ANNEX B

Pyraflufen-ethyl

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)

Acute oral toxicity study in bobwhite quail with ET-751 (van Dreumel *et al.*, 1995a)
Guidelines :
US EPA TSCA Pesticides Assessment Guidelines (Subdivision E, Paragraph 797.2175, 1991), US EPA FIFRA Pesticides Assessment Guidelines (Subdivision E, Paragraph 71-1, 1982) and US EPA FIFRA Accelerated Reregistration Phase 3 Technical Guidance (Subdivision E, Guideline Ref. No. 71-1, December 1989).
GLP :
Yes
Material and Methods :
Test Substance: pyraflufen-ethyl, purity : 97%
Test species : Colinus virginianus
Sex, weight, age : 5 _ and 5 _ by treatment group, _ : 167-217 g, _ : 154-202 g, 28 weeks old
Applied concentrations : untreated control, 2000 mg a.s. /kg body weight;
Type of application : oral by gavage using corn oil
Time of exposure : one single application, monitoring during 14 days
Findings :
Mortality : No mortality occurred
Body weight - food consumption : No substance-related change
Feed consumption :
Clinical signs : macroscopic post mortem examination realized at termination revealed no abnormalities
Conclusions :
LD_{50} (*Colinus virginianus*) > 2000 mg a.s./kg bw
NOEL (*Colinus virginianus*) = 2000 mg a.s./kg bw

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

5-day dietary toxicity study in bobwhite quail with ET-751 techn. (van Dreumel, I.F., Reijnders, J.B.J., 1996):
Guidelines :
OECD Guidelines for Testing of Chemicals (Method 205, adopted 4 April 1984), US EPA TSCA Pesticides Assessment Guidelines (Subdivision E, Paragraph 797.2050, 1991), US EPA FIFRA Accelerated Reregistration Phase 3 Technical Guidance (Subdivision E, Guideline Ref. No. 71-2, December 1989) and US EPA Pesticide Assessment Guidelines (Subdivision E, Paragraph 71-2, 1982).
GLP :
Yes
Material and Methods :
Test Substance: Pyraflufen-ethyl, chemical purity : 97.7%
Test species : Colinus virginianus
Sex, weight, age : 10 birds for each treatment (20 for control), not sexed, 11.5- 26.2 g, 10 days old
Applied concentrations : untreated control, 20, 78, 313, 1250, 5000 mg a.s./kg feed;
Type of application : The birds received th a.s. mixed to the diet. Homogeneity (92-108% of the nominal concentration) and stability of the a.s. in diet at ambient temperature over 6 days (97-100% of the nominal concentration at day 1) were determined.
Time of exposure : Short-term feeding test (3 days acclimatisation, 5 days with exposure by the feed + 3 days observations)
Findings :
Mortality : no substance-related mortality (1 chick of the control group and one chick of the 313 mg/kg group died)
Body weight and food consumption : no substance-related change.
Clinical signs :
- Clinical signs observed during the study period (abnormal posture, abnormal gait, wounds at the beak and the legs...) in the control and treated groups were not substance-releated and could be attributed to excessive agression between cage mates.

- Macroscopic post-mortem examination of the birds died not reveal any anomaly (except dead chick of the control group with dark red foci of the live, fracture of the tibia/fibula)
Conclusions :
LC_{50} (*Colinus virginianus*, 5d) > 5000 mg a.s./kg feed
NOEC (*Colinus virginianus*, 5d) = 5000 mg a.s./kg feed

5-day dietary toxicity study in mallard duck with ET-751 techn. (van Dreumel, Leopold, M.A. 1995):
Guidelines :
OECD Guidelines for Testing of Chemicals (Method 205, adopted 4 April 1984), US EPA TSCA Pesticides Assessment Guidelines (Subdivision E, Paragraph 797.2050, 1991), US EPA FIFRA Accelerated Reregistration Phase 3 Technical Guidance (Subdivision E, Guideline Ref. No. 71-2, December 1989) and US EPA Pesticide Assessment Guidelines (Subdivision E, Paragraph 71-2, 1982).
GLP :
Yes
Material and Methods :
Test Substance: Pyraflufen-ethyl, chemical purity : 97%
Test species : Anas platyrhynchos
Sex, weight, age : 10 birds for each treatment (20 for control), not sexed, 46.7-138.0 g, 10 days old
Applied concentrations : untreated control, 20, 78, 313, 1250, 5000 mg a.s./kg feed;
Type of application : The birds received th a.s. mixed to the diet. Homogeneity (98-102% of the nominal concentration) and stability of the a.s. in diet at ambient temperature over 8 days (85-102% of the nominal concentration at day 1) were determined.
Time of exposure : Short-term feeding test (3 days acclimatisation, 5 days with exposure by the feed + 3 days observations)
Findings :
Mortality : no substance-related mortality (1 bird in the 20 mg/kg group and 2 birds in the 78 mg/kg group died; No mortality in the other groups and control)
Body weight and food consumption : no substance-related change.
Clinical signs : Any abnormality was observed neither during the study period nor during the post-mortem examination
Conclusions :
LC_{50} (*Anas platyrhynchos*, 5d) > 5000 mg a.s./kg feed
NOEC (*Anas platyrhynchos*, 5d) = 5000 mg a.s./kg feed

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

Reproduction study in mallard duck with ET-751 techn. (Standens-Peek, W.M.M., Leopold, M.A. ,1997)
Guidelines :
US EPA TSCA Pesticides Assessment Guidelines (Subdivision E, Paragraph 797.2150, 1 July 1991), US EPA FIFRA Accelerated Reregistration Phase 3 Technical Guidance (Subdivision E, Guideline Ref. No. 71-4, 1989), OECD Guidelines for Testing of Chemicals (Method 206, adopted 4 April 1984) and Standard Evaluation Procedure, Avian Reproduction Test FDA (540/9-86-139, July 1986)
GLP :
Yes
Material and Methods :
Test substance : pyraflufen-ethyl, purity : 97.7%
Test species : Anas plathyrhynchos
Sex, weight, age : 16 pens of 1 _ and 1 _ each per treatment group; _ : 943 to 1244 g, _ : 875 to 1146 g; 47 weeks old
Applied concentrations : untreated control, 50, 500, 5000 mg a.s. /kg in the feed
Type of application : The birds received th a.s. mixed to the diet. Homogeneity was measured (98-104% of the nominal concentration). For the main study, stability was proven over a period of 24 days at -20°C.
Time of exposure :

10 weeks : pre-egg production period
10 weeks : egg production period
Findings :
Table B.8.1.3-1 : Major effects of pyraflufen-ethyl observed during the reproduction study of mallard duck

Endpoints	**Concentrations (mg a.s./kg diet)**			
	0	**50**	**500**	**5000**
Parent birds				
Mortality	No mortality			
Body weight	-	-	-	î bw _ î bw gain of _ and _
Food consumption	-	-	-	î (weeks 8 to 19)
Clinical observations	-	-	-	abnormal gait of 2_
Organ weight	-	-	-	ì (r) liver weight of _ and _ ì (r) spleen weight of _ î (r) testes weight
Reproduction parameters				
No. of eggs laid	881	923	859	541*
No. of eggs laid per female duck	55.1	57.7	53.7	33.8*
Mean egg weight (g) SD	59.4 4.6	57.8* 4.5	61.4* 4.8	48.7* 6.2
Mean eggshell thickness (mm) SD	0.368 0.018	0.373 0.022	0.376 0.031	0.359 0.042
Eggs cracked of eggs laid (%)	2.7	1.1	0.3	4.3
Eggs broken of eggs laid (%)	3.9	2.4	1.3	7.6*
Fertile eggs of eggs set (%)	98.4	95.3	97.0	85.9*
Infertile eggs of eggs set (%)	1.4	4.6	3.0	13.6*
Live 14-day old embryos of eggs set (%)	91.9	84.8*	91.4	47.1*
Live 21-day old embryos of eggs set (%)	89.3	81.2*	89.8	44.3*
Early embryonic death of fertile eggs (%)	6.5	11.0	5.8	44.8*
Late embryonic death of fertile eggs (%)	2.6	3.5*	1.6	2.8*
Live 21-day old embryos of fertile eggs (%)	90.8	85.2*	92.6	51.5*
Post 21-day embryonic death of fertile eggs (%)	32.6	39.6	39.2	22.0
Normal hatchlings of eggs set (%)	57.3	42.8*	51.1*	24.9*
Normal hatchlings of fertile eggs (%)	58.2	44.9*	52.7*	29.0*
Normal hatchlings of live 14-day old embryos (%)	62.3	50.4*	55.9	52.8*
Normal hatchlings of live 21-day old embryos (%)	64.1	52.7*	56.9	56.2
14-day old survivors of normal hatchlings (%)	95.7	95.7	93.6	86.5*
No. of normal hatchlings per female duck	26.4	21.6	24.5	6.5*
No. of 14-day old survivors per female duck	25.3	20.7	22.9	5.6*
Mean body weight 1-day old hatchlings (g) SD	36.7 3.5	35.8* 3.7	38.2 3.8	31.2* 3.8
Mean body weight 14-day old survivors (g) SD	147.7 40.2	142.2 35.2	144.1 36.8	121.9* 35.5
Mean growth Rate (g/day) SD	7.9 2.8	7.6 2.5	7.6 2.6	6.5 2.5

* = statistically significant (p£0.05) when compared to the control group.

Conclusions :

The notifier considered that the effects observed at 50 and 500 mg/kg were not substance-related. This interpretation was rather abusive.
NOEC (*Anas plathyrhynchos*, 20 weeks)< 50 mg a.s./kg feed was estabhished.

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

No study was performed with the formulation EXP 31279A (SC containing 9.57 g/l pyraflufen-ethyl and 519 g/l bifenox). However the notifier provided toxicity data on bifenox (acute toxicity data for two species, short-term toxicity data for two species).

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

The study is not required since the TERa > 10 and TERlt > 5.

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

This study is not required.

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

The active substance and its major metabolite E-1 are not bioaccumulable. The study is therefore not necessary..

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 pyraflufen-ethyl to birds.

Test species	**Test System**	**Duration of exposure**	**Results**	**References**
Colinus virginianus	acute	single appl.	LD_{50} > 2000 mg a.s./kg bw	van Dreumel, 1995a
Colinus virginianus	short-term	5 days	LC_{50} > 5000 mg a.s./kg food	van Dreumel *et al.* 1996
Anas plathyrhynchos	short-term	5 days	LC_{50} > 5000 mg a.s./kg food	van Dreumel *et al.* 1995
Anas plathyrhynchos	reproductio n	20 weeks	NOEC < 50 mg a.s./kg food	Standens-Peek *et al.* 1997

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 13.5 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 pyraflufen-ethyl in potential feed of birds

Target crop	**Application rate (g a.s./ha)**	**Estimated initial residues (mg a.s./kg food)**	
		Small insects	**Leaves and leafy crops**
Cereals	13.5	0.39	0.42

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

Target crop	**Bird type**	**food consumed**	**food consumption (% bw)**	**Max. daily intake (mg ai.s. bw / day)**
cereals	small bird (<100 g)	small insects	30	0.117
		leaves and leafy crop	30	0.126

Tab. B.8.1.8-4 : Toxicity exposure ratios for birds exposed to pyraflufen-ethyl - worst cases

Application rate (kg a.s./ha)	Crop	Organism	Time-scale	TER	Annex VI trigger
pyraflufen-ethyl					
0.0135	Cereals	small insectivorous bird	acute	17029	10
		small foliage-eating bird	acute	15930	10
		small insectivorous bird	short-term	12771	10
		small foliage-eating bird	short-term	11947	10
		small insectivorous bird	long-term	128	5
		small foliage-eating bird	long-term	119	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)

ET-751: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*). (Caunter, J.E., Magor, S.E., Croudace, C.P., 1995a)
Guidelines :
EEC Directive 92/69/EEC. Method for the determination of ecotoxicity, Acute toxicity for fish, L383A, Part C1, 29 December 1992
GLP :
Yes
Material and Methods :
Test substance : pyraflufen-ethyl, chemical purity : 97.6%, solubility : 50 µg/l
Test species : Rainbow trout (Oncorhyncus mykiss)
Number of organisms, weight, length, loading : 10 fish/treatment; 2.34 g (range 1.78 to 3.43 g); 54 mm (range 47 to 62 mm); 0.52 g/l
Type of test : 96 h flow through test, limit test
Applied and measured concentrations :
nominal : control, 100 µg a.s./l
measured concentrations ranging from 88-120 % of the nominal concentrations
Test conditions :
temperature : 14.7 to 14.9 °C,
pH : 7.48 to 7.82
oxygen content : 8.6 to 10.0 mg/l
total hardness : 52.3 to 55.7 mg $CaCO_3$/l
Photoperiod : 16 h light
Analytical methods : HPLC
Findings :
No mortalities or symptoms of toxicity were noted in this study.
Conclusions :
LC_{50} (a.s., 96h) > 100 µg a.s./l
NOEC (a.s., 96h) = 100 µg a.s./l

ET-751: Acute toxicity to bluegill sunfish (*Lepomis macrochirus*) (Caunter, J.E., Magor, S.E., Croudace, C.P., 1995b)
Guidelines :
OECD Guidelines for Testing of Chemicals, Method 203. Adopted 17 July 1992
GLP :
Yes
Material and Methods :
Test substance : pyraflufen-ethyl, chemical purity : 97.6%, solubility : 50 µg/l
Test species : bluegill sunfish (*Lepomis macrochirus*)
Number of organisms, weight, length, loading : 10 fish/treatment; 0.50 g (range 0.29 to 0.79 g); 28 mm (range 25 to 31 mm); 0.22 g/l
Type of test : 96 h flow through test, limit test
Applied and measured concentrations :
nominal : control, 100 µg a.s./l
The analytical recoveries were variable and consistently higher than the nominal concentrations (290%). 'Such variability is to be expected when working at concentration exceeding the limit of solubility'
Test conditions :
temperature : 21.2 to 21.4 °C,
pH : 7.60 to 7.88
oxygen content : 7.8 to 8.4 mg/l
total hardness : 52.3 to 56.3 mg $CaCO_3$/l

Photoperiod : 16 h light
Analytical methods : HPLC
Findings :
No mortalities or symptoms of toxicity were noted in this study.
Conclusions :
LC_{50} (a.s., 96h) > 100 µg a.s./l
NOEC (a.s., 96h) = 100 µg a.s./l

E-1: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) (Caunter, J.E., Johnson, P.A., Croudace, C.P., 1996a)
Guidelines :
OECD Guidelines for Testing of Chemicals, Method 203.
GLP :
Yes
Material and Methods :
Test substance : 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetic acid (E-1), chemical purity : 96.6%
Test species : Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms, weight, length, loading : 2 replicates of 10 fish per treatment; 1.09 g (range 0.67-1.30 g); 44 mm (range 39-47 mm); 0.73 g/l
Type of test : 96 h static test, limit test
Applied and measured concentrations :
nominal : control, 120 mg /l
measured concentrations ranging from 92 to 100% of the nominal concentrations
Test conditions :
temperature : 14.3 to 14.8 °C,
pH : 6.95 to 7.43
oxygen content : 9.0 to 10.4 mg/l
total hardness : 11.7 mg $CaCO_3$/l
Photoperiod : 16 h light
Analytical methods : HPLC
Findings :
Only one fish of the treated group died.
Conclusions :
LC_{50} (E-1), 96h) > 120 mg/l
NOEC (E-1, 96h) = 120 mg /l

E-1: Acute toxicity to bluegill sunfish (*Lepomis macrochirus*) (Caunter, J.E., Cornish, S.K., Croudace, C.P., 1996b)
Guidelines :
OECD Guidelines for Testing of Chemicals, Method 203.
GLP :
Yes
Material and Methods :
Test substance : 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetic acid (E-1), chemical purity : 96.6%
Test species : bluegill sunfish (*Lepomis macrochirus*)
Number of organisms, weight, length, loading : 10 fish per treatment; 2.15 g (range 1.16-3.50 g); 45 mm (range 38-53 mm); 0.72 g/l
Type of test : 96 h static test, limit test
Applied and measured concentrations :
nominal : control, 100 mg /l
measured concentrations ranging from 81 to 90% of the nominal concentrations
Test conditions :

temperature : 21.3 to 22.3 °C,
pH : no adjustement : 4.33 to 5.11; adjusted : 6.95 to 7.94
oxygen content : 8.0 to 8.4 mg/l
total hardness : 12.7 mg $CaCO_3$/l
Photoperiod : 16 h light
Analytical methods : HPLC
Findings :
no pH adjustment : 100% fish died
adjusted pH : no mortality
Conclusions :
LC_{50} (E-1, 96h) > 100 mg/l
NOEC (E-1, 96h) = 100 mg/l

B.8.2.2 Fish early life stage toxicity (Annex IIA 8.2.2.1)

E-1: Chronic toxicity to fathead minnow (*Pimephales promelas*) embryos and larvae. Croudace, C.P., Caunter, J.E., Wallace, S.J. (1996a)
Guidelines :
OECD Guidelines for Testing of Chemicals, Method 210. Adopted 17 July 1992
GLP :
Yes
Material and Methods :
Test substance : 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetic acid (E-1), chemical purity : 97.1%
Test species : fathead minnow (*Pimephales promelas*)
Number of organisms, age: 4 replicates of 20 organisms /treatment, eggs less than 24 hours old
Type of test : flow-throught test
Applied and measured concentrations :
nominal : water control, solvent control, 12.5 mg/l
measured concentrations were 10 mg/l (80 % of the nominal concentrations)
Test conditions :

Dissolved oxygen, pH and temperature were measured twice weekly
temperature : 24.7 to 25.6°C
pH : 7.28 to 7.99
oxygen content : 5.4 to 8.2 mg/l
total hardness (as $CaCO_3$) : 44.6 to 54.0 mg/l
Photoperiod : 16 hours light
Analytical methods : HPLC
Findings :
Egg hatching and survival at day 32 post-hatch were similar in control and treated groups (100% survival in the treated group)
No statistically significant differences in larval length and larval weight were observed.
Conclusions :
NOEC (*Pimephales promelas* embryos and larvae) = 10 mg a.s./l

B.8.2.3 Fish juvenile growth test (Annex IIA 8.2.2.2)

Not required. Early life stage test provided.

B.8.2.4 Fish life cycle test (Annex IIA 8.2.2.3)

Not required. Early life stage test provided.

B.8.2.5 Bioaccumulation potential in fish (Annex IIA 8.2.3)

As pyraflufen-ethyl is readily degraded to E-1 (pyraflufen) with DT_{50} value of up to 2 hours (data from the laboratory water/sediment systems, Point 7.2.1.3.2), there will be no long-term exposure of aquatic organisms to pyraflufen-ethyl. For this reason, a study has been conducted to assess the bioconcentration and elimination of the pyraflufen-ethyl metabolite, E-1 in rainbow trout.

Since the active substance and its 3 metabolites (E-1, E-2, E-3) present negligible risk to aquatic arthropod and fish, the determination of the bioconcentration potential of E-3 was not required, although its log Pow is 3.66

E-1: Determination of the accumulation and elimination of [^{14}C]E-1 in rainbow trout (*Oncorhynchus mykiss*). (Grinell, A.J., Croudace, C.P., Caunter, J.E., Gillings, E.,1996)
Guidelines :
OECD Guidelines for Testing of Chemicals, Method 305E
GLP :
Yes
Material and Methods :
Test substance : 14C-cyclopropyl radiolabeled 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetic acid (E-1), radiochemical purity : 99.98 %
Test species : rainbow trout (*Oncorhynchus mykiss*)
Number of organisms, weight, length, loading : 60 fish per test concentration; 2.19 g (range 0.63-4.01 g); 50 mm (range 36-62 mm); 0.37g/l
*Type of test :*continuous flow-through system (7 days exposure, 14 days depuration)
Applied and measured concentrations :
nominal : control, 0.1 and 1 mg a.s./l
measured concentrations ranging from 98-100 % of the nominal concentrations
Test conditions :
3 following parameters were recorded at day 0, 1, 2 then twice weekly
temperature : 14.8-15.3 °C,
pH : 6.32-6.89
oxygen content : 7.2-10.0 mg/l
total hardness : 14.0-22.0 mg/l $CaCO_3$
Photoperiod : 12 hours light
Analytical methods : LSC and TLC
Findings :
Table B.8.2.5-1 : Mean tissue concentration and BCF of metabolite E-1 in rainbow trout

Portion	Mean measured concentration in water			
	0.10 mg/l		0.98 mg/l	
	Mean tissue concentration (ng/g)	BCF	Mean tissue concentration (ng/g)	BCF
Flesh	<190	<1.9	<6700	<6.8
Carcass	<100	<1.0	<1700	<1.7
Whole body	<180	<1.8	<2400	<2.4

During the depuration phase, the level of [^{14}C]E-1 in the whole fish were below the limit of detection after 12 hours. Consequently no significant residues were considered to remain in the fish flesh or carcass. Since the [^{14}C]E-1 levels in the tissue were not quantifiable it was not possible to calculate the depuration rate constant, K_2.
Conclusion :
[^{14}C]E-1 showed no significant bioconcentration, with maximum BCF values in whole fish of less than 2.4. Any residues remaining in the fish throughout the depuration phase were below the level of quantification.

B.8.2.6 Acute toxicity to invertebrates (Annex IIA 8.2.4)

ET-751: Acute toxicity to *Daphnia magna* (Croudace, C.P., Banner, A.J., Johnson, P.A. , 1996b)
Guidelines :
OECD Guidelines 202, and EEC method C2 (Directive 92/69/EEC)
GLP :
Yes
Material and Methods :
Test substance : pyraflufen-ethyl, chemical purity : 97.6%, solubility : 82 µg/l at 20°C
Test species : Daphnia magna
Number of organisms, age : 20 organisms/test group, <24 hours old
Type of test : 48 hours semi-static, limit test
Applied and measured concentrations :
nominal : water and solvent control, 100 µg a.s./l
measured concentrations ranging from 61 to 95% of the nominal concentrations
Test conditions :
temperature : 20.3 to 20.5 °C
pH : 7.97 to 8.12
oxygen content : 8.8 to 9.0 mg/l
total hardness (as $CaCO_3$) : 224 mg/l
Photoperiod : 16 hours light
Analytical methods : HPLC
Findings :
No mortality was observed in the control and treatment (100 µg a.s./l) groups
Conclusions :
LC_{50} (*Daphnia magna*, 48 h) > 100 µg a.s./l
NOEC (*Daphnia magna*, 48 h) = 100 µg a.s./l

E-1: Acute toxicity to *Daphnia magna* (Croudace, C.P., Banner, A.J., Johnson, P.A., 1996c)
Guidelines :
OECD Guidelines 202, and EEC method C2 (Directive 92/69/EEC)
GLP :
Yes
Material and Methods :
Test substance : 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetic acid (E-1), chemical purity : 97.7%
Test species : Daphnia magna
Number of organisms, age : 20 organisms/test group, <24 hours old
Type of test : 48 hours static, limit test
Applied and measured concentrations :
nominal : water and solvent control, 120 mg E-1 /l
measured concentrations ranging from 100 to 101% of the nominal concentrations
Test conditions :
temperature : 20.3 to 20.5 °C
pH : 6.78 to 8.02
oxygen content : 8.8 to 9.0 mg/l
total hardness (as $CaCO_3$) : 246 mg/l
Photoperiod : 16 hours light
Analytical methods : HPLC
Findings :
No mortality was observed in the control and treatment (120 mg E-1/l) groups
Conclusions :
LC_{50} (*Daphnia magna*, 48 h) > 120 mg E-1/l
NOEC (*Daphnia magna*, 48 h) = 120 mg E-1/l

B.8.2.7 Chronic toxicity to aquatic invertebrates (Annex IIA 8.2.5)

E-1: Chronic toxicity to *Daphnia magna* (Croudace, C.P., Williams, N.J., Shearing, J.M. ,1996d)
Guidelines :
OECD Guidelines 202 (part II)
GLP :
Yes
Material and Methods :
Test substance : 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetic acid (E-1), chemical purity : 97.1%
Test species : Daphnia magna
Number of organisms, age : 10 adults /test group, <24 hours old
Type of test : 21 days reproduction test
Applied and measured concentrations :
nominal : water control, 18, 32, 56, 100, 120 mg E-1 /l
measured concentrations ranging from 99 to 100% of the nominal concentrations
Test conditions :
temperature : 20.0 to 20.6 °C
pH : 6.83 to 8.13
oxygen content : 8.0 to 9.1 mg/l
total hardness (as $CaCO_3$) : 267 mg/l
Photoperiod : 16 hours light
Analytical methods : HPLC
Findings :
Table B.8.2.7-1 : Effects of metabolite E-1 observed during the reproduction study of *Daphnia magna*

	Nominal concentrations (mg E-1/l)					
	0	18	32	56	100	120
Number dead parents (of 10 tested)	1	0	0	0	0	0
Total offspring per parent	126	117	128	127	119	62*
Parents length in mm	4.2	4.2	4.3	4.2	4.3	4.2

Conclusions :
A significant reduction of offspring mean was observed at 120 mg E-1/l .
NOEC (*Daphnia magna*, 21 d) = 100 mg E-1/l

B.8.2.8 Effects on algal growth (Annex IIA 8.2.6)

Algal toxicity studies performed with the active substance

ET-751: Toxicity to the green alga *Selenastrum capricornutum* . (Morgan, D.J., Croudace, C.P., Magor, S.E., 1996)
Guidelines :
OECD Guideline 201 and EEC method C3 (Directive 92/69/EEC)
GLP :
Yes
Material and Methods :
Test substance : pyraflufen-ethyl, chemical purity : 97.6%
Test species : green alga, *Selenastrum capricornutum*
Number of replicates, initial cell density : 3 replicates/ treatment group , 1.03 10^4 cells/ml
Type of test : 72 hours static
Applied and measured concentrations :
nominal : water and solvent control, 0.0091, 0.020, 0.044, 0.097, 0.21, 0.46, 1.0 and 2.2 µg a.s./l
Corresponding measured concentrations were 0.012, 0.017, 0.037, 0.082, 0.28, 0.43, 0.79 and 1.8 µg a.s./l (79-133% of nominal concentrations)
Test conditions :
temperature : 23.6 to 24.0 °C
pH : 7.4-7.5 at start; 7.5 to 9.0 at end : shift of 1.6 unit, however the shift was < 1 unit in the concentrations closest to the EC_{50}.
Light : continuous, 8200 lux » 101.0 µE/m^2s
Analytical methods : HPLC
Findings and conclusions :
based on areas under the growth curve
EbC_{50} = 0.23 µg a.s./l (95% conf.limits : 0.13 to 0.48 µg a.s./l)
NOEC = 0.037 µg a.s. /l
based on logarithmic growth rate
ErC_{50} = 0.65 µg a.s./l (95% conf.limits : 0.29 to > 1.8 µg a.s./l)
NOEC = 0.037 µg a.s. /l

ET-751 : Toxicity to the green alga *Selenastrum capricornutum* (Smyth, D.V., Croudace, C.P., Wallace, 1996)
Guidelines :
OECD Guideline 201 and EEC method C3 (Directive 92/69/EEC)
GLP :
Yes
Material and Methods :
Test substance :pyraflufen-ethyl, chemical purity : 97.6%
Test species : green alga, *Selenastrum capricornutum*
Number of replicates, initial cell density : 3 replicates/ treatment group ,1.01 10^4 cells/ml
Type of test : exposure over 3 days (72 hours - static), followed by a recovery phase of 6 days (144 hours)
Applied and measured concentrations :
Nominal concentrations : water and solvent control, 0.040, 0.088, 0.19, 0.43, 0.94, 2.1, 4.5, 10 µg a.s./l
corresponding measured concentrations were 0.079, 0.14, 0.24, 0.56, 1.2, 2.1, 3.1, 9.2 (69 to 198 % of nominal concentrations)
Test conditions :
temperature : 24 ± 1 °C
pH : 7.2-7.3 at start; 7.3-9.4 at end ; pH shift > 1 was observed in the concentrations groups up 0.19 µg/l where algal growth was important.
Light : continuous, 8670 lux » 103.8 µE/m^2s
Analytical methods : HPLC
Findings and conclusions :
Exposure phase :

based on areas under the growth curve
EbC_{50} = 0.31 µg a.s./l (95% conf.limits : 0.10 to 0.68 µg a.s./l)
NOEC = 0.040 µg a.s. /l
based on logarithmic growth rate
ErC_{50} = 1.0 µg a.s./l (95% conf.limits : 0.44 to > 3.3 µg a.s./l)
NOEC = 0.088 µg a.s. /l
Recovery phase :
In order to avoid toxic effects inoculum volumes were reduced at the highest test concentrations (2.1, 4.5 and 10 µg/l) . Re-start densities were therefore lower for for these 3 test groups. Algae of the concentration groups up to 2.1 recovered and had growth rates similar to the control (or even higher for the 2.1 µg/l group). Growth rate in the 4.5 µg/l group was lower than in control. No recovery in growth was observed at 10 µg/l.

ET-751: Toxicity to the freshwater diatom *Navicula pelliculosa* . (Smyth, Croudace, C.P., Johnson, 1996a)
Guidelines :
OECD Guideline 201
GLP :
Yes
Material and Methods :
Test substance : pyraflufen-ethyl, chemical purity : 97.6%
Test species : freshwater diatom, *Navicula pelliculosa*
Number of replicates, initial cell density : 3 replicates/ treatment group , 1.07 10^4 cells/ml
Type of test : 72 hours static
Applied and measured concentrations :
nominal : water and solvent control, 0.15, 0.30, 0.60, 1.2, 2.4, 4.8, 9.6 µg a.s./l
Measured concentrations were in the range from 74 to 87% of the nominal values
Test conditions :
temperature : 24 ± 1 °C
pH : 8.0-8.3 at start; 7.7-7.8 at end
Light : continuous, 4330 lux » 53.1 µE/m^2s
Analytical methods : HPLC
Findings and conclusions :
based on areas under the growth curve
EbC_{50} = 1.6 µg a.s./l (95% conf.limits : 0.84 to 4.6 µg a.s./l)
NOEC = 0.25 µg a.s. /l
based on logarithmic growth rate
ErC_{50} = 5.4 µg a.s./l (95% conf.limits : 2.0 to > 7.3 µg a.s./l)
NOEC = 0.89 µg a.s. /l

Algal toxicity studies performed with the metabolite E-1

E-1: Toxicity to the green alga *Selenastrum capricornutum* (Smyth, D.V., Croudace, C.P., Wallace, S.J. , 1996b)
Guidelines :
OECD Guideline 201 and EEC method C3 (Directive 92/69/EEC)
GLP :
Yes
Material and Methods :
Test substance : 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetic acid (E-1), chemical purity : 97.7%
Test species : green alga, *Selenastrum capricornutum*
Number of replicates, initial cell density : 3 replicates/ treatment group , 1.04 10^4 cells/ml
Type of test : 72 hours static
Applied and measured concentrations :
Nominal concentrations : water and solvent control, 1.0, 1.8, 3.2, 5.6, 10 , 18 µg E-1/l
Corresponding measured concentrations were 3.4, 5.7, 8.5 and 20 µg E-1/l (83-117% of nominal concentrations)
Analytical limit of detection was £ 1.8 µg/l; it was therefore impossible to measure the actual concentrations in the 1.0 and 1.8 µg/l vessels
Test conditions :
temperature : 23.3 to 24.6°C
pH :7.4-7.5 at start; 7.5 to 9.0 at end : shift of 1.6 unit, however the shift was < 1 unit in the concentrations closest to the EC_{50}.
Light : continuous, 7600 lux » 92.3 µE/m^2s
Analytical methods : HPLC
Findings and conclusions :
based on areas under the growth curve
EbC_{50} = 5.5 µg E-1/l (95% conf.limits : 3.6 to 9.1 µg a.s./l)
NOEC = 1.8 µg E-1 /l
based on logarithmic growth rate
ErC_{50} = 10 µg E-1 /l (95% conf.limits : 5.8 to > 18 µg a.s./l)
NOEC = 3.2 µg E-1 /l

E-1: Toxicity to the green alga *Selenastrum capricornutum* (Smyth, D.V., Croudace, C.P., Magor, S.E., 1997b)
Guidelines :
OECD Guideline 201 and EEC method C3 (Directive 92/69/EEC)
GLP :
Yes
Material and Methods :
Test substance : 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetic acid (E-1), chemical purity : 97.1%
Test species : green alga, *Selenastrum capricornutum*
Number of replicates, initial cell density : 3 replicates/ treatment group , 0.996 10^4 cells/ml
Type of test : exposure over 3 days (72 hours - static), followed by a recovery phase of 6 days (144 hours)
Applied and measured concentrations :
Nominal concentrations : water and solvent control, 0.91, 2.0, 4.5, 10, 23, 50, 110, 240, 520 µg E-1/l
Measured concentrations were in the range of 106 to 126 % of nominal concentrations
Analytical limit of quantification was 2 µg/l; it was therefore impossible to measure the actual concentrations in the 0.91 and 2.0 µg/l vessels
Test conditions :
temperature : 24 ± 1 °C
pH :7.3-7.4 at start; 7.2 to 9.3 at end : shift to pH> 9 was only observed in control and 0.91µg/l treatment group.
Light : continuous, 81700 lux » 97.3 µE/m^2s
Analytical methods : HPLC
Findings and conclusions :
Exposure phase :

based on areas under the growth curve
EbC_{50} = 2.2 µg E-1/l (95% conf.limits : 0.85 to 3.8 µg a.s./l)
NOEC = 0.91 µg E-1 /l
based on logarithmic growth rate
ErC_{50} = 4.0 µg E-1 /l (95% conf.limits : 2.2 to 11 µg a.s./l)
NOEC = 0.91 µg E-1 /l
Recovery phase :
In order to avoid toxic effects inoculum volumes were reduced at the highest test concentrations. Re-start densities were therefore unequal for the different test groups. The results of the recovery phase are questionable. Lag phases were observed for the algae previously exposed at 4.5 µg/l and above. Growth occurred for the algae previously exposed to 2.0 to 50 µg/l. No recovery in growth at 110, 240 and 520 µg/l

E-1: Toxicity to the freshwater diatom *Navicula pelliculosa* . (Smyth, Croudace, C.P., Johnson, 1996c)
Guidelines :
OECD Guideline 201
GLP :
Yes
Material and Methods :
Test substance : 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetic acid (E-1), chemical purity : 97.1%
Test species : freshwater diatom, *Navicula pelliculosa*
Number of replicates, initial cell density : 3 replicates/ treatment group , 1.02 10^4 cells/ml
Type of test : 72 hours static
Applied and measured concentrations :
nominal : water and solvent control, 75, 150, 300 600, 1200, 2400, 4800, 9600 µg a.s./l
Measured concentrations were in the range from 93 to 102% of the nominal values
Test conditions :
temperature : 24 ± 1 °C
pH :7.7-8.0 at start; 7.6-7.8 at end
Light : continuous, 4180 lux » 50.8 $\mu E/m^2s$
Analytical methods : HPLC
Findings and conclusions :
based on areas under the growth curve
EbC_{50} = 1700 µg a.s./l (95% conf.limits : 940 to 3900 µg a.s./l)
NOEC = 600 µg a.s. /l
based on logarithmic growth rate
ErC_{50} = 3600 µg a.s./l (95% conf.limits : 1700 to > 9600 µg a.s./l)
NOEC = 600 µg a.s. /l

Algal toxicity studies performed with the metabolite E-2

Freshwater algal growth inhibition study (72 hours) *(Selenastrum capricornutum)* (Odin-Feurtet, 1998)
Guidelines :
OECD Guideline 201
GLP :
No
Material and Methods :
[^{14}C-pyrazole] 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenol (E-2), purity 99.7%
Test species : green alga, *Selenastrum capricornutum*
Number of replicates, initial cell density : 2 replicates/ treatment group , 4 10^4 cells/ml
Type of test : exposure over 3 days (72 hours - static)
Applied and measured concentrations :
Nominal concentrations : water and solvent control, 0.01, 0.03, 0.07, 0.16, 0.35 µg E-2/l
Concentrations were not measured
Test conditions :
temperature : 23 ± 2 °C
pH : 8.0 ± 0.3 at start;
Light : 16 hours light , 6000-10000 lux
Analytical methods :-
Findings and conclusions :
Exploratory study : no chemical analyses, very brief report
based on areas under the growth curve
EbC_{50} = 0.16-0.35 µg E-2/l
NOEC = 0.16 µg E-2 /l
based on logarithmic growth rate
ErC_{50} > 0.35 µg E-2/l
NOEC = 0.16 µg E-2 /l

B.8.2.9 Effects on sediment dwelling organisms (Annex IIA 8.2.7)

E-2 is the only metabolite present in large amounts in sediment. As the arthropods tests had shown that these animals were not at risk, Chironomid test was not required.

B.8.2.10 Effects on aquatic plants (Annex IIA 8.2.8)

E-1: Toxicity to duckweed *Lemna gibba* (Smyth, Croudace, C.P., Magor, 1996c)
Guidelines :
US EPA Pesticide Assessment Guidelines (Subdivision J, EPA 540/09-82-020, October 1982. NTIS No. PB83-153940).
GLP :
Yes
Material and Methods :
Test substance : 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetic acid (E-1), chemical purity : 97.1%
Test species : Lemna gibba
Number of replicates,number of organisms : 3 replicates of 3 plants, each consisting of 4 fronds / treatment group
Type of test : 14 day static test, with renewal on day 7
Applied and measured concentrations :
nominal : water and solvent control, 0.032, 0.10, 0.32, 1.0, 3.2, 10, 32 , 100 µg E-1/l
On the basis of analyses made on day 0 and 7 for the highest concentration groups, a geometric mean recovery of 84% was calculated which take into account the test substance dissipation.
Test conditions :
temperature : 24.5 to 25.1 °C
pH : 4.6-4.8 freshly prepared solutions ; 5.0-5.9 'old' solutions
Light : continuous, 5110-5080 lux
Analytical methods : HPLC
Findings and conclusions :
based on the increase in number of fronds
EC_{50} = 4.7 µg E-1/l (95% conf.limits : 3.7 to 6.0 µg E-1/l)
NOEC = 2.7 µg E-1/l
based on the increase in weight of the plants
EC_{50} = 2.6 µg E-1/l (95% conf.limits : 2.0 to 3.6 µg E-1/l)
NOEC = 0.84 µg E-1/l
There were no observed symptoms (frond colouration, reduction in root growth, small frond size) at concentrations at or below 0.27 µg E-1/l (or 0.32)

B.8.2.11 Acute toxicity of the preparations (Annex IIIA 10.2.1)

EXP31279A : Acute toxicity (96 hours) to rainbow trout (*Oncorhynchus mykiss*) under semi-static conditions (Suteau, 1997a)
Guidelines :
OECD Guidelines 203, EEC method C1 (Directive 92/69/EEC)
GLP :
Yes
Material and Methods :
Test substance : EXP31279A (SC containing 9 g/l pyraflufen-ethyl and 500 g/l bifenox)
Test species : Rainbow trout (*Oncorhynchus mykiss*)
*Number of organisms, weight, length, loading :*10 fish per treatment group; 1.032 g (range 0.91-1.2 g); 50 mm (range 46-52 mm); 0.17 g/l
Type of test : 96 h semi- static test
Applied and measured concentrations :
nominal : control, 4, 8, 15, 30, 60 mg EXP31279A /l (60 mg/l is in excess of the visual limit of solubility)
Measured concentrations after a 48 h period were in the range 20-81 % and 59-83 % of nominal concentrations for pyraflufen-ethyl and bifenox, respectively.
Test conditions :
temperature : 15.4-16.0 °C,
pH : 7.6-8.1
oxygen content : 8.2 mg/l

total hardness : 49 mg $CaCO_3$/l
Photoperiod : 16 h light
Analytical methods : gas chromatography
Findings :
Mortality : No mortality was observed in any treatment group.
Clinical signs : At 72 and 96 hours, fishes with erratic swimming, accelerated respiration, pigementation disorders or excited were observed in all the treatment groups. Effects were dose-related.
Conclusions :
LC_{50} (*Oncorhynchus mykiss*, 96 h) > 60 mg EXP31279A/l
NOEC (*Oncorhynchus mykiss*, 96 h) <4 mg EXP31279A/l

EXP31279A : Acute toxicity (48 hours) to daphnids (*Daphnia magna*) under semi-static conditions (Suteau, 1997b)
Guidelines :
OECD Guidelines 202, EEC method C2 (Directive 92/69/EEC), EPA FIFRA guideline 72-2
GLP :
Yes
Material and Methods :
*Test substance :*EXP31279A (SC containing 9 g/l pyraflufen-ethyl and 500 g/l bifenox)
Test species : Daphnia magna
Number of organisms, age : 20 organisms/test group, <24 hours old
Type of test : 48 hours semi-static
Applied and measured concentrations :
nominal : water control, 0.8, 1.4, 2.6, 4.6, 8.3, 15 mg EXP31279A/l (15 mg/l concentration above limit of solubility)
Measured concentrations after a 24 h period were in the range 50-67 % and 28-87% of nominal concentrations for pyraflufen-ethyl and bifenox, respectively.
Test conditions :
temperature : 19.7-21.0 °C
pH : 7.97 to 8.12
oxygen content : 8.3 mg/l
total hardness (as $CaCO_3$) : 170 mg/l
Photoperiod : 16 hours light
Analytical methods : gas chromatography
Findings :
No mortality was observed in the treatment groups ; 5% mortality in the control.
Conclusions :
LC_{50} (*Daphnia magna*, 48 h) > 15 mg EXP31279A/l
NOEC (*Daphnia magna*, 48 h) = 0.8 mg EXP31279A/l ?? 15

EXP31279A : Freshwater algal growth inhibition study (72 hours) (*Selenastrum capricornutum*) (Odin-Feurtet, 1997b)
Guidelines :
OECD Guideline 201 and EEC method C3 (Directive 92/69/EEC)
GLP :
Yes
Material and Methods :
*Test substance :*EXP31279A (SC containing 9 g/l pyraflufen-ethyl and 500 g/l bifenox)
Test species : green alga, *Selenastrum capricornutum*
Number of replicates, initial cell density : 3 replicates/ treatment group , 1.90-2.10 10^4 cells/ml
Type of test : exposure over 3 days (72 hours - static), followed by a recovery phase of 4 days (96 hours)
Applied and measured concentrations :
Nominal concentrations : water and solvent control, 0.08, 0.17, 0.38, 0.84, 1.82, 4.00 µg EXP31279A/l
Measured concentrations of bifenox were in the range of 55-94 % of nominal concentrations, indicating that bifenox was unstable under the test conditions. Pyraflufen-ethyl was not analyzed. Unstability of bifenox could explain the

decreasing inhibition percentage.
Test conditions :
temperature : 21.0-22.1°C
pH :7..7-7.8 at start; 7.5 to 7.9 at end.
Light : continuous, 7000 lux
Analytical methods : gas chromatography
Findings and conclusions :
Exposure phase :
based on areas under the growth curve
EbC_{50} (24h) = 0.77 µg EXP31279A/l ; EbC_{50} (72h) = 1.84 µg EXP31279A/l; following 24 h exposure the percentage inhibition decreased through to test termination for all the test concentrations
based on logarithmic growth rate
ErC_{50} (24h) = 3.24 µg EXP31279A/l
NOEC = 0.17 µg EXP31279A/l
Recovery phase :
After 96 hours recovery was observed for all the test groups.

EXP31279A : Freshwater algal growth inhibition study (72 hours) (*Scenedesmus subspicatus*) (Odin-Feurtet, 1997a)
Guidelines :
OECD Guideline 201 and EEC method C3 (Directive 92/69/EEC)
GLP :
Yes
Material and Methods :
Test substance : EXP31279A (SC containing 9 g/l pyraflufen-ethyl and 500 g/l bifenox)
Test species : green alga, *Scenedesmus subspicatus*
Number of replicates, initial cell density : 3 replicates/ treatment group , 2 10^4 cells/ml
Type of test : exposure 72 hours - static
Applied and measured concentrations :
Nominal concentrations : water and solvent control, 0.08, 0.17, 0.38, 0.84, 1.82, 4.0 µg EXP31279A/l
Measured concentrations were in the range 59-68% and 94-145% of nominal concentrations for pyraflufen-ethyl and bifenox, respectively.
Test conditions :
temperature : 22.7-24.5 °C
pH :7.7-8.0 at start; 7.6-8.2 at end
Light : continuous, 9200 lux
Analytical methods : gas chromatography
Findings and conclusions :
based on areas under the growth curve
EbC_{50} = 0.48 µg EXP31279A/l
based on logarithmic growth rate
ErC_{50} = 0.68 µg EXP31279A /l
NOEC = 0.38 µg EXP31279A /l

EXP31279A : Toxitcity to the freshwater diatom *Navicula pelliculosa* (Smyth, et al., 1996)
Guidelines :
OECD Guideline 201 and EEC method C3 (Directive 92/69/EEC)
GLP :
Yes
Material and Methods :
Test substance : EXP31279A (SC containing 9 g/l pyraflufen-ethyl and 500 g/l bifenox)
*Test species :*freshwater diatom, *Navicula pelliculosa*
Number of replicates, initial cell density : 3 replicates/ treatment group ,1.02 10^4 cells/ml
Type of test : exposure over 3 days (72 hours - static), followed by a recovery phase of 6 days (144 hours)
Applied and measured concentrations :

Nominal concentrations : water and solvent control,0.73, 1.6,3.5, 7.7, 17, 37, 82, 180, 400 µg EXP31279A/l
Measured concentrations were in the range 73-100% of nominal concentrations for bifenox. The calculated concentrations were only based on the bifenox analysis.
Test conditions :
temperature : 23.9-24.2 °C
pH : 7.9-8.5 at start; 7.9-8.5 at end
Light : continuous, 4470 lux » 53.4 µE/m^2s
Analytical methods : gas chromatography
Findings and conclusions :
Exposure phase :
based on areas under the growth curve
EbC_{50} = 20 µg EXP31279A/l (95% conf.limits : 10 to 42 µg EXP31279A/l)
NOEC = 16 µg EXP31279A/l
based on logarithmic growth rate
EbC_{50} = 84 µg EXP31279A/l (95% conf.limits : 35 to 330 µg EXP31279A/l)
NOEC = 16 µg EXP31279A/l
Recovery phase :
Recovery was measured for the groups 37 to 400 µg/l. In order to avoid toxic effects re-start densities were lower for for these test groups. Algae of all test groups recovered and their densities were at the same level as the control after 6 days.

B.8.2.12 Microcosm and mesocosm study (Annex IIIA 10.2.2)

The study is not required.

B.8.2.13 Residue data in fish (Annex IIIA 10.2.3)

The study is not required. The water half-life of the a.s. is very short. The main metabolite E-1 is not bioaccumulable.

B.8.2.14 Supplementary studies of toxicity to fish and aquatic invertebrates (Annex IIIA 10.2.4)

Not required.

B.8.2.15 Summary of effects to water organisms (Annex IIA 8.2, Annex IIIA 10.2)

Table B.8.2.15-1 : Summary of effects of pyraflufen-ethyl and metabolite E-1 to water organisms

Type of test	Exposure period	Species	NOEC (µg/l)	LC_{50}/EC_{50} (µg/l)	Reference
Pyraflufen-ethyl					
Acute	96 hours	*Oncorhynchus mykiss*	100	**>100**	Caunter *et al.* (1995a)
Acute	96 hours	*Lepomis macrochirus*	100	>100	Caunter *et al.* (1995b)
Acute	48 hours	*Daphnia magna*	100	**>100**	Croudace *et al.* (1996b)
Exposure	72 hours	*Selenastrum capricornutum*	0.037[a], 0.037[b]	**0.23**[a], 0.65[b]	Morgan *et al.* (1996)
Exposure/ recovery	72 hours/ 144 hours	*Selenastrum capricornutum*	0.040[a], 0.088[b] (exposure)	0.31[a], 1.0[b] (exposure)	Smyth *et al.* (1997a)
Exposure	72 hours	*Navicula pelliculosa*	0.25[a], 0.89[b]	1.6[a], 5.4[b]	Smyth *et al.* (1996a)
Metabolite E-1					
Acute	96 hours	*Oncorhynchus mykiss*	120000	>120000	Caunter *et al.* (1996a)
Acute	96 hours	*Lepomis macrochirus*	100000	**>100000**	Caunter *et al.* (1996b)
Chronic	36 days	*Pimephales promelas*	**10000**	-	Croudace *et al.* (1996a)
Acute	48 hours	*Daphnia magna*	120000	**>120000**	Croudace *et al.* (1996c)
Chronic	21 days	*Daphnia magna*	**100000**	>120000	Croudace *et al.* (1996d)
Exposure	72 hours	*Selenastrum capricornutum*	1.8[a], 3.2[b]	5.5[a], 10[b]	Smyth *et al.* (1996b)
Exposure/ recovery	72 hours/ 144 hours	*Selenastrum capricornutum*	0.91[a], 0.91[b] (exposure)	**2.2**[a], 4.0[b] (exposure)	Smyth *et al.* (1997b)
Exposure	72 hours	*Navicula pelliculosa*	600[a], 600[b]	1700[a], 3600[b]	Smyth *et al.* (1996c)
Exposure	14 days	*Lemna gibba*	2.7[c], 0.84[d]	4.7[c], 2.6[d]	Smyth *et al.* (1996d)
Metabolite E-2					
Exposure	72 hours	*Selenastrum capricornutum*	0.16[a], 0.16[b]	**0.16-0.35**[a], >0.35[b]	Odin-Feurtet (1998)

a: figures based on area under growth curve (biomass)
b: figures based on growth rate
c: figures based on increase in number of fronds
d: figures based on increase in weight of plants
In bold, figures taken into account in the risk assessment

Table B.8.2.15-2 : Summary of effects of formulation EXP31279A (SC containing 9 g/l pyraflufen-ethyl and 500 g/l bifenox) to water organisms

Type of test	Exposure period	Species	NOEC (µg/l formulation)	LC_{50}/EC_{50} (µg/l formulation)	Reference
Acute	96 hours	*Oncorhynchus mykiss*	<4000	>60000	Suteau (1997a)
Acute	48 hours	*Daphnia magna*	800	>15000	Suteau (1997b)
Exposure/ recovery	72 hours/ 96 hours	*Selenastrum capricornutum*	0.17[a]	1.84a, ND[b]	Odin-Feurtet (1997b)
Exposure	72 hours	*Scenedesmus subspicatus*	0.38	0.48[a], 0.68[b]	Odin-Feurtet (1997a)

Exposure/ recovery	72 hours/ 144 hours	*Navicula pelliculosa*	16[a], 16b	20[a], 84[b]	Smyth *et al.* (1996)

B.8.2.16 Exposure and risk assessment for aquatic organisms (Annex IIIA 10.2)

The following assumptions were made to assess the risks for water organisms :

- toxicity figures which were taken into account are indicated in bold in table B.8.2.15-1
- the DT50 of the a.s. in water is very short (1-2h) : Only acute TER calculation is necessary
- the metabolite E-1 reached rapidly a maximum level in the water phase. Its DT50 is 50-100 days : TER acute and chronic were determined for fish, daphnia and algae.
- the metabolite E-2 is mainly present in sediment. However the TER for the most sensitive organism (alga) was determined.
- the metabolite E-3 is found at low level only in the sediment phase : No TER was calculated for the water organisms.
- 30 cm water depth,
- spray drift according to Ganzelmeir (1992),

We considered for the calculations of the TERmetabolites that metabolites E-1 and E-2 had the same molecular weight as the a.s., that the transformation of the a.s. into its metabolites was complete and instantaneous.

The TER calculations reveal that
- fish and aquatic invertebrates are not at risk.
- the a.s. and metabolite E-2 present a similar toxicity to algae. Mitigation techniques such as buffer zones should be applied in order to reduce the risk to algae and aquatic plants.

Table B.8.2.16-2 : Toxicity/Exposure Ratios for the most sensitive aquatic organisms exposed to pyraflufen-ethyl

Application rate (kg a.s./ha)	Crop	Organism	Time-scale	Distance (m)	TER	Annex VI trigger
pyraflufen-ethyl						
0.0135	Cereals	*Oncorhynchus mykiss*	acute	1	556	100
		Daphnia magna	acute	1	556	100
		Selenastrum capricornutum	acute	1	**1**	10
				5	**9**	10
				10	13	10
Metabolite E-1						
0.0135	Cereals	*Lepomis macrochirus*	acute	1	556000	100
		Daphnia magna	acute	1	667000	100
		Selenastrum capricornutum	acute	1	12	10
		Pimephales promelas	chronic (36 d)	1	55500	10
		Daphnia magna	chronic (21 d)	1	555000	10
Metabolite E-2						
0.0135	Cereals	*Selenastrum capricornutum*	acute	1	**1**	10
				5	**6**	10
				10	**9**	10
				15	18	10

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

Table B.8.3-1 : Toxicological data taken into consideration in the risk assessment for mammals

Test species	**Test System**	**Duration of exposure**	**Results**	**References**
rat	acute	single appl.	LD_{50} > 5000 mg a.s./kg bw	Amanuma 1995a
rat	short-term	28 days	NOAEL = 2000 mg a.s./kg food	Broadmeadow, 1994a
rabbit	teratogenicity	single application	NOAEL = 20 mg/kg bw/d (provisional information)	Burns, 1996

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 13.5 g a.s./ha

The TER reveal that the acute, short-term and long-term risk to mammals is negligible .
In absence of clear guidance provisional long-term TER was calculated. These long-term TER are probably superfluous for pyraflufen-ethyl

Table B.8.3-2 : Estimated initial concentration of pyraflufen-ethyl in potential feed of mammals

Target crop	**Application rate (g a.s./ha)**	**Estimated initial residues (mg a.s./kg food)**	
		Small insects	**Leaves and leafy crops**
Cereals	13.5	0.39	0.42

Table B.8.3-3 : Estimated oral uptake of pyraflufen-ethyl by mammals

Target crop	**Mammal type**	**food consumed**	**food consumption (% bw)**	**Max. daily intake (mg ai.s. bw / day)**
cereals	small mammal (<100 g)	small insects	30	0.117
		leaves and leafy crop	30	0.126

Table B.8.3-4 : Toxicity exposure ratios for mammals exposed to pyraflufen-ethyl - worst cases

Application rate (kg a.s./ha)	Crop	Organism	Time-scale	TER	Annex VI trigger
pyraflufen-ethyl					
0.0135	Cereals	small insectivorous mammal	acute	42571	10
		small foliage-eating mammal	acute	39825	10
		small insectivorous mammal	short-term	5109	10
		small foliage-eating mammal	short-term	4779	10

		small insectivorous mammal	long-term	20/0.117= 170	5
		small foliage-eating mammal	long-term	20/0.126 = 159	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)

Assessment of side effects of ET-751 Technical to the honey bee, *Apis mellifera* L. in the laboratory following the EPPO guideline No.170. (Nengel, S., 1996)
Guidelines :
EPPO guideline No. 170: Guideline on test methods for evaluating the side-effects of plant protection products on honey bees (1992)
GLP :
Yes
Material and Methods :
Test substance : pyraflufen-ethyl, chemical purity : 97.7%
Test species : Honeybees (*Apis mellifera L.*); worker bees
Number of organisms : 10 bees X 5 replicates/ concentration
Type of test : Acute and contact toxicity test (48 h)
Applied concentrations : 6.25, 25.00, 50.00, 100.00 µg a.s./bee (nominal); solvent control; positive control : dimethoate
Exposure route :
Oral test : a.s. dissolved in acetone was added to the 50% sucrose suspension. 200µl of sucrose solution containing the a.s.were offered to each replicate of 10 bees.
Contact test : CO_2 immobilized bees were treated by topical applications of 2µl suspension of the ventral side of the thorax.
Feeding : 50% aqueous sucrose solution
Test conditions :
28.0-31.0 °C, relative humidity of 42-56%
Findings and conclusions :
LD_{50} (48h) contact > 100 µg a.s./bee (nominal) (highest concentration tested)
NOEL (48h) contact = 100 µg a.s./bee (nominal)
LD_{50} (48h) oral > 111.99 µg a.s./bee
NOEL (48h) = 111.99 oral µg a.s./bee

EXP31279A: Laboratory Oral and Contact test with the Honeybee, *Apis mellifera*, based on the EPPO Guideline 170 (1992) (Candolfi, M.P., 1996a)
Guidelines :
EPPO guideline No. 170: Guideline on test methods for evaluating the side-effects of plant protection products on honey bees (1992)
GLP :
Yes
Material and Methods :
*Test substance :*EXP 31279A (SC containing 9.57 g/l pyraflufen-ethyl and 519 g/l bifenox)
Test species : Honeybees (*Apis mellifera L.*); worker bees
Number of organisms : 10 bees X 3 replicates/ concentration
Type of test : Acute and contact toxicity test (48 h)
Applied concentrations : 200 µg formulation/bee (nominal); solvent control; positive control : dimethoate
Exposure route :
Oral test : the formulation was dissolved in acetone was added to the 20% sucrose suspension. 100µl of sucrose solution containing the test substance were offered to each replicate of 10 bees.
Contact test : CO_2 immobilized bees were treated by topical applications of 1µl solution of the ventral side of the thorax.
Feeding : 50% aqueous sucrose solution
Test conditions :
23.0-25.0 °C, relative humidity of 60-79%
Findings and conclusions :
LD_{50} (48h) contact >200 µg formulation/bee (nominal)
NOEL (48h) contact = 200 µg firomulation/bee (nominal)
LD_{50} (48h) oral > 190 µg a.s./bee

NOEL (48h) = 190 oral µg 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 study on the formulation containing bifenox and pyraflufen-ethyl was performed.

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 (0.12-0.13).

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

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

Test species	**Test system**	**Results**	**References**
pyraflufen-ethyl			
Honeybee *(Apis mellifera L)*	Acute oral toxicity test	LD_{50} >111.99 µg a.s./bee.	Nengel (1996)
	Acute contact toxicity test	LD_{50} > 100 µg a.s./bee.	
EXP31279A (SC containing 9 g/l pyraflufen-ethyl and 500 g/l bifenox)			
Honeybee *(Apis mellifera L)*	Acute oral toxicity test	LD_{50} > 190 µg formulation /bee.	Candolfi (1996)
	Acute contact toxicity test	LD_{50} > 200 µg formulation /bee.	

Hazard quotients reveal that the bees are not at risk. As the formulation is applied on cereals crop up to tillering it is unlikely that bees will be exposed (no flowering plants in the field).

Table B.8.4.8-2 : Hazard quotients for honeybees

Application rate (kg a.s./ha)	Crop	Route	Hazard quotient	Annex VI trigger
0.0135	Cereals	oral	0.12	50
		contact	0.13	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)

No study was performed with the active substance. The effects on the non-target arthropods were evaluated by studies performed with the formulation EXP31279A (SC containing 9.57 g/l pyraflufen-ethyl and 519 g/l bifenox)

Studies of the effects of the a.s. on non-target arthropods have to be required only to assess the own impact of the substance. The requirement of these studies should be further discussed.

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

EXP31279A: Laboratory Toxicity Test with the Parasitic Wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) based on the IOBC Method of Polgar (1988) (Candolfi, M.P. ,1996)
Guidelines :
IOBC Method of Polgar (1988)
GLP :
Yes
Material and Methods :
Test substance : EXP 31279A (SC containing 9.57 g/l pyraflufen-ethyl and 519 g/l bifenox)
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, 10 replicates with 1 wasp each for the reproduction phase.
Type of test : Laboratory test
Applied and measured concentrations :
Positive control (Perfekthion), water control, treatment at a concentration equivalent to the maximum field application rate of 1.33 l/ha (200l/ha) , corresponding to 12 g pyraflufen-ethyl/ha and 665 g bifenox/ha
Exposure route :
24 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 11 days.
Test conditions :
temperature : 18.5-21.0°C, relative humidity : 68-94%, light intensity : 852 and 2318 lux for the exposure and reproduction phase, photoperiod : 16 hours light/8 hours dark.
Findings :
Table B.8.5.2-1 : Effects of formulation EXP 31279A on *Aphidius rhopalosiphi*

Evaluation criteria	**Control**	**Treatment**	**Endpoints**
exposure phase (% mortality)	0%	0%	
parasitization phase (mean No of mummies/female)	46.6 ± 26.5	45.6 ± 17.0	
			Reduction of beneficial capacity = 2.1 %

Conclusion :
The formulation EXP31279A is ‘harmless’ to *Aphidius rhopalosiphi* at the application rate of 1.33 l/ha

EXP31279A: Laboratory Contact Toxicity Test with the Predacious Mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae), based on the IOBC Approved Method of Overmeer (1988), (Candolfi, M.P., 1996)
Guidelines :
IOBC Method of Overmeer (1988)
GLP :
Yes
Material and Methods :
Test substance : EXP 31279A (SC containing 9.57 g/l pyraflufen-ethyl and 519 g/l bifenox)
Test species : Typhlodromus pyri (predaceous mite), protonymphs
Number of organisms : 5 replicates per treatment each with 20 protonymphs
Type of test : Laboratory test
Applied and measured concentrations :
Positive control (Ethyl Parathion), water control, treatment at a concentration equivalent to the maximum field application rate of 1.33 l/ha (200l/ha) , corresponding to 12 g pyraflufen-ethyl/ha and 665 g bifenox/ha
Exposure route :
protonymphs were maintained on glass plates for 14 days. Mortality was assessed on days 3, 7, 10 and 14. Eggs and hatched larvae were counted on days 7, 10 and 14.
Test conditions :
temperature : 23.5-26.0°C, relative humidity :70-82%, light intensity : 1237.4 lux , photoperiod : 16 hours light/8 hours dark.
Findings :
Table B.8.5.2-2 : Effects of formulation EXP 31279A on *Typhlodromus pyri*

Evaluation criteria	**Control**	**Treatment**	**Endpoints**
Mortality on day 14	5%	100%	
Egg production (No of eggs /female)	0.80	-	
			Reduction of beneficial capacity = 100 %

Conclusion :
The formulation EXP31279A is "harmful" to *Typhlodromus pyri* at the application rate of 1.33 l/ha

EXP31279A: Laboratory Contact Toxicity Test with Spiders, *Pardosa sp.* (Aranae: Lycosidae), based on the BBA Method of Wehling and Heimbach (1994) (Candolfi, M.P. , 1996d)
Guidelines :
BBA Method of Wehling and Heimbach (1994)
GLP :
Yes
Material and Methods :
Test substance : EXP 31279A (SC containing 9.57 g/l pyraflufen-ethyl and 519 g/l bifenox)
Test species :Pardosa amentata (spider), adults
Number of organisms : 20 replicates per treatment each with 1 spider
Type of test : Laboratory test
Applied and measured concentrations :
Positive control (lambda-cyhalothrin), water control, treatment at a concentration equivalent to the maximum field application rate of 1.33 l/ha (500l/ha) , corresponding to 12 g pyraflufen-ethyl/ha and 665 g bifenox/ha
Exposure route :
Spiders maintained in sand containing boxes were sprayed with the test solutions. Mortality, behaviour and feed consumption were monitored for 14 days.
Test conditions :
temperature : 18.5-22.0°C, relative humidity : 65-83%, light intensity : 1180 lux , photoperiod : 16 hours light/8 hours dark.
Findings :

Table B.8.5.2-3 : Effects of formulation EXP 31279A on *Pardosa amentata*

Evaluation criteria	**Control**	**Treatment**	**Endpoints**
Mortality after 14 days	15.0 ± 36.6	40.0 ± 50.3	Corrected mortality : 29.4%
Mean feeding rate (number flies/spider/d)	0.35 ± 0.23	0.52 ± 0.15	

Conclusion :
The formulation EXP31279A is 'harmless' to *Pardosa amentata* at the application rate of 1.33 l/ha

EXP31279A: Laboratory Acute Toxicity Test with the Ground Beetle *Poecilus cupreus* L. (Coleoptera: Carabidae), based on the IOBC Approved Method of Heimbach (1992) (Candolfi, M.P. , 1997a)
Guidelines :
IOBC Method of Heimbach (1992)
GLP :
Yes
Material and Methods :
Test substance : EXP 31279A (SC containing 9.57 g/l pyraflufen-ethyl and 519 g/l bifenox)
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 and measured concentrations :
Positive control (pyrazophos), water control, treatment at a concentration equivalent to the maximum field application rate of 1.33 l/ha (500l/ha) , corresponding to 12 g pyraflufen-ethyl/ha and 665 g bifenox/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 : 19.0-21.5°C, relative humidity : 58-83%, light intensity : 893 lux , photoperiod : 16 hours light/8 hours dark.
Findings :
Table B.8.5.2-4 : Effects of formulation EXP 31279A on *Poecilus cupreus*

Evaluation criteria	**Control**	**Treatment**	**Endpoints**
Mortality after 14 days _ _	 0 0	 0 6.7 ± 14.9	Corrected mortality = 3.3%
Mean feeding rate (number fly pupae /beetle/d)	0.17 ± 0.03	0.18 ± 0.02	

Conclusion :
The formulation EXP31279A is 'harmless' to *Poecilus cupreus* at the application rate of 1.33 l/ha

EXP31279A: Laboratory Toxicity Test with the Green Lacewing *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae), based on the IOBC Approved Method of Bigler (1988) (Candolfi, M.P., 1997b)

Guidelines :

IOBC Approved Method of Bigler (1988)

GLP :

Yes

Material and Methods :

Test substance : EXP 31279A (SC containing 9.57 g/l pyraflufen-ethyl and 519 g/l bifenox)

Test species : Chrysoperla carnea, 24 hour old larvae

Number of organisms : 4 replicates per treatment each with 10 larvae

Type of test : Laboratory test

Applied and measured concentrations :

Positive control (Perfekthion), water control, treatment at a concentration equivalent to the maximum field application rate of 1.33 l/ha (200l/ha) , corresponding to 12 g pyraflufen-ethyl/ha and 665 g bifenox/ha

Exposure route :

Lacewings larvae were exposed to the a.s. via treated glass plates with dry spray deposit for a period of 44 days. All insects reaching adult stage were transferred to untreated reproduction units to assess oviposition and egg viability (4 weeks).

Test conditions :

temperature : 21.0-24.0°C, relative humidity : 50-77%, light intensity : 2829 lux , photoperiod : 16 hours light/8 hours dark.

Findings :

Table B.8.5.2-5 : Effects of formulation EXP 31279A on *Chrysoperla carnea*

Evaluation criteria	**Control**	**Treatment**	**Endpoints**
Exposure phase - Mortality after 44 days (%)	 20.0 ± 8.2	 30.0 ± 14.1	Corrected mortality = 12.5%
Reproduction phase - Number of eggs per female - Hatching rate (%) - Mean mortality of adults (%)	 26.9 ± 6.9 94.6 ± 3.8 3.6 ± 5.1	 20.8 ± 0.3 94.4 ± 1.3 3.8 ± 5.4	Reproduction parameter = 0.77%
			Reduction of beneficial capacity = 32.6 %

Conclusion :

The formulation EXP31279A is ‘slightly harmful’ to *Chrysoperla carnea* at the application rate of 1.33 l/ha

EXP31279A: Laboratory Contact Toxicity Test with the Seven-Spotted Lady Beetle *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), based on the Method of Pinsdorf (1989) (Candolfi, M.P., 1997c)

Guidelines :

Method of Pinsdorf (1989)

GLP :

Yes

Material and Methods :

Test substance : EXP 31279A (SC containing 9.57 g/l pyraflufen-ethyl and 519 g/l bifenox)

Test species : Coccinella septempunctata, 3 day old larvae (second instar)

Number of organisms : 5 replicates per treatment each with 10 larvae

Type of test : Laboratory test

Applied and measured concentrations :

Positive control (pyrazophos), water control, treatment at a concentration equivalent to the maximum field application rate of 1.33 l/ha (200l/ha) , corresponding to 12 g pyraflufen-ethyl/ha and 665 g bifenox/ha

Exposure route :

Coccinella larvae were exposed to the a.s. via treated with dry spray deposit for a period of 22 days. All insects reaching adult stage were transferred to untreated reproduction units to assess oviposition and egg viability (5

weeks).
Test conditions :
temperature : 21.0-24.5°C, relative humidity : 48-77%, light intensity : 1297 lux , photoperiod : 16 hours light/8 hours dark.
Findings :
Table B.8.5.2-6 : Effects of formulation EXP 31279A on *Coccinella septempunctata*

Evaluation criteria	**Control**	**Treatment**	**Endpoints**
Exposure phase - Mortality after 22 days (%)	 12.0 ± 13.0	 14.0 ± 11.4	Corrected mortality = 2.27%
Reproduction phase - Number of eggs per female per day - Hatching rate (%) - No. of viable eggs per female (%)	 19.5 ± 11.1 56.9 ± 15.4 245.0	 18.1 ± 9.3 42.1 ± 20.8 183.4	Reproduction parameter = 31.5%
			Reduction of beneficial capacity = 33.04 %

Conclusion :
The formulation EXP31279A is 'slightly harmful' to *Coccinella septempunctata* at the application rate of 1.33 l/ha

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

The results of the laboratory tests indicate that no further testing in the field 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 EXP 31279A (SC containing 9.57 g/l pyraflufen-ethyl and 519 g/l bifenox) to non-target terrestrial arthropods - laboratory tests

Species	**Stage**	**Test substance**	**Dose (l/ha)**	**Endpoint**	**Effect**	**Annex VI trigger**	**References**
Aphidius rhopalosiphi	adults	formulation	1.33l/ha	beneficial capacity	2.1%	30%	Candolfi, 1996
Typhlodromus pyri	protonymphs	formulation	1.33l/ha	beneficial capacity	**100%**	30%	Candolfi, 1996
Pardosa amentata	adults	formulation	1.33l/ha	mortality	29.4%	30%	Candolfi, 1996d
Poecilus cupreus	adults	formulation	1.33l/ha	mortality	3.3%	30%	Candolfi, 1997a
Chrysoperla carnea	24 hour old larvae	formulation	1.33l/ha	beneficial capacity	**32.6%**	30%	Candolfi, 1997b
Coccinella septempunctata	3 day old larvae	formulation	1.33l/ha	beneficial capacity	**33.04%**	30%	Candolfi, 1997c

The formulation is harmful to *Typhlodromus pyri* and slightly harmful to both plant dwelling organisms (*Chrysoperla carnea, Coccinella septempunctata*). No further testing was required as the exposure of these arthropods is very limited under the conditions of use (herbicide sprayed at tillering of the cereals). The formulation is harmless to soil dwelling organisms (*Pardosa amentata, Poecilus cupreus*)

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)

Acute toxicity of ET-751 Technical on earthworms, *Eisenia foetida* using an artificial soil test (Wachter, S., 1996)
Guidelines :
OECD guideline No 207
GLP :
Yes
Material and Methods :
Test substance : pyraflufen-ethyl, chemical purity : 97.7%
Test species : Earthworms (*Eisenia foetida*)
Number of organisms, weight, age : 4 replicates each with 10 worms per treatment group, 273-389 g at start, 2 month old with clitellum
Type of test : laboratory test (14 days)
Applied concentrations : 100, 178, 316, 562, 1000 mg a.s./kg (nominal); water control, positive control : 2-cloro-acetamide
Soil type and test conditions :
Test substrate : 10% sphagnum peat, 20% kaolinite clay, 69% industrial sand, 1% calcium carbonate
water content : 35% water content
Temperature : 20 ± 2°C
Light regime : continuous, 400-800 lux
Analytical methods : not performed
Findings :
Mortality : no mortality was observed.
Observations : body weight changes were similar in the control and treatment groups
Conclusion :
LC_{50} (14d) > 1000 mg a.s./kg substrate
NOEC (14d) = 1000 mg a.s./kg substrate

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

The determination of sublethal effects of the a.s. is not required since the degradation of pyraflufen-ethyl in soil is very rapid (DT90 =3-23 days in the field).

Due to the very high margin of safety observed with the acute TER for the a.s. and the formulation EXP31279A, further testing with metabolites is not required.

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

EXP31279A: Acute Toxicity (14-Day) to Earthworms (*Eisenia foetida*), Artificial Soil Method (Suteau, P., 1996)
Guidelines :
OECD guideline No 207
GLP :
Yes
Material and Methods :
Test substance :
Test species : Earthworms (*Eisenia foetida*)
Number of organisms, weight, age : 4 replicates each with 10 worms per treatment group, 304-326 g at start, 2 month old with clitellum
Type of test : laboratory test (14 days)
Applied concentrations : 95, 171, 309, 556, 1000 mg formulation/kg (nominal); water control
Soil type and test conditions :
Test substrate : 10.1% sphagnum peat, 20.1% kaolinite clay, 69.4% industrial sand, 0.4% calcium carbonate

water content : 34% water content
Temperature : 20 °C
Light regime : continuous, 459-557 lux
Analytical methods : not performed
Findings :
Mortality : 2.5% mortality in the treatment groups 309, 556, 1000 mg/kg
Observations : body weight changes were similar in the control and treatment groups
Conclusion :
LC_{50} (14d) > 1000 mg formulation/kg substrate
NOEC (14d) = 171 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 EXP31279A, further testing of 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 since the TER >5.

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 pyraflufen-ethyl for earthworms

Test species	**Test system**	**Duration of exposure**	**Results (mg/kg soil)**	**References**
Pyraflufen-ethyl				
Earthworm *(Eisenia foetida)*	acute test	14 days	LC_{50} > 1000 mg a.s./kg NOEC = 1000 mg a.s./kg	Wachter, 1996
EXP31279A (SC containing 9 g/l pyraflufen-ethyl and 500 g/l bifenox)				
Earthworm *(Eisenia foetida)*	acute test	14 days	LC_{50} > 1000 mg formulation/kg NOEC = 171 mg formulation/kg	Suteau, 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^3

Due to the very high margin of safety observed with the acute TER for the a.s. and the formulation EXP31279A, further testing of metabolites and/or 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**
Pyraflufen-ethyl				
0.0135 kg a.s./ha	Cereals	acute	> 55556	10
EXP31279A (SC containing 9 g/l pyraflufen-ethyl and 500 g/l bifenox)				
1.33 l/ha	Cereals	acute	> 564	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, 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)

Assessment of the side effects of ET-751 technical on the activity of the soil microflora (Dengler, D., 1997)
Guidelines :
BBA-Guideline for the Official Testing of Pesticides, Part VI, 1-1, 2nd edition, dated March 1990.
GLP :
Yes
Material and Methods :
Test substance : pyraflufen-ethyl, chemical purity : 97.7%
Soils : 3 replicates per treatment group
LUFA Speyer soils
Applied concentrations :
'application rate of 20 g a.s. /ha and 100 g a.s./h at a penetration of 5 cm and soil gravity of 1.5 g/cm3' . No information on the actual concentration in mg a.s./kg soil
untreated control, positive control (dinoseb-acetate)
Type of test : 28-day nitrogen turnover test, 28 -day short time respiration
Test conditions :
Soil moisture : 40 % of its water holding capacity, 0.5% lucenre meal was used in the nitrogen turnover test.
Soil samples were incubated at 20°C ± 2 °C in the dark.
Findings :
Table 8.8.1-1 : Effects of pyraflufen-ethyl on the nitrogen turnover

	% Deviation of mineral nitrogen from control*		
	Pyraflufen-ethyl		Dinoseb-acetate
	20 g a.s./ha	100 g a.s./ha	26.2 mg/kg soil
Sandy soil, BBA type 2.1, 0.63 % OC, pH 6.0, CEC :-			
3 hours	2.79	1.12	3.91
14 days	4.56	0	-44.21
28 days	3.55	2.96	1.78
Sandy loam soil, BBA type 2.3, 1.35 % OC, pH 6.7, CEC :-			
3 hours	-18.96	-17.14	-16.62
14 days	-3.21	-0.46	-25.69
28 days	-10.49	-2.62	-45.88

* Negative values mean stimulating effects.

Soil 1 : Although all values of the individual nitrogen compounds (NH4+, NO3-) were significantly different from the control values, the content of N mineralized of the dinoseb-acetate group were equivalent to that of the control and substance group.
Soil 2 : Effects on the NH4+ (increase) and NO3- (decrease) were observed in the positive control at 14 days. At this stage both pyraflufen-ethyl treatments were similar to the untreated control. At day 28 all the groups untreated control, dinoseb-acetate, both treatment groups) showed similar NH4+ and NO3- concentrations.
The nitrogen turnover test is therefore acceptable for both soils.

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

	% Deviation of respiration rate from control*		
	Pyraflufen-ethyl		Dinoseb-acetate
	20 g a.s./ha	100 g a.s./ha	26.2 mg/kg soil
Sandy soil, BBA type 2.1			
3 hours	-44.19	-36.63	-36.63
14 days	-3.22	16.11	9.95
28 days	5.34	3.05	29.01
Sandy loam soil, BBA type 2.3			
3 hours	-18.89	-18.57	10.1
14 days	2.03	0.68	39.66
28 days	-8.82	-1.31	44.7

* Negative values mean stimulating effects

Conclusions :
The impact on the soil nitrogen turnover and on the respiration rate is negligible at the application rates of 20 and 100 g a.s./ha

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

A Laboratory Assessment of the Effects of EXP31279A on Soil Microflora Respiration and Nitrogen Transformations According to BBA Guidelines VI 1-1 (1990) (Forster, 1997)
Guidelines :
BBA-Guideline for the Official Testing of Pesticides, Part VI, 1-1, 2nd edition, dated March 1990.
GLP :
Yes
Material and Methods :
Test substance :EXP31279A (SC containing 9 g/l pyraflufen-ethyl and 500 g/l bifenox)
Soils : 3 replicates per treatment group
Origin of the soils is not mentionned (agricultural ?)
Applied concentrations :
The application rates were 2.07 mg formulation/kg soil and 10.36 mg formulation/kg soil corresponding to 1.5535 kg formulation/ha and 7.8 times the single application rate.
The treatment groups were compared to an untreated control.
Aretit flüssig had an enhancing effect on the ammonium and nitrate production.
Type of test : 28-day nitrogen turnover test, 28 -day short time respiration
Test conditions :
Soil moisture : 45 % 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 8.8.2-1 : Effects of EXP31279A (SC containing 9 g/l pyraflufen-ethyl and 500 g/l bifenox) on the nitrogen turnover

	% Deviation of mineral nitrogen from control*	
	1.5 kg/ha	7.5 kg/ha
Sandy loamy silt, 4.0 % OM, pH 6.5, CEC :-		
3 hours	-2.18	-10.79
14 days	+9.25	+8.47
28 days	+4.45	-0.82
Loamy sand, , 1.7 % OM, pH 6.6, CEC :-		
3 hours	?	?
14 days	?	?
28 days	?	?

* Negative values mean stimulating effects.

Table 8.8.2-2 : Effects of EXP31279A (SC containing 9 g/l pyraflufen-ethyl and 500 g/l bifenox) on the short term respiration

	% Deviation of respiration rate from control*	
	1.5 kg/ha	7.5 kg/ha
Sandy loamy silt, 4.0 % OM, pH 6.5, CEC :-		
3 hours	-22	-4.5
14 days	5.6	-1.0
28 days	6.0	6.6
Loamy sand, , 1.7 % OM, pH 6.6, CEC :-		
3 hours	?	?
14 days	?	?
28 days	?	?

* Negative values mean stimulating effects

Conclusions :
Due to numerous deviations (rates of 1 and 5 times the application rate, missing raw data, positive control effect) the study is not acceptable.

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

The study is not required since the effects on the soil microbial activity are less than 25% after 100days.

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

The impact on the soil nitrogen turnover and on the respiration rate is negligible at the application rates of 20 and 100 g a.s./ha. These rates are equivalent to 1.5 and 7.4 the GAP rate of 13.5 g a.s./ha. The test should have been performed at 1 and 10 times the GAP rate. Nevertheless, We consider that the test is sufficient to assess the effects of pyraflufen-ethyl on soil micro-organisms

The test with the representative formulation presents numerous deviations. A new test should be provided.

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)

‘ Pyraflufen-ethyl is a broad-leaved, non-persistent contact herbicide, selective for cereals.

The following list gives an overview of the susceptibility of the species tested when pyraflufen-ethyl is applied upland and/or paddy field.

Susceptible species

Crops and weeds:	*Abutilon theophrasti, Cucumis sativus, Cyperus serotinus, Galium aparine, Lycopersicon esculentum, Sagittaria pygmaea, Scirpus juncoides, Veronica persica, Xanthium strumarium*
Insects:	None
Microorganisms:	None

Moderately susceptible species

Crops and weeds:	*Echinochloa crus-galli, Glycine max, Oryza sativa*
Insects:	None
Microorganisms:	*Micronectriella nivalis, Rhizopus nigricans, Rhizoctonia solani* (AG-4)

Non-susceptible species

Crops and weeds:	*Avena fatua, Triticum aestivum*
Insects:	Species belonging to the following genera: *Adoxophyes, Cnaphalocrocis, Myzus, Nilaparvata, Plutella, Spodoptera, Tetranychus*
Microorganisms:	Fungal species belonging to the following genera; *Alternaria, Botrytis, Cochliobolus, Erysiphe, Fusarium, Phytophthora, Pseudoperonospora, Pseudocercosporella, Rhizoctonia, Rosellinia, Trichoderma, Typhula, Venturia, Verticillium* Bacterial species belonging to the following genera: *Erwinia, Pseudomonas, Xanthomonas* ‘

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 pyraflufen-ethyl will contaminate sewage treatment plant

B.8.11 References relied on

Ecotoxicology of the active substance (Annex IIA 8)

Author(s)	Year	Annex IIA Point Title Company, Report No.	GLP GEP Y/N	Published or not Y/N	Owner
Candolfi, M.P.	1996a	IIA, 8.3.2/01 EXP31279A: Laboratory Toxicity Test with the Parasitic Wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) based on the IOBC Method of Polgar (1988) Rhône-Poulenc Agrochimie, Report no: 96-032-1013	Y	N	RPA
Candolfi, M.P.	1996b	IIA, 8.3.2/02 EXP31279A: Laboratory Contact Toxicity Test with the Predacious Mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae), based on the IOBC Approved Method of Overmeer (1988) Rhône-Poulenc Agrochimie, Report no: 96-037-1013	Y	N	RPA
Caunter, J.E. Magor, S.E. Croudace, C.P.	1995a	IIA, 8.2.1/01 ET-751: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) Nihon Nohyaku, Report No: W-5003	Y	N	NN
Caunter, J.E. Magor, S.E. Croudace, C.P.	1995b	IIA, 8.2.1/02 ET-751: Acute toxicity to bluegill sunfish (*Lepomis macrochirus*) Nihon Nohyaku, Report No: W-5002	Y	N	NN
Caunter, J.E. Johnson, P.A. Croudace, C.P.	1996a	IIA, 8.2.1/03 E-1: Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) Nihon Nohyaku, Report No: W-5017	Y	N	NN
Caunter, J.E. Cornish, S.K. Croudace, C.P.	1996b	IIA, 8.2.1/04 E-1: Acute toxicity to bluegill sunfish (*Lepomis macrochirus*) Nihon Nohyaku, Report No: W-5018	Y	N	NN
Croudace, C.P. Caunter, J.E. Wallace, S.J.	1996a	IIA, 8.2.2.2 E-1: Chronic toxicity to fathead minnow (*Pimephales promelas*) embryos and larvae Nihon Nohyaku, Report No: W-5029	Y	N	NN
Croudace, C.P. Banner, A.J. Johnson, P.A.	1996b	IIA, 8.2.4/01 ET-751: Acute toxicity to *Daphnia magna* Nihon Nohyaku, Report No: W-5020	Y	N	NN
Croudace, C.P. Banner, A.J. Johnson, P.A.	1996c	IIA, 8.2.4/02 E-1: Acute toxicity to *Daphnia magna* Nihon Nohyaku, Report No: W-5016	Y	N	NN
Croudace, C.P. Williams, N.J. Shearing, J.M.	1996d	IIA, 8.2.5 E-1: Chronic toxicity to *Daphnia magna* Nihon Nohyaku, Report No: W-5026	Y	N	NN
Dengler, D.	1997	IIA, 8.5 Assessment of the side effects of ET-751 technical on the activity of the soil microflora	Y	N	NN

Author(s)	Year	Annex IIA Point Title Company, Report No.	GLP GEP Y/N	Published or not Y/N	Owner
		Nihon Nohyaku, Report No: N-5011			
Grinell, A.J. Croudace, C.P. Caunter, J.E. Gillings, E.	1996	IIA, 8.2.3 E-1: Determination of the accumulation and elimination of [^{14}C]E-1 in rainbow trout (*Oncorhynchus mykiss*) Nihon Nohyaku, Report No: W-5030	Y	N	NN
Morgan, D.J. Croudace, C.P. Magor, S.E.	1996	IIA, 8.2.6/01 ET-751: Toxicity to the green alga *Selenastrum capricornutum* Nihon Nohyaku, Report No: W-5024	Y	N	NN
Nengel, S.	1996	IIA, 8.3.1.1 Assessment of side effects of ET-751 Technical to the honey bee, *Apis mellifera* L. in the laboratory following the EPPO guideline No.170 Nihon Nohyaku, Report No: N-5010	Y	N	NN
Odin-Feurtet, M.	1998	IIA 8.2.6 E2 : Freshwater algal growth inhibition study (72 h) (*Selenastrum capricornutum*) Rhône-Poulenc, Report No : 603459	N	N	RPA
Pascual, J.	1998	IIA 8.2 Position paper : Pyraflufen-ethyl : Risk to fish, daphnids and algae from metabolite E2	N	N	RPA
Smyth, D.V. Croudace, C.P. Johnson, P.A.	1996a	IIA, 8.2.6/03 ET-751: Toxicity to the freshwater diatom *Navicula pelliculosa* Nihon Nohyaku, Report No: W-5021	Y	N	NN
Smyth, D.V. Croudace, C.P. Wallace, S.J.	1996b	IIA, 8.2.6/04 E-1: Toxicity to the green alga *Selenastrum capricornutum* Nihon Nohyaku, Report No: W-5015	Y	N	NN
Smyth, D.V. Croudace, C.P. Johnson, P.A.	1996c	IIA, 8.2.6/06 E-1: Toxicity to the freshwater diatom *Navicula pelliculosa* Nihon Nohyaku, Report No: W-5022	Y	N	NN
Smyth, D.V. Croudace, C.P. Magor, S.E.	1996d	IIA, 8.2.8 E-1: Toxicity to duckweed (*Lemna gibba*) Nihon Nohyaku, Report No: W-5025	Y	N	NN
Smyth, D.V. Croudace, C.P. Wallace, S.J.	1997a	IIA, 8.2.6/02 ET-751: Toxicity to the green alga *Selenastrum capricornutum* Nihon Nohyaku, Report No: W-5031	Y	N	NN
Smyth, D.V. Croudace, C.P. Magor, S.E.	1997b	IIA, 8.2.6/05 E-1: Toxicity to the green alga *Selenastrum capricornutum* Nihon Nohyaku, Report No: W-5034	Y	N	NN
Standens-Peek,	1997	IIA, 8.1.3	Y	N	NN

Author(s)	Year	Annex IIA Point Title Company, Report No.	GLP GEP Y/N	Published or not Y/N	Owner
W.M.M. Leopold, M.A.		Reproduction study in mallard duck with ET-751 techn. Nihon Nohyaku, Report No.: W-5032			
van Dreumel, I.F. Leopold, M.A.	1995a	IIA, 8.1.1 Acute oral toxicity study in bobwhite quail with ET-751 techn. Nihon Nohyaku, Report No.: W-5010	Y	N	NN
van Dreumel, I.F. Leopold, M.A.	1995b	IIA, 8.1.2/02 5-day dietary toxicity study in mallard duck with ET-751 techn. Nihon Nohyaku, Report No.: W-5011	Y	N	NN
van Dreumel, I.F. Reijnders, J.B.J.	1996	IIA, 8.1.2/01 5-day dietary toxicity study in bobwhite quail with ET-751 techn. Nihon Nohyaku, Report No.: W-5019	Y	N	NN
Wachter, S.	1996	IIA, 8.4.1 Acute toxicity of ET-751 Technical on earthworms, *Eisenia foetida* using an artificial soil test Nihon Nohyaku, Report No: N-5007	Y	N	NN

Ecotoxicology of the formulation MILAN (Annex IIIA 10)

Author(s)	Year	Annex IIA Point Title Company, Report No.	GLP GEP Y/N	Published or not Y/N	Owner
Candolfi, M.P.	1996a	Annex IIIA, 10.4.1 EXP31279A: Laboratory Oral and Contact Test with the Honeybee, *Apis mellifera*, based on the EPPO Guideline 170 (1992) Report n°:96-040-1013, 17 December 1996 Springborn Laboratories (Europe), Horn, Switzerland	Y	N	RPA
Candolfi, M.P.	1996b	Annex IIIA, 10.5.1/01 EXP31279A: Laboratory Toxicity Test with the Parasitic Wasp *Aphidius rhopalosiphi* (Hymenoptera: Braconidae) based on the IOBC Method of Polgar (1988) Report n°:96-032-1013, 5 September 1996 Springborn Laboratories (Europe), Horn, Switzerland	Y	N	RPA
Candolfi, M.P.	1996c	Annex IIIA, 10.5.1/02 EXP31279A: Laboratory Contact Toxicity Test with the Predacious Mite, *Typhlodromus pyri* Scheuiten (Acari: Phytodeiidae), based on the IOBC Approved Method of Overmeer (1988) Report n°:96-037-1013, 9 December 1996 Springborn Laboratories (Europe), Horn, Switzerland	Y	N	RPA
Candolfi, M.P.	1996d	Annex IIIA, 10.5.1/03 EXP31279A: Laboratory Contact Toxicity Test with Spiders, *Pardosa* sp. (Araneae: Lycosidae), based on the BBA Method of Wehling and Heimbach (1994) Report n°:96-042-1013, 17 December 1996 Springborn Laboratories (Europe), Horn, Switzerland	Y	N	RPA
Candolfi, M.P.	1997a	Annex IIIA, 10.5.1/04 EXP31279A: Laboratory Acute Toxicity Test with the Ground Beetle, *Poecilus cupreus* L. (Coleoptera: Carabidae) based on the IOBC Approved Method of Heimbach (1992) Report n°:96-046-1013, 14 January 1997 Springborn Laboratories (Europe), Horn, Switzerland	Y	N	RPA
Candolfi, M.P.	1997b	Annex IIIA, 10.5.1/05 EXP31279A: Laboratory Toxicity Test with the Green Lacewing, *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae), based on the IOBC Approved Method of Bigler (1988) Report n°:96-047-1013, 29 January 1997 Springborn Laboratories (Europe), Horn, Switzerland	Y	N	RPA
Candolfi, M.P.	1997c	IIIA, 10.5.1/06 EXP31279A: Laboratory Contact Toxicity Test with the Seven-Spotted Lady Beetle, *Coccinella Septempunctata* L. (Coleoptera: Coccinellidae), based on the Method of Pinsdorf (1989) Report n°:96-050-1013, 6 February 1997	Y	N	RPA

Author(s)	Year	Annex IIA Point Title Company, Report No.	GLP GEP Y/N	Published or not Y/N	Owner
		Springborn Laboratories (Europe), Horn, Switzerland			
Forster, J.	1997	Annex IIIA, 10.7.1. A Laboratory assessment of the effects of EXP31279A on soil microflora respiration and nitrogen transformation according to BBA guidelines VI 1-1 (1990) Report n°: ELL/1251, 10 February 1997 Eurolaboratories Ltd., Bedfordshire, UK	Y	N	RPA
Odin-Feurtet, M.	1997a	Annex IIIA, 10.2.1.3/01 EXP31279A: Freshwater Algal Growth Inhibition Study (72 hours) (*Scenedesmus subspicatus*) Report n°:SA 96365, 6 January 1997 Rhône-Poulenc Agro, Sophia Antipolis, France	Y	N	RPA
Odin-Feurtet,M.	1997b	Annex IIIA, 10.2.1.3/03 EXP31279A: Freshwater Algal Growth Inhibition Study (72 hours) (*Selenastrum capricornutum*) Report n°: SA 96435, 6 January 1997 Rhône-Poulenc Agro, Sophia Antipolis, France	Y	N	RPA
Smyth, D.V. et al	1996	Annex IIIA, 10.2.1.3/02 EXP31279A: Toxicity to the Freshwater Diatom *Navicula pelliculosa* Report n°:BL5877/B5, December 1996 Brixham Environmental Laboratory, Zeneca Ltd., UK	Y	N	RPA
Suteau, P.	1996	Annex IIIA, 10.6.1.1 EXP31279A: Acute Toxicity (14-Day) to Earthworms (*Eisenia foetida*), Artificial Soil Method Report n°: SA96269, 14 November 1996 Rhône-Poulenc Agro, Sophia Antipolis, France	Y	N	RPA
Suteau, P.	1997a	Annex IIIA, 10.2.1.1 EXP31279A: Acute Toxicity (96 hours) to Rainbow Trout (*Oncorhynchus mykiss*) under Semi-Static Conditions Report n°: SA 96298, 16 January 1997 Rhône-Poulenc Agro, Sophia Antipolis, France	Y	N	RPA
Suteau, P.	1997b	Annex IIIA, 10.2.1.2 EXP31279A: Acute Toxicity (48 hours) to Daphnids (*Daphnia magna*) under Semi-Static Conditions Report n°: SA 96296, 15 January 1997 Rhône-Poulenc Agro, Sophia Antipolis, France	Y	N	RPA