic acid, ASTCA)	> 99%
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M3 soil metabolite (N-(2,6-difluorophenyl)-5-aminosulfonyl-1H-1,2,4-triazole-3-carboxylic acid, DFP-ASTCA)	> 99%
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M1 or “decarboxylated M3” soil metabolite (N-(2,6-difluorophenyl)-1H-1,2,4-triazole-3-sulfonamide, DFP-TSA)	> 99%
---	-------

Material and methods :

- The intrinsic herbicidal activity of florasulam and its soil metabolites was evaluated in an hydroponic assay on whole plants. Bentgrass seeds (*Agrostis palustris*) and duckweed fronds (*Lemna minor*) were placed in plates and grown in a growth chamber. Plates were visually assessed 9 days after treatment (visual injury rating : 0 representing no injury; 100 representing complete necrosis).

- The activity of florasulam and its soil metabolites on acetolactate synthase was compared. Enzyme activity was assayed colorimetrically by measuring the acetoin formed from acetolactate using the method of Westerfield (1945)

Findings :

Table B.8.2.8-1 : Herbicidal activity of florasulam and its soil metabolites

Substances	Activity on <i>Lemna minor</i> I <sub>50</sub> (ppm)	Activity on <i>Agrostis palustris</i> I <sub>50</sub> (ppm)	Activity on the acetolactate synthase I <sub>50</sub> (ppm)
florasulam	0.00001	0.6	0.004
metabolite 5-hydroxy-DE-570	0.75	> 100	15.5
M4 soil metabolite, ASTCA	> 100	> 100	> 27
M3 soil metabolite, DFP-ASTCA	83	> 100	13.5
M1 soil metabolite, DFP-TSA	9	> 100	0.54

**Conclusions :**

This study shows that the 4 metabolites are less active than the active substance : the metabolite 5-hydroxy-DE-570 is 75000 times less active than the a.s. on *Lemna*; other metabolites have even less activity. The *in vitro* acetolactate synthase assays confirm these results.

**B.8.2.9 Acute toxicity of the preparations (Annex IIIA 10.2.1)**

EF-1343 : Acute toxicity to rainbow trout (Jenkins, 1996a)

**Guidelines :**

**EC Method C1, Directive 92/69 ≡ OECD Guideline No. 203**

**GLP :**

Yes

**Material and Methods :**

*Test substance* : EF-1343 (SC containing 50 g/l florasulam)

*Test species* : rainbow trout (*Oncorhynchus mykiss*)

*Number of organisms, weight, length, loading* : 20 fish/dose; 820 mg; 48 mm; 0.59 g fish/l

*Type of test* : 96-hour static limit test

*Nominal and measured concentrations* :

nominal : water control, 100 mg formulation/l

measured concentrations ranging from 109 to 123% of the nominal concentrations

*Test conditions* :

temperature : 13.0-14.2 °C,

pH :7.8-8.4

oxygen content : 79-103 % saturation

total hardness (as CaCO<sub>3</sub>): 216-252 mg/l

Photoperiod : 16 h light/8 h dark

*Analytical methods* : HPLC/UV

**Findings :**

*Mortality* : none

*Behavioral observations* : none

**Conclusions :**

LC<sub>50</sub> (96h, formulation) > 100 mg/l

NOEC (96h, formulation) = 100 mg/l

EF-1343 : Acute toxicity to *Daphnia magna* (Jenkins, 1996b)

**Guidelines :**

**EC Method C2, Directive 92/69 ≡ OECD Guideline No. 202, Part 1**

**GLP :**

Yes

Material and Methods :

*Test substance* : EF-1343 (SC containing 50 g/l florasulam)

*Test species* : daphnid, *Daphnia magna*

*Number of organisms, age* : 6 replicates each with 5 animals/concentration, < 24 h old

*Type of test* : 48 h static limit test

*Nominal and measured concentrations* :

nominal : water control, 100 mg formulation/l

Measured concentrations ranging from 108 to 112% of the nominal concentrations

*Test conditions* :

temperature : 18.7-19.8 °C,

pH : 7.6-8.0

oxygen content : 95-100% saturation

total hardness (as CaCO<sub>3</sub>) : 236 mg/l

Photoperiod : 16 h light/8 h dark

*Analytical methods* : HPLC

Findings and conclusions :

LC<sub>50</sub> (48h, formulation) > 100 mg/l

NOEC (48h, formulation) = 100 mg/l

EF-1343 : determination of 72-hour EC<sub>50</sub> to *Selenastrum capricornutum* (Jenkins, 1996c)

Guidelines :

EC Method C3, Directive 92/69 ≡ OECD Guideline No. 201

GLP :

Yes

Material and Methods :

*Test substance* : EF-1343 (SC containing 50 g/l florasulam)

*Test species* : green alga, *Selenastrum capricornutum*

*Number of organisms number of replicates* : 10000 cells/ml, 3 replicates per test concentration

*Type of test* : 72 h static test

*Nominal and measured concentrations* :

Nominal concentrations : control, 7.81, 15.6, 31.3, 62.5, 125, 250 µg/l

Measured concentrations ranging from 81 to 153% of the nominal concentrations. Concentration inaccuracies (138 and 153%) at the 2 lower concentrations which were below the NOEC.

*Test conditions* :

temperature : 22.6-24.1 °C,

pH : 7.4-8.5

oxygen content : -

total hardness : -

Photoperiod : continuous, 8000 lux

*Analytical methods* : HPLC with UV detection

Findings and conclusions :

EbC<sub>50</sub> (72 h, formulation) = 61.1 µg/l (confidence limits : 53.5-9.6)

NOEC (72 h, formulation) = 31.3 µg/l

**B.8.2.10 Microcosm and mesocosm study (Annex IIIA 10.2.2)**

The study is not required.

**B.8.2.11 Residue data in fish (Annex IIIA 10.2.3)**

The study is not required. A bioconcentration study was provided.

**B.8.2.12 Supplementary studies of toxicity to fish and aquatic invertebrates (Annex IIIA 10.2.4)**

Not required.

**B.8.2.13 Summary of effects to water organisms (Annex IIA 8.2, Annex IIIA 10.2)**

Table B.8.2.13-1 : Summary of effects of florasulam to water organisms

Test species	Test system	Duration of exposure	Results	References
<i>Oncorhynchus mykiss</i>	acute	96 h	LC <sub>50</sub> > <b>100</b> mg a.s./l NOEC = 100 mg a.s./l	Kirk, et al., 1995a
<i>Lepomis macrochirus</i>	acute	96 h	LC <sub>50</sub> > 100 mg a.s./l NOEC = 100 mg a.s./l	Kirk, et al., 1995b
<i>Menidia beryllina</i>	acute	96 h	LC <sub>50</sub> > 122 mg a.s./l NOEC = 122 mg a.s./l	Ward, et al., 1995c
<i>Oncorhynchus mykiss</i>	chronic	28 d	LC <sub>50</sub> > 119mg a.s./l NOEC = <b>119</b> mg a.s./l	Landre, et al., 1996
<i>Oncorhynchus mykiss</i>	bioconcentration	28 d	BCF = 0.8-2.2	Rick, et al., 1997
<i>Daphnia magna</i>	acute	48h	LC <sub>50</sub> > <b>292</b> mg a.s./l NOEC = 174 mg a.s./l	Kirk, et al., 1995
<i>Palaemonetes pugio</i>	acute	96 h	LC <sub>50</sub> > 120 mg a.s./l NOEC = 120 mg a.s./l	Ward, et al., 1995a
<i>Crassostrea virginica</i>	shell deposition	96 h	EC <sub>50</sub> > 125 mg a.s./l NOEC = 125 mg a.s./l	Ward, et al., 1995b
<i>Daphnia magna</i>	chronic	21 d	EC <sub>50</sub> (adult mortality) = 169.2 mg a.s./l NOEC (reproduction) = 64.8 mg a.s./l NOEC (length, weight) = <b>38.9</b> mg a.s./l	Kirk, et al., 1996
<i>Selenastrum capricornutum</i>	acute	72 h	EbC <sub>50</sub> = 9.42 µg a.s./l ErC <sub>50</sub> = <b>8.94</b> µg a.s./l NOEC <0.788 µg a.s./l	Milazzo, et al., 1995
<i>Navicula pelliculosa</i>	acute	120 h	EbC <sub>50</sub> = 1.38 mg a.s./l NOEC < 0.0493 mg a.s./l	Milazzo, et al., 1996
<i>Skeletonema costatum</i>	acute	120 h	EbC <sub>50</sub> = 43.1 mg a.s./l ErC <sub>50</sub> = 47.6 mg a.s./l NOEC < 12.1 mg a.s./l	Hughes, et al., 1995
<i>Anabaena flos-aquae</i>	acute	120 h	EC <sub>50</sub> = 363 µg a.s./l	Milazzo, et al., 1995
<i>Chironomus riparius</i>	emergence	28 d	EC <sub>50</sub> > 10 mg a.s./l NOEC = 10 mg a.s./l	Kelly, 1997
<i>Lemna gibba</i>	growth	14 d	EC <sub>50</sub> = <b>1.18</b> µg a.s./l NOEC = 0.62 µg a.s./l	Milazzo, et al., 1995



Table B.8.2.13-2 : Summary of effects of metabolite 5-hydroxy DE-570 to water organisms

Test species	Test system	Duration of exposure	Results	References
<i>Oncorhynchus mykiss</i>	acute	96 h	LC <sub>50</sub> > 91 mg/l NOEC = 91 mg/l	Kirk, et al. , 1996
<i>Daphnia magna</i>	acute	48h	LC <sub>50</sub> > 96.7 mg/l NOEC = 96.7 mg/l	Kirk, et al. , 1996
<i>Selenastrum capricornutum</i>	acute	72 h	EbC <sub>50</sub> = 21.32 mg a.s./l ErC <sub>50</sub> = 21.57 mg a.s./l NOEC = 6.64 mg a.s./l	Milazzo, et al., 1996
<i>Lemna minor</i> Acetolactate synthase <i>Agrostis palustris</i>	herbicidal activity	-	metabolite is 75000 times less active than the a.s. metabolite is 3900 times less active than the a.s. -	Ehr, et al., 1997

Table B.8.2.13-3 : Summary of effects of EF-1343 (SC containing 50 g/l florasulam) to water organisms

Test species	Test system	Duration of exposure	Results	References
<i>Oncorhynchus mykiss</i>	acute	96 h	LC <sub>50</sub> > <b>100</b> mg/l NOEC = 100 mg/l	Jenkins, 1996a
<i>Daphnia magna</i>	acute	48h	LC <sub>50</sub> > <b>100</b> mg/l NOEC = 100 mg/l	Jenkins, 1996b
<i>Selenastrum capricornutum</i>	acute	72 h	EbC <sub>50</sub> = <b>61.1</b> µg/l NOEC = 31.3 µg/l	Jenkins, 1996c

#### B.8.2.14 Exposure and risk assessment for aquatic organisms (Annex IIIA 10.2)

##### Active substance

The following assumptions were made to assess the risk to water organisms :

- toxicity figures which were taken into account are indicated in bold in tables B.8.2.15-1 and B.8.2.15-3.
- 30 cm water depth,
- spray drift according to Ganzelmeir (1992),

The results of the studies with the a.s. and with the formulation were used to calculate the acute TER.

The TER calculations with both types of data reveal that the aquatic organisms (fish, aquatic invertebrates, algae, aquatic plants) are not at acute risk. Algae (*Selenastrum capricornutum*, *Anabaena flos-aquae*) and aquatic plants (*Lemna minor*) are the most sensitive aquatic organisms.

Chronic TER are based on a conservative assumption : NOEC of the chronic trout and daphnid studies were compared to the initial PEC (1 m, 4% drift) . The TER calculations reveal the absence of chronic risk to aquatic organisms.

##### Metabolites

The main metabolite 5-hydroxy DE-570 presents a low toxicity to fish and aquatic invertebrates. It presents a low toxicity to the most sensitive alga *Selenastrum capricornutum*.

The herbicidal activity of the metabolites (metabolite 5-hydroxy-DE-570, ASTCA, DFP-ASTCA, DFP-TSA) is negligible in comparison with the activity of the a.s.

Table B.8.2.14-1 : Toxicity/Exposure Ratios for the most sensitive aquatic organisms exposed to florasulam

Application rate (kg a.s./ha)	Crop	Organism	Time-scale	Distance (m)	TER	Annex VI trigger
florasulam						
0.0075	Cereals	<i>Oncorhynchus mykiss</i>	acute	1	1000000	100
		<i>Daphnia magna</i>	acute	1	2920000	100
		<i>Selenastrum capricornutum</i>	acute	1	89	10
		<i>Lemna gibba</i>	acute	1	12	10
0.0075	Cereals	<i>Oncorhynchus mykiss</i>	chronic	1	1190000	10
		<i>Daphnia magna</i>	chronic	1	389000	10
calculations based on the results of the studies with the formulation EF-1343						
0.0075	Cereals	<i>Oncorhynchus mykiss</i>	acute	1	50000	100
		<i>Daphnia magna</i>	acute	1	50000	100
		<i>Selenastrum capricornutum</i>	acute	1	31	10

### B.8.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3.1)

Table B.8.1.8-1 : Toxicological endpoints chosen to assess the effects of florasulam to mammals.

Test species	Test System	Duration of exposure	Results	References
rat	acute	single appl.	LD <sub>50</sub> = 5000 mg a.s./kg bw	Brooks, 1997
rat	reprotox (systemic tox)		NOAEL = 100 mg a.s./kg bw or NOAEL = 2000 mg a.s./kg food	Liberacki et al., 1997

The risk assessment for mammals is based on the following assumptions :

- Food consumption of 30% bw for small mammals
- The initial residue is estimated according to Hoerger and Kenaga (1972)
- the maximum application rate is 7.5 g a.s./ha

The TER reveal that the acute and long-term risk to mammals is negligible.

Table B.8.1.8-2 : Estimated initial concentration of florasulam in potential feed of mammals

Target crop	Application rate (kg a.s./ha)	Estimated initial residues (mg a.s./kg food)	
		Small insects	Grass
Cereals	0.0075	0.2175	0.615

Table B.8.1.8-3 : Estimated oral uptake of florasulam by mammals

Target crop	Mammal type	food consumed	food consumption (% bw)	Max. daily intake (mg a.s. bw / day)
cereals	small mammal (<100 g)	small insects	30	0.065
		grass	30	0.184

Table B.8.1.8-4 : Toxicity exposure ratios for mammals exposed to florasulam - worst cases

Application rate (kg a.s./ha)	Crop	Organism	Time-scale	TER	Annex VI trigger
florasulam					
0.0075	Cereals	small insectivorous mammal	acute	76628	10
		small grass-eating mammal	acute	27100	10
		small insectivorous mammal	long-term	9195	5
		small grass-eating mammal	long-term	3252	5

#### **B.8.4 Effects on bees (Annex IIA 8.3.1; Annex IIIA 10.3.2)**

##### **B.8.4.1 Acute toxicity to bees (Annex IIA 8.3.1.1)**

XDE-570: An acute contact study with the honey bee (Palmer, et al., 1994)

Guidelines :

FIFRA Guideline No. 141-1  $\equiv$  EPPO Guideline 170, 1992

GLP :

Yes

Material and Methods :

*Test substance* : florasulam, purity : 99.2%

*Test species* : Honeybees (*Apis mellifera* L.); worker bees

*Number of organisms* : 4 replicates of 25 bees per test concentration

*Type of test* : 48 h contact test

*Applied concentrations* : 6.3, 12.5, 25.0, 50.0, 100  $\mu\text{g}$  a.s./bee (nominal); solvent control; negative control

*Exposure route :*

The bees were dosed by administering a 2 $\mu\text{l}$  droplet of the dosing solution to the abdomen and/or thorax.

*Feeding* : 50% aqueous sucrose solution

*Test conditions :*

32-35  $^{\circ}\text{C}$ , relative humidity : -

Findings and conclusion:

LD<sub>50</sub> (48h) contact > 100  $\mu\text{g}$  a.s./bee

NOEL (48h) contact = 100  $\mu\text{g}$  a.s./bee

A determination of the oral LD<sub>50</sub> for XDE-570 against the honey bee, *Apis mellifera* (Beech, 1996)

Guidelines :

EPPO Guideline 170, 1992

GLP :

Yes

Material and Methods :

*Test substance* : florasulam, purity : 99.2%

*Test species* : Honeybees (*Apis mellifera* L.); worker bees

*Number of organisms* : 2 replicates of 10 bees for the 100  $\mu\text{g}$ / bee group; 1 replicate of 10 bees for the 80  $\mu\text{g}$ / bee group ; 3 replicates of 10 bees for the controls.

*Type of test* : 48 h oral toxicity

*Applied concentrations* : 80, 100  $\mu\text{g}$  a.s./bee (nominal); solvent control; positive control : dimethoate

*Exposure route* : a.s. dissolved in DMF was added to the 50% sucrose suspension. 200 $\mu\text{l}$  of sucrose solution containing the a.s.were offered to each replicate of 10 bees.

*Feeding* : 50% aqueous sucrose solution

*Test conditions :*

26  $\pm$  1  $^{\circ}\text{C}$ , relative humidity of 60-80%

Findings and conclusion :

LD<sub>50</sub> (48h) oral > 100  $\mu\text{g}$  a.s./bee

NOEL (48h) oral < 80  $\mu\text{g}$  a.s./bee

##### **B.8.4.2 Bee brood feeding test (Annex IIA 8.3.1.2)**

The study is not required since the active substance is not an insect growth regulator.

##### **B.8.4.3 Acute toxicity of the preparations to bees (Annex IIIA 10.4.1)**

A Determination of Topical and Oral LD<sub>50</sub>s for EF-1343 (a 50 g/l SC Formulation of XDE-570) against the Honey Bee, *Apis mellifera* (Beech, 1996)

Assessment of Side Effects of EF-1343 to the Honeybee, *Apis Mellifera* L. in the Laboratory following the EPPO Guideline (Nengel, 1996)

Assessment of Side Effects of EF-1343 on Honeybees (*Apis Mellifera* L.) Laboratory Test (Mahlen, et al., 1997)

Testing Toxicity to Honeybee - *Apis Mellifera* L. (Laboratory) according to BBA Guideline, VI, 2301 (1991) (Kleiner et al., 1996)

4 laboratory studies were performed to assess the toxicity of the formulation EF-1343 (SC containing 50 g/l florasulam). The studies were made according to the EPPO guideline 170 or the BBA guideline. These tests confirm the low toxicity of florasulam to bees observed in the test performed with the a.s. The main results are presented in the table B.8.4.8-1.

**B.8.4.4 effects on bees of residues on crops (Annex IIIA 10.4.2)**

**B.8.4.5 Cage tests (Annex IIIA 10.4.3)**

**B.8.4.6 Field tests to investigate special effects (Annex IIIA 10.4.4)**

**B.8.4.7 Tunnel testing to investigate effects of feeding on contaminated honey (Annex IIIA 10.4.5)**

Those studies are not required since the hazard quotients are < 50 (0.075).

#### B.8.4.8 Exposure and risk assessment for bees (Annex IIIA 10.4)

Table B.8.4.8-1 : Summary of effects of florasulam to honeybees

Test species	Test system	Results	References
Florasulam			
Honeybee ( <i>Apis mellifera</i> L)	Acute oral toxicity test	LD <sub>50</sub> > 100 µg a.s./bee.	Palmer, et al., 1994
Honeybee ( <i>Apis mellifera</i> L)	Acute contact toxicity test	LD <sub>50</sub> > 100 µg a.s./bee.	Beech, 1996
EF-1343 (SC containing 50 g/l florasulam)			
Honeybee ( <i>Apis mellifera</i> L)	Acute oral toxicity test (24 h)	LD <sub>50</sub> > 100 µg a.s./bee.	Beech, 1996
	Acute contact toxicity test (24 h)	LD <sub>50</sub> > 100 µg a.s./bee.	
Honeybee ( <i>Apis mellifera</i> L)	Acute oral toxicity test (48 h)	LD <sub>50</sub> > 70.25 µg a.s./bee.	Nengel, 1996
	Acute contact toxicity test (48 h)	LD <sub>50</sub> > 100 µg a.s./bee.	
Honeybee ( <i>Apis mellifera</i> L)	Respiratory effects, residual effects, direct contamination, oral toxicity (72h)	no respiratory effects, no residual effects, no effects by direct contamination, no effects on behaviour and food uptake oral LD <sub>50</sub> > 0.1µl formulation/bee.	Mahlen, et al., 1997
Honeybee ( <i>Apis mellifera</i> L)	Respiratory effects, residual effects, direct contamination, oral toxicity (72h)	The test substance caused negligible or no mortality of the bees in the 4 tests; oral LD <sub>50</sub> > 200 µg formulation/bee.	Kleiner et al., 1996

Hazard quotients reveal that the bees are not at risk.

Table B.8.4.8-2 : Hazard quotients for honeybees

Application rate (kg a.s./ha)	Crop	Route	Hazard quotient	Annex VI trigger
0.0075	Cereals	oral	<0.075	50
		contact	<0.075	50

### B.8.5 Effects on other arthropods species (Annex IIA 8.3.2; Annex IIIA 10.5)

#### B.8.5.1 Effects of the active substance on non-target terrestrial arthropods (Annex IIA 8.3.2)

Effects of the a.s. on non-target arthropods were assessed in laboratory tests performed with a representative formulation : EF-1343 (SC containing 50 g/l florasulam).

#### B.8.5.2 Effects of the formulations on non-target terrestrial arthropods (laboratory, semi-field tests) Annex IIIA 10.5.1)

A laboratory study to evaluate the effects of XDE-570 on the predatory mite, *Typhlodromus pyri* (Austin, 1997a)

##### Guidelines :

IOBC/WPRS Bulletin 1988/XI/4 pp 65 - 70 ≡ SETAC ≡ ESCORT

##### GLP :

Yes

##### Material and Methods :

*Test substance* : EF-1343 (SC containing 50 g/l florasulam)

*Test species* : *Typhlodromus pyri* (predaceous mite), protonymphs

*Number of organisms* : 5 replicates per treatment each with 20 protonymphs

*Type of test* : Laboratory test

*Applied concentrations* :

Positive control (fenpropathrin), water control, treatment at concentrations equivalent to the field application rates of 7.5 and 15 g a.s./ha (200l/ha)

*Exposure route* :

protonymphs were maintained on glass plates for 7 days. Mortality was assessed on days 1, 3, 7. Eggs and hatched larvae were counted on days 10 and 13.

*Test conditions* :

temperature : 24.4-25.3°C, relative humidity : 69-77%, light intensity : 898-1200 lux , photoperiod : 16 hours light/8 hours dark.

##### Findings :

Table B.8.5.2-1 : Effects of formulation EF-1343 on *Typhlodromus pyri*

Evaluation criteria	Control	7.5 g a.s./ha	15 g a.s./ha
Mortality at day 7 (%)	22 %	40 %	50 %
Egg production (No of eggs /female)	5.0	5.7	4.4
Reduction of beneficial capacity		12.3 %	43.6 %

##### Conclusion :

The formulation EF-1343 (SC containing 50 g/l florasulam) is 'harmless' to *Typhlodromus pyri* at the application rate of 7.5 g a.s./ha and 'slightly harmful' at the rate of 15 g a.s./ha

A laboratory study to evaluate the effects of XDE-570 on the parasitic wasp, *Aphidius rhopalosiphi* (Austin , 1997b)

##### Guidelines :

Mead-Briggs, M (1992) Aspects of Applied Biology 31, 179 - 189 ≡ SETAC ≡ ESCORT

##### GLP :

Yes

##### Material and Methods :

*Test substance* : EF-1343 (SC containing 50 g/l florasulam)

*Test species* : *Aphidius rhopalosiphi* (parasitic wasp), adult females, less than 48 hours old

*Number of organisms* : 3 replicates per treatment each with 10 wasps for the exposure phase, 5 replicates with 2 wasps each for the reproduction phase.

*Type of test* : Laboratory test

*Applied concentrations* :

Positive control (dimethoate), water control, treatment at concentrations equivalent to the field application rates of 7.5 and 15 g a.s./ha (200l/ha)

*Exposure route* :

48 hours exposure on glass plate. For the parasitization phase, females were individually transferred to potted barley plants infested with aphids. *Aphidius* were removed after 24 hours. The number of mummies were assessed after 12 days.

*Test conditions* :

temperature : 19.3-20.5°C, relative humidity : 64-78 %, light intensity : 752-1250 lux , photoperiod : continuous low intensity

Findings :

Table B.8.5.2-2 : Effects of formulation EF-1343 on *Aphidius rhopalosiphi*

Evaluation criteria	Control	7.5 g a.s./ha	15 g a.s./ha
exposure phase (% mortality)	13	7	27
parasitization phase (mean No of mummies/female)	9.8	6.9	6.3
Reduction of beneficial capacity		25.2%	49.7%

Conclusion :

The formulation EF-1343 (SC containing 50 g/l florasulam) is 'harmless' to *Aphidius rhopalosiphi* at the application rate of 7.5 g a.s./ha and 'slightly harmful' at the rate of 15 g a.s./ha

A laboratory study to evaluate the effects of XDE-570 on the carabid beetle *Poecilus cupreus* (Austin, 1996)

Guidelines :

IOBC/WPRS Bulletin 1992/XV/3 pp 103-109 = SETAC = ESCORT

GLP :

Yes

Material and Methods :

*Test substance* :EF-1343 (SC containing 50 g/l florasulam)

*Test species* : *Poecilus cupreus*, 5-6 weeks old adults beetles

*Number of organisms* : 5 replicates per treatment each with 3 \_ and 3 \_ beetles

*Type of test* : Laboratory test

*Applied concentrations* :

Positive control (dimethoate), water control,treatment at concentrations equivalent to the field application rates of 7.5 and 15 g a.s./ha (400l/ha)

*Exposure route* :

Beetles maintained in sand containing boxes were sprayed with the test solutions. Mortality, behaviour and feed consumption were monitored for 14 days.

*Test conditions* :

temperature : 19.8-20.0 °C, relative humidity : 82-89%, light intensity : 729-1239 lux , photoperiod : 16 hours light/8 hours dark.

Findings :

Table B.8.5.2-3 : Effects of formulation EF-1343 on *Poecilus cupreus*

Evaluation criteria	Control	7.5 g a.s./ha	15 g a.s./ha
Mortality after 14 days (%)	0	0	0
Mean feeding rate throughout the study (number fly pupae /beetle)	1.07	1.12	1.33
Total effect (E)		0	0

Conclusion :



The formulation EF-1343 (SC containing 50 g/l florasulam) is 'harmless' to *Poecilus cupreus* at the application rates of 7.5 and 15 g a.s./ha.

A laboratory study to evaluate the effects of XDE-570 on the green lacewing, *Chrysoperla carnea* (Nickless, 1996)

Guidelines :

IOBC/WPRS Bulletin 1988/XI/4 pp 71 - 77 ≡ SETAC ≡ ESCORT

GLP :

Yes

Material and Methods :

*Test substance* : EF-1343 (SC containing 50 g/l florasulam)

*Test species* : *Chrysoperla carnea*, first instar larvae

*Number of organisms* : 30 replicates each with 1 larva per treatment

*Type of test* : Laboratory test

*Applied concentrations :*

Positive control (dimethoate), water control, treatment at concentrations equivalent to the field application rates of 7.5 and 15 g a.s./ha (200l/ha)

*Exposure route :*

Lacewings larvae were exposed to the a.s. via treated glass plates with dry spray deposit for a period of 18 days. All insects reaching adult stage were transferred to untreated reproduction units to assess oviposition and egg viability (31 days).

*Test conditions :*

temperature : 21.3-24.0°C, relative humidity : 68-76%, light intensity : 2120-3470 lux , photoperiod : 16 hours light/8 hours dark.

Findings :

Table B.8.5.2-4 : Effects of formulation EF-1343 on *Chrysoperla carnea* - laboratory test

Evaluation criteria	Control	7.5 g a.s./ha	15 g a.s./ha
Exposure phase			
- Larval mortality (%)	10.7	6.9	17.2
- Pupae mortality (%)	4.0	0.0	4.2
- Total juvenile mortality (%)	14.7	6.9	21.4
Reproduction phase			
- Number of eggs per female	19.8	4.4	0.0
Total effect (E)		77.55%	100%

Conclusion :

The formulation EF-1343 is 'slightly harmful' to *Chrysoperla carnea* at the application rate of 7.5 g a.s./ha and 'harmful' at the rate of 15 g a.s./ha

An extended laboratory test to evaluate the effects of the herbicide EF-1343, a 50 g/l suspension concentrate formulation of XDE-570, on the green lacewing, *Chrysoperla carnea* (Thompson, 1998)

Guidelines :

A field methnod for testing the effects of pesticides on larvae of the green lacewing *Chrysoperla carnea* (Neuroptera , Chrysopidae) (Vogt et al., 1992)

GLP :

Yes

Material and Methods :

*Test substance* : EF-1343 (SC containing 50 g/l florasulam)

*Test species* : *Chrysoperla carnea*, 3-4 day old larvae

*Number of organisms* : 40 larvae/concentration

*Type of test* : Extended laboratory test

*Applied concentrations :*

Positive control (dimethoate), water control, treatment at concentration equivalent to the field application rate of 7.5 g a.s./ha (400l/ha)

Exposure route :

Maize seedlings, cultivated in pots, were treated. 2 hours after treatment, a single larva was placed into each pot and confined using a clear acetate sheeting. Larvae were fed with aphids.

When larvae had pupated, they were transferred to emergence boxes. Adults were then transferred to oviposition boxes. Egg laying was monitored over a 22 day period.

Test conditions :

temperature :19-24 °C, relative humidity : 16-63%, light intensity : 5800 lux , photoperiod : 16 hours light/8 hours dark.

**Egg laying :** temperature 21-26 °C, relative humidity : 31-60%, light intensity : 5830 lux , photoperiod : 16 h light/8 h dark.

Findings :

Table B.8.5.2-5 : Effects of formulation EF-1343 on *Chrysoperla carnea* - Extended laboratory test

Evaluation criteria	Control	7.5 g a.s./ha
Exposure phase		
- Larvae pupating (%)	52	60
- Larvae reaching adulthood (%)	48	48
Reproduction phase		
- Number of eggs per female per day	9.4	10.0
Total effect (E)		0%

Conclusion :

The formulation EF-1343 is 'harmless' to *Chrysoperla carnea* at the application rate of 7.5 g a.s./ha (extended laboratory test)

**B.8.5.3 Effects of the formulations on non-target terrestrial arthropods (field tests) Annex IIIA 10.5.2)**

No study is required.

#### B.8.5.4 Summary of effects, exposure and risk assessment for non-target terrestrial arthropods

Table B.8.5.4-1 : Summary of effects of EF-1343 (SC containing 50 g/l florasulam) to non-target terrestrial arthropods

Species	Stage	Test substance	Dose g a.s./ha	Endpoint	Effect	Annex VI trigger	References
Laboratory tests							
<i>Typhlodromus pyri</i>	protonymphs	formulation	7.5	beneficial capacity	12.3%	30%	Austin, 1997a
			15		<b>43.6 %</b>		
<i>Aphidius rhopalosiphi</i>	adults	formulation	7.5	beneficial capacity	25.2%	30%	Austin, 1997b
			15		<b>49.7%</b>		
<i>Poecilus cupreus</i>	adults	formulation	7.5	mortality	0%	30%	Austin, 1996
			15		0%		
<i>Chrysoperla carnea</i>	first instar larvae	formulation	7.5	beneficial capacity	<b>77.55%</b>	30%	Nickless, 1996
			15		<b>100%</b>		
Extended laboratory test							
<i>Chrysoperla carnea</i>	first instar larvae	formulation	7.5	beneficial capacity	0%	30%	Thompson, 1998

Florasulam is a post emergence broadleef weed herbicide used in cereals at the maximum rate of 7.5 g a.s./ha. Florasulam can be applied from stage BBCH 12 (2 leaves) to stage BBCH 49 (stem elongation).

At the application rate of 7.5 g a.s./ha florasulam is harmless to *Typhlodromus pyri*, *Aphidius rhopalosiphi* and *Poecilus cupreus*.

Florasulam is slightly harmful to *Chrysoperla carnea* in laboratory test. Florasulam is harmless to *Chrysoperla* in an extended laboratory test performed at the application of 7.5 g a.s./ha

### **B.8.6 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.3.6)**

#### **B.8.6.1 Acute toxicity to earthworms (Annex IIA 8.4.1)**

XDE-570 herbicide: acute toxicity to the earthworm, *Eisenia foetida* (Boeri, et al., 1994)

Guidelines :

OECD Method No. 207

GLP :

Yes

Material and Methods :

*Test substance* : florasulam, purity : 99.2%

*Test species* : Earthworms (*Eisenia foetida*)

*Number of organisms, weight, age* : 4 replicates each with 10 worms per treatment group, 0.2305-0.3498 g at start, with developed clitellum

*Type of test* : laboratory test (14 days)

*Nominal and measured concentrations* :

Measured : 95.7, 200, 336, 690, 1320 mg a.s./kg; water control

Measured concentrations ranging from 93 to 105 % of the nominal concentrations

*Soil type and test conditions* :

Test substrate : 10% sphagnum peat, 20% kaolinite clay, 69% industrial sand

water content : 27%, pH : 6.2

Temperature : 19.3-22.0 °C

Light regime : continuous, 750 lux

*Analytical methods* : HPLC with UV

Findings :

*Mortality* : no mortality was observed.

*Observations* : body weight changes were similar in the control and treatment groups

Conclusion :

LC<sub>50</sub> (14d) > 1320 mg a.s./kg substrate

NOEC (14d) = 336 mg a.s./kg substrate

5-Hydroxy-XDE-570: acute toxicity to the earthworm, *Eisenia foetida* (Ward, et al., 1996)

Guidelines :

OECD Method No. 207

GLP :

Yes

Material and Methods :

*Test substance* : metabolite **5-hydroxy DE-570**, purity : 97.0 %

*Test species* : Earthworms (*Eisenia foetida*)

*Number of organisms, weight, age* : 4 replicates each with 10 worms per treatment group, 0.2491-0.3317 g at start, with developed clitellum

*Type of test* : laboratory test (14 days)

*Nominal and measured concentrations* :

Measured : 141, 225, 399, 678, 1120 mg a.s./kg; water control

Measured concentrations ranging from 83 to 89 % of the nominal concentrations

*Soil type and test conditions* :

Test substrate : 10% sphagnum peat, 20% kaolinite clay, 69% industrial sand,

water content : 26%, pH : 6.1

Temperature : 20.7-21.5 °C

Light regime : continuous, 420 lux

*Analytical methods* : HPLC with UV

Findings :

*Mortality* : no mortality was observed.

*Observations* : body weight changes were similar in the control and treatment groups

Conclusion :

LC<sub>50</sub> (14d, metabolite 5-hydroxy) > 1120 mg/kg substrate

NOEC (14d, metabolite 5-hydroxy) = 1120mg/kg substrate

**B.8.6.2 Sublethal effects on earthworms (Annex IIA 8.4.2)**

Due to the very high margin of safety observed with the acute TER for the a.s. and the formulation EF-1343, further testing of the sublethal effects is not required.

**B.8.6.3 Acute toxicity of the formulations to earthworms (Annex IIIA 10.6.1.1)**

EF-1343: acute toxicity study in the earthworm (artificial soil test) (Rees, 1996)

Guidelines :

OECD Method No. 207

GLP :

Yes

Material and Methods :

*Test substance* : EF-1343 (SC containing 50 g/l florasulam)

*Test species* : Earthworms (*Eisenia foetida*)

*Number of organisms, weight* : 4 replicates each with 10 worms per treatment group, 306-598 mg at start

*Type of test* : laboratory test (14 days)

*Nominal and measured concentrations* : 1033 mg/kg; water control

*Soil type and test conditions :*

Test substrate : 9.5% sphagnum peat, 18.7% kaolinite clay, 63.6% fine quartz sand, 4.8% rabbit faeces, 0.7% calcium carbonate

Water content : 35%, pH : 6.0

Temperature : 18-22 °C

Light regime : continuous, 600 lux

*Analytical methods :-*

Findings :

*Mortality* : no mortality was observed.

*Observations* : body weight changes were similar in the control and treatment groups

Conclusion :

LC<sub>50</sub> (14d) > 1033 mg formulation /kg substrate

NOEC (14 d) = 1033 mg formulation /kg substrate

**B.8.6.4 Sublethal effects of the formulation on earthworms (Annex IIIA 10.6.1.2)**

Due to the very high margin of safety observed with the acute TER for the a.s. and the formulation EF-1343, further testing of the sublethal effects is not required.

**B.8.6.5 Field tests - residue content of earthworms (Annex IIIA 10.6.1.3)**

The study is not required.

### B.8.6.6 Summary and risk assessment for earthworms (Annex III, 10.6.1.1)

Table B.8.6.6-1 : Summary of effects of florasulam for earthworms

Test species	Test system	Duration of exposure	Results (mg/kg soil)	References
Florasulam				
Earthworm ( <i>Eisenia foetida</i> )	acute test	14 days	LC <sub>50</sub> > 1320 mg a.s./kg NOEC (14d) = 336 mg a.s./kg	Boeri, et al., 1994
metabolite <b>5-hydroxy DE-570</b>				
Earthworm ( <i>Eisenia foetida</i> )	acute test	14 days	LC <sub>50</sub> > 1120 mg formulation/kg NOEC = 1120 mg formulation/kg	Ward, et al., 1996
EF-1343 (SC containing 50 g/l florasulam)				
Earthworm ( <i>Eisenia foetida</i> )	acute test	14 days	LC <sub>50</sub> > 1033 mg formulation/kg NOEC = 1033 mg formulation/kg	Rees, 1996

The following assumptions were made to assess the acute risk for earthworms :

- 100% of the spray reaches the soil surface.
- The substance (or formulation) is distributed in a 5 cm soil layer with a soil density of 1.5 g/cm<sup>3</sup>

Due to the very high margin of safety observed with the acute TER for the a.s. and the formulation EF-1343, further testing of the sublethal effects is not required.

Table B.8.6.6-2 : Toxicity/exposure ratios for earthworms

Application rate	Crop	Time-scale	TER	Annex VI trigger
Florasulam				
0.0075 kg a.s./ha	Cereals	acute	> 132000	10
EF-1343 (SC containing 50 g/l florasulam)				
0.150 l/ha	Cereals	acute	>5165	10

### B.8.7 Effects on other soil non-target macro-organisms (Annex IIIA 10.6.2)

As the absence of risk has been demonstrated for soil non-target arthropods (*Poecilus cupreus*), earthworms and soil non-target micro-organisms, these studies are not necessary.

### B.8.8 Effects on soil non-target micro-organisms (Annex IIA 8.5; Annex IIIA 10.7)

#### B.8.8.1 Impact of the active substance on soil microbial activity (Annex IIA 8.5)

A laboratory assessment of the effects of XDE-570 on soil microflora respiration and nitrogen turnover according to BBA Guidelines VI 1-1 (1990) (Forster, 1997)

Guidelines :

BBA Guideline VI, 1-1 (1990)

GLP :

Yes

Material and methods :

*Test Substance:* florasulam, purity : 99.3%

*Soils :* 3 and 4 replicates per treatment group, respectively for the respiration and nitrogen tests

The soils were collected from grassland sites that had not received pesticide or fertilizer treatments within the previous 12 months.

*Applied concentrations :*

The a.s. was applied at the concentration of 0.01 and 0.05 mg/kg soil, equivalent to the application rates of 7.5 g a.s./ha and 37.5 g a.s./ha, at a penetration of 5 cm and soil gravity of 1.5 g/cm<sup>3</sup>. Untreated control, positive control (dinoseb-acetate)

*Type of test :* 28-day nitrogen turnover test, 28 -day short time respiration

*Test conditions :*

Soil moisture : 45.0-46.3% of its water holding capacity, 0.5% lucerne meal was used in the nitrogen turnover test.

Soil samples were incubated at 21°C ± 2 °C in the dark.

Findings :

Table 8.8.1-1 : Effects of florasulam on the nitrogen turnover

	% Deviation from control								
	Florasulam						Dinoseb-acetate		
	0.01 mg/kg soil			0.05 mg/kg soil			mg/kg soil		
	NH <sup>4+</sup>	NO <sup>3-</sup>	N-min	NH <sup>4+</sup>	NO <sup>3-</sup>	N-min	NH <sup>4+</sup>	NO <sup>3-</sup>	N-min
Silty sand soil, BBA type 96/269, 1.7 % OM, pH 6.3, CEC :-									
3 hours	- 18.13	- 6.09	- 9.12	- 20.88	- 1.85	- 6.63	(- 1.76)	(+ 9.03)	(+ 6.02)
14 days	- 3.45	+ 18.02	+ 17.58	+ 6.90	+ 15.90	+ 15.72	(-)	(- 125.01)	(+ 51.26)
28 days	0	- 5.52	- 5.45	- 3.33	+ 4.78	+ 4.67	(+ 3227.12)	(+ 6.49)	(+ 71.31)
Sandy loamy silt soil, BBA type 96/270, 4.0 % OC, pH 6.3, CEC :-									
3 hours	+ 29.92	+ 1.18	+ 6.23	+ 20.78	- 9.04	- 3.80	(-21.40)	(- 4.97)	(- 9.19)
14 days	- 4.35	+ 1.99	+ 1.89	+ 1.45	+ 15.33	+ 15.09	(+ 882.14)	(+ 29.80)	(+ 36.84)
28 days	+ 8.62	- 8.45	- 8.26	+ 5.17	- 4.16	- 4.05	(+ 108.11)	(+ 54.78)	(+ 55.66)

Table 8.8.1-2 : Effects of florasulam on the short term respiration

	% Deviation of respiration rate from control		
	Florasulam		Dinoseb-acetate
	0.01 mg/kg soil	0.05 mg/kg soil	mg/kg soil
Silty sand soil, BBA type 96/269, 1.7 % OM, pH 6.3, CEC :-			
3 hours	- 8.70	- 5.80	(- 30.77)
14 days	0	0	(- 24.19)
28 days	- 8.93	- 5.36	(- 43.64)
Sandy loamy silt soil, BBA type 96/270, 4.0 % OC, pH 6.3, CEC :-			
3 hours	+ 4.13	- 1.65	(- 17.54)
14 days	+ 3.77	+ 5.66	(- 54.29)
28 days	+ 17.20	+ 20.43	(- 50.00)
60 days	- 6.06	+ 1.01	

**Conclusions :**

*Nitrogen turnover :*

After 28 days, no statistically significant effects were observed on mineral nitrogen concentrations at both application rates (0.01 and 0.05 mg/kg a.s. soil)

*Soil respiration :*

In silty sand, soil no statistically significant effects were observed after 28 days at both application rates (0.01 and 0.05 mg/kg a.s. soil).

In sandy loamy silt soil, statistically significant effects (> 15%) were observed after 28 days at both application rates. After 60 days no statistically significant effects were observed at both application rates (0.01 and 0.05 mg/kg a.s. soil).

**B.8.8.2 Impact of the formulations on soil microbial activity (laboratory) (Annex IIIA 10.7.1)**

No study is required.

**B.8.8.3 Further laboratory, glasshouse or field testing to investigate impact on soil microbial activity (Annex IIIA 10.7.2)**

No study is required.

**B.8.8.4 Summary of studies on non-target micro-organisms - exposure and risk assessment for non-target micro-organisms**

The impact of florasulam on the nitrogen turnover and the soil respiration was evaluated in 2 soils. Florasulam was applied at the concentration of 0.01 and 0.05 mg/kg soil, equivalent to the application rates of 7.5 g a.s. /ha and 37.5 g a.s./ha, at a penetration of 5 cm and soil gravity of 1.5 g/cm<sup>3</sup>.

Effects on the nitrogen turnover (<15% after 28 days) and on the soil respiration (< 15% after 60 days) are below the trigger values established in the Annex of Directive 91/414 (<25% after 100 days) and indicate a low risk of the a.s. to soil microorganisms.



### **B.8.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex IIA 8.6; Annex IIIA 10.8)**

The activity of DE-570 in herbicide, insecticide and fungicide screening tests and the herbicidal activity of DE-570 soil metabolites (Ehr, et al., 1997)

#### **Herbicide screen**

Florasulam was screened post-emergence against 25 plant species including 13 broadleaf species, 11 grass species and 1 sedge species. Florasulam demonstrated potent herbicidal activity on numerous broadleaf species at rates of 1 mg/l or less. No broadleaf crop selectivity was observed. As a group, grass species were about 30 times less sensitive than broadleaf species. Wheat seedlings exhibited a high degree of tolerance, as did the cool season grass weeds, wild oats and blackgrass, with little sensitivity to Florasulam at rates up to 31 mg/l, the highest concentration tested.

Florasulam was also evaluated for pre-emergence activity against 20 plant species including 10 broadleaf species, 9 grass species and 1 sedge species. All broadleaf species were moderately to highly sensitive but, overall, were less sensitive to pre-emergence applications than to post-emergence treatments. Grass species were much less sensitive to Florasulam with pre-emergence applications with wheat and wild oats showing high tolerance to rates equivalent to 140 g/ha.

The results of the post- and pre-emergence screening tests demonstrated that, in general, 5-hydroxy-DE570 was much less phytotoxic than Florasulam and that M3, decarboxyl M3 and M4 demonstrated no phytotoxicity at rates much higher than effective rates of Florasulam. In post-emergence tests, 5-hydroxy-DE-570 caused low level symptomology on broadleaf plants, characterised by transient stunting and chlorosis. 5-Hydroxy-DE-570, however, did not affect grass species and M3, decarboxyl M3 and M4 produced no phytotoxic effects on any plant species. In pre-emergence tests, 5-hydroxy-DE-570 was over 100 times less phytotoxic to broadleaf species than Florasulam with only minimal transient effects observed at 18 and 35 g/ha. No significant phytotoxicity was observed on grasses at 70 g/ha and M3, decarboxyl M3 and M4 produced no phytotoxic effects on any plant species following pre-emergence application.

#### **Insecticide screen**

Florasulam was tested at very high rates compared with rates which are herbicidal. The insecticide test systems provided contact or ingestive exposures as well as application to eggs, larvae or adult insects. No harmful effects were observed on any of the six species of phytophagous insects (*Aphis gossypii*, *Diabrotica undecimpunctata*, *Spodoptera exigua*, *Heliothis virescens*, *Macrostes severini*) or one species of phytophagous mite (*Tetranychus urticae*) at 800 mg/l, the highest concentration tested (50 mg/l for *Diabrotica*).

#### **Fungicide screen**

Florasulam was screened on three diseases of wheat and two diseases of broadleaf crops. Only negligible activity was observed against *Septoria nodorum* and *S. tritici* at rates up to 100 mg/l. Moderate effects were recorded against *Erysiphe graminis tritici* at 25 and 100 mg/l and significant control of *Phytophthora infestans* at 100 mg/l and *Plasmopara viticola* at 25 and 100 mg/l. The apparent disease control in grape and tomato, however, may have been due in part to the phytotoxic effect of Florasulam on the host plant hindering the infection process.

#### **Conclusion**

Florasulam demonstrates very high levels of activity on broadleaf weeds and low activity against grasses. Pre-emergence activity is appreciably less than post-emergence activity and the rapid degradation of Florasulam to metabolites of low phytotoxicity will ensure that the risk to non-target plants is minimal once the active substance is degraded in the soil. Florasulam shows no appreciable activity against insects and only weak fungicidal activity at rates considerably higher than those exhibiting herbicidal properties.'

### **B.8.10 Effects on biological methods of sewage treatment (Annex IIA 8.7)**

Under the normal conditions of practical use (herbicide in cereals) it is not expected that florasulam will contaminate sewage treatment plant.



### B.8.11 References relied on

#### Ecotoxicology of the active substance (Annex IIA 8)

Annex point(s) 91/414/EEC	Author, Title, Test institute, Report number/Study ID, Date of report For publications: reference	Dow AgroScience s Report No.	GLP GEP	Published Protected
IIA 8.3.2	Austin, HM, A Laboratory Study to Evaluate the Effects of XDE-570 on the Carabid Beetle <i>Poecilus cupreus</i> Ecotox Limited, Tavistock, Devon, UK ER-96-01, March 1996	GHE-P-6709	Yes	Unpublished Protected
IIA 8.3.2	Austin, HM, A Laboratory Study to Evaluate the Effects of XDE-570 on the Predatory Mite, <i>Typhlodromus pyri</i> Ecotox Limited, Tavistock, Devon, UK ER-96-31, February 1997a	GHE-P-6706	Yes	Unpublished Protected
IIA 8.3.2	Austin, HM, A Laboratory Study to Evaluate the Effects of XDE-570 on the Parasitic Wasp, <i>Aphidius rhopalosiphii</i> Ecotox Limited, Tavistock, Devon, UK ER-96-34, February 1997b	GHE-P-6707	Yes	Unpublished Protected
IIA 8.1.3	Beavers, JB, Jaber, MJ, XDE-570 Herbicide: A Pilot Reproduction Study with the Mallard Wildlife International Ltd, Easton, Maryland, USA 103-409, January 1995a	DECO-ES-2821	No	Unpublished Protected
IIA 8.1.3	Beavers, JB, Jaber, MJ, XDE-570 Herbicide: A Pilot Reproduction Study with the Northern Bobwhite Wildlife International Ltd, Easton, Maryland, USA 103-408, January 1995b	DECO-ES-2820	No	Unpublished Protected
IIA 8.3.1.1	Beech, P, A Determination of the Oral LD <sub>50</sub> s for XDE-570 against the Honey Bee, <i>Apis mellifera</i> Agrochemical Evaluation Unit, Department of Biology, The University, Southampton, UK DOW-96-3, November 1996	GHE-P-6705	Yes	Unpublished Protected
IIA 8.4.1	Boeri, RL, Magazu, JP, Ward, TJ, XDE-570 Herbicide: Acute Toxicity to the Earthworm, <i>Eisenia foetida</i> TR Wilbury Laboratories Inc, Marblehead, Massachusetts, USA 464-DO, July 1994	DECO-ES-2798	Yes	Unpublished Protected
IIA 8.1.1	Campbell, SM, Beavers, JB, XDE-570: An Acute Oral Toxicity Study with the Japanese Quail Wildlife International Ltd, Easton, Maryland, USA 103-403, December 1994	DECO-ES-2799	Yes	Unpublished Protected
IIA 8.6	Ehr, RJ, Alexander, AL, The Activity of DE-570 in Herbicide, Insecticide and Fungicide Screening Tests and the Herbicidal Activity of DE-570 Soil Metabolites DowElanco, Indianapolis, Indiana, USA None stated, July 1997	DERBI # 60600	No	Unpublished Protected
IIA 8.2.8	Ehr, RJ, Schmitzer, PR, Gray, JA, The Activity of DE-570	DERBI # 60700	No	Unpublished Protected

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IIA 8.5	Forster, J, A Laboratory Assessment of the Effects of XDE-570 on Soil Microflora Respiration and Nitrogen Turnover According to BBA Guidelines VI 1-1 (1990) Euro Laboratories Limited, Sandy, Bedfordshire, UK ELL/1186, January 1997	GHE-T-713	Yes	Unpublishe dProtected
IIA 8.1.3	Gallagher, SP, Beavers, JB, Jaber, M, XDE-570: A Reproduction Study with the Northern Bobwhite ( <i>Colinus virginianus</i> ) Wildlife International Ltd, Easton, Maryland, USA 103-411, December 1995a	DECO-ES-2911	Yes	Unpublishe dProtected
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IIA 8.2.6	Hastings, M, Preparation of Soil Extracts for Determination of the Algal Toxicity of XDE-570 Metabolites DowElanco Europe, Letcombe Regis, Oxon, UK 13U, November 1997	GHE-P-6616	Yes	Unpublishe dProtected
IIA 8.1.2	Helsten, BR, Solatycki, AM, XDE-570 Herbicide: 8-day Acute Dietary LC <sub>50</sub> Study in Japanese Quail Bio-Life Associates Ltd, Neillsville, WI, USA BLAL No. 128-004-01, July 1994b	DECO-ES-2797	Yes	Unpublishe dProtected
IIA 8.1.2	Helsten, BR, Solatycki, AM, XDE-570 Herbicide: 8-day Acute Dietary LC <sub>50</sub> Study in Mallard Ducklings Bio-Life Associates Ltd, Neillsville, WI, USA BLAL No. 128-005-02, July 1994a	DECO-ES-2796	Yes	Unpublishe dProtected
IIA 8.2.6	Hughes, JS, Williams, TL, Conder, LA, The Toxicity of XDE-570 to <i>Skeletonema costatum</i> Carolina Ecotox Inc, Durham, North Carolina, USA 10-03-1, September 1995	DECO-ES-3021	Yes	Unpublishe dProtected
IIA 8.2.6	Jenkins, CA, Two Aqueous Soil Extracts Containing XDE-570 Metabolites: Growth Inhibition of <i>Selenastrum capricornutum</i> (Preliminary Toxicity Screen) Huntingdon Life Sciences Ltd, Eye, Suffolk, UK DWC828/970371, October 1997	GHE-T-837	Yes	Unpublishe dProtected
IIA 8.7	Jenkins, WR, XDE-570 (Pure): Assessment of Ready Biodegradability Pharmaco::LSR, Eye, Suffolk, UK 94/DES180/0468, July 1994	GHE-P-3736	Yes	Unpublishe dProtected
IIA 8.7	Jenkins, WR, XDE-570 5-Hydroxy Metabolite: Assessment of Ready Biodegradability	GHE-P-4552	Yes	Unpublishe dProtected

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IIA 8.2.5 IIA 8.2.7	Kelly, CR, XDE-570: To Assess the Toxicity to the Sediment Dwelling Phase of the Midge, <i>Chironomus riparius</i> Huntingdon Life Sciences Ltd, Huntingdon, Cambridgeshire, UK. DWC 843/973949, October 1997	GHE-T-838	Yes	Unpublishe dProtected
IIA 8.2.1	Kirk, HD, Hugo, JM, Evaluation of the Acute Toxicity of 5-hydroxy XDE-570 to the Rainbow Trout, <i>Oncorhynchus mykiss</i> Walbaum The Dow Chemical Company, Midland, Michigan, USA DECO-ES-3118, August 1996	DECO-ES-3118	Yes	Unpublishe dProtected
IIA 8.2.4	Kirk, HD, Landre, AM, Hugo, JM, Evaluation of the Acute Toxicity of 5-Hydroxy XDE-570 to the Daphnid, <i>Daphnia magna</i> Straus The Dow Chemical Company, Midland, Michigan, USA DECO-ES-3117, August 1996	DECO-ES-3117	Yes	Unpublishe dProtected
IIA 8.2.5	Kirk, HD, Landre, AM, Hugo, JM, Stahl, DC, Evaluation of the Chronic Toxicity of XDE-570 Herbicide to the Daphnid, <i>Daphnia magna</i> Straus. The Dow Chemical Company, Midland, Michigan, USA DECO-ES-2944, January 1996	DECO-ES-2944	Yes	Unpublishe dProtected
IIA 8.2.4	Kirk, HD, Landre, AM, Massaro, LM, Hugo, JM, Stahl, DC, Evaluation of the Acute Toxicity of XDE-570 Herbicide to the Daphnid, <i>Daphnia magna</i> Straus. The Dow Chemical Company, Midland, Michigan, USA DECO-ES-2938, May 1995	DECO-ES-2938	Yes	Unpublishe dProtected
IIA 8.2.1	Kirk, HD, Miller, JA, Hugo, JM, Stahl, DC, Evaluation of the Acute Toxicity of XDE-570 Herbicide to the Rainbow Trout, <i>Oncorhynchus mykiss</i> Walbaum. The Dow Chemical Company, Midland, Michigan, USA DECO-ES-2940, January 1995a	DECO-ES-2940	Yes	Unpublishe dProtected
IIA 8.2.1	Kirk, HD, Miller, JA, Hugo, JM, Stahl, DC, Evaluation of the Acute Toxicity of XDE-570 Herbicide to the Bluegill, <i>Lepomis macrochirus</i> Rafinesque. The Dow Chemical Company, Midland, Michigan, USA DECO-ES-2939, January 1995b	DECO-ES-2939	Yes	Unpublishe dProtected
IIA 8.2.2.1	Landre, AM, Martin, MD, Weinberg, JT, Evaluation of the Prolonged (28-day) Toxicity of XDE-570 Herbicide to the Rainbow Trout, <i>Oncorhynchus mykiss</i> Walbaum. The Dow Chemical Company, Midland, Michigan, USA DECO-ES-2973, January 1996	DECO-ES-2973	Yes	Unpublishe dProtected
IIA 8.2.6	Milazzo, DP, Hugo, JM, McFadden, L, XDE-570 5-Hydroxy: The Toxicity to the Freshwater Green Alga, <i>Selenastrum capricornutum</i> Printz The Dow Chemical Company, Midland, Michigan, USA	ES-3115	Yes	Unpublishe dProtected

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IIA 8.2.8	Milazzo, DP, Martin, MD, Kirk, HD, Hugo, JM, The Toxicity of XDE-570 Herbicide to the Aquatic Plant, Duckweed, <i>Lemna gibba</i> L. G-3 The Dow Chemical Company, Midland, Michigan, USA ES-2988, November 1995	ES-2988	Yes	Unpublished Protected
IIA 8.2.6	Milazzo, DP, Landre, AM, Rick, DL, Martin, MD, XDE-570 Herbicide: The Toxicity to the Blue-Green Alga, <i>Anabaena flos-aquae</i> The Dow Chemical Company, Midland, Michigan, USA DECO-ES-3005, December 1995	DECO-ES-3005	Yes	Unpublished Protected
IIA 8.2.6	Milazzo, DP, Landre, AM, Hugo, JM, Martin, MD, XDE-570 Herbicide: The Toxicity to the Freshwater Diatom, <i>Navicula pelliculosa</i> . The Dow Chemical Company, Midland, Michigan, USA DECO-ES-3045, March 1996	DECO-ES-3045	Yes	Unpublished Protected
IIA 8.2.6	Milazzo, DP, Humbert, LM, Hugo, JM, Martin, MD, XDE-570 Herbicide: The Toxicity to the Green Alga, <i>Selenastrum capricornutum</i> Printz. The Dow Chemical Company, Midland, Michigan, USA DECO-ES-2946, February 1995	DECO-ES-2946	Yes	Unpublished Protected
IIA 8.3.2	Nickless, A, A Laboratory Study to Evaluate the Effects of XDE-570 on the Green Lacewing, <i>Chrysoperla carnea</i> Ecotox Limited, Tavistock, Devon, UK ER-96-24, November 1996	GHE-P-6708	Yes	Unpublished Protected
IIA 8.3.1.1	Palmer, SJ, Beavers, JB, XDE-570: An Acute Contact Study with the Honey Bee Wildlife International Ltd, Easton, Maryland, USA 103-407, August 1994	DECO-ES-2819	Yes	Unpublished Protected
IIA 8.2.3	Rick, DL, Miller, JA, Kirk, HD, The Bioconcentration of XDE-570 by the Rainbow Trout, <i>Oncorhynchus mykiss</i> Walbaum The Dow Chemical Company, Midland, Michigan, USA ES-3038, October 1997	ES-3038	Yes	Unpublished Protected
IIA 8.2.4	Ward, TJ, Magazu, JP, Boeri, RL, XDE-570: Acute Toxicity to the Grass Shrimp, <i>Palaemonetes pugio</i> TR Wilbury Laboratories Inc, Marblehead, Massachusetts, USA 643-DO, September 1995a	DECO-ES-2922	Yes	Unpublished Protected
IIA 8.2.4	Ward, TJ, Magazu, JP, Boeri, RL, XDE-570: Acute Flow-Through Mollusc Shell Deposition Test TR Wilbury Laboratories Inc, Marblehead, Massachusetts, USA 644-DO, September 1995b	DECO-ES-2923	Yes	Unpublished Protected
	Ward, TJ, Magazu, JP, Boeri, RL, XDE-570: Acute Toxicity	DECO-ES-		Unpublished

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IIA 8.4.1	Ward, TJ, Magazu, JP, Boeri, RL, 5-Hydroxy-XDE-570: Acute Toxicity to the Earthworm, <i>Eisenia foetida</i> . TR Wilbury Laboratories Inc, Marblehead, Massachusetts, USA 1022-DO, December 1996	DECO-ES- 3120	Yes	Unpublishe dProtected

Ecotoxicology of the formulation EF-1343 (Annex IIIA 10)

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IIIA 10.5.1 (see IIA 8.3.2)	Austin, HM, A Laboratory Study to Evaluate the Effects of XDE-570 on the Parasitic Wasp, <i>Aphidius rhopalosiphi</i> Ecotox Limited, Tavistock, Devon, UK ER-96-34, February 1997b	GHE-P-6707	Yes	Unpublishe dProtected
IIIA 10.4.1	Beech, P, A Determination of Topical and Oral LD <sub>50</sub> s for EF-1343 (a 50 g/l SC Formulation of XDE-570) against the Honey Bee, <i>Apis mellifera</i> Agrochemical Evaluation Unit, Department of Biology, The University, Southampton, UK DOW-96-2, September 1996	GHE-P-5251	Yes	Unpublishe dProtected
IIIA 10.8 (see IIA 8.6)	Ehr, RJ, Alexander, AL, The Activity of DE-570 in Herbicide, Insecticide and Fungicide Screening Tests and the Herbicidal Activity of DE-570 Soil Metabolites DowElanco, Indianapolis, Indiana, USA None stated, July 1997	DERBI # 60600	No	Unpublishe dProtected
IIIA 10.2.1	Jenkins, CA, EF-1343: Acute Toxicity to Rainbow Trout Huntingdon Life Sciences Ltd, Eye, Suffolk, UK 96/DES345/0351, October 1996a	GHE-T-654	Yes	Unpublishe dProtected
IIIA 10.2.1	Jenkins, CA, EF-1343: Acute Toxicity to <i>Daphnia magna</i> Huntingdon Life Sciences Ltd, Eye, Suffolk, UK 96/DES346/0352, October 1996b	GHE-T-655	Yes	Unpublishe dProtected
IIIA 10.2.1	Jenkins, CA, EF-1343: Determination of 72-hour EC <sub>50</sub> to <i>Selenastrum capricornutum</i> Huntingdon Life Sciences Ltd, Eye, Suffolk, UK 96/DES366/0353, October 1996c	GHE-T-656	Yes	Unpublishe dProtected
IIIA 10.4.1	Kleiner, R, Haussmann, Brenner, P, Testing Toxicity to Honeybee ( <i>Apis mellifera</i> L.) According to BBA Guideline VI, 23-1 (1991) Biochem, D-76185 Karlsruhe, Germany 96 10 48 041, June 1996	GHE-T-833	Yes	Unpublishe dProtected
IIIA 10.4.1	Mühlen, Ackemeier, Rieger, Assessment of Side Effects of EF-1343 on Honey Bees ( <i>Apis mellifera</i> L.) Laboratory Test IPSAB, D-48135 Münster, Germany BL608, September 1996	GHE-T-835	Yes	Unpublishe dProtected



Annex point(s) 91/414/EEC	Author, Title, Test institute, Report number/Study ID, Date of report For publications: reference	Dow AgroScience s Report No.	GLP GEP	Published Protected
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IIIA 10.6.1.1	Rees, PB, EF-1343: Acute Toxicity Study in the Earthworm Huntingdon Life Sciences Ltd, Eye, Suffolk, UK 96/DES349/0479, October 1996	GHE-T-671	Yes	Unpublishe dProtected
IIIA 10.5.1	Thompson, B, An extended laboratory test to evaluate the effects of the herbicide EF-1343 A 50 G/L suspension concentrate formulation oof XDE-570, on the green lacewing, <i>Chrysoperla carnea</i> Dow AgroSciences, Letcombe Laboratory, Letcombe Regis, Wantage, Oxon, UK. February 1998	EA97D5A06 1	Yes	Unpublishe d Protected