

ANNEX B

Active substance: Bensulfuron methyl

B.9 Ecotoxicology

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Table of content

B.9.	Ecotoxicological studies.....	4
B.9.1	Effects on birds (Annex IIA 8.1; Annex IIIA 10.1)	4
B.9.1.1	Acute oral toxicity (Annex IIA 8.1.1)	4
B.9.1.2	Short-term dietary toxicity (Annex IIA 8.1.2).....	5
B.9.1.3	Subchronic toxicity and reproduction (Annex IIA 8.1.3).....	7
B.9.1.4	Acute oral toxicity of the preparations (Annex IIIA 10.1.1)	11
B.9.1.5	Supervised cage or field tests (Annex IIIA 10.1.2)	11
B.9.1.6	Acceptance of bait, granules or treated seeds by birds (Annex IIIA 10.1.3).....	11
B.9.1.7	Effects of secondary poisoning (Annex IIIA 10.1.4).....	11
B.9.1.8	Summary of effects, exposure and hazard assessment for birds.....	11
B.9.2	Effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2).....	15
B.9.2.1	Acute toxicity to fish (Annex IIA 8.2.1)	15
B.9.2.2	Chronic toxicity to fish (Annex IIA 8.2.2)	23
B.9.2.3	Bioconcentration in fish (Annex IIA 8.2.3).....	27
B.9.2.4	Acute toxicity to aquatic invertebrates (Annex IIA 8.2.4)	35
B.9.2.5	Chronic toxicity to aquatic invertebrates (Annex IIA 8.2.5).....	43
B.9.2.6	Effects on algal growth (Annex IIA 8.2.6).....	46
B.9.2.7	Effects on sediment dwelling organisms (Annex IIA 8.2.7)	58
B.9.2.8	Aquatic Plants (Annex IIA 8.2.8).....	59
B.9.2.9	Acute toxicity of the preparations (Annex IIIA 10.2.1)	67
B.9.2.10	Microcosm or mesocosm studies (Annex IIIA 10.2.2).....	74
B.9.2.11	Residue data in fish (Annex IIIA 10.2.3)	74
B.9.2.12	Additional studies (Annex IIIA 10.2.4).....	74
B.9.2.13	Summary of effects, exposure and hazard assessment for aquatic organisms.....	74
B.9.3	Effects on terrestrial vertebrates other than birds (Annex IIIA 10.3)	83
B.9.4	Effects on bees (Annex IIA 8.3.1; Annex IIA 10.4).....	87
B.9.4.1	Acute toxicity to bees (Annex IIA 8.3.1.1)	87
B.9.4.2	Bees brood feeding test (Annex IIA 8.3.1.2).....	89
B.9.4.3	Acute toxicity of the preparation to bees (Annex IIIA 10.4.1).....	89
B.9.4.4	Residue test (Annex IIIA 10.4.2)	89
B.9.4.5	Cage test (Annex IIIA 10.4.3)	89
B.9.4.6	Field tests (Annex IIIA 10.4.4).....	89
B.9.4.7	Tunnel test (Annex IIIA 10.4.5).....	89
B.9.4.8	Summary of effects, exposure and hazard assessment for bees.....	89
B.9.5	Effects on other arthropod species (Annex IIA 8.3.2; IIIA 10.5).....	90
B.9.5.1	Effects of the active substance (Annex IIA 8.3.2).....	90
B.9.5.2	Effects of the formulation (Annex IIIA, 10.5).....	93
B.9.5.3	Summary of effects, exposure and hazard assessment for other arthropods species	93
B.9.6	Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.6.1).....	94
B.9.6.1	Acute toxicity (Annex IIA 8.4.1)	94
B.9.6.2	Sublethal effects (Annex IIA 8.4.2).....	100
B.9.6.3	Acute toxicity of the formulation (Annex IIIA 10.6.1.1)	100
B.9.6.4	Sublethal effects of the formulation (Annex IIIA 10.6.1.2)	100
B.9.6.5	Field studies of the formulation (Annex IIIA 10.6.1.3).....	101

B.9.6.6	Summary of effects, exposure and hazard assessment for earthworms.....	101
B.9.7	Effects on other soil non-target macro-organisms (Annex IIIA 10.6.2).....	102
B.9.8	Effects on soil non-target micro-organisms (Annex IIA 8.5; Annex IIIA 10.7).....	102
B.9.8.1	Effects of active substance	102
B.9.8.2	Effects of metabolites	109
B.9.8.3	Effects of formulations: laboratory testing (Annex IIIA 10.7.1).....	118
B.9.8.4	Effects of formulations: additional testing (Annex IIIA 10.7.2)	119
B.9.8.5	Summary of effects, exposure and hazard assessment for non-target micro-organisms	119
B.9.9	Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex IIA 8.6) ...	120
B.9.10	Summary of effects, exposure and hazard assessment for other non-target organisms (flora and fauna).....	122
B.9.11	Effects on biological methods of sewage treatment (Annex IIA 8.7).....	123
B.9.12	Reference relied on.....	126

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

B.9. Ecotoxicological studies

B.9.1 Effects on birds (Annex IIA 8.1; Annex IIIA 10.1)

B.9.1.1 Acute oral toxicity (Annex IIA 8.1.1)

1) *An acute oral toxicity study in the mallard with H #15,190. Beavers, J.B., 1984.*

Cross reference IIA, 8.1.1./01

Guidelines:

USEPA 71-1

GLP:

No

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical Batch: F5384-52 Purity: 95.9%

Bensulfuron methyl technical was administered in corn oil by single oral dose to fasted mallards (*Anas platyrhynchos*). Five mallards/sex/dose received doses of 0, 398, 631, 1000, 1590, and 2510 mg a.i./kg bw. Birds were observed for clinical signs of toxicity, body weight effects, and mortality for 14 days after dosing.

Findings:

No treatment-related mortalities or clinical signs of toxicity were observed (Table B.9.1). There were no test substance-related body weight or food intake effects noted.

Table B.9.1: Summary of toxicological responses of mallards following a single oral dose of Bensulfuron methyl technical

Dose (mg a.i./kg body wt.)	Sex	Toxicological results ^a	Duration of clinical signs	Time of death
0	M	0/0/5	-	-
0	F	0/0/5	-	-
398	M	0/0/5	-	-
398	F	0/0/5	-	-
631	M	0/0/5	-	-
631	F	0/0/5	-	-
1000	M	0/0/5	-	-
1000	F	0/0/5	-	-
1590	M	0/0/5	-	-
1590	F	0/0/5	-	-
2510	M	0/0/5	-	-
2510	F	0/0/5	-	-

^anumber of animals which died/number of animals with clinical signs/number of animals used

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.2: Acute oral toxicity to mallards - Summary of endpoints

Test substance	Bensulfuron methyl technical
Test object	Mallards male and female
LD ₅₀	>2510 mg a.i./kg bw
Lowest observed effect level (LOEL)	>2510 mg a.i./kg bw
Highest tested dose without toxic effect (NOEL)	>2510 mg a.i./kg bw

Conclusions:

The acute oral LD₅₀ value for mallards exposed to Bensulfuron methyl technical by single oral dose was >2510 mg a.i./kg bw, the highest concentration tested.

B.9.1.2 Short-term dietary toxicity (Annex IIA 8.1.2)

2) *A dietary LC₅₀ study in the mallard with H # 15,190. Beavers, J.B., 1984a.*

Cross reference IIA, 8.1.2./01

Guidelines:

USEPA 71-2

GLP:

No

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-52

Purity: 95.9%

Mallard ducklings (*Anas platyrhynchos*; 10 birds/dose) received Bensulfuron methyl technical in the feed at concentrations of 0, 562, 1000, 1780, 3160, and 5620 ppm (nominal concentration) for 5 days. Birds were observed for a total of 8 days (including 3 days post-treatment) for abnormal behaviour, mortality, signs of toxicity, and changes in body weight and food intake.

Findings:

No treatment-related mortalities or clinical signs of toxicity were observed (Table B.9.3). At 1780 ppm and above there were slight reductions in body weight gain and in food consumption noted for the Day 6 through Day 8 observation period.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.3: Summary of toxicological responses, body weight, and food intake of mallard ducklings following a 5-day dietary exposure to Bensulfuron methyl technical

Treatment (ppm)	Toxicological results ^a	Duration of clinical signs	Time of death	Mean body mass (g/bird)			Mean intake (g/bird/day)	
				Day 0	Day 5	Day 8	Days 0-5	Days 6-8
0	0/0/10	-	-	130	254.6	313.4	57.4	69.2
562	0/0/10	-	-	134	250	300	51	71
1000	0/0/10	-	-	145	269	324	59	76
1780	0/0/10	-	-	147	274	302	60	61
3160	0/0/10	-	-	143	272	303	59	62
5620	0/0/10	-	-	128	251	282	57	60

^a number of animals which died/number of animals with clinical signs/number of animals used

Table B.9.4: Short-term dietary toxicity to mallards - Summary of endpoints

Test substance	Bensulfuron methyl technical	
Test object	Mallard ducklings	
	Endpoint based on ppm in feed	Endpoint based on mg a.i./kg bw/day
LC ₅₀	>5620 ppm	>1690
Lowest observed effect concentration (LOEC)	1780 ppm	507
Highest tested dose without toxic effect (NOEC)	1000 ppm	285

Conclusions:

The short-term dietary LC₅₀ value for mallard ducklings exposed to Bensulfuron methyl technical in the diet for 5 days was >5620 ppm (>1690 mg a.i./kg body weight/day), the highest concentration tested. The no mortality dosage was >5620 ppm (>1690 mg a.i./kg body weight/day).

The short-term dietary NOEC was 1000 ppm based on slight body weight gain effects at 1780 ppm and above.

3) *A dietary LC₅₀ study in the bobwhite with H # 15,190. Beavers, J.B., 1984b.*

Cross reference IIA, 8.1.2./02

Guidelines:

USEPA 71-2

GLP:

No

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-52

Purity: 95.9%

Northern bobwhite chicks (*Colinus virginianus*; 10 birds/dose) received Bensulfuron methyl technical in the feed at concentrations of 0, 562, 1000, 1780, 3160, or 5620 ppm (nominal concentration) for 5 days. Birds were observed for

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

a total of 8 days (including 3 days post-treatment) for abnormal behaviour, mortality, signs of toxicity, and changes in body weight and food intake.

Findings:

No treatment-related mortalities or clinical signs of toxicity were observed (Table B.9.5). There were no test substance-related body weight or food intake effects noted.

Table B.9.5: Summary of toxicological responses, body weight, and food intake of northern bobwhite chicks following a single oral dose of Bensulfuron methyl technical

Treatment (nominal ppm)	Toxicological results ^a	Duration of clinical signs	Time of death	Mean body mass (g/bird)			Mean intake (g/bird/day)	
				Day 0	Day 5	Day 8	Days 0-5	Days 6-8
0	0/0/10	-	-	27.8	40.8	50.2	8.2	11
562	0/0/10	-	-	28	41	51	8	10
1000	0/0/10	-	-	27	40	49	8	10
1780	0/0/10	-	-	29	42	51	8	10
3160	0/0/10	-	-	29	43	53	8	11
5620	0/0/10	-	-	27	40	49	10	11

^a Number of animals which died/number of animals with clinical signs/number of animals used

Table B.9.6: Short-term dietary toxicity to northern bobwhite quail - Summary of endpoints

Test substance	Bensulfuron methyl technical	
Test object	Northern bobwhite quail chicks	
	Endpoint based on ppm in feed	Endpoint based on mg a.i./kg bw/day
LC ₅₀	>5620 ppm	>1678
Lowest observed effect concentration (LOEC)	>5620 ppm	>1678
Highest tested dose without toxic effect (NOEC)	>5620 ppm	>1678

Conclusions:

The short-term dietary LC₅₀ value for northern bobwhite quail exposed to Bensulfuron methyl technical in the diet for 5 days was >5620 ppm (>1678 mg/kg body weight/day), the highest concentration tested. The no mortality dosage was >5620 ppm (>1678 mg/kg body weight/day).

The short-term dietary NOEC was 5620 ppm (1678 mg/kg body weight/day) based on no effects in any concentration tested.

B.9.1.3 Subchronic toxicity and reproduction (Annex IIA 8.1.3)

- 4) *Bensulfuron methyl (DPX-F5384) technical: A one generation reproduction study with the Mallard.*
Gallagher, S.P., Beavers, J.B., Jaber, M., 2002.

Cross reference IIA, 8.1.3./01

Guidelines:

USEPA 71-4, OECD 206

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-333

Purity: 98.66%

Each treatment and control group contained sixteen pairs of mallards (*Anas platyrhynchos*), one pair (one male and one female) per pen. Mallards were fed Bensulfuron methyl in the feed at concentrations of 0, 35, 140, and 560 ppm for 21 weeks. Reproductive parameters were measured beginning at the onset of egg laying. Adult birds were observed daily for abnormal behaviour, mortality, signs of toxicity, and periodically for changes in body weight and food intake. Necropsies were performed on all adults that died during the test and all adults surviving until test termination. In addition, egg shell thickness, effects on egg production and quality, and hatchling health and survivability were examined. Upon completion of the test, Dunnett's multiple comparison method was used to determine statistically significant differences between the control and each of the treatment groups. Sample units were the individual pens within each experiment group except for adult body weight where the sample unit was the individual bird.

Findings:

Analytical results: None of the control samples contained detectable levels of Bensulfuron methyl. Diet samples collected from the 35, 140, and 560 ppm test concentrations contained 35.7 ± 1.0 ppm, 142 ± 4.8 ppm, and 519 ± 29 ppm, respectively.

General observations:

- No mortalities occurred during the test and no overt signs of toxicity were observed other than incidental clinical observations normally associated with injuries and pen wear. Such signs included foot and head lesions, neck abrasions and feather loss. In addition, lameness and ruffled appearance also were observed. Except for incidental findings, all birds appeared normal throughout the test.
- All surviving adults were subjected to gross necropsy following adult termination. All findings observed were considered unrelated to treatment.
- There were no treatment-related effects on adult body weight at any of the concentrations tested. No statistically significant differences between the control group and the 35, 140, and 560 ppm treatment groups were observed at any of the body weight intervals.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.7: Summary of mean body weight (g) of mallards exposed to Bensulfuron methyl in the diet

Dose	Sex	Week of the test						Total weight change (g)
		0	2	4	6	8	Adult termination	
Control	Males	1116	1120	1118	1119	1086	1177	62
	Females	973	975	984	992	969	1152	179
35 ppm	Males	1124	1131	1129	1124	1112	1164	40
	Females	982	996	996	998	991	1141	159
140 ppm	Males	1116	1114	1120	1119	1104	1149	33
	Females	972	965	969	977	972	1068	95
560 ppm	Males	1109	1121	1113	1105	1101	1181	72
	Females	991	1011	1007	992	1003	1120	129

Note: Only surviving birds were included in the calculations for each body weight interval.

Differences between control and each treatment group were not statistically significant ($p > 0.05$).

- There were no apparent treatment-related effects on adult feed consumption measured at the 35 and 140 ppm test concentrations and any differences from the control were not statistically significant at any of the feed consumption intervals. At the 560 ppm test concentration there were slight increases in feed consumption particularly during the first 10 and last 3 weeks of the study. The differences observed, although slight, were statistically significant at $p < 0.05$ during Weeks 1, 8, 9, 19, and 20. However, excessive feed wastage noted during cage-side observations in two pens (Pens 962 and 964) contributed to the slight increase observed. While possibly related to palatability of the diet containing the test substance, the increase in feed wastage observed was not considered to be biologically meaningful. When data from the two pens were eliminated, except for Week 1 (which remained statistically different at $p < 0.05$), the differences observed were no longer statistically significant.

Reproductive parameters:

When compared to the control group, there were no statistically significant differences in any of the reproductive parameters measured in the 35, 140, or 560 ppm treatment groups. At the 35 ppm a.i. test concentration, slight reductions were noted in mean number of viable embryos per numbers of eggs set, live 3 week embryos per total number of viable embryos and number of hatchlings per number of live three week embryos. However, in all cases the slight reductions observed at 35 ppm were the result of data from two pens (Pens 922 and 928). Of the 37 eggs laid in Pen 928, 21 (57%) were cracked, three were incubated and one egg hatched. Of the 28 eggs set from Pen 922, only 2 (7%) were viable and none hatched. When data from those two pens were eliminated, values for all three parameters were comparable to the control group (viability - $94 \pm 9\%$, live 3-week embryos - $100 \pm 1\%$, hatchability - $86 \pm 11\%$).

While not statistically significant, there was a slight reduction in hatchability in both the 140 and 560 ppm treatment groups ($76 \pm 23\%$ and $76 \pm 16\%$, respectively) when compared to the control group ($88 \pm 9\%$). In both instances, the reductions observed were not isolated to one or two pens. However, the differences observed were not concentration responsive, and all values were comparable to the historical control value for this parameter of $74 \pm 12\%$. When compared to the mean value for the historical control groups, the hatchability values for the 35, 140, or 560 ppm a.i. treatment groups were not statistically significant ($p = 0.78, 0.95$, and 0.99 , respectively). Therefore, the differences observed were not considered to be treatment related. Summaries of the reproductive data are presented in Table B.9.8 and Table B.9.9.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.8: Summary of reproductive effects of Bensulfuron methyl on mallards

Reproductive parameter	Test group (dietary concentration in ppm a.i.)			
	0	35	140	560
Number of replicates	16	16	16	16
Total eggs laid/group	747	704	734	686
Eggs cracked	15	33	7	3
Eggs set	662	577	651	614
Viable embryos	631	514	610	585
Live three-week embryos	627	511	597	582
Hatchlings	566	445	453	445
14-Day-old survivors	564	445	450	440
Eggs laid/hen	47	44	46	43
Eggs laid/hen/day ^a	0.54	0.51	0.53	0.50
14-Day-old survivors/hen	35	28	28	18

^a Based on 86 days of egg production**Table B.9.9: Summary of reproductive effects, normalised as percentages, of Bensulfuron methyl on mallards**

Reproductive parameters ^a	Test Group (dietary concentration in ppm a.i.)			
	0	35	140	560
Number of replicates	16	16	16	16
Total eggs laid ^b	747	704	734	686
Eggs laid/maximum laid	65	61	64	60
Eggs cracked/eggs laid	2	5	1	0
Viable embryos/eggs set	95	86	93	94
Live three-week embryos/viable embryos	99	96	98	100
Hatchlings/Live 3-week embryos	88	78	76	76
14-Day old survivors/hatchlings	98	100	99	99
Hatchlings/eggs set	84	72	70	72
14-Day-old survivors/eggs set	82	72	69	71
Hatchlings/maximum set	54	42	43	42
14-Day-old survivors/maximum set	53	42	43	42

^a Parameters are standardised based on relative performance within treatments to enable statistical comparisons. Values represent pen means for experimental group.^b Represents the total number of eggs laid in each group.Differences between the control and each treatment groups were not statistically significant (Dunnett's method, $p > 0.05$).**Table B.9.10: Subchronic toxicity and reproduction in mallards exposed to Bensulfuron methyl summary of endpoints**

Test substance	Bensulfuron methyl (DPX-F5384)	
Test object	Mallards male and female	
	Endpoint based on ppm in feed	Endpoint based on mg a.i./kg bw/day
Lowest observed effect concentration (LOEC)	560	83
Highest tested dose without toxic effect (NOEC)	560	83
Toxic threshold effect level, TEL (mean LOEC-NOEC)	560	83

Conclusions:

The NOEC for mallards exposed to Bensulfuron methyl in the diet for 21 weeks was 560 ppm (83 mg a.i./kg body weight/day).

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

B.9.1.4 Acute oral toxicity of the preparations (Annex IIIA 10.1.1)

An acute oral toxicity study with the formulated product Bensulfuron methyl 60WG is not required because the TER_a and TER_{st} for the active substance (Bensulfuron methyl) are >100 (see B.9.1.8, Table B.9.12). In addition, results from testing in mammals indicated that toxicity of the formulation was not significantly greater than the active substance.

B.9.1.5 Supervised cage or field tests (Annex IIIA 10.1.2)

This test is not required because the TER_a and TER_{st} are >100 , and the TER_{it} is >5 for the active substance (see B.9.1.8, Table B.9.12, Table B.9.13 and Table B.9.14). In addition, there is no evidence of risk from any further study on the active substance (e.g., reproduction study).

B.9.1.6 Acceptance of bait, granules or treated seeds by birds (Annex IIIA 10.1.3)

This test is not required because formulated product Bensulfuron methyl 60WG is not intended for use as bait, granules, or seed treatments and the TER_a for the active substance is >10 (see B.9.1.8, Table B.9.12).

B.9.1.7 Effects of secondary poisoning (Annex IIIA 10.1.4)

The avian TER_a and TER_{st} and TER_{it} for Bensulfuron methyl are >100 , >100 and >5 , respectively (see B.9.1.8, Table B.9.12, Table B.9.13 and Table B.9.14). Therefore, the herbicide Bensulfuron methyl 60WG is predicted to pose low risk to birds and mammals. Given the low toxicity to birds and the low bioaccumulation risk ($\log K_{ow} = 2.2$), the risk of secondary poisoning at multiple levels in the terrestrial vertebrate food chain is very low and further studies are not requested.

B.9.1.8 Summary of effects, exposure and hazard assessment for birds**B.9.1.8.1 Toxicity**

Bensulfuron methyl is of low acute toxicity to birds, producing no mortality at the maximum dose levels used in the single oral and short-term dietary tests. No significant treatment-related effects were observed on reproductive parameters in the test with mallards at the highest dose tested. A summary of the toxicity of Bensulfuron methyl and Bensulfuron methyl 60WG to birds is shown in Table B.9.11.

Table B.9.11: Summary of avian toxicity endpoints for Bensulfuron methyl

Study (species)	LD_{50} , LC_{50} , NOEC	LD_{50} , LC_{50} , NOEC (converted to daily dose)	Lowest Lethal Dose	Cross Reference
Acute Oral Toxicity (mallard duck)	>2510 mg/kg bw	-	No mortality	8.1.1/01
Short-term Dietary Toxicity (5 day) (bobwhite quail)	>5620 ppm	>1678 mg a.i./kg bw/d	No mortality	8.1.2/02
Short-term Dietary Toxicity (5 day) (mallard duck)	>5620 ppm	>1690 mg a.i./kg bw/d	No mortality	8.1.2/01
Subchronic and Reproductive Toxicity (147 day) (mallard duck)	560 ppm	83 mg a.i./kg bw/d	No mortality	8.1.3/01

Values in **bold** were selected for risk assessment.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

The risk of Bensulfuron methyl 60WG to birds was assessed according to Guidance Document on Risk Assessment for Birds and Mammals under Council Directive 91/414/EEC (SANCO/4145/2000, draft September 2002). This assessment includes the use of generic indicator species that are crop-relevant, and assumes realistic worst-case exposure. Where multiple toxicity endpoints were available, only the results of the most conservative endpoint (lowest LD₅₀, LC₅₀, and NOEC) were used to estimate risk.

B.9.1.8.2 Risk assessment

Consumption of contaminated food

According to the risk assessment scheme for birds and mammals, the major exposure route for the Tier 1 risk assessment for birds is considered to be intake of residues via contaminated feed. Estimated exposure is expressed in terms of daily dose for all time scales considered.

Calculation of ETE (mg/kg bw/day) for acute, short-term & long-term exposure for birds.

Scenarios for first Tier acute and short-term exposure estimate (see Table B.9.12, and Table B.9.13) were based on the following assumptions:

- application rate in rice is 0.06 kg Bensulfuron methyl/ha (max. 1 application per season)
- the contaminated diet is not avoided (AV Avoidance factor 1 = no avoidance)
- animals satisfy their entire food demand in the treated area (PT Fraction of diet obtained in treated area = 1)
- animals feed on a single food type (PD Fraction of food type in diet = 1; one type)
- the relevant bird indicator species according to crop and crop stage (cereals, early and late) are:
 - large herbivorous bird (goose, 3000g bw), and
 - insectivorous bird (wren, tit: 10 g bw)
- MAF = 1 (based on a single application per season)
- foliar DT₅₀ (residue decline) for the first Tier used the default value of 10 days
- the acute and short-term ETE (estimated theoretical exposure) of a compound is calculated with the following equation:

$$\text{ETE} = (\text{FIR} / \text{bw}) * \text{RUD} * \text{Application rate} * \text{MAF} * \text{AV} * \text{PT} * \text{PD} \text{ (mg/ kg bw/ day)}$$

FIR Food intake rate of indicator species (gram fresh weight per day)

bw Body weight (g)

RUD (Residues per unit dose) residues in vegetation & insects 90th percentile of the initial concentration

MAF 1 for residues in foliage (based on a single application per season)

No MAF is applied for residues in insects

AV, PT, PD All factors become 1 and can be omitted

Scenario for first Tier long-term exposure estimate (see Table B.9.14) is based on the following assumptions:

- the same assumptions are made to estimate long-term ETE as for acute and short-term exposure except that a time-weighted average estimate of residues is used
- time-weighted (twa) residues are used to reflect the long-term exposure. The f_{twa} factor is based on the following two default values: 1) time window (averaging time): 21 days (3 weeks), and 2) a foliar DT₅₀ = 10 days resulting in a f_{twa} value of 0.53 which is used in the calculations for long-term exposure

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

- in case of insects as food type no default f_{twa} factor is used for the first Tier as the time course of residue level is unknown

The long-term estimated daily uptake (ETE) of a compound is given by:

$$\text{ETE} = (\text{FIR} / \text{bw}) * \text{RUD} * \text{Application rate} * \text{MAF} * f_{\text{twa}} * \text{AV} * \text{PT} * \text{PD} \text{ (mg/ kg bw/ day)}$$

FIR	Food intake rate of indicator species (gram fresh weight per day)
bw	Body weight (g)
RUD	(Residues per unit dose) residues in vegetation & insects 90 th percentile of the initial concentration
MAF	1 for foliage (based on a single application per season) No MAF is applied for residues in insects
f_{twa}	Time-weighted residues are used, so it is assumed that over a period of 3 weeks the average concentration is about half the initial concentration – default value: 0.53
AV, PT, PD	All factors become 1 and can be omitted

Acute, short-term and long-term TERs exceed relevant Annex VI criteria of 10, 10 and 5, respectively (Table B.9.12, Table B.9.13, and Table B.9.14).

Table B.9.12: Tier 1 acute TERs for birds based on dietary exposure to Bensulfuron methyl after application of Bensulfuron methyl 60WG in rice (cereals scenario)

Indicator species	LD ₅₀ (mg a.i./kg bw)	FIR/ bw	RUD	Rate applied (kg a.i./ha)	MAF	ETE	TER _a	Trigger value
Large herbiv. bird	>2510	0.44	142	0.06	1	3.75	>669	10
Insect. bird	>2510	1.04	52	0.06	1	3.25	>772	10

Table B.9.13: Tier 1 short-term TERs for birds based on dietary exposure to Bensulfuron methyl after application of Bensulfuron methyl 60WG in rice (cereals scenario)

Indicator species	LC ₅₀ (mg a.i./kg bw)	FIR/ bw	RUD	Rate applied (kg a.i./ha)	MAF	ETE	TER _{st}	Trigger value
Large herbiv. bird	>1678	0.44	76	0.06	1	2.01	>835	10
Insect. bird	>1678	1.04	29	0.06	1	1.81	>927	10

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.14: Tier 1 long term TER_{lt} for birds based on dietary exposure to Bensulfuron methyl after application of Bensulfuron methyl 60WG in rice (cereals scenario)

Indicator species	NOEC (mg a.i./kg bw)	FIR/bw	RUD	Rate applied (kg a.i./ha)	MAF	f_{twa}	ETE	TER_{lt}	Trigger value
Large herbiv. bird	83	0.44	76	0.06	1	0.53	1.06	78.3	5
Insect. bird	83	1.04	29	0.06	1	1	1.81	45.9	5

Bioaccumulation and food chain behaviour

According to the SANCO/4145/2002 (Chapter 4) for organic chemicals with a $\log P_{ow} > 3$ a potential for bioaccumulation should be assessed. The $\log K_{ow}$ at pH 5 was 2.18 (highest measured $\log K_{ow}$) for Bensulfuron methyl and therefore an assessment of potential risk to earthworm- and fish-eating birds is not necessary. However, in order to fully assess the potential risk to birds from exposure to residues contained in food items not covered in the standard exposure scenarios, the potential risk to birds consuming earthworms or fish was assessed.

Food chain from earthworm to earthworm eating birds

TER_{lt} calculations for food chain exposure of birds to Bensulfuron methyl in relation to consumption of earthworms are provided in Table B.9.15.

The Tier 1 TER_{lt} for worm-eating birds are acceptable (>5), based on worst-case modelling of PEC_{soil} and assuming a maximum bioconcentration factor.

Table B.9.15: Avian long-term TER for food chain exposure of earthworm eating birds to Bensulfuron methyl

Inputs	Value	Comments
Octanol-water partitioning coefficient, K_{ow} , pH 5.0	151	From Physical-Chemical Section (Annex IIA, point 2)
$\log K_{ow}$, pH 5.0	2.18	
Organic carbon content of soil, f_{oc}	0.02	Given in SANCO/4145/2000
Organic carbon adsorption coefficient, K_{oc} (mL/g)	315	Average value for 3 Italian paddy soils, from Fate and Behaviour Section
Estimated worm bioconcentration factor	0.37 ^a	$BCF_{worm} = (0.84 + 0.01K_{ow}) / (f_{oc} * K_{oc})$ Assume worst-case $BCF_{worm} = 1.6^a$
Bird Daily Dose conversion factor	1.1	Given in SANCO/4145/2000
Bird chronic NOEC (mg a.i./kg bw*d)	83	Cross reference IIA, 8.1.3/01
<i>TER Calculations—Rice, Tier 1</i>		
Maximum initial PEC_{soil} (mg/kg soil)	0.0782	Maximum initial PEC_{soil} based on 1 application of 60 g a.i./ha, instantaneous sorption to soil, 0% foliar interception (Med-Rice, 2003), from Fate and Behaviour Section
Worm residues (mg/kg worm)	0.125	$PEC_{worm} = PEC_{soil} * BCF_{worm}$; assuming $BCF_{worm} = 1.6$
Bird daily dose (mg a.i./kg bw*d)	0.138	$DD_{bird} = PEC_{worm} * 1.1$
Worm-eating bird TER	603	$TER > 5$

^a Because measured BCF values are available for aquatic species, and the measured values exceed the estimated worm bioconcentration factor, the highest measured BCF (1.6) will be used as a worst-case estimate of BCF_{worm}

Food chain from fish to fish-eating birds

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

TER_{it} calculations for food chain exposure of fish-eating birds to Bensulfuron methyl based on consumption of residues in fish are provided in Table B.9.16.

The TER_{it} for fish-eating birds are acceptable (>5), based on Tier 1 modelling of PEC_{sw}.

Table B.9.16 Avian long-term TER for food chain exposure of fish-eating birds to Bensulfuron methyl

Inputs	Value	Comments
Fish BCF	1.6	Assumed maximum measured BCF (red swamp cray fish, <i>Procambrus clarki</i>), Cross reference IIA, 8.2.3/04
Bird NOEC (mg a.i./kg bw*d)	83	Cross reference IIA, 8.1.3/01
Bird Daily Dose conversion factor	0.21	Given in SANCO/4145/2000
Higher-Tier average maximum PEC _{sw} (mg/L)	0.0192	Assumes PEC _{sw} = PEC _{paddy water} , one application of Bensulfuron methyl (60 g a.i./ha) (from Fate and Behaviour Section)
<i>Calculations—Rice, Tier 1</i>		
PEC _{fish} (mg a.i./kg bw)	0.03	PEC _{fish} = PEC _{sw} * BCF
Bird daily dose (mg a.i./kg bw*d)	0.006	DD _{bird} = PEC _{fish} * Bird Daily Dose conversion factor
Fish-eating bird TER	12865	TER = NOEC/DD _{bird} The TER >5

Avian exposure via drinking water

Inputs, assumptions and TER_{it} calculations for exposure to birds through consumption of Bensulfuron methyl residues in drinking water are provided in Table B.9.17.

Tier 1 TER_{it} for birds potentially exposed to Bensulfuron methyl through drinking water are acceptable (>5), based on conservative modelling of PEC_{sw}.

Table B.9.17: Avian long-term TERs for birds exposed to Bensulfuron methyl in drinking water sources

Inputs	Value	Comments
Higher-Tier average maximum PEC _{sw} (mg/L)	0.0192	Maximum PEC _{sw} (assume PEC _{sw} = PEC _{paddy water} = PEC _{drinking water}), based on one application of Bensulfuron methyl (60 g a.i./ha) (from Fate and Behaviour Section)
Bird NOEC [mg a.i./kg bw*day]	83	Cross reference IIA, 8.1.3/01
<i>Calculations—Rice, Tier 1</i>		
Daily water intake (insectivorous bird) (L/day)	0.0027	Total water ingestion rate (L/day) = 0.059W ^{0.67} where W is body weight in kg (assume 0.010 kg tit)
Bird daily dose [mg a.i./kg bw*day]	0.0052	DD = (PEC _{drinking water} * Total water ingestion rate) / W (assuming PEC _{drinking water} = PEC _{paddy water})
Bird TER _{it} for drinking water exposure	16010	TER _{it} = NOEC / DD _{bird} TER >5

Conclusions

A Tier 1 assessment of potential risk to birds suggests that Bensulfuron methyl 60WG poses a low risk to birds.

B.9.2 Effects on aquatic organisms (Annex IIA 8.2; Annex IIIA 10.2)

B.9.2.1 Acute toxicity to fish (Annex IIA 8.2.1)

Acute toxicity to fish – Bensulfuron methyl

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

- 5) *Static acute toxicity of Haskell sample number 16,985 to the sheepshead minnow, Cyprinodon variegatus. Boeri, R.L., 1988.*

Cross reference IIA 8.2.1./01

Guidelines:

USEPA 72-3

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-52

Purity: 94.9%

The acute toxicity of Bensulfuron methyl to unfed juvenile sheepshead minnows, *Cyprinodon variegatus*, was determined in an unaerated, static, 96-hour test following procedures described in Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians (ASTM, 1980). Treatments consisted of a dilution water control, and nominal concentrations of 100, 115, 125, 140, and 150 mg Bensulfuron methyl/L. The test design consisted of 4 replicates of 5 fish for a total of 20 fish for each treatment and control. Test media was natural, filtered (5 µm) seawater and solutions were maintained at 22°C throughout the test. The number of surviving fish and occurrence of sublethal effects were assessed 24, 48, 72, and 96 h after test initiation.

Findings:

Summaries of cumulative mortality and sublethal effects are presented in Table B.9.18 and Table B.9.19, respectively. The mean, measured concentrations of Bensulfuron methyl were not detected at dilution water control, 78.4, 92.5, 101, 113, and 123 mg a.i./L and ranged from 78.4 to 82% of nominal concentrations. Bensulfuron methyl concentrations remained stable throughout the 96 h test period, varying from 0 to +4% of initial concentrations. Loading in controls at test end was 0.05 g/L.

No mortality or sublethal effects were seen in the control or any test concentration up to 123 mg a.i./L at the end of the 96 hour test. The 96 hour LC₅₀, based on the mean, measured concentration of Bensulfuron methyl, was greater than 123 mg a.i./L.

Table B.9.18: Observed mortality of sheepshead minnow, *Cyprinodon variegatus*, exposed to Bensulfuron methyl for 96 hours in an unaerated, static, acute test

Mean, measured concentration of Bensulfuron methyl (mg a.i./L)	Cumulative mortality (No. dead / No. at test start)			
	24 h	48 h	72 h	96 h
Water control	0/5	0/5	0/5	0/5
78.4	0/5	0/5	0/5	0/5
92.5	0/5	0/5	0/5	0/5
101	0/5	0/5	0/5	0/5
113	0/5	0/5	0/5	0/5
123	0/5	0/5	0/5	0/5

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.19: Observed sublethal effects of sheephead minnow, *Cyprinodon variegatus*, exposed to Bensulfuron methyl for 96 hours in an unaerated, static, acute test

Mean, measured concentration of Bensulfuron methyl (mg a.i./L)	Sublethal effects (No. affected / No. at test start)			
	24 h	48 h	72 h	96 h
Water control	0/5	0/5	0/5	0/5
78.4	0/5	0/5	0/5	0/5
92.5	0/5	0/5	0/5	0/5
101	0/5	0/5	0/5	0/5
113	0/5	0/5	0/5	0/5
123	0/5	0/5	0/5	0/5

Conclusions:

The 96-hour LC₅₀ in the sheephead minnow was greater than 123 mg Bensulfuron methyl/L.

6) *Static acute 96-hour LC₅₀ of IN-F5384-52 to channel catfish (Ictalurus punctatus). Hall, C.L., 1986.*

Cross reference IIA, 8.2.1./03

Guidelines:

Not specified

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-52

Purity: 95.9%

The acute toxicity of Bensulfuron methyl to unfed channel catfish, *Ictalurus punctatus*, was determined in an unaerated, static, 96-hour test. Treatments consisted of a dilution water control, a pH adjusted (pH 9.0) dilution water control, and a nominal concentrations of 98, 109, 121, 135, and 150 mg Bensulfuron methyl/L. Test concentrations were not verified by analysis, however based on the solubility observed in similar aquatic test systems reported in this document, the maximum test concentration was approximately 120 mg/L. A single test chamber was used for each treatment and control. Ten juvenile catfish (3.1 cm mean standard length and 0.43 g mean wet weight) were randomly placed into each test vessel. Test solutions were maintained between 22.0 and 22.1°C throughout the study. Mortality and sublethal effects were assessed every 24 h during the 96 h exposure period.

Findings:

A summary of cumulative mortality is presented in Table B.9.20. No mortality or sublethal effects were seen at any test concentration or in either control at the end of the 96 hour limit test. Dissolved oxygen and pH in Bensulfuron methyl treatments were within acceptable limits and similar to the controls throughout the test. The 96 hour LC₅₀, based on nominal concentrations of Bensulfuron methyl, was greater than 150 mg/L.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.20: Observed mortality of channel catfish, *Ictalurus punctatus*, exposed to Bensulfuron methyl for 96 hours in an unaerated, static, acute test

Mean, measured concentration of Bensulfuron methyl (mg a.i./L)	Cumulative mortality (No. dead / No. at test start)			
	24 h	48 h	72 h	96 h
Water control	0/10	0/10	0/10	0/10
pH adjusted water control	0/10	0/10	0/10	0/10
98	0/10	0/10	0/10	0/10
109	0/10	0/10	0/10	0/10
121	0/10	0/10	0/10	0/10
135	0/10	0/10	0/10	0/10
150	0/10	0/10	0/10	0/10

Conclusions:

The 96-hour LC₅₀ in the channel catfish was greater than 150 mg Bensulfuron methyl/L.

- 7) *Bensulfuron methyl (DPX-F5384) technical: Static, acute, 96-hour, limit test to rainbow trout, Oncorhynchus mykiss. Samel, A., 2001a.*

Cross reference IIA, 8.2.1./05

Guidelines:

USEPA 72-1 (1982); OECD 203 (1992); Directive 92/69/EEC; EEC Method C.1 (1992)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-333

Purity: 98.66%

The acute toxicity of Bensulfuron methyl to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, was determined in an unaerated, static, 96-hour test. Treatments consisted of a dilution water control, a pH 9.0 dilution water control (adjusted to increase the solubility of Bensulfuron methyl), and a nominal concentration of 80 mg Bensulfuron methyl/L. A maximum test concentration of 80 mg/L was chosen because the maximum measured concentration of Bensulfuron methyl in the test system after stirring for 24 hours was 82 mg a.i./L. Two water control chambers, 2 pH adjusted water control chambers, and 6 replicate limit test concentration chambers with 5 fish in each chamber were used (total of 10 fish in each of the controls and 30 fish in the limit test concentration). Test solutions were maintained between 12.2 and 12.3°C throughout the study.

Findings:

Summaries of cumulative mortality and sublethal effects are presented in Table B.9.21 and Table B.9.22, respectively. The mean, measured concentration of Bensulfuron methyl was 66 mg a.i./L (mean recovery of 83% of nominal concentrations). No mortality was seen at 66 mg a.i./L Bensulfuron methyl or in the controls at the end of

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

the 96-hour limit test. The 96-hour LC₅₀, based on the mean, measured concentration of Bensulfuron methyl, was greater than 66 mg/L.

Table B.9.21: Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to Bensulfuron methyl for 96 hours in an unaerated, static, acute test

Mean, measured concentration of Bensulfuron methyl (µg a.i./L)	Cumulative mortality (No. dead / No. at test start)							
	24 h		48 h		72 h		96 h	
	A ^a	B ^a	A ^a	B ^a	A ^a	B ^a	A ^a	B ^a
Water control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
pH adjusted water control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
66-1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
66-2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
66-3	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

^a A and B represent replicates; each replicate contained 5 fish (total 30 fish per test concentration) at test start.

Table B.9.22: Observed sublethal effects of rainbow trout, *Oncorhynchus mykiss*, exposed to Bensulfuron methyl for 96 hours in an unaerated, static, acute test

Mean, measured concentration of Bensulfuron methyl (µg a.i./L)	Sublethal effects (No. affected / No. at test start)							
	24 h		48 h		72 h		96 h	
	A ^a	B ^a	A ^a	B ^a	A ^a	B ^a	A ^a	B ^a
Water control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
pH adjusted water control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
66	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
66	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
66	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

^a A and B represent replicates; each replicate contained 10 fish (total 20 fish per test concentration) at test start.

Conclusions:

The 96-hour LC₅₀ for the rainbow trout was greater than 66 mg Bensulfuron methyl/L.

- 8) *Bensulfuron methyl (DPX-F5384) technical: Static, acute, 96-hour, limit test to bluegill sunfish, Lepomis Macrochirus. Samel, A., 2001b.*

Cross reference IIA, 8.2.1./06

Guidelines:

USEPA 72-1 (1982); OECD 203 (1992); Directive 92/69/EEC; EEC Method C.1 (1992)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-333

Purity: 98.66%

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

The acute toxicity of Bensulfuron methyl to unfed juvenile bluegill sunfish, *Lepomis macrochirus*, was determined in an unaerated, static, 96-hour test. Treatments consisted of a dilution water control, a pH adjusted (pH 9.0) dilution water control, and a nominal concentration of 120 mg Bensulfuron methyl/L. Two water control chambers, 2 pH adjusted water control chambers, and 6 replicate limit test concentration chambers with 5 fish in each chamber were used for testing. Test solutions were maintained between 22.7 and 23.1°C throughout the test.

Findings:

Summaries of cumulative mortality and sublethal effects are presented in Table B.9.23 and Table B.9.24, respectively. The mean, measured concentration of Bensulfuron methyl during the test was 120 mg a.i./L and was 100% of nominal concentration. Loading in both sets of controls at test end was 0.2 g/L. No mortality was seen at the 120 mg/L Bensulfuron methyl or in the controls at the end of the 96 hour limit test. The 96 hour LC₅₀, based on the mean, measured concentration of Bensulfuron methyl, was greater than 120 mg/L.

Table B.9.23: Observed mortality of bluegill sunfish, *Lepomis macrochirus*, exposed to Bensulfuron methyl for 96 hours in an unaerated, static, acute test

Mean, measured concentration of Bensulfuron methyl (mg a.i./L)	Cumulative mortality (No. dead / No. at test start)			
	24 h	48 h	72 h	96 h
Water control	0/5	0/5	0/5	0/5
pH adjusted water control	0/5	0/5	0/5	0/5
120-1	0/5	0/5	0/5	0/5
120-2	0/5	0/5	0/5	0/5
120-3	0/5	0/5	0/5	0/5
120-4	0/5	0/5	0/5	0/5
120-5	0/5	0/5	0/5	0/5
120-6	0/5	0/5	0/5	0/5

Table B.9.24: Observed sublethal effects of bluegill sunfish, *Lepomis macrochirus*, exposed to Bensulfuron methyl for 96 hours in an unaerated, static, acute test

Mean, measured concentration of Bensulfuron methyl (mg a.i./L)	Sublethal effects (No. affected / No. at test start)			
	24 h	48 h	72 h	96 h
Water control	0/5	0/5	0/5	0/5
pH adjusted water control	0/5	0/5	0/5	0/5
120-1	0/5	0/5	0/5	0/5
120-2	0/5	0/5	0/5	0/5
120-3	0/5	0/5	0/5	0/5
120-4	0/5	0/5	0/5	0/5
120-5	0/5	0/5	0/5	0/5
120-6	0/5	0/5	0/5	0/5

Conclusions:

The 96-hour LC₅₀ in the bluegill sunfish was greater than 120 mg Bensulfuron methyl/L.

Acute toxicity to fish – metabolites of Bensulfuron methyl

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

9) *IN-N5297: Static, acute, 96-hour, himit test to rainbow trout, Oncorhynchus mykiss. Gries, T., 2002.*

Cross reference IIA, 8.2.1./02

Guidelines:

USEPA 72-1 (1982); OECD 203 (1992); EEC Method C.1 (1992)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-N5297 technical metabolite

Batch: N5297-004

Purity: 99.5%

The acute toxicity of IN-N5297 to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, was determined in an unaerated, static, 96-hour limit test. Treatments consisted of a dilution water control, and a single nominal concentration of 120 mg IN-N5297/L. One water control chamber and 3 replicate limit test concentration chambers with 10 fish in each chamber were used for testing (total of 10 fish in the control and 30 fish in the limit test concentration). Test solutions were maintained between 11.0 and 12.9°C throughout the test.

Findings:

Summaries of cumulative mortality and sublethal effects are presented in Table B.9.25. Mean, measured concentrations of IN-N5297 were 118 mg a.i./L and ranged from 97 to 100% of nominal concentrations. There were no mortality or sublethal effects in the control and the treated test solutions.

Table B.9.25: Summary of mortality and sublethal effects of IN-N5297 on rainbow trout, *Oncorhynchus mykiss*, exposed for 96 hours in an unaerated, static, acute test

Mean, measured concentrations ^a of IN-N5297 (mg a.i./L)	Cumulative mortality (No. dead / No. at test start)				Sublethal effects (No. affected / No. at test start)			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Water control	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
118 A ^b	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
118 B ^b	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
118 C ^b	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

^a Average of mean, measured IN-N5297 concentrations in the 3 replicate test chambers

^b A, B, and C represent replicates. There were 10 fish in each replicate chamber at test start.

Conclusions:

The 96-hour LC₅₀ in rainbow trout, *Oncorhynchus mykiss* was >118 mg IN-N5297/L.

10) *IN-J0290: Static, acute, 96-hour LC₅₀ to rainbow trout, Oncorhynchus mykiss. Hoke, R.A., 2002.*

Cross reference IIA, 8.2.1./04

Guidelines:

USEPA 72-1 (1982); OECD 203 (1992); Directive 92/69/EEC, EEC Method C.1 (1992)

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

GLP:

Yes

*The study is acceptable*Materials and methods:

Test Substance: IN-J0290 technical metabolite

Batch: J0290-17

Purity: 99.6%

The acute toxicity of IN-J0290 to unfed fingerling rainbow trout, *Oncorhynchus mykiss*, was determined in an unaerated, static, 96-hour test. Treatments consisted of a dilution water control and nominal concentrations of 6.3, 12.5, 25, 50, and 100 mg IN-J0290/L. One replicate containing 10 fish was used for each treatment concentration and control. Test solutions were maintained between 11.2 and 11.4°C throughout the study.

Findings:

Summaries of cumulative mortality and sublethal effects are presented in Table B.9.26 and Table B.9.27, respectively. Mean, measured concentrations of IN-J0290 were not detected at control, 6.1, 12, 23, 48, and 97 mg a.i./L and ranged from 92 to 97% of nominal concentrations. Exposure of rainbow trout to mean, measured IN-J0290 concentrations of 6.1, 12, 23, 48, and 97 mg/L resulted in 0% mortality in all concentrations at the end of 96 hours. No mortality or sublethal effects were seen in the water control fish. The highest mean, measured concentration causing no mortality at test end was 97 mg/L. Two fish exhibited transient lethargy after 24 h exposure to 97 mg a.i./L, but had recovered by 48 hours.

Table B.9.26: Observed mortality of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-J0290 for 96 hours in an unaerated, static, acute test

Mean, measured concentration of IN-J0290 (mg a.i./L)	Cumulative mortality (Number dead / Number at test start)			
	24 h	48 h	72 h	96 h
Water control	0/10	0/10	0/10	0/10
6.1	0/10	0/10	0/10	0/10
12	0/10	0/10	0/10	0/10
23	0/10	0/10	0/10	0/10
48	0/10	0/10	0/10	0/10
97	0/10	0/10	0/10	0/10

One test chamber containing 10 fish (total 10 fish per test concentration) at test start

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.27: Observed sublethal effects of rainbow trout, *Oncorhynchus mykiss*, exposed to IN-J0290 for 96 hours in an unaerated, static acute test

Mean, measured concentration of IN-J0290 (mg a.i./L)	Sublethal effects (Number with effects / Number alive)			
	24 h	48 h	72 h	96 h
Water control	0/10	0/10	0/10	0/10
6.1	0/10	0/10	0/10	0/10
12	0/10	0/10	0/10	0/10
23	0/10	0/10	0/10	0/10
48	0/10	0/10	0/10	0/10
97	2 ^a /10	0/10	0/10	0/10

One test chamber containing 10 fish (total 10 fish per test concentration) at test start.

^alethargic

Conclusions:

The 96-hour LC₅₀ for the rainbow trout was >97 mg IN-J0290/L.

B.9.2.2 Chronic toxicity to fish (Annex IIA 8.2.2)

B.9.2.2.1 Chronic toxicity test on juvenile fish

A study to examine the chronic toxicity to juvenile fish is not required because two fish early life stage toxicity tests were performed and the results are presented below.

B.9.2.2.2 Fish early life stage toxicity test

11) *Early life-stage toxicity of IN F5384-122 to fathead minnows (Supplement No. 1). Hutton, D.G., 1988b.*

Cross reference IIA, 8.2.2.2./01

Guidelines:

USEPA 72-4

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-122

Purity: 97.3%

The effects of Bensulfuron methyl on the early life stages of the fathead minnow, *Pimephales promelas* were determined under unaerated, continuous-flow conditions for 36 days. A dilution water control and nominal test substance concentrations of 3.8, 7.5, 15, 30, 60, and 120 mg/L were used during the study. A total of 80 embryos were exposed per concentration (20 embryos per embryo cup, 2 cups per replicate, 2 replicates per concentration) at test start. The test consisted of 2 phases, an embryo phase where the survival and hatchability of larvae were observed and a larval phase where the survival and growth of the larvae were observed. The first larvae hatched on

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Day 3. As the larvae hatched from embryos, they were transferred from the embryo cup to the appropriate aquarium up to 20 larvae per duplicate. By Day 4 most of the larvae had hatched and hatching was considered complete. Larval survival was observed for the next 32 days.

Analytical verification of Bensulfuron methyl concentrations was made on test solutions sampled at 0, 7, 14, 21, 28, and 35 days of exposure. Test solutions were supplied to each replicate test chamber by an all glass, modified Mount and Brungs proportional diluter at a turnover rate of approximately 6 aquarium volumes (7.5 L) per day. Test solutions were maintained at an average $24.8 \pm 1^\circ\text{C}$. Observations were made for assessment of number of dead eggs at hatching, first and last day of hatching, first day of swim-up, survival and abnormalities from hatching to thinning, and survival and abnormalities from thinning to test end. Standard length and blotted wet weight of surviving fingerlings were determined at test end.

Findings:

Mean, measured concentrations of Bensulfuron methyl were less than the limit of quantitation (LOQ = 1 mg/L) (control), 5.0, 8.8, 14, 34, 65, and 120 mg a.i./L and ranged from 93 to 130% of nominal concentrations. All test solutions were clear and colourless throughout the study. All chemical and physical parameters for the 36-day study were within acceptable ranges.

A summary of embryo hatching, and survival and abnormalities from hatching to thinning is presented in Table B.9.28. A summary of survival and abnormalities from thinning to test end, and mean length and wet weight (blotted dry) values at test end are presented in Table B.9.29.

All surviving fish looked normal at the end of the test. No statistically significant effect was observed on any of the evaluated endpoints.

Table B.9.28: Observed embryo hatching for fathead minnows, *Pimephales promelas*, exposed to Bensulfuron methyl

Mean, measured concentration of Bensulfuron methyl (mg a.i./L)	Hatching (%)				
	A ^a	B ^a	C ^a	D ^a	Mean ^b
Water Control	55	75	65	80	69
5.0	70	80	60	60	68
8.8	60	75	70	85	72
14	55	55	80	70	65
34	60	65	70	70	66
65	60	80	70	60	68
120	65	55	65	70	64

^a Replicates

^b None of the values were statistically different from the control at the $p < 0.05$ level.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.29: Observed larval survival for fathead minnows, *Pimephales promelas*, exposed to Bensulfuron methyl

Mean measured Bensulfuron methyl (mg a.i./L)	Survival (%)		
	A ^a	B ^a	Mean ^b
0	100	90	95
5.0	100	100	100
8.8	100	95	98
14	100	80	90
34	100	95	98
65	100	100	100
120	100	100	100

^a Replicates

^b None of the values were statistically different from the control at the $p < 0.05$ level.

Conclusions:

The 36-day NOEC for Bensulfuron methyl in a fathead minnows early life stage test was 120 mg a.i./L, based on mean measured concentrations of Bensulfuron methyl and embryo hatching percentage and larval survival.

12) *Bensulfuron methyl (DPX-F5384) technical: early life-stage toxicity to rainbow trout, Oncorhynchus mykiss. Samel, A., 2003.*

Cross reference IIA, 8.2.2.2./02

Guidelines:

OECD 210; USEPA 72-4

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-333

Purity: 98.66%

The effects of Bensulfuron methyl on the early life stages of rainbow trout (*Oncorhynchus mykiss*) were determined under unaerated, continuous-flow conditions for 90 days. A dilution water control and nominal test substance concentrations of 0.75, 1.5, 3.0, 6.0, and 12.0 mg/L were used during the study. A total of 80 embryos were exposed per concentration (20 embryos per embryo cup, 2 cups per replicate, 2 replicates per concentration) at test start. On Day 46, after swim-up had begun in the controls, the fingerlings were thinned to a total of 30 fish per concentration (15 fish per replicate, 2 replicates per concentration).

Analytical verification of Bensulfuron methyl concentrations was made on test solutions sampled on Days 0 and 6, once weekly, and at test end (Day 90). Test solutions were supplied to each replicate test chamber by a continuous flow metering system at a rate of approximately 1.25 L per hour (approximately five, 6-L test solution volume additions per day). Embryos and alevins were held in relative darkness until Day 40, and then held under a photoperiod of 16 hours light and 8 hours darkness (which included 30 minutes of transitional light between light and

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

dark intervals) for the remainder of the study. After thinning, the fingerlings were fed newly-hatched brine shrimp (Days 46-53) or newly-hatched brine shrimp and trout chow (Days 54-89). Test solutions were maintained between 11.0 and 12.0°C (mean 11.4°C). Observations were made for assessment of number of dead eggs at hatching, first and last day of hatching, first day of swim-up, survival and abnormalities from hatching to thinning, and survival and abnormalities from thinning to test end. Standard length and blotted wet weight of surviving fingerlings were determined at test end.

Findings:

Mean, measured concentrations of Bensulfuron methyl were less than the limit of detection (LOD 0.004 mg/L, control), 0.82, 1.5, 3.4, 6.6, and 12 mg/L and ranged from 100 to 113% of nominal concentrations. All test solutions were clear and colourless throughout the study. All chemical and physical parameters for the 90-day study were within acceptable ranges.

A summary of hatching, swim-up, and survival and abnormalities from hatching to thinning is presented in Table B.9.30. A summary of survival and abnormalities from thinning to test end, and mean length and wet weight (blotted dry) values at test end are presented in Table B.9.31.

Table B.9.30: Summary of hatching, survival and sublethal effects (hatch to thinning) of Bensulfuron methyl in an early life stage test with rainbow trout

Mean measured concentrations of Bensulfuron methyl (mg a.i./L)	Mean hatching day			Hatching to thinning				First day of swim-up
				Survival		Abnormalities ^b		
	First	Last ^a	Mean percent hatch	Mean no. alive/ mean no. hatching	Mean %	Mean no. affected/ mean no. alive	Mean %	
Water Control	27	30	95	19/19	100	0/19	0	44
0.82	27	30	90	18/18	99	0.25/18	1.3	43
1.5	27	30	95	19/19	100	0.25/19	1.3	44
3.4	27	29 ^c	98	19/20	99	0/19	0	41 ^c
6.6	26	29 ^c	96	19/19	98	0/19	0	41 ^c
12	25	28 ^c	93	18/19	97	0/18	0	41 ^c

^a Last day of hatching is defined as the last day that remaining live eggs (or live embryos) hatch to yield live alevins.

^b Based on abnormalities still present at the time of thinning.

^c Statistically different from controls (Jonckheere's trend test).

Table B.9.31: Summary of survival, abnormalities, and growth (thinning to test end) of Bensulfuron methyl in an early life stage test with rainbow trout

Mean measured concentrations of Bensulfuron methyl (mg a.i./L)	Thinning to test end				Mean length (cm)	Mean wet weight (g)
	Survival		Abnormalities			
	Mean no. alive/ mean total	%	Mean no. affected/ mean no. alive	%		
Water Control	15/15	97	0/15	0	4.5	1.36
0.82	15/15	100	0.5/15	4	4.6	1.38
1.5	15/15	97	0.5/15	4	4.6	1.45
3.4	15/15	100	0.5/15	4	4.5	1.37
6.6	15/15	100	0.5/15	4	4.5	1.37
12	15/15	100	0.5/15	4	4.5	1.37

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Conclusions:

The 90-day NOEC of Bensulfuron methyl in a trout 90-day early life stage test was 1.5 mg/L, based on mean measured concentrations of Bensulfuron methyl and last day of hatching and first day of swim-up. *Fish life cycle test*

A fish life cycle study was not performed based on the following criteria:

- Bensulfuron methyl has low acute toxicity to fish (lowest measured $LC_{50} = >66$ mg a.i./L).
- The LogP for Bensulfuron methyl is <3 (0.79 at pH 7).
- Bioconcentration potential for Bensulfuron methyl is low. The measured bioconcentration factors for fish and crustaceans (rainbow trout, channel catfish, carp, and red swamp crayfish) were all less than 2.
- In laboratory water/sediment studies, Bensulfuron methyl is moderately persistent (the range for combined aquatic/sediment DT_{50} was 50–87 days; the corresponding range of DT_{90} was 167–289 days). In the field, these rates are expected to be faster due to additional dissipation mechanisms such as photolysis (Bensulfuron methyl EU Dossier, Annex IIA, Document M-II, Section 5, DuPont-6185 EU).

B.9.2.3 Bioconcentration in fish (Annex IIA 8.2.3)

Bioconcentration potential for Bensulfuron methyl is predicted to be low. It does not persist in water or sediment and it has a $\text{LogP} < 3$. However, BCF tests with Bensulfuron methyl were conducted on a variety of species for the US EPA and summarised below to support the conclusion of low bioconcentration potential for this active substance.

13) DPX-F5384 [^{14}C -phenyl(U)] flowthrough bioconcentration study with carp, *Cyprinus carpio*. Larkin, J.C., 1984a.

Cross reference IIA, 8.2.3./01

Guidelines:

Not specified

GLP:

No

The study is acceptable

Materials and methods:

Test Substance: ^{14}C -DPX-F5384 radiolabeled technical

Batch: [^{14}C -phenyl(U)]Bensulfuron methyl HOTC 981

Purity: 94.7%

Bioconcentration potential of [phenyl- ^{14}C (U)]Bensulfuron methyl (^{14}C -DPX-F5384) was determined in the carp (*Cyprinus carpio*). Fish were exposed to mean, measured water concentrations of 5 mg/L ^{14}C -Bensulfuron in a flow-through system, based on radiochemical analysis during the uptake phase. The flow rate during the test was adjusted to deliver 2 litres of treatment media every 8 minutes and resulted in a turnover rate of 5 chamber volumes (72.9 litres) per day. Total hardness was 100 mg/L as CaCO_3 , total alkalinity was 135 mg/L as CaCO_3 , pH was 7.0–7.3, temperature was $22 \pm 2^\circ\text{C}$, and O_2 concentration was 7.9 ± 0.2 mg/L.

The study was initiated by placing 80 fish into each of three test chambers (control and 2 treatment replicates) consisting of 108 L glass aquaria. The average weight of the fish was 1.64 g, the static loading rate was 1.80 g/L.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Daily observations were made to check for fish mortality and/or sublethal effects. Treated fish were exposed for 28 days followed by a 14-day depuration phase. Samples of water and fish for radiometric analysis were collected on Days 0, 1, 3, 7, 10, 14, 21, and 28. Four fish were sampled from each tank at each sampling point. For each sample, 2 whole fish were analysed and 2 were divided into edible (raw agricultural product, lateral musculature with overlying skin) and non-edible (remainder of the carcass including head and viscera) tissues. During depuration, control and exposure water samples and fish were sampled on Days 1, 3, 7, 10, and 14. The steady-state bioconcentration factor (BCF) was determined at the end of the 28 day exposure phase and after 14 day of depuration. Bioconcentration factors (BCFs) were calculated by dividing ^{14}C residues in whole fish or tissues by ^{14}C concentration in the water phase.

Findings:

The nominal ^{14}C -Bensulfuron methyl concentration selected for the bioconcentration study was 5 mg/L. The mean, measured concentration based on radiochemical analysis was 5.0 mg/L, range: 4.1 to 5.7 mg/L during the uptake phase and <1.0 mg/L (water phase LOD = 1.0 mg/L) during the depuration phase of the study. Although the concentration of Bensulfuron methyl was not measured during the study except by analysis of ^{14}C , Bensulfuron methyl is stable at the pH of the test solution in this study (pH 7–7.3). (For example, in a guideline hydrolysis study, only 1.8% Bensulfuron methyl hydrolysed at pH 7 in 30 days, DuPont-4499). It is anticipated therefore that the ^{14}C analysed during the exposure phase of the study was intact Bensulfuron methyl.

Test organism observations: No adverse effects were observed in control or treated fish at 5 mg/L ^{14}C Bensulfuron methyl during the course of this study.

Bioconcentration: Bensulfuron methyl concentrations in edible, and non-edible fish tissues and whole fish remained near or below the limit of detection throughout the study (Table B.9.32). The maximum BCF for edible and non-edible fish tissues and whole fish were 1.0, 2.5, and <0.9, respectively. Bioconcentration of Bensulfuron methyl did not occur in the carp.

Table B.9.32: Mean Bensulfuron methyl concentrations in exposure water, edible and non-edible tissues, and whole fish during uptake and depuration phases of a bioconcentration study with the carp

Study Day	Exposure / Depuration phases (d)		Water (Mean mg/L) ^a	Edible fish tissue (Mean mg/kg) ^b	Non-edible fish tissue (Mean mg/kg) ^b	Whole fish (Mean mg/kg) ^b
	Exp	Dep				
0	0	-	5.4	<4.4	<4.4	<4.4
1	1	-	5.6	<4.4	<4.4	<4.4
3	3	-	5.4	<4.4	<4.4	<4.4
7	4	-	5.1	<4.4	<4.4	<4.4
10	10	-	5.0	<4.4	<4.4	<4.4
14	14	-	5.0	<4.4	<4.4	<4.4
21	21	-	4.6	<4.4	5.4	<4.4
28	28	-	4.2	<4.4	8.4	<4.4
29	-	1	<1.0	<4.4	<4.4	<4.4
31	-	3	<1.0	4.6	5.3	<4.4
35	-	7	<1.0	<4.4	<4.4	<4.4
38	-	10	<1.0	<4.4	<4.4	<4.4
42	-	14	<1.0	<4.4	<4.4	<4.4

^a Limit of detection = 1 mg/L; mean of 2 replicates

^b Limit of detection for tissues = 4.4 mg/kg; mean of 2 replicates

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9. 33: Summary of Bensulfuron methyl bioconcentration factors (BCF) for the carp (*Cyprinus carpio*)

Tissue	Maximum BCF	
	Exposure	Depuration
Edible	<0.9	1.0
Non-edible	1.7	1.1
Whole Fish	<0.9	<0.9

Conclusions:

The maximum whole-fish bioconcentration factor for Bensulfuron methyl in the carp was <1, indicating that Bensulfuron methyl has low potential to bioconcentrate in fish.

14) DPX-F5384 [^{14}C -phenyl(U)] Flow-through bioconcentration study with rainbow trout, *Salmo gairdneri*. Larkin, J.C., 1984b.

Cross reference IIA, 8.2.3./02

Guidelines:

Not specified

GLP:

No

The study is acceptable

Materials and methods:

Test Substance: ^{14}C -DPX-F5384 technical

Batch: [^{14}C -phenyl(U)] HOTC 981

Purity: 98%

Bioconcentration potential of [phenyl- ^{14}C (U)]Bensulfuron methyl (^{14}C -DPX-F5384) was determined in the rainbow trout, *Salmo gairdneri*. Fish were exposed to mean, measured water concentrations of 3.9 mg/L ^{14}C -Bensulfuron in a flow-through system, based on radiochemical analysis during the uptake phase. The flow rate during the test was adjusted to deliver 2 litres of treatment media every 7.9 minutes and resulted in a turnover rate of 5.2 chamber volumes (72.9 litres) per day. Total hardness was 124 mg/L as CaCO_3 , total alkalinity was 158 mg/L as CaCO_3 , pH was 6.8–7.2, temperature was $11 \pm 1^\circ\text{C}$, and O_2 concentration was 9.2 ± 0.2 mg/L.

The study was initiated by placing 80 fish into each of three test chambers (1 control and 2 treatment replicates) consisting of 108 L glass aquaria. The average weight of the fish was 1.74 g, the static loading rate was 1.91 g/L. Daily observations were made to check for fish mortality and/or sublethal effects. Treated fish were exposed for 28 days followed by a 14-day depuration phase. Samples of water and fish for radiometric analysis were collected on Days 0, 1, 3, 7, 10, 14, 21 and 28; from control fish were sampled on days 0, 1 and 28. Four fish were sampled from each tank each day. For each sample, 2 fish were analysed whole and 2 were divided into edible (raw agricultural product, lateral musculature with overlying skin) and non-edible (remainder of the carcass including head and viscera) tissues. During depuration, control and exposure water samples and exposure fish were sampled on Days 1, 3, 7, 10, and 14; from control fish were sampled only on days 14. The steady-state bioconcentration factor (BCF) was

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

determined at the end of the 28 day exposure phase and after 14 day of depuration. Bioconcentration factors (BCFs) were calculated by dividing ^{14}C residues in whole fish or tissues by ^{14}C concentration in the water phase.

Findings:

The mean measured ^{14}C -Bensulfuron methyl concentration based on radiochemical analysis was 3.9 mg/L, range: 2.5 to 4.5 mg/L, during the uptake phase and <0.8 mg/L (water phase LOD = 0.8 mg/L) during the depuration phase of the study. Although the concentration of Bensulfuron methyl was not measured during the study except by analysis of ^{14}C , Bensulfuron methyl is stable at the pH used in this study pH 6.8–7.2 (For example, in a guideline hydrolysis study, only 1.8% Bensulfuron methyl hydrolysed at pH 7 in 30 days, DuPont-4499) and, therefore, the ^{14}C analysed during the exposure phase of the study is expected to be intact Bensulfuron methyl.

Test organism observations: No adverse effects were observed in control or treated fish at 3.9 mg/L ^{14}C Bensulfuron methyl during the course of this study.

Bioconcentration: Bensulfuron methyl concentrations in edible, and non-edible fish tissues and whole fish remained near or below the limit of detection throughout the study (Table B.9.34). The maximum BCF for edible and non-edible fish tissue and whole fish were 2.2, 1.1 and 1.0, respectively. Bioconcentration of Bensulfuron methyl did not occur in rainbow trout.

Table B.9.34: Mean Bensulfuron methyl concentrations in exposure water, rainbow trout edible and non-edible tissues, and whole fish during uptake and depuration phases

Study Day	Exposure / Depuration phases (d)		Water (Mean mg/L) ^a	Edible fish tissue (Mean mg/kg) ^b	Non-edible fish tissue (Mean mg/kg) ^b	Whole fish (Mean mg/kg) ^b
	Exp	Dep				
0	0	-	4.5	<3.2	<3.2	<3.2
1	1	-	4.1	3.2	3.3	<3.2
3	3	-	4.0	<3.2	<3.2	<3.2
7	4	-	3.9	<3.2	<3.2	3.4
10	10	-	3.9	<3.2	<3.2	<3.2
14	14	-	4.1	<3.2	<3.2	<3.2
21	21	-	4.3	<3.2	<3.2	<3.2
28	28	-	2.5	<3.2	<3.2	<3.2
29	-	1	<0.8	6.1	4.4	<3.2
31	-	3	<0.8	8.4	<3.2	<3.2
35	-	7	<0.8	<3.2	<3.2	3.6
38	-	10	<0.8	<3.2	<3.2	<3.2
42	-	14	<0.8	<3.2	<3.2	<3.2

^a Limit of detection = 0.8 mg/L; mean of 2 replicates

^b Limit of detection for tissues = 3.2 mg/kg; mean of 2 replicates

Table B.9.35: Summary of Bensulfuron methyl bioconcentration factors (BCF) for rainbow trout (*Salmo gairdneri*)

Tissue	Maximum BCF	
	Exposure	Depuration
Edible	<0.8	2.2
Non-edible	<0.8	1.1
Whole fish	0.9	1.0

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Conclusions:

The maximum whole-fish bioconcentration factor of Bensulfuron methyl in rainbow trout was 1.0, indicating that Bensulfuron methyl has low potential to bioconcentrate in fish.

15) *Residues of [¹⁴C]Bensulfuron methyl in channel catfish. McComish, M.F., Marquis, J.K., 1991.*

Cross reference IIA, 8.2.3/03

Guidelines:

USEPA 165-4 & 171-4

GLP:

Yes

The study is not acceptable. The guideline is not met in several points: for example 1) depuration after exposure in missing; 2) number of fish sampling less than 5; 3) only one concentration was tested, instead of 2; 4) static instead of flow-through or semi-static conditions.

Materials and methods:

Test Substance: ¹⁴C-DPX-F5384 technical

Batch: [pyrimidine-2-¹⁴C] HOTC 319; [phenyl(U)-¹⁴C] HOTC 320

Purity: [phenyl-¹⁴C(U)]Bensulfuron methyl (DPX-F5384-1) = 98.9%

[pyrimidine-2-¹⁴C(U)]Bensulfuron methyl (DPX-F5384-2) = 97.4%

Bioconcentration potential of [phenyl-¹⁴C(U)]Bensulfuron methyl (¹⁴C-DPX-F5384-1) and [pyrimidine-2-¹⁴C(U)]Bensulfuron methyl (¹⁴C-DPX-F5384-2) was determined in the channel catfish (*Ictalurus punctatus*). Fish were exposed for 3 days to mean, measured water concentrations of 10.7 µg/L [phenyl-¹⁴C(U)]Bensulfuron methyl and 10.2 µg/L [pyrimidine-2-¹⁴C(U)]Bensulfuron methyl in a static test system. The test item was diluted into acetone and administered to the individual test vessels. The final concentration of acetone in the treatment solutions and solvent control was 0.2%. Total hardness was 40–48 mg/L as CaCO₃, total alkalinity was 198–204 mg/L as CaCO₃, pH was 8.0–8.5, temperature was 19.9–20.5°C, and O₂ concentration was 7.7–9.0 mg/L. The study was initiated by transferring fish to 20 litre test vessels that contained 15 litres of treatment solution. One fish was indiscriminately distributed to each of 6 replicates of the solvent control and the two test substances, and each of 2 dilution water control test vessels. The number of surviving organisms and the occurrence of sublethal effects were determined visually and were recorded initially and after 24, 48, and 72 hours.

Catfish were harvested and processed from each treatment group on Day 3. Each fish was separated into the following fractions: filet, deboned meat, edible, skin, head and rack, viscera, inedible tissues, and whole body. All fractions were analysed separately. Bioconcentration factors (BCFs) were calculated by dividing ¹⁴C residues in whole fish or tissues by ¹⁴C concentration in the water phase.

Findings:

The mean measured ¹⁴C-Bensulfuron methyl concentrations over the 3 day exposure period based on HPLC and radiometric analysis were 10.7 ± 0.6 µg/L for [phenyl-¹⁴C(U)]Bensulfuron methyl and 10.2±0.6 µg/L for [pyrimidine-2-¹⁴C(U)]Bensulfuron methyl.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Test organism observations: No mortality or sublethal effects were observed in control or treated fish during the course of this study.

Bioconcentration: Mean Bensulfuron methyl concentrations and BCFs in the various fish tissues and whole fish are shown in Table B.9.36.

Table B.9.36: Mean residue concentration (μg equivalents/g) ($\pm$ SD) of [phenyl- ^{14}C (U)]Bensulfuron methyl (DPX-F5384-1) and [pyrimidine-2- ^{14}C (U)]Bensulfuron methyl (DPX-F5384-2) equivalents and BCF in tissues collected following a 3-day static exposure catfish study

Tissue	Test item 1		Test item 2	
	DPX-F5384-1	BCF	DPX-F5384-2	BCF
Filet ^a	0.32 ± 0.11	0.03	0.44 ± 0.12	0.043
Deboned meat ^a	0.32 ± 0.10	0.03	0.45 ± 0.10	0.044
Edible ^a	0.32 ± 0.10	0.03	0.44 ± 0.12	0.043
Skin ^a	0.73 ± 0.11	0.068	1.15 ± 0.33	0.112
Head and rack ^a	0.41 ± 0.11	0.038	0.45 ± 0.07	0.044
Viscera ^a	8.59 ± 1.44	0.803	38.4 ± 19.12	3.765
Inedible ^a	1.19 ± 0.13	0.112	3.97 ± 2.02	0.391
Whole body ^a	0.98 ± 0.11	0.091	3.11 ± 1.47	0.305

^a Values are means of all 6 replicates

Conclusions:

The maximum BCF for any catfish tissue after 3 days exposure to Bensulfuron methyl was 0.8 to 3.8 (viscera). The mean whole-fish bioconcentration factor for Bensulfuron methyl in channel catfish was 0.09 to 0.3. These results demonstrate that Bensulfuron methyl has low potential to bioconcentrate in fish.

16) *Flow-through crayfish bioconcentration study with ^{14}C -Bensulfuron methyl. Romaine, R.P., Kleinow, K.M., 1989.*

Cross reference IIA, 8.2.3./04

Guidelines:

USEPA 165-4

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: ^{14}C -DPX-F5384 technical

Batch: [pyrimidine-2- ^{14}C] HOTC number not specified; [phenyl(U)- ^{14}C] HOTC number not specified

Purity: [phenyl- ^{14}C (U)]Bensulfuron methyl (DPX-F5384-1) = 98.1%

[pyrimidine-2- ^{14}C (U)]Bensulfuron methyl (DPX-F5384-2) = 98.0%

A flow-through bioconcentration study was conducted with [phenyl- ^{14}C (U)]Bensulfuron methyl (^{14}C -DPX-F5384-1) and [pyrimidine-2- ^{14}C (U)]Bensulfuron methyl (^{14}C -DPX-F5384-2) using the red swamp crayfish *Procambarus clarkii*. The test consisted of a 28 day uptake exposure period followed by a 14 day depuration period. Sexually

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

mature freshwater crayfish were exposed for 28 days to nominal water concentrations of [phenyl-¹⁴C(U)]Bensulfuron methyl and [pyrimidine-2-¹⁴C(U)]Bensulfuron methyl of 1.0 mg/L. Test item stocks were made in 98% acetone and the final acetone concentration in control and Bensulfuron methyl treatments was 0.1%.

The test system consisted of 3 108-L all-glass test units, one for the solvent control and one for each of the 2 test items. Each test unit was divided into 3 30-L exposure chambers (9 total chambers). To separate crayfish and minimise cannibalism, 30 cubicles of polyethylene mesh were placed into each exposure chamber.

The flow rate during the test was adjusted to deliver 500 mL of treatment media every 8-10 minutes and resulted in a turnover rate of 2–2.6 chamber volumes per day. Total hardness was 110 ± 17 mg/L as CaCO₃, total alkalinity was 98 ± 20 mg/L as CaCO₃, total ammonia <0.46 mg/L as N, pH was 7.2-8.5, mean temperature was 21.8°C (range: 20 to 23°C), and O₂ concentration was >5.1 mg/L.

The study was initiated by placing 35 crayfish into each exposure chamber. Treatments were 0.1% acetone (control) and 1 mg/L (nominal) concentrations of [phenyl-¹⁴C(U)]Bensulfuron methyl, or [pyrimidine-2-¹⁴C(U)]Bensulfuron methyl. The animal loading rate was 13.2 g/L based on a mean crayfish weight of 11.3 g and chamber volume of 30 litres. Crayfish were fed 2-3 times per week with a formulated crustacean ration.

Water samples were taken daily during the exposure and depuration phases for radiometric analysis. Crayfish were sampled on Days 0, 1, 3, 7, 14, 21 and 28 during uptake and on Days 1, 3, 7, 10 and 14 during depuration. Five crayfish were harvested, hemolymph collected and then dissected to remove edible tissues (abdominal muscle and hepatopancreas).

Tissue residues were analysed and the steady-state bioconcentration factor (BCF), and uptake and depuration rate constants were calculated. Bioconcentration factors (BCFs) were calculated by dividing ¹⁴C residues in whole crayfish or tissues by ¹⁴C concentration in the water phase.

Findings:

Water quality parameters during the bioconcentration study were satisfactory to the general well being of the crayfish. Natural mortality during the study was 43% in the acetone controls, 41% in [phenyl-¹⁴C(U)]Bensulfuron methyl, and 45% in [pyrimidine-2-¹⁴C(U)]Bensulfuron methyl. Natural mortality was attributed to physiological stress associated with molting and cannibalism of the molted crayfish by intermolt animals. The crayfish mortality, which averaged 7% per week was not unusual considering the size, maturity, and age of the animals used in this study. The surviving crayfish appeared to be in good physical condition at the termination of the study.

The mean measured water concentrations of ¹⁴C-Bensulfuron methyl during the exposure phase of the study were 1.00 mg/L for [phenyl-¹⁴C(U)]Bensulfuron methyl and 0.98 mg/L for [pyrimidine-2-¹⁴C(U)]Bensulfuron methyl. HPLC analysis of exposure water revealed that the percentage of Bensulfuron methyl present during uptake ranged from 95.8 to 100% for [phenyl-¹⁴C(U)]Bensulfuron methyl and 97.7 to 100% for [pyrimidine-2-¹⁴C(U)]Bensulfuron methyl. The mean residue concentrations in the hepatopancreas, abdominal muscle, non-edible remaining carcass, and the whole crayfish are presented in Table B.9.37. The steady state BCFs, half-lives, and tissue concentrations of ¹⁴C radioactivity in the various tissues are presented in Table B.9. 38. The highest BCF for any crayfish tissue is 5.1 indicating that Bensulfuron methyl has a very low potential to bioconcentrate.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.37: Mean residue concentrations of [phenyl-¹⁴C(U)]Bensulfuron methyl equivalents in edible and non-edible crayfish tissues during uptake and depuration phases of a 42 day bioconcentration study

Study Day	Exposure / Depuration phases (d)		Hepatopancreas (µg/g) ^a	Abdominal (µg/g) ^a	Carcass (µg/g) ^a	Whole body (µg/g) ^a
	Exp	Dep				
0	0	-	nd	nd	nd	nd
1	1	-	0.5	nd	nd	nd
3	3	-	2.1	nd	nd	nd
7	4	-	2.4	nd	0.4	0.4
14	14	-	2.6	nd	0.6	0.7
21	21	-	4.9	nd	0.7	0.8
28	28	-	5.5	nd	0.8	1.0
29	-	1	4.2	nd	0.5	0.6
31	-	3	2.6	nd	0.5	0.6
35	-	7	5.3	nd	0.7	0.9
38	-	10	2.0	nd	0.4	0.4
42	-	14	1.0	nd	nd	nd

^a mean of all replicates (5 crayfish/sampling time for exposure and 3 crayfish/sampling time for depuration)

^b nd == not detectable (residue LOD ≈ 0.44 µg/g).

Table B.9. 38: Mean residue concentrations of [pyrimidine-¹⁴C(U)]Bensulfuron methyl equivalents in edible and non-edible crayfish tissues during uptake and depuration phases of a 42 day bioconcentration study

Study day	Exposure / Depuration phases (d)		Hepatopancreas (µg/g) ^a	Abdominal (µg/g) ^a	Carcass (µg/g) ^a	Whole body (µg/g) ^a
	Exp	Dep				
0	0	-	nd	nd	nd	nd
1	1	-	1.1	nd	nd	nd
3	3	-	1.2	nd	nd	nd
7	4	-	2.0	nd	0.6	0.6
14	14	-	3.1	nd	0.5	0.6
21	21	-	4.7	nd	nd	0.5
28	28	-	2.9	nd	0.5	0.6
29	-	1	2.7	nd	0.4	0.5
31	-	3	1.1	nd	nd	nd
35	-	7	1.5	nd	nd	nd
38	-	10	1.4	nd	nd	nd
42	-	14	0.7	nd	nd	nd

^a Mean of all replicates (5 crayfish/sampling time for exposure and 3 crayfish/sampling time for depuration)

^b nd = not detectable (residue LOD ≈ 0.44 µg/g).

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.39: Maximum bioconcentration factors, depuration half-life estimates and test item concentration (C_{ss}) at apparent steady state of crayfish tissues exposed to [phenyl-¹⁴C(U)]Bensulfuron methyl (DPX-F5384-1) or [pyrimidine-¹⁴C(U)]Bensulfuron methyl (DPX-F5384-2)

Tissue	¹⁴ C-DPX-F5384-1			¹⁴ C-DPX-F5384-2		
	BCF	t _{1/2} (h)	C _{ss} (µg/g)	BCF	t _{1/2} (h)	C _{ss} (µg/g)
Edible						
Hepatopancreas	5.1	172	5.1	4.9	237	4.8
Muscle	nd ^a	nd	nd	nd	nd	nd
Weighted mean	1.8	200	1.8	1.5	218	1.5
Non-edible						
Carcass	1.5	377	1.5	1.1	225	1.1
Whole body	1.6	327	1.6	1.0	244	1.0

^and = not detectable because residue concentrations were <LOD (0.44 µg/g)

Conclusions:

Bensulfuron methyl has low potential to bioconcentrate in the crayfish. The maximum steady-state BCF in edible tissue was 5.1 and in whole body was 1.6.

B.9.2.4 Acute toxicity to aquatic invertebrates (Annex IIA 8.2.4)

B.9.2.4.1 Acute toxicity to aquatic invertebrates – Bensulfuron methyl

17) Static Acute toxicity of Haskell sample number 16,985 to embryos and larvae of the eastern oyster *Crassostrea virginica*. Boeri, R.L., 1988.

Cross reference IIA, 8.2.4./01

Guidelines:

Not specified

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-52

Purity: 94.9%

The acute toxicity of Bensulfuron methyl to the embryos and larvae of the eastern oyster, *Crassostrea virginica*, was determined in a static, 48-hour test. Embryos of the eastern oyster were obtained by spawning conditioned adult oysters. Dilution water was natural seawater treated by filtering through a sand bed filter, a UV irradiator, and an activated carbon filter. Oysters were spawned by removing them from the water for 2 hours, placing individual an oyster in a culture dish containing dilution water, raising the temperature from 20 to 27°C over 1 hour and adding oyster sperm suspension to each dish. Within 45 minutes of sperm addition, the oysters spawned. Eggs and sperm were combined within 15 minutes of spawning and embryos were used immediately for the test. The test design

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

consisted of 3 replicates for Bensulfuron methyl treatments and 4 replicates for the controls, each containing approximately 30,000 oyster embryos/L. Nominal test concentrations were 0 (control), 40, 60, 100, 125, and 142 mg a.i./L. The test was conducted at 20±1°C and the numbers of normal and abnormal oyster larvae were assessed visually after 48 hours. The results of the test were used to calculate the percentage reduction of normal oyster larvae from each test vessel when compared to control. All data analyses are based on nominal concentrations.

Findings:

A summary of the findings is presented in Table B.9.40 and Table B.9.41.

Table B.9.40: Summary of the number of normal larval eastern oyster *Crassostrea virginica* exposed to Bensulfuron methyl for 48 hours in an unaerated, static, acute test

Nominal concentrations of Bensulfuron methyl (mg a.i./L)	Mean number of oyster larvae/mL after 48 h exposure							
	Normal				Abnormal			
	1 ^a	2	3	4 ^b	1 ^a	2	3	4 ^b
Dilution water control	30.0	30.4	32.4	31.6	0.0	0.0	0.0	0.0
40	31.4	32.0	30.4	-	0.0	0.6	0.2	-
60	31.2	29.4	32.4	-	0.0	0.0	0.4	-
100	31.0	30.4	32.0	-	0.2	0.2	0.2	-
125	30.4	30.0	29.4	-	0.2	0.0	0.0	-
142	29.4	30.4	30.8	-	0.0	0.0	0.0	-

^a 1-4 represent replicate test vessels containing 5 daphnids each (total 20 daphnids per concentration) at test start.

^b the controls had 4 replicates

Table B.9.41: Summary of percent reduction of normal larval eastern oyster *Crassostrea virginica* exposed to Bensulfuron methyl for 48 hours in an unaerated, static, acute test

Nominal concentrations of Bensulfuron methyl (mg a.i./L)	Mean number of normal larvae/mL ^a	Percentage reduction of normal test larvae ^b
Dilution water control	31.1	-
40	31.3	0.6
60	31.0	0.3
100	31.1	0.0
125	29.9	3.9
142	30.2	2.9

^a mean of 4 replicates for control and 3 replicates for each treatment

^b % reduction is the mean number of normal larvae/mL minus the mean number of normal control larvae/mL divided by the mean number of normal control larvae/mL x 100

Conclusions:

The 48-hour EC₅₀ based on percentage reduction of normal oyster larvae was >142 mg Bensulfuron methyl/L.

18) *Static acute toxicity of Haskell sample number 16,985 to the mysid, Mysidopsis bahia. Boeri, R.L., 1988.*

Cross reference IIA, 8.2.4./02

Guidelines:

USEPA 72-3

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

GLP:

Yes

*The study is acceptable*Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-52

Purity: 94.9%

The acute toxicity of Bensulfuron methyl to mysid shrimp, *Mysidopsis bahia* was determined in a static, 96-hour test following procedures described in Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians (ASTM, 1980). Treatments consisted of a dilution water control and nominal concentrations of 100, 115, 125, 140, and 150 mg Bensulfuron methyl/L. The test design consisted of 2 replicates of 10 mysids for a total of 20 mysids for each treatment and control. Test media was natural, filtered (5 µm) seawater and solutions were maintained at 22±1°C. Mysids were fed daily during the test. Aeration was required 72 h after the start of the test to maintain dissolved oxygen concentrations above acceptable limits. The number of surviving mysids and occurrence of sublethal effects were assessed 24, 48, 72, and 96 h after test initiation.

Findings:

A summary of the findings is presented in Table B.9.42 and Table B.9.43.

All test vessels that contained Bensulfuron methyl were initially slightly cloudy. Test vessels containing mean measured concentrations of 85.4, 105, and 112 mg a.i./L remained cloudy throughout the test. Mean, measured concentrations of Bensulfuron methyl were not detected, 85.4, 105, 112, 124, and 130 mg a.i./L and ranged from 85 to 91% of nominal concentrations. The concentrations of Bensulfuron methyl remained stable throughout the test and varied from +6 to +14% of initial concentrations at the end of 96 hours. Loading during the test was approximately 0.002 g/L. The 24, 48, 72, and 96 h LC₅₀s for mysids exposed to Bensulfuron methyl are >130 mg a.i./L. The NOEC (concentration which produced no statistically significant difference in survival when compared to the control) was 130 mg a.i./L. No sublethal effects were noted during the course of the study.

Table B.9.42: Summary of observed mortality of *Mysidopsis bahia* exposed to Bensulfuron methyl for 96 hours in a static, acute test

Mean, measured concentrations of Bensulfuron methyl (mg a.i./L)	Cumulative mortality (No. dead/No. at test start)							
	24h		48h		72h		96h	
	1 ^a	2 ^a	1 ^a	2 ^a	1 ^a	2 ^a	1 ^a	2 ^a
Dilution Water Control	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
85.4	0/10	0/10	0/10	0/10	0/10	2/10	0/10	3/10
105	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10
112	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
124	0/10	0/10	0/10	0/10	3/10	0/10	3/10	0/10
130	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10

^a 1 and 2 represent replicate test vessels containing 10 mysids each (total 20 mysids per concentration) at test start.

There were no statistical differences between treatments and control (Dunnett's, p >0.05)

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.43: Summary of observed mortality of *Mysidopsis bahia* exposed to Bensulfuron methyl for 96 hours in a static, acute test

Mean, measured concentrations of Bensulfuron methyl (mg a.i./L)	Sublethal effects (No. affected/No. at test start)							
	24h		48h		72h		96h	
	1 ^a	2 ^a	1 ^a	2 ^a	1 ^a	2 ^a	1 ^a	2 ^a
Dilution Water Control	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
85.4	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
105	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
112	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
124	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
130	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

^a 1 and 2 represent replicate test vessels containing 10 mysids each (total 20 mysids per concentration) at test start.

Conclusions:

The 96-hour LC₅₀ based on mean, measured test concentrations in *Mysidopsis bahia* was >130 mg Bensulfuron methyl/L.

19) *Acute toxicity of H-16,441 to the Crayfish (Procambarus clarki). Johnson, I.C., Drotter, K.R., 1986.*

Cross reference IIA, 8.2.4./05

Guidelines:

USEPA 72-2

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-52

Purity: 95.9%

The acute toxicity of Bensulfuron methyl to the freshwater crayfish, *Procambarus clarki* was determined in a static, 96-hour test. Test crayfish were 30 ± 5 mm telson to rostrum length and 0.64 ± 0.3 g wet weight. The test was conducted in 44 L glass test containers, each containing a final volume of 35 L of test solution or control freshwater at a depth of approximately 24 cm. The test design consisted of 3 replicates containing 10 crayfish each for the control and single treatment concentration (total of 30 per treatment or control). A single nominal treatment concentration of 100 mg Bensulfuron methyl/L was used. The test temperature was maintained at 22°C. The number of surviving crayfish was assessed 24, 48, 72, and 96 h after test initiation.

Findings:

A summary of the findings is presented in Table B.9.44.

All test vessels that contained nominally 100 mg Bensulfuron methyl/L were cloudy. Mean, measured concentrations of Bensulfuron methyl were not detected (control), 62 to 69 mg/L on Day 0, and 72 to 76 mg/L on Day 4. The mean measured concentration of Bensulfuron methyl for all replicates and sampling times was 71 mg a.i./L or 71% of

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

nominal. Loading density during the test was 0.18 g/L. Crayfish were not fed during the test. After 96 h exposure to 71 mg Bensulfuron methyl/L, there were no mortalities observed in any control or treatment. The 96 h LC₅₀ for crayfish exposed to Bensulfuron methyl is >71 mg a.i./L. The NOEC (concentration which produced no statistically significant difference in survival when compared to the control) was 71 mg a.i./L.

Table B.9.44: Summary of observed mortality of crayfish exposed to Bensulfuron methyl for 96 hours in an static, acute test

Mean, measured concentrations of Bensulfuron methyl (mg a.i./L)	Cumulative Mortality (No. dead/No. at test start)			
	24h	48h	72h	96h
Control A	0/10	0/10	0/10	0/10
Control B	0/10	0/10	0/10	0/10
Control C	0/10	0/10	0/10	0/10
71A ^a	0/10	0/10	0/10	0/10
71B ^a	0/10	0/10	0/10	0/10
71C ^a	0/10	0/10	0/10	0/10

^a replicate test vessels containing 10 crayfish each (total 30 crayfish per treatment) at test start

Conclusions:

The 96-hour LC₅₀ based on mean, measured test concentrations in the crayfish, *Procambarus clarki*, was >71 mg Bensulfuron methyl/L.

20) *Bensulfuron methyl (DPX-F5384) technical: static, acute 48-hour toxicity test to Daphnia magna.* Samel, A., 2001.

Cross reference IIA, 8.2.4./06

Guidelines:

USEPA 72-2 (1982); OECD 202 (1984); Directive 92/69/EEC; EEC Method C.2 (1992)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-333

Purity: 98.66%

The acute toxicity of Bensulfuron methyl to unfed *Daphnia magna* (<24-hour old) was determined in an unaerated, static, 48-hour test. Treatments consisted of a dilution water control, a pH adjusted (pH 9) dilution water control, and nominal concentrations of 7.5, 15, 30, 60, and 120 mg Bensulfuron methyl/L. Five daphnids were used per replicate with four replicates per test concentration and control.

Findings:

A summary of the findings is presented in Table B.9.45 and Table B.9.46. Mean, measured concentrations of Bensulfuron methyl were not detected, 7.2, 15, 30, 61, and 130 mg a.i./L and ranged from 96 to 108% of nominal

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

concentrations. No immobility or sublethal effects were noted during the course of the study. The highest concentration causing no immobility was 130 mg a.i./L.

Table B.9.45: Summary of observed immobility of unfed *Daphnia magna* exposed to Bensulfuron methyl for 48 hours in an unaerated, static, acute test

Mean, measured concentrations of Bensulfuron methyl (mg a.i./L)	Immobility (No. immobile/No. at test start)							
	24 hours				48 hours			
	Aa	B ^a	C ^a	D ^a	A ^a	B ^a	C ^a	D ^a
Dilution Water Control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
pH 9.0 Water Control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
7.2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
15	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
30	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
61	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
130	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

^a A – D represent replicate test vessels containing 5 daphnids each (total 20 daphnids per concentration) at test start.

Table B.9.46: Summary of observed sublethal effects of unfed *Daphnia magna* exposed to Bensulfuron methyl for 48 hours in an unaerated, static, acute test

Mean, measured concentrations of Bensulfuron methyl (mg a.i./L)	Immobility (No. affected/No. alive start)							
	24 hours				48 hours			
	Aa	B ^a	C ^a	D ^a	A ^a	B ^a	C ^a	D ^a
Dilution Water Control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
pH 9.0 Water Control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
7.2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
15	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
30	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
61	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
130	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

^a A – D represent replicate test vessels containing 5 daphnids each (total 20 daphnids per concentration) at test start.

Conclusions:

The 48-hour EC₅₀ based on mean, measured test concentrations in *Daphnia magna* was >130 mg Bensulfuron methyl/L.

B.9.2.4.2 Acute toxicity to aquatic invertebrates – metabolites of Bensulfuron methyl

21) IN-15297: Static, acute, 48-hour EC₅₀ to *Daphnia magna*. Gries, T., 2002.

Cross reference IIA, 8.2.4./03

Guidelines:

USEPA 72-2 (1982); OECD 202 (1984); Directive 92/69/EEC; EEC Method C.2 (1992)

GLP:

Yes

The study is acceptable

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Materials and methods:

Test Substance: IN-N5297 technical metabolite

Batch: N5297-004

Purity: 99.5%

The acute toxicity of IN-N5297 to unfed *Daphnia magna* (<24-hour old) was determined in an unaerated, static, 48-hour test. Treatments consisted of a dilution water control and nominal concentrations of 7.5, 15, 30, 60, and 120 mg IN-N5297/L. Five daphnids were used per replicate with four replicates per test concentration and control.

Findings:

A summary of the findings is presented in Table B.9.47. Mean, measured concentrations of IN-N5297 were 7.04, 15.0, 30.0, 59.3, and 117 mg IN-N5297/L and ranged from 94 to 101% of nominal concentrations. No immobility or sublethal effects were seen in the dilution water control. Immobilisation after 48 hours of exposure was 10, 10, 0, 0, and 10% at mean, measured concentrations of 7.04, 15.0, 30.0, 59.3, and 117 mg IN-N5297/L, respectively. After 2 days of exposure, one erratic swimming daphnid was observed in the 30.0 mg IN-N5297/L treatment level and one pale daphnid was observed at the mean, measured concentration of 59.3 mg IN-N5297/L. These effects showed no dose response relationship and were thus not considered to be test item related.

Table B.9.47: Summary of observed immobility and sublethal effects of unfed *Daphnia magna* exposed to IN-N5297 for 48 hours in an unaerated, static, acute test

Mean, measured concentrations of IN-N5297 (mg a.i./L)	Immobility (No. immobile/No. at test start)							
	24 hours				48 hours			
	A ^a	B ^a	C ^a	D ^a	A ^a	B ^a	C ^a	D ^a
Dilution water control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
7.04	0/5	0/5	0/5	1/5	0/5	0/5	0/5	2/5
15.0	1/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5
30.0	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0 ^{1-b} /5
59.3	0/5	0/5	0/5	0/5	0/5	0/5	0 ^{1-c} /5	0/5
117	0/5	1/5	1/5	0/5	0/5	1/5	1/5	0/5

^a A – D represent replicate test vessels containing 5 daphnids.

^b Daphnid with erratic swimming, superscript numbers indicate the number of daphnids with this sublethal effect.

^c Daphnid pale in colour, superscript numbers indicate the number of daphnids with this sublethal effect.

Conclusions:

The 48-hour EC₅₀ in *Daphnia magna* was >117 mg IN-N5297/L.

22) IN-J0290: Static, acute, 48-hour EC₅₀ to *Daphnia magna*. Hoke, R.A., 2002.

Cross reference IIA, 8.2.4./04

Guidelines:

USEPA 72-2 (1982); OECD 202 (1984); Directive 92/69/EEC, EEC Method C.2 (1992)

GLP:

Yes

The study is acceptable

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Materials and methods:

Test Substance: IN-J0290 technical metabolite

Batch: J0290-17

Purity: 99.6%

The acute toxicity of IN-J0290 to unfed *Daphnia magna* (<24-hour old) was determined in an unaerated, static, 48-hour test. Treatments consisted of a dilution water control and nominal concentrations of 6.3, 12.5, 25, 50, and 100 mg IN-J0290/L. Five daphnids were used per replicate with four replicates per test concentration and control.

Findings:

A summary of the findings is presented in Table B.9.48 and Table B.9.49. Mean, measured concentrations of IN-J0290 were not detected, 6.4, 13, 26, 52, and 100 mg a.i./L and ranged from 100 to 104% of nominal concentrations. No immobility or sublethal effects were observed in the water control daphnids. The highest mean, measured concentration causing no immobility at test end was 100 mg/L. There was 10% immobility in the 26 mg/L mean, measured test concentration group. At test end, floating test organisms were observed at the 13 and 26 mg/L mean, measured test concentrations and lethargic test organisms were observed in the 26, 52, and 100 mg/L mean, measured test concentrations.

Table B.9.48: Summary of observed immobility of unfed *Daphnia magna* exposed to IN-J0290 for 48 hours in an unaerated, static, acute test

Mean, measured concentrations of IN-J0290 (mg a.i./L)	Number immobile/Number at test start							
	24 hours				48 hours			
	A ^a	B ^a	C ^a	D ^a	A ^a	B ^a	C ^a	D ^a
Dilution water control	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
6.4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
13	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
26	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5
52	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
100	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

^aReplicate test chambers containing 5 daphnids each (total 20 daphnids per concentration) at test start.

Table B.9.49: Summary of observed sublethal effects of unfed *Daphnia magna* exposed to IN-J0290 for 48 hours in an unaerated, static, acute test

Mean, measured concentrations of IN-J0290 (mg a.i./L)	Number affected / Number alive							
	24 hours				48 hours			
	A ^a	B ^a	C ^a	D ^a	A ^a	B ^a	C ^a	D ^a
Dilution water control	0/5	2 ^b /5	0/5	0/5	0/5	0/5	0/5	0/5
6.4	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
13	0/5	0/5	0/5	2 ^b /5	0/5	0/5	0/5	3 ^b /5
26	3 ^b /5	5 ^b /5	0/5	0/5	1 ^b /3	1 ^c /5	0/5	0/5
52	0/5	0/5	0/5	1 ^c /5	0/5	0/5	0/5	1 ^c /5
100	0/5	0/5	0/5	0/5	0/5	0/5	1 ^c /5	0/5

^aReplicate test chambers containing 5 daphnids each (total 20 daphnids per concentration) at test start.

^bDaphnid floating at surface

^cDaphnid lethargic

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Conclusions:

The 48-hour EC₅₀ for *Daphnia magna* was >100 mg IN-J0290/L.

B.9.2.5 Chronic toxicity to aquatic invertebrates (Annex IIA 8.2.5)

B.9.2.5.1 Chronic toxicity to aquatic invertebrates – Bensulfuron methyl

23) *Bensulfuron methyl (DPX-F5384) technical: 21 day chronic, static-renewal toxicity to Daphnia magna. Samel, A., 2002.*

Cross reference IIA, 8.2.5./01

Guidelines:

OECD 211; EPA 850.1300

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-333

Purity: 98.66%

The effects of Bensulfuron methyl on the growth and reproduction of *Daphnia magna* (<24-hour old) were assessed in an unaerated, static-renewal, 21-day test. Treatments consisted of a dilution water control and nominal concentrations of 3.0, 6.0, 12.5, 25, and 50 mg Bensulfuron methyl/L. Ten replicates were used per treatment with one daphnid per replicate. Test concentrations were renewed every Monday, Wednesday, and Friday or 48 hours. The daphnids were fed YCT (yeast-cereal leaves-trout chow) and *Selenastrum capricornutum* daily (<0.2 mg total organic carbon per daphnid daily). Test solutions were maintained between 19.7 and 20.4°C.

Findings:

Analytical verification of Bensulfuron methyl concentrations was made on test solutions sampled on Day 0 and at regular intervals during the study. Mean, measured concentrations of Bensulfuron methyl were 3.2, 6.1, 12, 24, and 47 mg a.i./L and ranged from 94 to 107% of nominal concentrations. All chemical and physical parameters for the 21-day study were within acceptable ranges.

A summary of percent adult survival, first day of reproduction, total live young produced per surviving female, total immobile young produced per surviving female, and length and dry weight of surviving adults is presented in Table B.9.50. The 21-day NOEC (no observable effect concentration) for Bensulfuron methyl using *Daphnia magna* neonates exposed for 21 days under static-renewal conditions based on mean, measured concentrations and adult immobility at the end of the study was 12 mg/L. The LOEC (lowest observed effect concentration) based on mean, measured concentrations and adult immobility at the end of the study was 24 mg/L. The EC₅₀ for adult survival was greater than 46.1 mg/L.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.50: Summary of test endpoints following exposure of *Daphnia magna* to bensulfuron methyl for 21 days

Mean, measured concentrations of DPX-F5384 (mg a.i./L)	Mean % adult survival ^a	Mean first day of reproduction ^b	Mean total live young ^c	Mean total immobile young ^d	Mean adult length (mm)	Mean adult dry weight (mg) ^e
Water control ^f	100	10.1 (0.9)	63.8 (46.4)	0	3.89 (0.84)	0.5 (0.2)
3.2	100	9.4 (0.9)	81.2 (30)	0	4.27(0.49)	0.5(0.1)
6.1	100	9.6 (0.9)	79.9 (42.4)	0	4.13(0.75)	0.6(0.1)
12	100	9.7 (1.1)	60.4 (33.4)	0	3.87(0.64)	0.4(0.1)
24	40 ^g	9.7 (1.0)	59.0 (51.0)	0	3.93(0.10)	0.4(0.1)
47	70 ^g	9.9 (0.8)	31.3 (14.2) ^g	0.3 (0.5) ^g	3.24(0.45) ^g	0.2(0.0) ^g

^a Percent of adult daphnids alive at the end of the test (immobility was synonymous with death) (SD)

^b First day that reproduction was observed in the replicates (SD)

^c Mean of live young produced per surviving female (SD)

^d Mean of immobile young produced per surviving female (SD)

^e Per surviving adult (SD)

^f Haskell laboratory well water

^g Statistically different from controls (Jonckheere-Terpstra was used for all except for adult mobility which used Cochran-Armitage)

Conclusions:

The 21-day NOEC (no observable effect concentration) for Bensulfuron methyl, based on mean, measured concentrations and adult immobility at the end of the study was 12 mg/L for *Daphnia magna* neonates exposed for 21 days under unaerated, static-renewal conditions.

B.9.2.5.2 Chronic toxicity to aquatic invertebrates – metabolites of Bensulfuron methyl

24) IN-J0290: 21-Day Chronic Toxicity to *Daphnia magna*. Samel, A., 2002.

Cross reference IIA, 8.2.5./02

Guidelines:

USEPA 72-4 (1982), OPPTS 850.1300 (1996), OECD 211 (1998)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-J0290 technical metabolite

Batch: J0290-17

Purity: 99.6%

The effects of IN-J0290 on the growth and reproduction of *Daphnia magna* (24-hour old) were assessed in an unaerated, static-renewal, 21-day test. Treatments consisted of a dilution water control and nominal concentrations of 3.0, 6.3, 12.5, 25, and 50 mg IN-J0290/L. Ten replicates were used per treatment, with one daphnid per replicate. Test concentrations were renewed every Monday, Wednesday, and Friday. The daphnids were fed YCT (yeast-cereal leaves-trout chow) and *Selenastrum capricornutum* daily (0.1-0.2 mg total organic carbon per daphnid daily). Test solutions were maintained between 20.3 and 21.0°C.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Findings:

Analytical verification of IN-J0290 concentrations was made on test solutions sampled on Day 0 and at regular intervals during the study. Mean, measured concentrations of IN-J0290 were 3.0, 6.3, 12.4, 24.9, and 49.9 mg a.i./L and ranged from 99.2 to 100% of nominal concentrations. All chemical and physical parameters for the 21-day study were within acceptable ranges.

A summary of percent adult survival, first day of reproduction, total live young produced per surviving female, total immobile young produced per surviving female, and length and dry weight of surviving adults is presented in Table B.9.51.

Table B.9.51: Summary of test endpoints following exposure of *Daphnia magna* to IN-J0290 for 21 days

Mean, measured concentrations of IN-J0290 (mg a.i./L)	Mean % adult survival ^a	Mean first day of reproduction ^b	Mean total live youngc	Mean total immobile youngd	Mean adult length (mm) ^e	Mean adult dry weight (mg) ^e
Water control	100	8.6	272.5	0.0	5.10	0.6
3.0	100	8.9	253.4	0.0	5.13	0.8
6.3	100	8.7	244.1	0.0	5.09	0.8
12.4	100	8.6	244.9	0.0	5.13	0.8
24.9	90	8.7	236.9	0.1	5.11	0.7
49.9	90	8.7	207.8 ^f	0.3 ^f	5.09	0.8

^a Percent of adult daphnids alive at the end of the test

^b First day that reproduction was observed

^c Live young produced per surviving adult in 21 days

^d Immobile young produced per surviving adult in 21 days

^e Per surviving adult

^f Statistically different from controls (Jonckheere-Terpstra)

Conclusions:

The 21-day NOEC (no observable effect concentration) and LOEC (lowest observable effect concentration) for IN-J0290, based on mean, measured concentrations and total and immobilised young at 21 days, were 24.9 and 49.9 mg/L, respectively, for *Daphnia magna* exposed to IN-J0290 for 21 days under unaerated, static-renewal conditions.

25) *IN-N5297: 21-day chronic toxicity to Daphnia magna. Samel, A., 2002.*

Cross reference IIA, 8.2.5./03

Guidelines:

OPPTS 850.1300 (1996), OECD 211 (1998)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-N5297 technical metabolite

Batch: N2597-004

Purity: 99.5%

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

The effects of IN-N5297 on the growth and reproduction of *Daphnia magna* (<24-hour old) were assessed in an unaerated, static-renewal, 21-day test. Treatments consisted of a dilution water control and nominal concentrations of 3.0, 6.3, 12.5, 25, and 50 mg/L IN-N5297/L. Ten replicates were used per treatment, 10 replicates contained 1 daphnid per replicate. Test concentrations were renewed every Monday, Wednesday, and Friday or 48 hours. The daphnids were fed YCT (yeast-cereal leaves-trout chow) and *Selenastrum capricornutum* daily (0.1-0.2 mg total organic carbon per daphnid daily). Test solutions were maintained between 20.5 and 20.9°C.

Findings:

Analytical verification of IN-N5297 concentrations was made on test solutions sampled on day 0 and at regular intervals during the study. Mean, measured concentrations of IN-N5297 were 3.09, 6.43, 12.7, 25.2, and 50.3 mg IN-N5297/L and ranged from 101 to 103% of nominal concentrations. All chemical and physical parameters for the 21-day study were within acceptable ranges.

A summary of percent adult survival, first day of reproduction, total live young produced per surviving female, total immobile young produced per surviving female, and length and dry weight of surviving adults is presented in Table B.9.52.

Table B.9.52: Summary of test endpoints following exposure of *Daphnia magna* to IN-N5297 for 21 days

Mean, measured concentrations of IN-N5297 (mg a.i./L)	Mean % adult survival ^a	Mean first day of reproduction ^b	Mean total live young ^c	Mean total immobile young ^d	Mean adult length (mm) ^e	Mean adult dry weight (mg) ^e
Water Control	90	8.0	214.7	0	5.21	1.0
3.09	100	8.1	188.9	0	5.15	1.0
6.43	90	8.1	209.0	0	5.16	0.9
12.7	90	8.2	195.6	0	5.16	1.1
25.2	90	8.0	188.8 ^f	0	5.07	0.8
50.3	100	8.6	181.2 ^f	0	4.99 ^f	0.9

^a Percent of adult daphnids alive at the end of the test

^b First day that reproduction was observed

^c Live young produced per surviving adult in 21 days

^d Immobile young produced per surviving adult in 21 days

^e Per surviving adult

^f Statistically different from controls (Jonckheere-Terpstra)

Conclusions:

The NOEC (no observable effect concentration) and LOEC (lowest observable effect concentration), for IN-N5297, based on mean, measured concentrations and total live young at 21 days, was 12.7 and 25.2 mg/L for *Daphnia magna* exposed to IN-N5297 for 21 days.

B.9.2.6 Effects on algal growth (Annex IIA 8.2.6)

B.9.2.6.1 Effects on algal growth – Bensulfuron methyl

- 26) Bensulfuron methyl (DPX-F5384) technical: Influence on growth and growth rate of the green alga *Selenastrum capricornutum*. Sloman, T.L., 2001a.

Cross reference IIA, 8.2.6./03

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Guidelines:

OECD 201 (1984); Directive 92/69/EC; EEC Method C3 (1992)

GLP:

Yes

*The study is acceptable*Materials and methods:

Test Substance: DPX-F5483 technical

Batch: F5384-333

Purity: 98.66%

The effect of Bensulfuron methyl on *Selenastrum capricornutum* was determined using algal cultures with AAP nutrient medium. Three replicates at 10, 20, 40, 80, and 160 µg a.i./L measured at test initiation, were incubated for 72 hours and cell counts were taken at 24-hour intervals.

Recovery: The ability of algae to recover from exposure to Bensulfuron methyl was assessed by transferring cells to fresh, untreated AAP media and monitoring cell growth for another 6 days. Recovery was evaluated for all concentrations exhibiting >50% inhibition after 120 h exposure in the definitive test.

Findings:

The effects of Bensulfuron methyl on the growth of *Selenastrum capricornutum* are shown in Table B.9.53.

Recovery: Algal cells previously exposed to media containing up to 160 µg a.i./L were able to resume logarithmic growth within 6 days of transferring them to fresh, un-treated media (Table B.9.54). This demonstrates that the effects of Bensulfuron methyl on algae are reversible.

Table B.9.53: Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to Bensulfuron methyl for 120 hours

Test concentration (µg a.i./L)	Mean cell density (cells/mL)	% Inhibition		
		Cell density	Growth rate	Area under the growth curve
Water control	2.6×10^6	—	—	—
10	2.0×10^6	21.34	4.32	24.92
20	1.3×10^6	48.71	12.05	48.92
40	8.0×10^5	69.15	21.20	71.47
80	3.3×10^4	98.71	78.45	97.43
160	3.0×10^4	98.84	83.33	98.97

Table B.9.54: Recovery of algal growth inhibition following exposure of *Selenastrum capricornutum* to Bensulfuron methyl for 120 hours

Previous exposure concentration (µg a.i./L)	Cell count (cells/mL)		
	Day 0	Day 3	Day 6
Water control	2.6×10^4	4.83×10^6	7.9×10^6
40	2.4×10^4	1.27×10^6	5.8×10^6
80	1000	4.2×10^5	5.58×10^6
160	900	6×10^4	5.08×10^6

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Conclusions:

Growth inhibition data obtained with Bensulfuron methyl on *Selenastrum capricornutum* were as follows:

Cell density:	72-hour EC ₅₀ = 21.4 µg a.i./L
	72-hour NOEC < 10 µg a.i./L
Area under the growth curve:	72-hour EC ₅₀ = 20.4 µg a.i./L
	72-hour NOEC < 10 µg a.i./L
Growth rate:	72-hour EC ₅₀ = 57.4 µg a.i./L
	72-hour NOEC < 10 µg a.i./L

The effects of Bensulfuron methyl on *Selenastrum capricornutum* are expected to be reversible at concentrations ≤160 µg a.i./L.

27) *Bensulfuron methyl (DPX-F5384) technical: Influence on growth and growth rate of the blue-green alga Anabaena flos-aquae. Sloman, T.L., 2001b.*

Cross reference IIA, 8.2.6./04

Guidelines:

OPPTS 850.5400 (1996)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-333

Purity: 98.66%

The effect of Bensulfuron methyl technical on *Anabaena flos-aquae* was determined using algal cultures with AAP nutrient medium. Three replicates at 31.25, 62.5, 125, 250, and 500 µg/L, measured at test initiation, were incubated for 96 hours and cell counts were taken at 24-hour intervals.

Recovery: The ability of *Anabaena* to recover from exposure to Bensulfuron methyl was assessed by transferring cells to fresh, untreated AAP media and monitoring cell growth for 6 days. Recovery was evaluated for all concentrations exhibiting >50% inhibition after 96 h exposure in the definitive test.

Findings:

The effects of Bensulfuron methyl technical on the growth of *Anabaena flos-aquae* are shown in Table B.9.55.

Recovery: *Anabaena* cells previously exposed to media containing up to 500 µg a.i./L were able to resume logarithmic growth within 6 days of transferring them to fresh, un-treated media. This demonstrates that the effects of Bensulfuron methyl on *Anabaena* are reversible.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.55: Summary of algal growth inhibition following exposure of *Anabaena flos-aquae* to Bensulfuron methyl technical for 96 hours

Test concentration (µg a.i./L)	Mean cell density (cells/mL)	% Inhibition		
		Cell density	Growth rate	Area under the growth curve
Water control	2.4×10^6	—	—	—
31.25	2.2×10^6	6.57	1.29	21.82
62.5	6.1×10^5	74.55	25.02	80.02
125	4.0×10^4	98.32	77.26	85.73
250	2.3×10^4	99.02	87.38	97.24
500	2.0×10^4	99.16	89.13	94.75

Conclusions:

Growth inhibition data obtained with Bensulfuron methyl technical on *Anabaena flos-aquae* were as follows:

Cell density:	96-hour $EC_{50} = 50.6 \mu\text{g a.i./L}$ 96-hour NOEC < $31.25 \mu\text{g a.i./L}$
Area under the growth curve:	96-hour $EC_{50} = 44.1 \mu\text{g a.i./L}$ 96-hour NOEC < $31.25 \mu\text{g a.i./L}$
Growth rate:	96-hour $EC_{50} = 88.5 \mu\text{g a.i./L}$ 96-hour NOEC < $31.25 \mu\text{g a.i./L}$

The effects of Bensulfuron methyl technical on *Anabaena flos-aquae* are expected to be reversible at concentrations $\leq 500 \mu\text{g a.i./L}$.

B.9.2.6.2 Effects on algal growth – metabolites of Bensulfuron methyl

- 28) *IN-D1R84: Influence on growth and growth rate of the green algal Selenastrum capricornutum. Boeri, R.L., Wyskiel, D.C., Ward, T.J., 2002.*

Cross reference IIA, 8.2.6./01

Guidelines:

OECD 201; EPA 123-2

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-D1R84 technical metabolite

Batch: D1R84-001

Purity: 98.9%

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

The effect of IN-D1R84 on *Selenastrum capricornutum* was determined using algal cultures with AAP nutrient medium. Three replicates at 3.20, 6.41, 12.8, 24.8, and 49.4 mg/L, measured at test initiation, were incubated for 72 hours and cell counts were taken at 24-hour intervals.

Recovery: The ability of algae to recover from exposure to IN-D1R84 was assessed by transferring cells to fresh, untreated AAP media and monitoring cell growth for 4 days. Recovery was evaluated for all concentrations exhibiting >50% inhibition after 72 h exposure in the definitive test.

Findings:

The effects of IN-D1R84 on the growth and growth rate of *Selenastrum capricornutum* are shown in Table B.9.56.

Recovery: Algal cells previously exposed to media containing 49.4 mg IN-D1R84/L were able to resume logarithmic growth within 4 days of transferring them to fresh, un-treated media. This demonstrates that the effects of IN-D1R84 on algae are reversible.

Table B.9.56: Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to IN-D1R84 for 72 hours

Test concentration (mg a.i./L)	Mean cell density (cells/mL)	% Inhibition		
		Cell density	Growth rate	Area under the growth curve
Water control	1.86×10^6	—	—	—
3.20	1.95×10^6	0	0	0
6.41	1.87×10^6	0	0	0
12.8	1.77×10^6	5	1	6
24.8	1.71×10^6	8	2	7
49.4	$<1.10 \times 10^4$	>99 ^a	>98 ^a	>99 ^a

^aSignificantly different from control by the ANOVA and Bonferroni's test, $p < 0.05$.

Conclusions:

Growth inhibition data obtained with IN-D1R84 on *Selenastrum capricornutum* were as follows:

Cell density:	72-hour $EC_{50} = 37.5$ mg a.i./L
	72-hour NOEC = 6.41 mg a.i./L
Area under the growth curve:	72-hour $EC_{50} = 37.5$ mg a.i./L
	72-hour NOEC = 6.41 mg a.i./L
Growth rate:	72-hour $EC_{50} = 36.2$ mg a.i./L
	72-hour NOEC = 6.41 mg a.i./L

The effects of IN-D1R84 on *Selenastrum capricornutum* are expected to be reversible at a concentrations ≤ 49.4 mg a.i./L.

- 29) *IN-N5297: Influence on growth and growth rate of the green alga Selenastrum capricornutum*. Gries, T., 2002.

Cross reference IIA, 8.2.6./02

Guidelines:

USEPA 122-2 & 123-2 (1982); OECD 201 (1984); Directive 92/69/EEC, EEC Method C3 (1992)

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

GLP:

Yes

*The study is acceptable*Materials and methods:

Test Substance: IN-N5297 technical metabolite

Batch: N5297-004

Purity: 99.5%

The effect of IN-N5297 on *Selenastrum capricornutum* was determined using algal cultures with AAP nutrient medium, incubated in an incubator at $24 \pm 2^\circ\text{C}$ for 72 hours. Because of the insensitivity of *Selenastrum capricornutum* to IN-N5297, six replicates at one nominal concentration, 120 mg/L, (129 mg a.i./L measured at test initiation), were tested. Cell counts were taken at 24-hour intervals.

Findings:

The effects of IN-N5297 on the growth and growth rate of *Selenastrum capricornutum* are shown in Table B.9.57.

Table B.9.57: Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to IN-N5297 for 72 hours

Test concentration (mg a.i./L)	Mean cell density (cells/mL)	% Inhibition relative to control		
		Cell density	Area under the growth curve	Growth rate
blank control	0.647×10^6	—	—	—
120	0.681×10^6	-5.2	-3.7	-4.6

Not statistically different from control (t-test, $\alpha = 0.05$)

Conclusions:

Growth inhibition values obtained with IN-N5297 on *Selenastrum capricornutum* were as follows:

Cell density: 72-hour $\text{EC}_{50} > 120 \text{ mg/L}$
 72-hour NOEC = 120 mg/L

Area under the growth curve: 72-hour $\text{EC}_{50} > 120 \text{ mg/L}$
 72-hour NOEC = 120 mg/L

Growth rate: 72-hour $\text{EC}_{50} > 120 \text{ mg/L}$
 72-hour NOEC = 120 mg/L

30) IN-J0290: Influence on growth and growth rate of the green alga *Selenastrum capricornutum*. Sloman, T.L., 2002a.

Cross reference IIA, 8.2.6./05

Guidelines:

OECD 201 (1984); Directive 92/69/EEC, EEC Method C3 (1992)

GLP:

Yes

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

The study is acceptable

Materials and methods:

Test Substance: IN-J0290 technical metabolite

Batch: J0290-17

Purity: 99.6%

The effect of IN-J0290 on *Selenastrum capricornutum* was determined using algal cultures with AAP nutrient medium. Three replicates at 7.5, 15, 30, 60, and 120 mg/L, measured at test initiation, were incubated in an incubator at $24 \pm 2^\circ\text{C}$ for 72 hours and cell counts were taken at 24-hour intervals.

Recovery: The ability of algae to recover from exposure to IN-J0290 was assessed by transferring cells to fresh, untreated AAP media and monitoring cell growth for 72 h. Recovery was evaluated for all concentrations exhibiting >50% inhibition after 72 h exposure in the definitive test.

Findings:

The effects of IN-J0290 on the growth and growth rate of *Selenastrum capricornutum* are shown in Table B.9.58.

Recovery: Algal cells previously exposed to media containing up to 120 mg IN-J0290/L were able to resume logarithmic growth within 72 hours of transferring them to fresh, un-treated media. This demonstrates that the effects of IN-J0290 on algae are reversible.

Table B.9.58: Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to IN-J0290 for 72 hours

Test concentration (mg a.i.)/L	Mean cell density (cells/mL)	% Inhibition relative to control		
		Cell density	Area under the growth curve	Growth rate
blank control	4.3×10^6	—	—	—
7.5	4.4×10^6	-4.23	8.05	-0.67
15	3.5×10^6	18.18 ^a	21.06 ^a	3.33 ^a
30	2.8×10^6	33.23 ^a	37.58 ^a	6.66 ^a
60	3.3×10^6	23.35 ^a	19.82 ^a	4.44 ^a
120	2.0×10^6	52.82 ^a	55.24 ^a	12.45 ^a

^aStatistically different from controls (ANOVA, Williams' test, $\alpha = 0.05$) based on analysis of mean cell density, growth rate, and area under the curve.

Conclusions:

Growth inhibition values obtained with IN-J0290 on *Selenastrum capricornutum* were as follows:

Cell density: 72-hour $\text{EC}_{50} > 60 \text{ mg/L}$
 72-hour NOEC = 7.5 mg/L

Area under the growth curve: 72-hour $\text{EC}_{50} > 60 \text{ mg/L}$
 72-hour NOEC < 7.5 mg/L

Growth rate: 72-hour $\text{EC}_{50} > 120 \text{ mg/L}$
 72-hour NOEC = 7.5 mg/L

The effects of IN-J0290 on *Selenastrum capricornutum* are expected to be reversible at concentrations $\leq 120 \text{ mg/L}$.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

31) *IN-R9419: Influence on growth and growth rate of the green alga Selenastrum capricornutum. Sloman, T.L., 2002b.*

Cross reference IIA, 8.2.6./06

Guidelines:

OECD Guideline Number 201 & Directive 92/69/EEC Method C3 (1992)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-R9419 technical metabolite

Batch: R9419-002

Purity: 98.5%

The effect of IN-R9419 on *Selenastrum capricornutum* was determined using algal cultures with AAP nutrient medium. Three replicates at 15, 25, 35, 45, and 55 mg IN-R9419/L, measured at test initiation, were incubated in an incubator at $24 \pm 2^\circ\text{C}$ for 72 hours and cell counts were taken at 24 hour intervals.

To assess recovery after the initial 72 hours exposure period, algae from concentrations 35, 45, and 55 mg/L exhibiting >50% inhibition were placed in nutrient medium without IN-R9419. Cell counts were taken every 3 days for up to 9 days.

Recovery: The ability of algae to recover from exposure to IN-R9419 was assessed by transferring cells to fresh, untreated AAP media and monitoring cell growth for 6 days. Recovery was evaluated for all concentrations exhibiting >50% inhibition after 72 h exposure in the definitive test.

Findings:

The effects of IN-R9419 on the growth and growth rate of *Selenastrum capricornutum* are shown in Table B.9. 59.

Recovery: Algal cells previously exposed to media containing up to 55 mg IN-R9419/L were able to resume logarithmic growth within 6 days of transferring them to fresh, un-treated media. This demonstrates that the effects of IN-R9419 on algae are reversible.

Table B.9. 59: Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to IN-R9419 for 72 hours

Test concentration (mg a.i./L)	Mean cell density (cells/mL)	% Inhibition relative to control		
		Cell density	Area under the growth curve	Growth rate
blank control	2.4×10^6	—	—	—
15	2.5×10^6	-2.60	-10.95	-0.52
25	1.5×10^6	40.44 ^a	40.74	9.44
35	2.1×10^5	91.53 ^a	89.87	45.00
45	5.7×10^4	97.68 ^a	96.32	68.63
55	2.0×10^4	99.18 ^a	98.06 ^a	89.12 ^a

^aStatistically different from controls (ANOVA, Williams' test, $\alpha = 0.05$) based on analysis of mean cell density, growth rate, and area under the curve.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Conclusions:

Growth inhibition values obtained with IN-R9419 on *Selenastrum capricornutum* were as follows:

Cell density:	72-hour EC ₅₀ = 26.3 mg/L
	72-hour NOEC = 15 mg/L
Area under the growth curve:	72-hour EC ₅₀ = 26.4 mg/L
	72-hour NOEC = 15 mg/L
Growth rate:	72-hour EC ₅₀ = 37.3 mg/L
	72-hour NOEC = 15 mg/L

The effects of IN-R9419 on *Selenastrum capricornutum* are expected to be reversible at concentrations ≤55 mg/L.

32) *IN-T5831: Influence on growth and growth rate of the green alga Selenastrum capricornutum. Sloman, T. L., 2002c.*

Cross reference IIA, 8.2.6./07

Guidelines:

OECD 201 (1984); Directive 92/69/EEC, EEC Method C3 (1992)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-T5831 technical metabolite

Batch: T5831-4

Purity: 93.7%

The effect of IN-T5831 on *Selenastrum capricornutum* was determined using algal cultures with AAP nutrient medium. Three replicates at 7.5, 15, 30, 60, and 120 mg IN-T5831/L, measured at test initiation, were incubated in an incubator at 24 ± 2°C for 72 hours and cell counts were taken at 24 hour intervals.

Recovery: The ability of algae to recover from exposure to IN-T5831 was assessed by transferring cells to fresh, untreated AAP media and monitoring cell growth for 6 days. Recovery was evaluated for all concentrations exhibiting >50% inhibition after 72 h exposure in the definitive test.

Findings:

The effects of IN-T5831 on the growth and growth rate of *Selenastrum capricornutum* are shown in Table B.9.60.

Recovery: Algal cells previously exposed to media containing up to 120 mg IN-T5831/L were able to resume logarithmic growth within 6 days of transferring them to fresh, un-treated media. This demonstrates that the effects of IN-T5831 on algae are reversible.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.60: Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to IN-T5831 for 72 hours

Test concentration (mg a.i./L)	Mean cell density (cells/mL)	% Inhibition relative to control		
		Cell density	Area under the growth curve	Growth rate
blank control	4.1×10^6	—	—	—
7.5	3.8×10^6	6.72	8.95	1.16
15	3.5×10^6	13.35	16.19	2.40
30	3.1×10^6	22.85	30.55	4.31
60	1.6×10^6	61.26 ^a	66.19 ^a	16.10 ^a
120	8.7×10^4	97.87 ^a	95.19 ^a	64.56 ^a

^a Statistically different from controls (ANOVA, Williams' test, $\alpha = 0.05$) based on analysis of mean cell density, growth rate, and area under the curve.

Conclusions:

Growth inhibition values obtained with IN-T5831 on *Selenastrum capricornutum* were as follows:

Cell density: 72-hour $EC_{50} = 46.9$ mg/L
72-hour NOEC < 7.5 mg/L

Area under the growth curve: 72-hour $EC_{50} = 41.1$ mg/L
72-hour NOEC < 7.5 mg/L

Growth rate: 72-hour $EC_{50} = 99.1$ mg/L
72-hour NOEC < 7.5 mg/L

The effects of IN-T5831 on *Selenastrum capricornutum* are expected to be reversible at concentrations ≤ 120 mg/L.

33) *IN-F7880: Influence on growth and growth rate of the green alga Selenastrum capricornutum. Sloman, T.L., 2002d.*

Cross reference IIA, 8.2.6./08

Guidelines:

OECD 201 (1984); Directive 92/69/EEC, EEC Method C3 (1992)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-F7880 technical metabolite

Batch: F7880-004

Purity: 97.3%

The effect of IN-F7880 on *Selenastrum capricornutum* was determined using algal cultures with AAP nutrient medium. Three replicates at 4, 8, 16, 32, and 64 mg IN-F7880/L, measured at test initiation, were incubated in an incubator at $24 \pm 2^\circ\text{C}$ for 72 hours and cell counts were taken at 24 hour intervals.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Recovery: The ability of algae to recover from exposure to IN-F7880 was assessed by transferring cells to fresh, untreated AAP media and monitoring cell growth for 6 days. Recovery was evaluated for all concentrations exhibiting >50% inhibition after 72 h exposure in the definitive test.

Findings:

The effects of IN-F7880 on the growth and growth rate of *Selenastrum capricornutum* are shown in Table B.9.61.

Recovery: Algal cells previously exposed to media containing up to 64 mg IN-F7880/L were able to resume logarithmic growth within 6 days of transferring them to fresh, un-treated media. This demonstrates that the effects of IN-F7880 on algae are reversible.

Table B.9.61: Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to IN-F7880 for 72 hours

Test concentration mg a.i./L	Mean cell density (cells/mL)	% Inhibition relative to control		
		Cell density	Area under the growth curve	Growth rate
blank control	2.7×10^6	—	—	—
4	3.4×10^6	-29.3	-20.8	-4.6
8	1.9×10^6	29.6	31.8	6.5
16	1.2×10^6	56.8 ^a	56.7	15.1
32	1.6×10^5	94.0 ^a	90.0	50.3 ^a
64	3.0×10^4	98.9 ^a	99.2 ^a	82.1 ^a

^aStatistically different from controls (ANOVA, Williams' test, $\alpha = 0.05$) based on analysis of mean cell density, growth rate, and area under the curve.

Conclusions:

Growth inhibition values obtained with IN-F7880 on *Selenastrum capricornutum* were as follows:

Cell density: 72-hour $EC_{50} = 13.5$ mg/L
72-hour NOEC = 4 mg/L

Area under the growth curve: 72-hour $EC_{50} = 13.5$ mg/L
72-hour NOEC = 4 mg/L

Growth rate: 72-hour $EC_{50} = 32.3$ mg/L
72-hour NOEC = 4 mg/L

The effects of IN-F7880 on *Selenastrum capricornutum* are expected to be reversible at concentrations less than or equal to 64 mg/L.

34) IN-DAT97: Influence on growth and growth rate of the green alga *Selenastrum capricornutum*. Sloman, T.L., 2003.

Cross reference IIA, 8.2.6./09

Guidelines:

OECD 201 (1984), EEC method C.2. (1992)

GLP:

Yes

The study is acceptable

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Materials and methods:

Test Substance: IN-DAT97 technical metabolite

Batch: DAT97-001

Purity: 96%

The effect of IN-DAT97 on *Selenastrum capricornutum* was determined using algal cultures with AAP nutrient medium. Three replicates at 6.25, 12.5, 25, 50, and 100 mg/L measured at test initiation, were incubated in an incubator at $24 \pm 2^\circ\text{C}$ for 72 hours and cell counts were taken at 24 hour intervals.

Recovery: The ability of algae to recover from exposure to IN-F7880 was assessed by transferring cells to fresh, untreated AAP media and monitoring cell growth for 6 days. Recovery was evaluated for all concentrations exhibiting >50% inhibition after 72 h exposure in the definitive test.

Findings:

The effects of IN-DAT97 on the growth and growth rate of *Selenastrum capricornutum* are shown in Table B.9.62.

Recovery: Algal cells previously exposed to media containing up to 100 mg IN-DAT97/L were able to resume logarithmic growth within 6 days of transferring them to fresh, un-treated media. This demonstrates that the effects of IN-DAT97 on algae are reversible.

Table B.9.62: Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to IN-DAT97 for 72 hours

Test concentration rate	Mean cell density (cells/mL)	% Inhibition relative to control		
		Cell density	Area under the growth curve	Growth rate
blank control	2.3×10^6	—	—	—
6.25	2.5×10^6	-9.70	-10.82	-1.68
12.5	2.4×10^6	-3.62	-6.91	-0.62
25	1.4×10^6	37.92 ^a	33.20	8.83 ^a
50	1.3×10^4	99.42 ^a	99.28 ^a	95.76 ^a
100	6.7×10^3	99.71 ^a	100.00 ^a	100.00 ^a

^a Statistically different from controls (Jonckheere-Terpstra, $\alpha = 0.05$)

Conclusions:

Growth inhibition values obtained with IN-DAT97 on *Selenastrum capricornutum* were as follows:

Cell density: 72-hour $\text{EC}_{50} = 26.4 \text{ mg/L}$

72-hour NOEC = 12.5 mg/L

Area under the growth curve: 72-hour $\text{EC}_{50} = 26.9 \text{ mg/L}$

72-hour NOEC = 12.5 mg/L

Growth rate: 72-hour $\text{EC}_{50} = 33.7 \text{ mg/L}$

72-hour NOEC = 12.5 mg/L

The effects of IN-DAT97 on growth and growth rate of *Selenastrum capricornutum* were found to be algistatic, i.e., logarithmic growth resumed within 144 hours (6 days), at concentrations less than or equal to 100 mg/L.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

B.9.2.7 Effects on sediment dwelling organisms (Annex IIA 8.2.7)

35) *Bensulfuron methyl/metsulfuron methyl (DPX-JM206) 60WG(DF) (29:1): static, acute, 96-hour limit test to the chironomid, Chironomus tentans. Hoke, R.A., 1997.*

Cross reference IIA, 8.2.7./01

Guidelines:

OECD 203 (1992)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-JM206 60WG

Batch: JM206-12

Purity: 600 g a.i./kg

The acute toxicity of DPX-JM206 60WG, containing 59.28% Bensulfuron methyl and 2.12% metsulfuron methyl, to fed chironomid larvae *Chironomus tentans* (approximately 12 days old) was determined in an unaerated, static, 96-hour limit test. Treatments consisted of a dilution water control, and a single nominal concentration of 200 mg DPX-JM206 60WG/L (125 mg Bensulfuron methyl/L). Glass beakers (250 mL) containing 200 mL of test solution (6.4 cm depth) were used as test chambers. Two replicate chambers were used for the control and 6 replicate chambers were used for the DPX-JM206 60WG test solution. Each of the replicate test chambers contained 5 chironomids for a total of 10 chironomids in the control treatment and 30 chironomids in the DPX-JM206 60WG treatment solutions. Mortality and behavioral observations were made every 24 hours. Total alkalinity, EDTA hardness, and conductivity at test start were 79 mg/L CaCO₃, 79 mg/L CaCO₃, and 200 µmhos/cm, respectively. Dissolved oxygen ranged from 7.8 to 8.8 mg/L, pH ranged from 7.7 to 8.2, and the mean temperature was 22.5°C (range 22.3–22.6°C).

Findings:

Mean, measured concentrations of Bensulfuron methyl in the test solutions were 0 (below the limit of detection) in the controls and 125 mg Bensulfuron methyl/L in the nominal, 200 mg DPX-JM206 60WG/L treatment solutions.

A summary of the findings is presented in Table B.9.63. Mean mortality was 20% in the control and 17% at 200 mg DPX-JM206 60WG/L (125 mg/L Bensulfuron methyl) during the 96 h limit test. No sublethal effects were observed in the control or the 200 mg DPX-JM206 60WG/L (125 mg Bensulfuron methyl/L) treatments.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.63: Mortality of the Chironomid, *Chironomus tentans*, exposed to DPX-JM206 60WG in an unaerated, static, acute 96-hour limit test

Mean, measured concentrations of Bensulfuron methyl in the test solutions (mg a.i./L)	Cumulative Mortality (%)							
	24 hours		48 hours		72 hours		96 hours	
	A ^a	B ^a	A ^a	B ^a	A ^a	B ^a	A ^a	B ^a
Dilution Water Control	0	0	0	0	0	0	20	20
125 (replicate C)	0	0	0	0	20	0	40	0
125 (replicate D)	0	0	0	0	0	20	0	40
125 (replicate E)	0	0	0	0	0	0	20	0

^a A and B represent replicate test vessels containing 5 chironomids (total 10 chironomids in the water control, 30 chironomids in the test concentration) at test start.

Conclusions:

The 96-hour EC₅₀ in *Chironomus tentans* was >200 mg DPX-JM206 60WG/L (>125 mg Bensulfuron methyl/L).

B.9.2.8 Aquatic Plants (Annex IIA 8.2.8)

B.9.2.8.1 Aquatic Plants – Bensulfuron methyl

36) *Bensulfuron methyl (DPX-F5384) technical: Influence on growth and reproduction of Lemna gibba G3. Sloman, T.L., 2001.*

Cross reference IIA, 8.2.8./03

Guidelines:

ASTM Standards, Volume 11.04, Method E1415-91 (1991)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-333

Purity: 98.66%

The effect of Bensulfuron methyl technical on *Lemna gibba* G3 was determined in 20X AAP nutrient medium. Four replicates, at nominal concentrations 0.25, 0.5, 1, 2, and 4 µg Bensulfuron methyl/L, were incubated in a chamber for 14 days at 25±2°C and frond counts were taken every 2 or 3 days. Frond count and biomass were determined after 14 days. Bensulfuron methyl was determined to be stable over the course of the definitive test as evidenced by the analytical recoveries obtained from the day 0 and day 14 test solutions.

Recovery: The ability of *Lemna* to recover from exposure to Bensulfuron methyl was assessed by transferring fronds to fresh, untreated nutrient media and monitoring growth for up to 14 days. Recovery was evaluated for all concentrations exhibiting >50% inhibition after 14 d exposure in the definitive test.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Findings:

The effects of Bensulfuron methyl technical on the growth and reproduction of *Lemna gibba* G3 are shown in Table B.9.64.

Recovery: *Lemna* fronds previously exposed to media containing up to 4 µg Bensulfuron methyl/L (the highest concentration tested) were able to resume logarithmic growth within 14 days of transferring them to fresh, un-treated media. This demonstrates that the effects of Bensulfuron methyl on *Lemna* are reversible.

Table B.9.64: Summary of growth inhibition following exposure of *Lemna gibba* G3 to Bensulfuron methyl technical for 14 days

Test concentration rate	14-Day mean frond number	% Inhibition relative to control	14-Day mean biomass (mg)	% Inhibition relative to control
blank control	480	—	42.0	—
0.25	469	2.19	41.5	1.35
0.5	432	10.06	37.1	11.82
1	129 ^a	73.22	22.8 ^a	45.68
2	52 ^a	89.27	15.2 ^a	63.92
4	33 ^a	93.07	12.6 ^a	69.94

^a Significantly different from control by the Williams' test criteria $\alpha = 0.05$.

Conclusions:

Growth inhibition values obtained with Bensulfuron methyl technical on *Lemna gibba* G3 were as follows:

Frond number: 14-day EC₅₀ = 0.8 µg/L

14-day NOEC < 0.25 µg/L

Frond biomass: 14-day EC₅₀ = 1.5 µg/L

14-day NOEC < 0.25 µg/L

The effects of Bensulfuron methyl technical on *Lemna gibba* G3 are expected to be reversible at concentrations up to at least 4 µg/L (the highest concentration tested).

B.9.2.8.2 Aquatic Plants – metabolites of Bensulfuron methyl

- 37) *IN-D1R84: Influence on growth and reproduction of Lemna gibba G3. Boeri, R.L., Wyskiel, D.C., Ward, T.J., 2002.*

Cross reference IIA, 8.2.8./01

Guidelines:

EPA 122-2; EPA 123-2; EPA 850.4400

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-D1R84 technical metabolite

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Batch: D1R84-001

Purity: 98.9%

The effect of IN-D1R84 on *Lemna gibba* G3 was determined in 20X AAP nutrient medium. Three replicates at nominal concentrations of 9.0, 17, 33, 65, and 130 mg/L were incubated for 14 days and frond counts were taken every 3 or 4 days. Frond count and biomass were determined after 14 days. IN-D1R84 was determined to be stable over the course of the definitive test as evidenced by the analytical recoveries obtained from the day 0 and day 14 test solutions. Frond count and biomass were determined for up to 14 days. Recovery after the initial 14-day exposure period was not assessed, as there was greater than 50% of the control growth at all tested concentrations.

Findings:

The effects of IN-D1R84 on the growth and reproduction of *Lemna gibba* are shown in Table B.9.65.

Table B.9.65: Effect of IN-D1R84 on the growth and reproduction of *Lemna gibba*

Nominal concentration (mg a.i./L)	14-Day mean frond number	% Inhibition relative to control	14-Day mean biomass (mg)	% Inhibition relative to control
0	553	—	41.2	—
9.0	836	0	104.9	0
17	1007	0	108.8	0
33	466	16	45.0	0
65	622	0	53.5	0
130	355	36	46.3	0

No treatment values were significantly different from controls by the Dunnett's test criteria, $p < 0.05$.

Conclusions:

Growth inhibition values obtained with IN-D1R84 on *Lemna gibba* were as follows:

Frond number: 14-day $EC_{50} \geq 130$ mg a.i./L

14-day NOEC = 17 mg/L

Frond biomass: 14-day $EC_{50} \geq 130$ mg a.i./L

14-day NOEC = 130 mg/L

38) IN-N5297: Influence on growth and reproduction of *Lemna gibba* G3. Gries, T., 2002c.

Cross reference IIA, 8.2.8./02

Guidelines:

USEPA 122-2 & 123-2 (1982); OPPTS 850.4400; ASTM Standards 11.04 (1991)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-N5297 technical metabolite

Batch: N5297-004

Purity: 99.5%

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

The effect of IN-N5297 on *Lemna gibba* G3 was determined in 20X AAP nutrient medium. Four replicates at 0 (blank control) and nominal 120 mg/L, 120 mg a.i./L measured at test initiation, were incubated in an incubator for 14 days at 25±2°C and frond counts were taken every 2 or 3 days. Frond count and biomass were determined after 14 days.

Findings:

The effects of IN-N5297 on the growth and reproduction of *Lemna gibba* G3 are shown in Table B.9.66.

Table B.9.66: Summary of growth inhibition following exposure of *Lemna gibba* G3 to IN-N5297 for 14 days

Test concentration (mg/L)	14-Day mean frond number	% Inhibition relative to control	14-Day mean biomass increase (mg)	% Inhibition relative to control
Blank control	261	—	24.5	—
120	255	2.4	26.6	-8.3

Conclusions:

Growth inhibition values obtained with IN-N5297 on *Lemna gibba* G3 were as follows:

Frond number: 14-day EC₅₀ > 120 mg/L
 14-day NOEC < 120 mg/L
 Frond biomass: 14-day EC₅₀ > 120 mg/L
 14-day NOEC = 120 mg/L

39) IN-R9419: Influence on growth and reproduction of *Lemna gibba* G3. Sloman, T.L., 2002a.

Cross reference IIA, 8.2.8./04

Guidelines:

ASTM E1415-91 (1991)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-R9419 technical metabolite

Batch: R4919-002

Purity: 98.5%

The effect of IN-R9419 on *Lemna gibba* G3 was determined in 20X AAP nutrient medium. Four replicates, at nominal concentrations of 1, 2, 4, 8, and 16 mg IN-R9419/Litre, were incubated in an incubator for 14 days at 25±2°C and frond counts were taken every 2 or 3 days. IN-R9419 was determined to be stable over the course of the definitive test as evidenced by the analytical recoveries obtained from the day 0 and day 14 test solutions. Frond count and biomass were determined after 14 days.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Recovery: The ability of *Lemna* to recover from exposure to IN-R9419 was assessed by transferring fronds to fresh, untreated nutrient media and monitoring growth for up to 14 days. Recovery was evaluated for all concentrations exhibiting >50% inhibition after 14 d exposure in the definitive test.

Findings:

The effects of IN-R9419 on the growth and reproduction of *Lemna gibba* G3 are shown in Table B.9.67.

Recovery: *Lemna* fronds previously exposed to media containing up to 16 mg Bensulfuron methyl/L were able to resume logarithmic growth within 14 days of transferring them to fresh, un-treated media. This demonstrates that the effects of IN-R9419 on *Lemna* are reversible.

Table B.9.67: Summary of growth inhibition following exposure of *Lemna gibba* G3 to IN-R9419 for 14 days

Test concentration (mg a.i./L)	14-Day mean frond number	% Inhibition relative to control	14-Day mean biomass (mg)	% Inhibition relative to control
blank control	495	—	40.6	—
1	521	-5.36	40.4	0.57
2	478	3.44	40.4	0.66
4	439 ^a	11.17	35.5 ^a	12.63
8	117 ^a	76.39	21.6 ^a	46.76
16	33 ^a	93.43	15.3 ^a	62.26

^aSignificantly different from control by the Williams' test criteria, $\alpha=0.05$.

Conclusions:

Growth inhibition values obtained with IN-R9419 on *Lemna gibba* G3 were as follows:

Frond number: 14-day $EC_{50} = 6.2$ mg/L
 14-day NOEC = 1 mg/L
 Frond biomass: 14-day $EC_{50} = 10.5$ mg/L
 14-day NOEC < 1 mg/L

The effects of IN-R9419 on *Lemna gibba* G3 are expected to be reversible at concentrations ≤ 16 mg/L.

40) IN-T5831: Influence on growth and reproduction of *Lemna gibba* G3. Sloman, T.L., 2002.

Cross reference IIA, 8.2.8./05

Guidelines:

ASTM E1415-91 (1991)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-T5831 technical metabolite

Batch: T5831-4

Purity: 93.7%

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

The effect of IN-T5831 on *Lemna gibba* G3 was determined in 20X AAP nutrient medium. Four replicates, at nominal concentrations of 10, 20, 40, 80 and 160 mg IN-T5831/L of nutrient medium (ppm), were incubated in an incubator for 14 days at 25±2°C and frond counts were taken every 2 or 3 days. IN-T5831 was determined to be stable over the course of the definitive test as evidenced by the analytical recoveries obtained from the day 0 and day 14 test solutions. Frond count and biomass were determined after 14 days.

Recovery: The ability of *Lemna* to recover from exposure to IN-T5831 was assessed by transferring fronds to fresh, untreated nutrient media and monitoring growth for up to 14 days. Recovery was evaluated for all concentrations exhibiting >50% inhibition after 14 d exposure in the definitive test.

Findings:

The effects of IN-T5831 on the growth and reproduction of *Lemna gibba* G3 are shown in Table B.9.68.

Recovery: *Lemna* fronds previously exposed to media containing up to 160 mg Bensulfuron methyl/L (the highest concentration tested) were able to resume logarithmic growth within 14 days of transferring them to fresh, un-treated media. This demonstrates that the effects of IN-T5831 on *Lemna* are reversible.

Table B.9.68: Summary of growth inhibition following exposure of *Lemna gibba* G3 to IN-T5831 for 14 days

Test concentration (mg a.i./L)	14-Day mean frond number	% Inhibition relative to control	14-Day mean biomass (mg)	% Inhibition relative to control
blank control	456	—	42.1	—
10	439	3.67	42.5	-0.95
20	426 ^a	6.69	42.9	-1.90
40	396 ^a	13.10	43.3	-2.93
80	349 ^a	23.46	41.5	1.43
160	149 ^a	67.27	19.5 ^a	53.65

^a Significantly different from control by the Williams' test criteria, $\alpha = 0.05$.

Conclusions:

Growth inhibition values obtained with IN-T5831 on *Lemna gibba* G3 were as follows:

Frond number: 14-day EC₅₀ = 120.9 mg/L

14-day NOEC < 10 mg/L

Frond biomass: 14-day EC₅₀ = 97.9 mg/L

14-day NOEC = 40 mg/L

The effects of IN-T5831 on *Lemna gibba* G3 are expected to be reversible at concentrations ≤160 mg/L.

41) IN-F7880: Influence on growth and reproduction of *Lemna gibba* G3. Sloman, T.L., 2002b.

Cross reference IIA, 8.2.8./06

Guidelines:

ASTM E1415-91 (1991)

GLP:

Yes

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

The study is acceptable

Materials and methods:

Test Substance: IN-F7880 technical metabolite

Batch: F7880-004

Purity: 97.3%

The effect of IN-F7880 on *Lemna gibba* G3 was determined in 20X AAP nutrient medium. Four replicates, at nominal concentrations of 0.5, 1, 1.5, 2.5, and 5 mg/L, were incubated in an incubator for 14 days at 25±2°C and frond counts were taken every 2 or 3 days. IN-F7880 was determined to be stable over the course of the definitive test as evidenced by the analytical recoveries obtained from the day 0 and day 14 test solutions. Frond count and biomass (plant dry weight) were determined after 14 days.

Recovery: The ability of *Lemna* to recover from exposure to IN-F7880 was assessed by transferring fronds to fresh, untreated nutrient media and monitoring growth for up to 14 days. Recovery was evaluated for all concentrations exhibiting >50% inhibition after 14 d exposure in the definitive test.

Findings:

The effects of IN-F7880 on the growth and reproduction of *Lemna gibba* G3 are shown in Table B.9.69.

Recovery: *Lemna* fronds previously exposed to media containing up to 5 mg Bensulfuron methyl/L (the highest concentration tested) were able to resume logarithmic growth within 14 days of transferring them to fresh, un-treated media. This demonstrates that the effects of IN-F7880 on *Lemna* are reversible.

Table B.9.69: Summary of growth inhibition following exposure of *Lemna gibba* G3 to IN-F7880 for 14 days

Test concentration (mg a.i./L)	14-Day mean frond number	% Inhibition relative to control	14-Day mean biomass (mg)	% Inhibition relative to control
blank control	476	—	43.0	—
0.5	487	-2.15	41.9	2.56
1	311 ^a	34.65	26.7 ^a	37.83
1.5	119 ^a	74.96	20.1 ^a	53.26
2.5	63 ^a	86.77	16.7 ^a	61.09
5	31 ^a	93.49	13.5 ^a	68.60

^aSignificantly different from control by the Williams' test criteria, $\alpha=0.05$.

Conclusions:

Growth inhibition values obtained with IN-F7880 on *Lemna gibba* G3 were as follows:

Frond number: 14-day EC₅₀ = 1.18 mg/L

14-day NOEC = 0.5 mg/L

Frond biomass: 14-day EC₅₀ = 1.82 mg/L

14-day NOEC < 0.5 mg/L

The effects of IN-F7880 on *Lemna gibba* G3 are expected to be reversible at concentrations up to at least 5 mg/L.

42) IN-J0290: Influence on growth and reproduction of *Lemna gibba* G3. Sloman, T.L., 2002.

Cross reference IIA, 8.2.8./07

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Guidelines:

ASTM E1415-91 (1991)

GLP:

Yes

*The study is acceptable*Materials and methods:

Test Substance: IN-J0290 technical metabolite

Batch: J0290-17

Purity: 99.6%

The effect of IN-J0290 on *Lemna gibba* G3 was determined in 20X AAP nutrient medium, incubated in an incubator for 14 days at 25±2°C. Because of the insensitivity of *Lemna gibba* G3 to IN-J0290, three replicates at one nominal concentration of 100 mg/L were tested. IN-J0290 was determined to be stable over the course of the definitive test as evidenced by the analytical recoveries obtained from the day 0 and day 14 test solutions. Frond counts were taken every 2 or 3 days. Frond count and biomass were determined after 14 days.

Findings:

The effects of IN-J0290 on the growth and reproduction of *Lemna gibba* G3 are shown in Table B.9.70.

Table B.9.70: Summary of growth inhibition following exposure of *Lemna gibba* G3 to IN-J0290 for 14 days

Test concentration mg/L	14-Day mean frond number	% Inhibition relative to control	14-Day mean biomass (mg)	% Inhibition relative to control
blank control	523	—	37.1	—
100	519	0.77	36.8	0.81

Conclusions:

Growth inhibition values obtained with IN-J0290 on *Lemna gibba* G3 were as follows:

Frond number: 14-day EC₅₀ > 100 mg/L
 14-day NOEC < 100 mg/L
 Frond biomass: 14-day EC₅₀ > 100 mg/L
 14-day NOEC < 100 mg/L

43) IN-DAT97: Influence on growth and reproduction of *Lemna gibba* G3. Leva, S., 2003.

Cross reference IIA, 8.2.8./08

Guidelines:

ASTM E1415-91 (1991)

GLP:

Yes

The study is acceptable

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Materials and methods:

Test Substance: IN-DAT97 technical metabolite

Batch: DAT97-001

Purity: 96%

The effect of IN-DAT97 on *Lemna gibba* G3 was determined in 20X AAP nutrient medium. Four replicates, at the nominal concentrations of 6.25, 12.5, 25, 50 and 100 mg IN-DAT97/L, were incubated in an incubator for 14 days at 25±2°C and frond counts were taken every 2 or 3 days. IN-DAT97 was determined to be stable over the course of the definitive test as evidenced by the analytical recoveries obtained from the day 0 and day 14 test solutions. Frond count and biomass were determined after 14 days.

Recovery: The ability of *Lemna* to recover from exposure to IN-DAT97 was assessed by transferring fronds to fresh, untreated nutrient media and monitoring growth for up to 14 days. Recovery was evaluated for all concentrations exhibiting >50% inhibition after 14 d exposure in the definitive test.

Findings:

The effects of IN-DAT97 on the growth and reproduction of *Lemna gibba* G3 are shown in Table B.9. 71.

Recovery: *Lemna* fronds previously exposed to media containing up to 100 mg Bensulfuron methyl/L (the highest concentration tested) were able to resume logarithmic growth within 14 days of transferring them to fresh, un-treated media. This demonstrates that the effects of IN-DAT97 on *Lemna* are reversible.

Table B.9. 71: Summary of growth inhibition following exposure of *Lemna gibba* G3 to IN-DAT97 for 14 days

Test concentration mg/L	14-Day mean frond number	Inhibition relative to control	14-Day mean biomass (mg)	% Inhibition relative to control
blank control	493	—	43.6	—
6.25	512	-3.85	43.0	1.30
12.5	487	1.37	41.9	3.90
25	506	-2.48	41.7	4.28
50	438 ^a	-11.15	37.7 ^a	13.53
100	138 ^A	72.07	22.0 ^a	49.62

^a Statistically different from controls, Jonckheere-Terpstra, $\alpha = 0.05$.

Conclusions:

Growth inhibition values obtained with IN-DAT97 on *Lemna gibba* G3 were as follows:

Frond number: 14-day EC₅₀ = 79.1 mg

14-day NOEC = 6.25 mg

Frond biomass: 14-day EC₅₀ ≥ 100 mg

14-day NOEC < 6.25 mg

The effects of IN-DAT97 on *Lemna gibba* G3 are expected to be reversible at concentrations up to at least 100 mg/L.

B.9.2.9 Acute toxicity of the preparations (Annex IIIA 10.2.1)

Testing is required if the toxicity of the formulation can not be predicted on the basis of the data for the active substance (Commission Directive 91/414/EEC) and the formulation contains a mixture of two active substances.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

44) *Static acute 48-Hour EC₅₀ of IN F5384-85 to Daphnia magna. Hutton, D.G., 1988a.*

Cross reference IIIA, 10.2.1./01

Guidelines:

USEPA 72-1

GLP:

Yes

The study is acceptable

Materials and methods:

Test substance: DPX-F5384 60WG

Batch: F5384-85

Purity: 600 g a.i./kg

The acute toxicity of Bensulfuron methyl 60WG to unfed *Daphnia magna* (<24-hour old at test initiation) was determined in an unaerated, static, 48-hour test. Treatments consisted of a dilution water control, and nominal concentrations of 47, 63, 84, 112, and 150 mg Bensulfuron methyl 60WG/L. Ten daphnids were used per replicate with two replicates per test concentration and control.

Findings:

A summary of the findings is presented in Table B.9. 72. Mean, measured concentrations of Bensulfuron methyl were 0, 30, 42, 56, 73, and 99 mg a.i./L. There were no test concentration-related immobility or sublethal effects below 99 mg a.i./L. The highest concentration causing no immobility was >99 mg a.i./L and the lowest concentration causing 100% immobility was >99 mg a.i./L. No sublethal effects were observed at any test concentration.

Table B.9. 72: Summary of observed immobility and sublethal effects of unfed *Daphnia magna* exposed to Bensulfuron methyl 60WG for 48 hours in an unaerated, static acute test

Mean, measured concentrations of Bensulfuron methyl (mg a.i./L)	Immobility (No. immobile/No. at test start)			
	24 hours		48 hours	
	A ^a	B ^a	A ^a	B ^a
Dilution water control	0/10	0/10	0/10	0/10
30	0/10	0/10	0/10	0/10
42	0/10	0/10	0/10	0/10
56	0/10	0/10	0/10	0/10
73	0/10	0/10	0/10	0/10
99	0/10	0/10	0/10	0/10

^a A, B represent replicate test vessels containing 10 daphnids.

Conclusions:

The 48-hour EC₅₀ in *Daphnia magna* was >99 mg Bensulfuron methyl a.i./L (>150 mg nominal Bensulfuron methyl 60WG/L).

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

45) *Static acute 96-hour LC₅₀ of IN-F5384-85 to rainbow trout (Salmo gairdneri). Hutton, D.G., 1988b.*

Cross reference IIIA, 10.2.1./02

Guidelines:

USEPA 72-1

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 60WG (60WG = 60DF)

Batch: F5384-85

Purity: 600 g a.i./kg

The acute toxicity of Bensulfuron methyl 60WG (wetttable granule formulation, purity: 60%) to unfed fingerling rainbow trout, *Salmo gairdneri* (*Oncorhynchus mykiss*), was determined in an unaerated, static, 96-hour test. Treatments consisted of a dilution water control, and nominal concentrations of 89, 130, 200, 300, and 450 mg Bensulfuron methyl 60WG/L. Two replicates containing 5 fish each were exposed to each treatment concentration and control. Test solutions were maintained between 12.2 and 12.5°C.

Findings:

A summary of cumulative mortality is presented in Table B.9. 73. Mean, measured concentrations of Bensulfuron methyl were 0, 56, 79, 130, 170, and 270 mg a.i./L. There were no mortality below 270 mg a.i./L, the highest dose tested. The lowest concentration causing 100% mortality was >450 mg a.i./L.

Table B.9. 73: Observed mortality of rainbow trout exposed to Bensulfuron methyl 60WG for 96 hours in an unaerated, static, acute test

Mean, measured concentration of Bensulfuron methyl (mg a.i./L)	Cumulative mortality (No. dead / No. at test start)							
	24 h		48 h		72 h		96 h	
	Aa	Ba	Aa	Ba	Aa	Ba	Aa	Ba
Water Control	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
56	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
79	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
130	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
170	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
270	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10

^a A and B represent replicates; each replicate contained 5 fish (total 10 fish per test concentration) at test start.

Conclusions:

The 96-hour LC₅₀ for the rainbow trout was >270 mg Bensulfuron methyl a.i./L (>450 mg nominal Bensulfuron methyl 60WG/L).

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

- 46) *Bensulfuron methyl (DPX-F5384) 60WG: Influence on growth and growth rate of green alga Selenastrum capricornutum. Sloman, T. L., 2001a.*

Cross reference IIIA, 10.2.1/03

Guidelines:

Directive 92/69/EEC

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 60WG

Batch: F5384-332

Purity: 600 g a.i./kg

The effect of Bensulfuron methyl 60WG on *Selenastrum capricornutum* was determined using algal cultures with AAP nutrient medium. Three replicates at 4, 8, 16, 32, and 64 µg Bensulfuron methyl 60WG/L of nutrient medium (ppb), measured at test initiation, were incubated in a chamber at 24±2°C for 72 hours and cell counts were taken at 24-hour intervals.

Recovery: after the initial 72 hours exposure period, algae from concentrations 16, 32, and 64 µg/L exhibiting >50% inhibition were placed in nutrient medium without Bensulfuron methyl 60WG. Cell counts were made every 3 days for up to 9 days.

Findings:

The effects of Bensulfuron methyl 60WG on the growth and growth rate of *Selenastrum capricornutum* are shown in Table B.9. 74.

Recovery: the ability of *Selenastrum capricornutum* to recover from prior exposure to Bensulfuron methyl 60WG is demonstrated in Table B.9. 75.

Table B.9. 74: Summary of algal growth inhibition following exposure of *Selenastrum capricornutum* to Bensulfuron methyl 60WG for 72 hours

Nominal concentration (µg product/L)	Mean cell density (cells/mL)	% Inhibition relative to control		
		Cell density	Area under the growth curve	Growth rate
blank control	2.1 x 10 ⁶	—	—	—
4	2.1 x 10 ⁶	0.79	-4.42	0.04
8	1.4 x 10 ⁶	31.32 ^a	19.69	6.97
16	6.2 x 10 ⁵	70.27 ^a	67.30 ^a	22.65
32	1.4 x 10 ⁵	93.32 ^a	89.86 ^a	51.01 ^a
64	1.7 x 10 ⁴	99.21 ^a	97.85 ^a	91.37 ^a

^a Statistically different from controls (ANOVA, Williams' test, α = 0.05) based on analysis of mean cell density, growth rate and area under the curve.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9. 75: Recovery of *Selenastrum capricornutum* from prior exposure to Bensulfuron methyl 60WG

Nominal concentration (µg product/L)	Day 0	Day 1	Day 6
Blank control	2.2×10^4	4.22×10^6	5.96×10^6
16	1.87×10^4	5.07×10^6	5.2×10^6
32	4.2×10^3	4.16×10^6	5.32×10^6
64	500	4.4×10^5	6.28×10^6

Conclusions:

Growth inhibition values obtained with Bensulfuron methyl 60WG on *Selenastrum capricornutum* were as follows:

Cell density: 72-hour $EC_{50} = 11.3 \mu\text{g/L}$ ($6.78 \mu\text{g a.i./L}$)

72-hour NOEC $< 4 \mu\text{g/L}$ ($2.4 \mu\text{g a.i./L}$)

Area under the growth curve: 72-hour $EC_{50} = 12.9 \mu\text{g/L}$ ($7.74 \mu\text{g a.i./L}$)

72-hour NOEC $= 4 \mu\text{g/L}$ ($4.8 \mu\text{g a.i./L}$)

Growth rate: 72-hour $EC_{50} = 28.8 \mu\text{g/L}$ ($17.28 \mu\text{g a.i./L}$)

72-hour NOEC $< 4 \mu\text{g/L}$ ($19.2 \mu\text{g a.i./L}$)

The effects of Bensulfuron methyl 60WG on *Selenastrum capricornutum* are expected to be reversible at concentrations of at least $64 \mu\text{g product/L}$.

47) *Bensulfuron methyl (DPX-F5384) 60WG: Influence on growth and growth rate of blue-green alga Anabaena flos-aquae. Sloman, T. L., 2001b.*

Cross reference IIIA, 10.2.1./04

Guidelines:

USEPA 850.5400 (1996)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 60WG

Batch: F5384-332

Purity: 600 g a.i./kg

The effect of Bensulfuron methyl 60WG on *Anabaena flos-aquae* was determined using algal cultures with AAP nutrient medium. Three replicates at 5, 10, 20, 40, and $80 \mu\text{g}$ Bensulfuron methyl 60WG/L of nutrient medium (ppb), measured at test initiation, were incubated in an incubator at $24 \pm 2^\circ\text{C}$ for 96 hours and cell counts were taken at 24-hour intervals.

Recovery: after the initial 96 hour exposure period, algae from concentrations 20, 40, and $80 \mu\text{g/L}$ exhibiting $>50\%$ inhibition were placed in nutrient medium without Bensulfuron methyl 60WG. Cell counts were taken every 3 days for up to 9 days.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Findings:

The effects of Bensulfuron methyl 60WG on the growth and growth rate of *Anabaena flos-aquae* are shown in Table B.9. 76.

Recovery: the ability of *Anabaena flos-aquae* to recover from prior exposure to Bensulfuron methyl 60WG is demonstrated in Table B.9. 77.

Table B.9. 76: Summary of algal growth inhibition following exposure of *Anabaena flos-aquae* to Bensulfuron methyl 60WG for 96 hours

Nominal concentration (µg product/L)	Mean cell density (cells/mL)	% Inhibition relative to control		
		Cell density	Area under the growth curve	Growth µg/L
blank control	9.8×10^5	—	—	—
5	9.7×10^5	0.68	9.21	0.14
10	7.8×10^5	20.48	39.33 ^a	5.17
20	4.2×10^5	57.34 ^a	65.69 ^a	18.72
40	6.3×10^4	93.52 ^a	91.21 ^a	59.92 ^a
80	2.7×10^4	97.27 ^a	96.44 ^a	81.98 ^a

^a Statistically different from controls (ANOVA, Williams' test, $\alpha = 0.05$) based on analysis of mean cell density, growth rate and area under the curve.

Table B.9. 77: Recovery of *Anabaena flos-aquae* from prior exposure to Bensulfuron methyl 60WG

Nominal concentration (µg product/L)	Day 0	Day 3	Day 6	Day 9
Blank control	9000	1.6×10^5	6.56×10^6	1.53×10^7
20	12500	8.0×10^4	6.24×10^6	1.5×10^7
40	1900	1.0×10^4	2.56×10^6	6.56×10^6
80	800	5.0×10^4	3.84×10^6	1.38×10^7

Conclusions:

Growth inhibition values obtained with Bensulfuron methyl 60WG on *Anabaena flos-aquae* were as follows:

Cell density: 96-hour $EC_{50} = 17.3 \mu\text{g/L}$ ($10.38 \mu\text{g a.i./L}$)

96-hour NOEC $< 5 \mu\text{g/L}$ ($6.0 \mu\text{g a.i./L}$)

Area under the growth curve: 96-hour $EC_{50} = 13.5 \mu\text{g/L}$ ($8.1 \mu\text{g a.i./L}$)

96-hour NOEC $< 5 \mu\text{g/L}$ ($3.0 \mu\text{g a.i./L}$)

Growth µg/L: 96-hour $EC_{50} = 35.9 \mu\text{g/L}$ ($21.54 \mu\text{g a.i./L}$)

96-hour NOEC $< 5 \mu\text{g/L}$ ($12 \mu\text{g a.i./L}$)

The effects of Bensulfuron methyl 60WG on *Anabaena flos-aquae* are expected to be reversible at concentrations of at least $80 \mu\text{g product/L}$.

48) Bensulfuron methyl (DPX-F5384) 60WG: Influence on growth and reproduction of *Lemna gibba* G3. Sloman, T., 2001c.

Cross reference IIIA, 10.2.1/05

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Guidelines:

ASTM method E1415-91 (1991)

GLP:

Yes

*The study is acceptable*Materials and methods:

Test Substance: DPX-F5384 60WG

Batch: F5384-332

Purity: 600 g a.i./kg

The effect of Bensulfuron methyl 60WG on *Lemna gibba* G3 was determined in 20X AAP nutrient medium. Four replicates at nominal concentrations of 0.25, 0.5, 1, 2 and 4 µg Bensulfuron methyl 60WG/liter of nutrient medium (ppb), were incubated in a chamber for 14 days at $25 \pm 2^\circ\text{C}$ and frond counts were taken every 2 or 3 days. Bensulfuron methyl 60WG was determined to be stable over the course of the definitive test as evidenced by the analytical recoveries obtained from the day 0 and day 14 test solutions. Frond count and biomass were determined after 14 days.

Recovery: after the initial 14-day exposure period, fronds from concentrations 2 and 4 µg/L exhibiting >50% inhibition were placed in nutrient medium without Bensulfuron methyl 60WG. Fronds were counted every 3 to 5 days for up to 14 days.

Findings:

The effects of Bensulfuron methyl 60WG on the growth and reproduction of *Lemna gibba* G3 are shown in Table B.9. 78.

Recovery: the ability of *Lemna gibba* to recover from prior exposure to Bensulfuron methyl 60WG is demonstrated in Table B.9. 79.

Table B.9. 78: Summary of growth inhibition following exposure of *Lemna gibba* G3 to Bensulfuron methyl 60WG for 14 days

Nominal concentration (µg product/L)	14-Day mean frond number	% Inhibition relative to control	14-Day mean biomass (mg)	% Inhibition relative to control
blank control	510	—	45.5	—
0.25	552	-8.24	43.7	3.95
0.5	504	1.23	45.4	0.37
1	422 ^a	17.31	38.9 ^a	14.49
2	100 ^a	80.43	21.1 ^a	53.66
4	40 ^a	92.15	15.3 ^a	66.40

^aStatistically different from controls (ANOVA, Williams' test, $\alpha = 0.05$) based on analysis of mean frond number and biomass

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9. 79: Recovery of *Lemna gibba* from prior exposure to Bensulfuron methyl 60WG

Nominal concentration (µg product/L) ^a	DAY 0	Day 3	DAY 7	Day 10	Day 14
Blank control	15	54	181	350	473
2	88	262	465	702	900
4	40	76	169	276	448

^aBensulfuron methyl 60WG concentration during exposure phaseConclusions:

Growth inhibition values obtained with Bensulfuron methyl 60WG on *Lemna gibba* G3 were as follows:

Frond number: 14-day EC₅₀ = 1.44 µg/L (0.864 µg a.i./L)
 14-day NOEC = 0.25 µg/L (0.30 µg a.i./L)
 Frond biomass: 14-day EC₅₀ = 2.29 µg/L (1.37 µg a.i./L)
 14-day NOEC < 0.25 µg/L (0.30 µg a.i./L)

The effects of Bensulfuron methyl 60WG on *Lemna gibba* G3 are expected to be reversible at concentrations of at least 4 µg product/L.

B.9.2.10 Microcosm or mesocosm studies (Annex IIIA 10.2.2)

Microcosm or mesocosm studies are not required for Bensulfuron methyl 60WG. The TER_a and TER_{it} for Bensulfuron methyl are >100 for fish and >10, respectively, and exceed the Annex VI decision making criteria (see point B.9.2.13).

B.9.2.11 Residue data in fish (Annex IIIA 10.2.3)

Residues in fish were not measured for Bensulfuron methyl since the potential for bioconcentration is low for Bensulfuron methyl (see B.9.2.3).

B.9.2.12 Additional studies (Annex IIIA 10.2.4)

No additional studies were performed with fish or *Daphnia* due to the low acute toxicity demonstrated with both Bensulfuron methyl and Bensulfuron methyl 60WG.

B.9.2.13 Summary of effects, exposure and hazard assessment for aquatic organisms**B.9.2.13.1 Toxicity**

Bensulfuron methyl has low acute and chronic toxicity to all freshwater and marine fish and aquatic invertebrates; however, it is toxic to algae and aquatic plants under the laboratory test conditions. Bensulfuron methyl has a very low potential for bioconcentration. The metabolites of Bensulfuron methyl demonstrated low toxicity to all aquatic species tested. A summary of the aquatic toxicity of Bensulfuron methyl, Bensulfuron methyl 60WG, and the major aquatic and soil metabolites are given in Table B.9. 80 and Table B.9. 81.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9. 80: Summary of toxicity studies of Bensulfuron methyl (active substance and formulation) for aquatic organisms

Test organism	Test Substance	Duration, conditions	Toxicological Endpoint (mg a.i./L)	Concentration n/m*	Dossier file number
Fish, acute					
<i>Cyprinodon variegatus</i>	a.i.	96 hr, static	LC ₅₀ > 123	m	IIA, 8.2.1/01
<i>Ictalurus punctatus</i>	a.i.	96 hr, static	LC ₅₀ > 150	n	IIA, 8.2.1/03
<i>Oncorhynchus mykiss</i>	a.i.	96 hr, static	LC ₅₀ > 66	m	IIA, 8.2.1/05
<i>Lepomis macrochirus</i>	a.i.	96 hr, static	LC ₅₀ > 120	n	IIA, 8.2.1/06
<i>Oncorhynchus mykiss</i>	DPX-F5384 60WG	96 hr, static	LC ₅₀ > 270	m	IIIA, 10.2.1/02
Fish, chronic					
<i>Pimephales promelas</i> ELS	a.i.	36 d, flow through	NOEC > 120	n	IIA, 8.2.2.2/01
<i>Oncorhynchus mykiss</i> ELS	a.i.	90 d, flow through	NOEC = 1.5	n	IIA, 8.2.2.2/02
Fish, Bioconcentration					
<i>Cyprinus carpio</i>	a.i.	flow through	BCF (whole) < 0.9		IIA, 8.2.3/01
<i>Oncorhynchus mykiss</i>	a.i.	flow through	BCF (whole) = 1.0		IIA, 8.2.3/02
<i>Procambarus clarki</i>	a.i.	flow through	BCF (whole) = 1.6		IIA, 8.2.3/04
Invertebrates, acute					
<i>Crassostrea virginica</i>	a.i.	48 hr, static	EC ₅₀ > 142	n	IIA, 8.2.4/01
<i>Mysidopsis bahia</i>	a.i.	96 hr, static	LC ₅₀ > 130	m	IIA, 8.2.4/02
<i>Procambarus clarki</i>	a.i.	96 hr, static	LC ₅₀ > 71	m	IIA, 8.2.4/05
<i>Daphnia magna</i>	a.i.	48 hr, static	EC ₅₀ > 130	m	IIA, 8.2.4/06
<i>Daphnia magna</i>	DPX-F5384 60WG	48 hr, static	EC ₅₀ > 99	m	IIIA, 10.2.1/01
Invertebrates, chronic					
<i>Daphnia magna</i>	a.i.	21 d, static renewal	NOEC = 12	m	IIA, 8.2.5/01
Sediment dwellings					
<i>Chironomus tentans</i>	DPX-JM206 60WG	96 hr, static	LC ₅₀ > 125	m	IIA, 8.2.7/01
Algae					
<i>Selenastrum capricornutum</i>	a.i.	72 hr, static	E _r C ₅₀ = 0.0574 E _b C ₅₀ = 0.0204	n	IIA, 8.2.6/03
<i>Anabaena flos-aquae</i>	a.i.	96 hr, static	E _r C ₅₀ = 0.0885 E _b C ₅₀ = 0.0441	n	IIA, 8.2.6/04
<i>Selenastrum capricornutum</i>	DPX-F5384 60WG	72 hr, static	E _r C ₅₀ = 0.0173 E _b C ₅₀ = 0.0077	n	IIIA, 10.2.1/03
<i>Anabaena flos-aquae</i>	DPX-F5384 60WG	96 hr, static	E _r C ₅₀ = 0.0215 E _b C ₅₀ = 0.0081	n	IIIA, 10.2.1/04
Aquatic plants					
<i>Lemna gibba</i>	a.i.	14 d, static	E _r C ₅₀ = 0.0008 E _b C ₅₀ = 0.0015	n n	IIA, 8.2.8/03
<i>Lemna gibba</i>	DPX-F5384 60WG	14 d, static	E _r C ₅₀ = 0.000864 E _b C ₅₀ = 0.00137	n n	IIIA, 10.2.1/05

Values in bold were selected for risk assessment

*n = nominal; m = measured

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9. 81: Summary of toxicity studies of metabolites of Bensulfuron methyl for aquatic organisms

Test organism	Test Substance	Duration, conditions	Toxicological Endpoint (mg/L)	Concentration n/m [#]	Dossier file number
<i>Oncorhynchus mykiss</i> Acute	IN-J0290	96 hr, static	LC₅₀ > 97	m	IIA, 8.2.1/04
<i>Daphnia magna</i> Acute		48 hr, static	EC₅₀ > 100	n	IIA, 8.2.4/04
<i>Daphnia magna</i> Chronic		21 days, static renewal	NOEC = 24.9	m	IIA, 8.2.5/02
<i>Selenastrum capricornutum</i> Acute		72 hr, static	E_bC₅₀ > 60 E_rC₅₀ > 120	n	IIA, 8.2.6/05
<i>Lemna gibba</i>		14 d, static	E_{fb}C₅₀ > 100	n	IIA, 8.2.8/07
<i>Oncorhynchus mykiss</i> Acute	IN-N5297	96 hr, static	LC₅₀ > 118	m	IIA, 8.2.1/02
<i>Daphnia magna</i> Acute		48 hr, static	EC₅₀ > 117	m	IIA, 8.2.4/03
<i>Daphnia magna</i> Chronic		21 days, static renewal	NOEC = 12.7	m	IIA, 8.2.5/03
<i>Selenastrum capricornutum</i> Acute		72 hr, static	E_{b/r}C₅₀ > 120	n	IIA, 8.2.6/02
<i>Lemna gibba</i>		14 d, static	E_{fb}C₅₀ > 120	n	IIA, 8.2.8/02
<i>Selenastrum capricornutum</i> Acute	IN-D1R84	72 hr, static	E_bC₅₀ = 37.5 E_rC₅₀ = 36.2	n n	IIA, 8.2.6/01
<i>Lemna gibba</i>		14 d, static	E_{fb}C₅₀ > 130	n	IIA, 8.2.8/01
<i>Selenastrum capricornutum</i> Acute	IN-R9419	72 hr, static	E_bC₅₀ = 26.4 E_rC₅₀ = 37.3	n n	IIA, 8.2.6/06
<i>Lemna gibba</i>		14 d, static	E_rC₅₀ = 6.2 E_bC₅₀ = 10.5	n n	IIA, 8.2.8/04
<i>Selenastrum capricornutum</i> Acute	IN-T5831	72 hr, static	E_bC₅₀ = 41.1 E_rC₅₀ = 99.1	n n	IIA, 8.2.6/07
<i>Lemna gibba</i>		14 d, static	E_rC₅₀ = 120.9 E_bC₅₀ = 97.9	n n	IIA, 8.2.8/05
<i>Selenastrum capricornutum</i> Acute	IN-F7880	72 hr, static	E_bC₅₀ = 13.5 E_rC₅₀ = 32.3	n n	IIA, 8.2.6/08
<i>Lemna gibba</i>		14 d, static	E_rC₅₀ = 1.18 E_bC₅₀ = 1.82	n n	IIA, 8.2.8/06
<i>Selenastrum capricornutum</i> Acute	IN-DAT97	72 hr, static	E_bC₅₀ = 26.9 E_rC₅₀ = 33.7	n n	IIA, 8.2.6/09
<i>Lemna gibba</i>		14 d, static	E_rC₅₀ = 79.1 E_bC₅₀ > 100	n n	IIA, 8.2.8/08

Values in bold were selected for risk assessment

n = nominal; m = measured

The approaches used to examine the potential risk of Bensulfuron methyl 60WG to aquatic organisms follow the recommendations given in the Guidance Document for Environmental Risk Assessments of Active Substances used on Rice in the EU for Annex I Inclusion, Sanco/1090/2000 rev.1, June 2003 (Med-Rice, 2003). Initially, a Med-Rice

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Tier 1 assessment was conducted to consider the risk of Bensulfuron methyl and its potential degradates to aquatic organisms within the rice paddy and adjacent receiving ditch. Predicted concentrations in water and soil within the paddy and water and sediment within the ditch were calculated using the Med-Rice (2003) calculator. Subsequently, a refined risk assessment based on higher-tiered exposure modelling using RICEWQ/RIVWQ was conducted for Bensulfuron methyl only. This higher-tiered assessment considered the risk of Bensulfuron methyl to the most sensitive aquatic organisms identified in Tier 1 assuming they inhabit paddies, ditches, canals and rivers receiving outflow from paddy fields.

B.9.2.13.2 Exposure

In Table B.9. 82 the maximum Tier 1 PEC_{pw}, PEC_{soil} values for rice paddy and the maximum Tier 1 PEC_{sw}, PEC_{sed} values for the receiving ditch are presented.

A summary of the RICEWQ/RIVWQ PEC_{sw} results for paddies, ditches, canals and the river aggregated across all meteorological scenarios and simulation years is presented in Table B.9. 83.

For details see the Fate and Behaviour Section.

Table B.9. 82: Maximum Tier 1 PEC values determined using the methods given in Med-Rice (2003) for paddy water, (PEC_{pw}), soil (PEC_{soil}), surface water (PEC_{sw}) and sediment (PEC_{sed}) for Bensulfuron methyl 60WG

Compound	Maximum PEC value from either the clay or sand scenario			
	Paddy water and soil		Ditch water and sediment	
	PEC _{pw} ^a (µg/L)	PEC _{soil} ^b (µg/kg)	PEC _{sw} ^c (µg/L)	PEC _{sed} ^d (µg/kg)
Bensulfuron methyl	19.2	78.2	1.75	7.19
IN-F7880	2.35	7.23	0.215	0.634
IN-R9419	6.39	20.8	0.371	1.17
IN-D1R84	5.39	4.18	0.359	0.0563
IN-J0290	2.54	13.6	0.210	1.14
IN-N5297	8.53	11.7	0.716	0.435
IN-T5831	3.73	2.90	0.296	0.0465
IN-DAT97	4.37	3.39	0.373	0.0585

^a PEC_{pw} = PEC in paddy water

^b PEC_{soil} = PEC in paddy soil (µg/kg dry soil)

^c PEC_{sw} = PEC in the receiving ditch surface water

^d PEC_{sed} = PEC in the receiving ditch sediment (µg/kg dry sediment)

Table B.9. 83: RICEWQ/RIVWQ PEC_{sw} summary statistics for treated paddies, ditches, canals, and the river for Bensulfuron methyl

Overall summary – data from all meteorological scenarios combined ^a								
Meteorological scenario	Water body	Number of simulation years	maximum daily PEC _{sw} (µg/L)					
			Minimum	25 th %	Average ^b	Median	75 th %	Maximum
France, Greece, Italy, Portugal, and Spain data combined	paddy	1000	12.2	27.5	34.0	32.2	37.2	256
	ditch	1000	1.97	2.85	4.22	3.77	5.36	26.3
	canal	1000	0.635	0.949	1.42	1.25	1.84	3.61
	river	100	0.152	0.221	0.365	0.338	0.437	1.13

^a Detailed results for all individual meteorological scenarios are described fully in DuPont Report Number DuPont-12165 and in Bensulfuron methyl EU Dossier, Annex IIIA, Document M-III, Section 5, DuPont-6197 EU

^b Maximum average PEC_{sw} values were selected for the higher-tiered risk assessment

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9. 84: RICEWQ/RIVWQ average distribution of the 90th percentile 14-day TWA PEC_{sw} values (µg/L) for Bensulfuron methyl

Overall summary – data from all meteorological scenarios combined ^a			
Meteorological scenario	Water body	Number of simulation years	average distribution of the 90 th percentile 14-day TWA PEC _{sw} (µg/L)
France, Greece, Italy, Portugal, and Spain data combined	paddy	1000	0.088
	ditch	1000	0.032 ^b
	canal	1000	0.006
	river	100	0.004

^a Detailed results for all individual meteorological scenarios are described fully in DuPont Report Number DuPont-12165 and in Bensulfuron methyl EU Dossier, Annex IIIA, Document M-III, Section 5, DuPont-6197 EU

^b Maximum average PEC_{sw} value was selected for the higher-tiered risk assessment

B.9.2.13.3 Risk assessment

Tier 1 acute aquatic risk within the rice paddy

A Tier 1 acute risk assessment was conducted for aquatic organisms assuming they inhabit the rice paddy fields during flood and are therefore exposed to the maximum initial PEC_{pw} following an application of 60 g a.i./ha of Bensulfuron methyl 60WG. Because concentrations of Bensulfuron methyl are the highest within the paddy immediately after application and decrease thereafter, the Tier 1 assessment may be used to exclude organisms that clearly are not at risk and focus refined risk assessments on those that fail to exceed the Annex VI regulatory triggers. Assuming exposure to maximum initial Tier 1 PEC_{pw}, the acute TERs for all tested aquatic organisms except algae and aquatic plants substantially exceed the Annex VI triggers (Table B.9. 85), as would be expected for a herbicide intended to control aquatic weed species. Tested species included the standard sensitive aquatic species normally tested for the EU and additional freshwater, marine and estuarine species tested for non-EU regulatory requirements. It may be concluded that Bensulfuron methyl poses a low acute risk to non-plant species within the paddy system.

Table B.9. 85: Acute Tier 1 TERs for aquatic organisms exposed to the maximum Med-Rice PEC in paddy water (PEC_{pw}) for Bensulfuron methyl

Test Substance	Test organism	EC ₅₀ or LC ₅₀ (mg a.i./L)	Max. PEC _{pw} (mg a.i./L) ^a	TER	Annex VI Trigger
Bensulfuron methyl	<i>Oncorhynchus mykiss</i>	>66	0.0192	>3438	100
Bensulfuron methyl	<i>Procambarus clarki</i>	>71	0.0192	>3698	100
DPX-JM206 60WG	<i>Chironomus tentans</i>	>125	0.0192	>6510	100
DPX-F5384 60WG	<i>Selenastrum capricornutum</i>	0.0173	0.0192	0.90	10
Bensulfuron methyl	<i>Lemna gibba</i>	0.0008	0.0192	0.04	10

^a Determined using the Tier 1 Med-Rice (2003) calculation method.

Bioconcentration: the endpoints from 3 bioconcentration studies on 2 fish species and a crustacean are presented in Table B.9. 80. The calculated bioconcentration factors (BCF) ranged from 0.09 to 1.6, indicating that Bensulfuron methyl does not bioconcentrate in aquatic organisms.

Metabolites: Tier 1 acute aquatic risk within the rice paddy

A Tier 1 acute risk assessment was conducted for all Bensulfuron metabolites potentially produced in soil or aquatic water/sediment systems. The assessment is based on the worst-case assumptions of assuming aquatic organisms inhabit the rice paddy fields during flood and are exposed to maximum concentrations of the metabolites. Maximum

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

metabolite concentrations were determined using the Tier 1 Med-Rice (2003) model and assumed maximum use rate of 60 g a.i./ha, no foliar interception, instantaneous conversion to the metabolite at the maximum percent formed in any environmental fate study, corrected for molecular weight conversion.

Assuming exposure to metabolites at the maximum initial PEC_{pw} calculated according to Med-Rice (2003), the acute Tier 1 TERs for all the metabolites and all tested aquatic organisms substantially exceeded the Annex VI triggers (Table B.9. 86). It may be concluded that Bensulfuron methyl metabolites pose a low acute risk to aquatic organisms including the most sensitive organisms to Bensulfuron methyl.

Table B.9. 86: Acute Tier 1 TERs for aquatic organisms exposed to maximum PEC in paddy water (PEC_{pw}) of metabolites of Bensulfuron methyl

Test Substance	Test organism	Toxicological endpoint (mg/L)	Max. PEC_{pw} (mg/L) ^a	TER	Annex VI Trigger
IN-J0290	<i>Oncorhynchus mykiss</i>	>97	0.00254	>38189	100
	<i>Daphnia magna</i>	>100	0.00254	>39370	100
	<i>Selenastrum capricornutum</i>	>120	0.00254	>47244	10
	<i>Lemna gibba</i>	>100	0.00254	>39370	10
IN-N5297	<i>Oncorhynchus mykiss</i>	>118	0.00853	>13833	100
	<i>Daphnia magna</i>	>117	0.00853	>13716	100
	<i>Selenastrum capricornutum</i>	>120	0.00853	>14068	10
	<i>Lemna gibba</i>	>120	0.00853	>14068	10
IN-D1R84	<i>Selenastrum capricornutum</i>	36.2	0.00539	6716	10
	<i>Lemna gibba</i>	>130	0.00539	>24119	10
IN-R9419	<i>Selenastrum capricornutum</i>	37.3	0.00639	5837	10
	<i>Lemna gibba</i>	6.2	0.00639	970	10
IN-T5831	<i>Selenastrum capricornutum</i>	99.1	0.00373	26568	10
	<i>Lemna gibba</i>	120.9	0.00373	32413	10
IN-F7880	<i>Selenastrum capricornutum</i>	32.3	0.00235	13745	10
	<i>Lemna gibba</i>	1.18	0.00235	502	10
IN-DAT97	<i>Selenastrum capricornutum</i>	33.7	0.00437	7712	10
	<i>Lemna gibba</i>	79.1	0.00437	18101	10

^a Determined using the Tier 1 Med-Rice (2003) model

Tier 1 chronic aquatic risk within the rice paddy

A Tier 1 chronic risk assessment was conducted for aquatic organisms assuming they inhabit the rice paddy fields during flood and are therefore exposed to the maximum initial PEC_{pw} following an application of 60 g a.i./ha of Bensulfuron methyl 60WG. Because concentrations of Bensulfuron methyl are the highest within the paddy immediately after application and decrease thereafter, the Tier 1 assessment may be used as a screen and only those organisms that fail to exceed regulatory triggers are considered in higher-tiered assessments.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Assuming worst-case exposure to maximum initial Tier 1 PEC_{pw}, the long-term TERs for rainbow trout and *Daphnia magna* substantially exceed the Annex VI triggers (Table B.9. 87). It may be concluded that Bensulfuron methyl poses a low chronic risk to fish and invertebrates, even when the highest potential exposure is considered.

Table B.9. 87: Summary of long-term Tier 1 TERs for aquatic organisms exposed to maximum PEC in paddy water (PEC_{pw}) of Bensulfuron methyl

Test Substance	Test organism	NOEC (mg a.i./L)	Max. PEC _{pw} (mg a.i./L) ^a	TER	Annex VI Trigger
Bensulfuron methyl	<i>Oncorhynchus mykiss</i>	1.5	0.0192	78	10
Bensulfuron methyl	<i>Daphnia magna</i>	12	0.0192	625	10

^a Determined using the Tier 1 Med-Rice (2003) model

Metabolites: Tier 1 chronic aquatic risk within the rice paddy

Two Bensulfuron methyl metabolites occurred in the sediment phase of the laboratory water/sediment degradation studies at levels exceeding 10% of applied radioactivity after 14 days. This finding triggers the need for an assessment of potential risk to sediment organisms. According to the Guidance Document on Aquatic Ecotoxicology in the context of Directive 91/414/EEC, Sanco/3268/2001 rev. 4 (final), 17 October, 2002, toxicity studies on sediment organisms are triggered by high chronic toxicity to *Daphnia magna* (for example, the 21-day NOEC must be <0.1 mg a.i./L to required direct testing on *Chironomus species*). The toxicity of IN-J0290 and IN-N5297 was evaluated in a 21-day chronic daphnid reproduction test. In order to assess the potential long-term risk of these metabolites to aquatic invertebrates (including sediment organisms), the long-term TERs were determined assuming worst-case maximum initial Med-Rice (2003) PEC_{pw} and using the 21-day NOECs from the daphnid reproduction studies.

A summary of the long-term TERs for IN-J0290 and IN-N5297 is presented in Table B.9. 88. The TERs far exceed Annex VI decision making criterion of 10 and suggest that neither metabolites poses a long-term risk to aquatic invertebrates in the water column or the sediment.

Table B.9. 88: Summary of long-term Tier 1 TERs for aquatic organisms exposed to maximum PEC in the paddy water (PEC_{pw}) of Bensulfuron methyl metabolites

Test Substance	Test organism	NOEC (mg a.i./L)	Max. PEC _{pw} (mg a.i./L) ^a	TER	Annex VI Trigger
IN-J0290	<i>Daphnia magna</i>	24.9	0.00254	9803	10
IN-N5297	<i>Daphnia magna</i>	12.7	0.00853	1489	10

^a Determined using the Tier 1 Med-Rice (2003) model

Tier 1 acute aquatic risk within the receiving ditches for algae and *Lemna*

Based on the results of the Tier 1 acute risk assessment, it was concluded that within the rice paddy, initial concentrations of Bensulfuron methyl may pose a risk to algae and aquatic plants. An assessment therefore was performed to determine the risk to algae and aquatic plants potentially inhabiting ditches receiving outflow from rice paddies treated with Bensulfuron methyl.

The Tier 1 PEC_{sw} in receiving ditches was determined using the Tier 1 Med-Rice (2003) method. The maximum initial PEC_{sw} estimated for ditches immediately after receiving outflow from Bensulfuron methyl-treated paddies was

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

0.00175 mg a.i./L. Acute TERs for algae and *Lemna* were calculated using this exposure value and are presented below (Table B.9. 89).

Table B.9. 89: Summary of Tier 1 TERs for algae and *Lemna* exposed to Bensulfuron methyl in ditches receiving outflow from treated rice paddies

Test substance	Test organism	EC ₅₀ (mg a.i./L)	Max. PEC _{sw} (mg a.i./L) ^a	TER	Annex VI trigger
Bensulfuron methyl	<i>Selenastrum capricornutum</i>	0.0173	0.00175	9.90	10
Bensulfuron methyl	<i>Lemna gibba</i>	0.0008	0.00175	0.46	10

^a Determined using the Tier 1 Med-Rice (2003) calculation method.

The resulting Tier 1 TER for algae is only just below the Annex VI trigger of 10 and therefore it may be concluded that Bensulfuron methyl poses a minimal risk to algae inhabiting receiving ditches. It is to be considered that inhibition of both algal species by Bensulfuron methyl was found to be reversible when cells previously exposed to Bensulfuron methyl were transferred to fresh, untreated medium. Moreover, Bensulfuron methyl dissipates rapidly in natural aquatic systems. Therefore, under actual field conditions, risk to algae in ditches adjacent to rice paddies is expected lower than the estimated.

The Tier 1 TER for *Lemna* shows an unacceptable risk of Bensulfuron methyl for aquatic plants. A refined risk assessment was conducted to investigate potential risk to aquatic plants under more realistic conditions than described in the Tier 1 Med-Rice exposure scenarios.

Higher-Tiered risk assessment for aquatic plants based on RICEWQ/RIVWQ modelling

The purpose of the higher tiered assessment was to evaluate the risk on more realistic exposure conditions compared to the Tier 1 calculations of Med-Rice (2003). Risk to aquatic plants inhabiting ditches, canals and rivers receiving outflow from treated rice paddies was considered. TERs were calculated using average maximum daily PECs from RICEWQ/RIVWQ simulations and the NOAEC (based on recovery).

Table B.9. 90: Higher-tiered risk assessment for aquatic plants based on RICEWQ/RIVWQ PEC values

Scenario	Average Max. PEC _{sw} (mg a.i./L)	NOAEC (mg a.i./L)	TER	Annex VI trigger
Ditch	0.0042	0.004	0.95	10
Canal	0.0014	0.004	2.9	10
River	0.00037	0.004	10.8	10

The resulting TERs for the river, but not the canal or ditch scenarios exceeded the trigger of 10. Risk assessment still need to be refined. Moreover, RMS has considered the NOAEC of 4 µg/L not acceptable: the recovery test gives information on the potential for recovery of *Lemna*, but does not allow to extrapolate a NOAEC value useful for the risk assessment.

Notifier has further refined the risk to aquatic plants

Higher-tiered modelling was used to obtain the 90th percentile 14-d TWA PEC_{sw} values for each of 20 simulation years and 5 meteorological scenarios. The TER, calculated on the basis of the average 90th percentile 14-d TWA

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

PEC_{sw} (worst case) and the lowest EC₅₀ for *Lemna*, resulted equal to 25 (above the EU Annex VI trigger of 10). Therefore, it can be concluded that the risk to aquatic plants in drainage ditches is low under more realistic exposure conditions.

Table B.9. 91: TERs for Lemna based on 14-d TWA PEC_{sw} in Ditches Outside Rice Paddies

Species	Endpoint	Endpoint value (µg a.i./L)	14-d TWA PEC _{sw} (µg/L) ^a	TER	Trigger value
<i>Lemna</i>	14-d EC ₅₀	0.8	0.032	25	10

^a 50th percentile of the 90th percentile values from each of 100 simulation years (20 simulation years × 5 meteorological scenarios × 1, 90th percentile 14-d TWA PEC_{sw} value per year = 100 values).

To strongly support this conclusion, results of biological greenhouse screening on 18 aquatic plant species were used to evaluate the potential range of sensitivities of aquatic plants. A comparison of EC₅₀s for all 21 aquatic plant species (Table B.9. 92) for which data are available (18 aquatic macrophytes, two algae and *Lemna*), demonstrates that *Lemna* is the most sensitive species.

Table B.9. 92: Summary of Regulatory Study Endpoints and Rice Herbicide Screening Data for Bensulfuron methyl

Group	Family	Genus species	Common name	EC ₅₀ (g/ha) ^a	EC ₅₀ (µg/L) ^b
Monocot	Lemnaceae	<i>Lemna gibba</i>	<i>Lemna</i>	-	0.8
Monocot	Pontederiaceae	<i>Monochoria vaginalis</i>	Monochoria	0.4	1.4
Monocot	Alismataceae	<i>Alisma plantago-aquatica</i>	American waterplantain	0.6	2.0
Fern	Marsileaceae	<i>Marsilea quadrifolia</i>	Waterclover	1.2	4.0
Monocot	Cyperaceae	<i>Scirpus juncooides</i>	Japanese bulrush	2.2	7.2
Dicot	Lythraceae	<i>Ammannia coccinea</i>	Redstem	4.3	14.5
Monocot	Cyperaceae	<i>Eleocharis kuroguwai</i>	Japanese waterchestnut	4.8	15.9
Dicot	Scrophulariaceae	<i>Lindernia pyxidaria</i> L.	False pimpernel	5.6	18.6
Algae	Chlorophyceae	<i>Selenastrum capricornutum</i>		-	20.4
Monocot	Cyperaceae	<i>Cyperus iria</i>	Rice flatsedge	6.4	21.4
Monocot	Cyperaceae	<i>Cyperus esculentus</i> L.	Yellow nutsedge	6.5	21.7
Monocot	Cyperaceae	<i>Scirpus mucronatus</i> L.	Ricefield bulrush	7.2	24.0
Monocot	Cyperaceae	<i>Eleocharis dulcis</i>	Chinese waterchestnut	9.0	30.1
Monocot	Alismataceae	<i>Sagittaria latifolia</i>	Broadleaf arrowhead	9.6	32.1
Monocot	Cyperaceae	<i>Cyperus serotinus</i>	Japanese nutsedge	10.0	33.4
Algae	Nostocaceae	<i>Anabaena flos-aquae</i>		-	44
Monocot	Poaceae	<i>Echinochloa phyllopogon</i>	Late watergrass	38.7	128.9
Monocot	Poaceae	<i>Echinochloa oryzoides</i>	Early watergrass	43.2	143.9
Monocot	Poaceae	<i>Echinochloa crus-galli</i> (L.) Beauv.	Barnyardgrass	46.8	155.8
Monocot	Poaceae	<i>Echinochloa colonum</i> (L.) Link	Junglerice	68.7	228.6
Monocot	Poaceae	<i>Leptochloa fascicularis</i> (L.) Nees	Bearded sprangletop	186.6	621.4

^a EC₅₀s were calculated based on surface area application rates (g/ha) and % visual injury to plants after 21 – 28d exposure to Bensulfuron methyl

^b EC₅₀s are calculated based on initial concentrations in 3cm deep flood water and % visual injury to plants after 21 – 28d exposure to Bensulfuron methyl

This suggests that a risk assessment based on laboratory studies with *Lemna*, in which test concentrations are maintained over the entire test period over-predicts risk to aquatic plants likely to be posed by Bensulfuron methyl under field conditions.

The inclusion of additional aquatic plant species in the risk assessment may well address uncertainty associated with inter-species sensitivity. Moreover, the availability of several tested species could allow a Species Sensitivity Distribution analysis and a trigger reduction, for national level authorizations.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Risk to sediment dwelling invertebrate species

Bensulfuron methyl was found to partition to sediment in a water/sediment fate study and therefore it is appropriate to assess its potential risk to sediment-dwelling organisms. A long-term study with *Chironomus* was not conducted because Bensulfuron methyl demonstrates very low acute and long-term/reproductive toxicity to aquatic invertebrates. Tests with *Daphnia magna* resulted in an 48-h EC₅₀ of >130 mg a.i./L and a 21-d NOEC of 12 mg a.i./L. In an acute, water-only test with the sediment organism *Chironomus tentans*, Bensulfuron methyl had a 96-h EC₅₀ >125 mg a.i./L.

The low toxicity to aquatic invertebrates, including sediment-dwelling invertebrates and moderately rapid dissipation of Bensulfuron methyl in sediment systems (mean, whole-system DT₅₀ = 48d) supports the conclusion that the risk of Bensulfuron methyl to sediment-dwelling invertebrate species is acceptable.

B.9.3 Effects on terrestrial vertebrates other than birds (Annex IIIA 10.3)

Toxicity testing with the formulated product, Bensulfuron methyl 60WG on terrestrial vertebrates was conducted and the endpoints used in the risk assessment are summarised in Table B.9.93.

Table B.9. 93: Summary of mammalian toxicity end-points for Bensulfuron methyl**B.9.3.1.1 Toxicity**

Test substance	Test organism	Exposure type	LD ₅₀ (mg/kg bw)	NOEL (mg s./kg bw/d)	Cross reference
Bensulfuron methyl	Rat	Acute oral	>5000	-	IIA, 5.2.1/01
Bensulfuron methyl	Rat	Two-generation reproduction	-	20.9 ^a (250) ^b	IIA, 5.6.1/01

^a NOEL in terms of daily dose is an average for F1 males based on slightly lower body weight in F1 adult males at 7500 ppm and females based on lower body weight in F1 litters at 7500 ppm.

^b NOEL in terms of dietary concentration of Bensulfuron methyl (mg a.i./kg feed).

B.9.3.1.2 Risk assessment*Consumption of contaminated food*

Risk of Bensulfuron methyl 60WG to wild mammals was assessed according to SANCO/4145/2000. The major exposure route for the Tier 1 risk assessment for mammals is considered to be intake of residues via contaminated food. Estimated exposure is expressed in terms of daily dose for all time scales considered.

Calculation of PEC_{food} (or ETE, mg/kg bw/day) for acute, and long-term exposure for wild mammals.

Scenarios for first Tier acute exposure estimate were based on the following assumptions:

- Application rate in rice is 0.06 kg Bensulfuron methyl/ha (max. 1 application per season)
- The contaminated diet is not avoided (AV Avoidance factor 1 = no avoidance)
- Animals satisfy their entire food demand in the treated area (PT Fraction of diet obtained in treated area = 1)
- Animals feed on a single food type (PD Fraction of food type in diet = 1; one type)

The relevant mammal indicator species according to crop and crop stage (cereals, early and late) are:

- Early: Small herbivorous mammal (vole, 25 g bw), and
- Late: Insectivorous mammal (shrew: 10 g bw)

- MAF = 1 (based on a single application per season)

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

- Foliar DT₅₀ (residue decline) for the first Tier used the default value of 10 days

The acute ETE (estimated theoretical exposure) of a compound is calculated with the following equation:

$$ETE = (FIR/bw) * RUD * Application\ rate * MAF * AV * PT * PD \text{ (mg/ kg bw/ day)}$$

FIR	Food intake rate of indicator species (gram fresh weight per day)
bw	Body weight (g)
RUD	(Residues per unit dose) residues in vegetation and insects 90 th percentile of the initial concentration
MAF	1 for residues in foliage (based on a single application per season) No MAF is applied for residues in insects
AV, PT, PD	All factors become 1 and can be omitted

Scenario for first Tier long-term exposure estimate is based on the following assumptions:

- The same assumptions are made to estimate long-term ETE as for acute and short-term exposure except that a time-weighted average estimate of residues is used.
- Time-weighted (twa) residues are used to reflect the long-term exposure. The f_{twa} factor is based on the following two default values: 1) time window (averaging time): 21 days (3 weeks), and 2) a foliar DT₅₀ = 10 days resulting in a f_{twa} value of 0.53 which is used in the calculations for long-term exposure
- In case of insects as food type no default f_{twa} factor is used for the first Tier as the time course of residue level is unknown

The long-term estimated daily uptake (ETE) of a compound is given by:

$$ETE = (FIR/bw) * RUD * Application\ rate * MAF * f_{twa} * AV * PT * PD \text{ (mg/kg bw/day)}$$

FIR	Food intake rate of indicator species (gram fresh weight per day)
bw	Body weight (g)
RUD	(Residues per unit dose) residues in vegetation and insects 90 th percentile of the initial concentration
MAF	1 for foliage (based on a single application per season) No MAF is applied for residues in insects
f_{twa}	Time-weighted residues are used, so it is assumed that over a period of 3 weeks the average concentration is about half the initial concentration – default value: 0.53
AV, PT, PD	All factors become 1 and can be omitted

Tier 1 risk assessments were conducted for mammals potentially exposed to dietary residues of Bensulfuron methyl after applications of Bensulfuron methyl 60WG to rice paddies in the European Union. The methods for selecting toxicity endpoints, calculating predicted exposure (ETE), and deriving toxicity to exposure ratios (TERs) were based upon SANCO/4145/2000. Acute and long-term TERs exceed relevant Annex VI criteria of 10 and 5, respectively (Table B.9. 94 and Table B.9. 95).

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9. 94: Acute Tier 1 TERs for wild mammals exposed to Bensulfuron methyl after application of Bensulfuron methyl 60WG in rice

Scenario	Indicator species	LD ₅₀ (mg a.i./ kg bw)	FIR/ bw	RUD	Rate applied (kg a.i./ha)	MAF	ETE	TER _a	Trigger value
Rice (early)	Small herbivorous mammal	>5000	1.39	142	0.06	1	11.84	>422	10
Rice (late)	Insectivorous mammal	>5000	0.63	14	0.06	1	0.53	>9434	10

Table B.9. 95: Long-term Tier 1 TERs for wild mammals exposed to Bensulfuron methyl after application of Bensulfuron methyl 60WG in rice

Scenario	Indicator species	NOEL (mg a.i./ kg bw/d)	FIR/ bw	RUD	Rate applied (kg a.i./ha)	MAF	F _{twa}	ETE	TER _{lt}	Trigger value
Rice (early)	Small herbiv. mammal	20.9	1.39	76	0.06	1	0.53	3.36	6.22	5
Rice (late)	Insectiv. mammal	20.9	0.63	5.1	0.06	1	1	0.19	108	5

Bioaccumulation and food chain behaviour

According to the Guidance Document on birds and mammals (Chapter 4 in SANCO/4145/2002) for organic chemicals with a $\log P_{ow} > 3$ the following assessment is used to indicate if there might be a potential for bioaccumulation. The $\log K_{ow}$ at pH 5 (highest measured $\log K_{ow}$) was 2.18 for Bensulfuron methyl and therefore an assessment of potential risk to earthworm and fish-eating mammals is not requested. However, in order to fully assess the potential risk to mammals from exposure to residues contained in food items including drinking water not covered in the standard exposure scenarios, the potential risk to mammals consuming residues contained in earthworms, fish and drinking water was assessed.

Food chain from earthworm to earthworm eating mammals

TER_{lt} calculations for food chain exposure of mammals to Bensulfuron methyl in relation to consumption of earthworms are provided in Table B.9. 96.

The Tier 1 TER_{lt} for worm-eating mammals are acceptable (>5), based on worst-case modelling of PEC_{soil} and assuming a maximum bioconcentration factor.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9. 96: Wild mammal long-term TER for food chain exposure of earthworm-eating mammals to Bensulfuron methyl

Inputs	Value	Comments
Octanol-water partitioning coefficient, K_{ow} , pH 5.0	151	From Physical-Chemical Section (Annex IIA, point 2)
$\log K_{ow}$, pH 5.0	2.18	
Organic carbon content of soil, f_{oc}	0.02	Given in SANCO/4145/2000
Organic carbon adsorption coefficient, K_{oc} (mL/g)	315	Average value for 3 Italian paddy soils (from Fate and Behaviour Section)
Estimated worm bioconcentration factor	0.37 ^a	$BCF_{worm} = (0.84 + 0.01K_{ow}) / (f_{oc} * K_{oc})$ assume worst-case $BCF_{worm} = 1.6$
Wild mammal Daily Dose conversion factor	1.4	Given in SANCO/4145/2000
Mammal chronic NOEL (mg a.i./kg bw day)	20.9	Cross reference IIA, 5.6.1/01
Maximum initial PEC_{soil} (mg/kg soil)	0.0782	Maximum initial PEC_{soil} based on 1 application of 60 g a.i./ha, instantaneous sorption to soil, 0% foliar interception (Med-Rice, 2003) (from Fate and Behaviour Section)
TER Calculations—Rice, Tier 1		
Worm residues (mg/kg worm)	0.125	$PEC_{worm} = PEC_{soil} * BCF_{worm}$, assuming $BCF_{worm} = 1.6$
Mammal daily dose (mg a.i./kg bw*d)	0.175	$DD_{mammal} = PEC_{worm} * 1.4$
Worm-eating Mammal TER_{it}	119	$TER_{it} = NOEL / DD_{mammal}$ $TER > 5$

^a Because measured BCF values are available for aquatic species, and the measured values exceed the estimated worm bioconcentration factor, the highest measured BCF (1.6) will be used as a worst-case estimate of BCF_{worm} .

Food chain from fish to fish-eating mammals

TER_{it} calculations for food chain exposure of fish-eating mammals to Bensulfuron methyl based on consumption of residues in fish are provided in Table B.9. 97.

Tier 1 TER_{it} for fish-eating mammals are acceptable (>5), based on Tier 1 estimates of PEC_{sw} .

Table B.9. 97: Wild mammal long-term TERs for food chain exposure of fish-eating mammals to Bensulfuron methyl

Inputs	Value	Comments
Fish BCF	1.6	Assumed maximum measured BCF (red swamp cray fish, <i>Procambrus clarki</i>), Cross reference IIA, 8.2.3/04
Mammal NOEL (mg a.i./kg bw*d)	20.9	Cross reference IIA, 5.2.1/01
Mammal Daily Dose conversion factor	0.13	Given in SANCO/4145/2000
Higher-Tier average maximum PEC_{sw} (mg/L)	0.0192	Assumes $PEC_{sw} = PEC_{paddy\ water}$, one application of Bensulfuron methyl (60 g a.i./ha) (from Fate and Behaviour Section)
Calculations—Rice, Tier 1		
PEC_{fish} (mg a.i./kg bw)	0.03	$PEC_{fish} = PEC_{sw} * BCF$
Mammal daily dose (mg a.i./kg bw*d)	0.004	$DD_{mammal} = PEC_{fish} * 0.13$
Fish-eating mammal TER_{it}	5359	$TER_{it} = NOEL / DD_{mammal}$ $TER_{it} > 5$

Exposure via drinking water

TER_{it} calculations for exposure to mammals through consumption of Bensulfuron methyl residues in drinking water are provided in Table B.9. 98.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Tier 1 TER_{it} for mammals potentially exposed to Bensulfuron methyl through drinking water are acceptable (>5), based on worst-case modelling of PEC_{sw} .

Table B.9. 98: TER_{it} for mammals exposed to Bensulfuron methyl in drinking water sources

Inputs	Value	Comments
Higher-Tier average maximum PEC_{sw} (mg/L)	0.0192	Maximum PEC_{sw} (assume $PEC_{sw} = PEC_{paddy\ water} = PEC_{drinking\ water}$), based on one application of Bensulfuron methyl (60 g a.i./ha) (from Fate and Behaviour Section)
Mammal NOEC [mg a.i./ (kg bw*day)]	20.9	Cross reference IIA, 5.6.1/01
<i>Calculations—Rice, Tier 1</i>		
Daily water intake (insectivorous mammals) (L/day)	0.0016	Total water ingestion rate (L/day) = $0.099W^{0.90}$ where W is body weight in kg (assume 0.010 kg shrew)
Mammal daily dose [mg a.i./ (kg bw*day)]	0.003	$DD = (PEC_{drinking\ water} * \text{Total water ingestion rate}) / W$ (assuming $PEC_{drinking\ water} = PEC_{paddy\ water}$, $0.034\ \mu\text{g a.i./L}$)
Mammal TER_{it} for drinking water exposure	6803	$TER_{it} = NOEL / DD_{mammal}$ $TER > 5$

Conclusion

All wild mammal acute and long-term TERs exceeded relevant Annex VI regulatory criteria, regardless of routes of exposure and therefore the risk of Bensulfuron methyl to wild mammals can be considered acceptable.

B.9.4 Effects on bees (Annex IIA 8.3.1; Annex IIA 10.4)

B.9.4.1 Acute toxicity to bees (Annex IIA 8.3.1.1)

49) *Bensulfuron Methyl (DPX-F5384): Acute oral and contact toxicity to the honey bee, Apis mellifera L. (Hymenoptera, Apidae). Wainwright, M.J., 2002.*

Cross reference IIA, 8.3.1.1./01

Guidelines:

OECD 213 (1998), OECD 214 (1998), EPPO 170 (OEPP/EPPO Bulletin 22, 203-215)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-333

Purity: 98.66%

The acute oral and contact toxicity test of Bensulfuron methyl to honey bees (*Apis mellifera* L.) were conducted as a limit test with a single test substance treatment rate of $100\ \mu\text{g a.i./bee}$ (oral and contact) (see Table B.9. 99 and Table B.9. 100) plus a control. Six replicates per treatment, each containing 10 honey bees per replicate total of 60 bees per treatment, are used. Controls consisted of untreated bees and bees provided oral 50% sucrose solution containing 5% N,N-dimethylformamide (DMF) (oral study) and untreated bees and bees to which DMF was applied (contact test). Dimethoate ("Pestanal", 99.8%) was the toxic standard used in these tests. In the oral test, each replicate bee cage

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

was provided 250 µL test solution. In the contact test, 2 µL of each treatment solution was applied to the dorsal thorax of each bee. Bees were observed 4, 24, and 48 hours after treatment for mortality and sublethal effects.

Findings:*Oral Exposure*

Oral repellence was observed at 100 µg Bensulfuron methyl/bee as doses were not fully consumed in the oral exposure study 6 hours after introduction. Only 51.41% of doses were consumed, giving a mean actual dose of 51.41 µg a.i./bee. A single bee died when exposed to 51.41 µg a.i./bee, resulting in a cumulative mortality of 1.7%. No sublethal effects (other than repellence) were observed. Control mortality was 3.3% in the untreated controls and 0% in the 50% sucrose/5% DMF controls.

Contact Exposure

A maximum mortality of 5% was observed 48 hours after contact application of Bensulfuron methyl at a rate equivalent to 100 µg a.i./bee (Table B.9. 100). There were no mortalities in any control at any time during the test. No sublethal effects were observed in any treatment.

The 48h oral and contact LD₅₀s for the toxic reference material were 0.12 and 0.19 µg a.i./bee, respectively. The study was considered valid since control mortality was acceptable and the toxic reference dimethoate generated LD₅₀ values were within the specified range of 0.1–0.35 µg a.i./bee.

Table B.9. 99: Acute oral toxicity of Bensulfuron methyl to honeybees

Treatment ^a (µg a.i./bee)	Test substance intake ^b (µg a.i./bee)	Mean cumulative mortality (%) ^c		
		4 hours	24 hours	48 hours
0 (untreated control)	N/A	0	3.3	3.3
0 (sucrose, DMF control)	N/A	0	0	0
100	51.41	1.7	1.7	1.7

^a Treatments are specified as intended uptake, in µg a.i./bee per treatment rate.

^b Test substance intake is specified as actual uptake, in mean µg a.i./bee per treatment rate.

^c Test mortality for treatments is corrected for control mortality (mortality of bees exposed to sucrose/DMF solution alone).

Table B.9. 100: Acute contact toxicity of Bensulfuron methyl to honeybees

Treatment ^a (µg a.i./bee)	Mean cumulative mortality ^b (%)		
	4 hours	24 hours	48 hours
0 (untreated control)	0	0	0
0 (DMF control)	0	0	0
100	0	1.7	5

^a Treatments are specified as mean µg a.i./bee per treatment rate, applied to the dorsal thorax of each bee.

^b Test mortality for treatments is corrected for control mortality (mortality of bees exposed to sucrose solution alone).

Conclusion:

Acute Endpoint	Benflusulfuron methyl (µg a.i./bee)
48-h Oral LD ₅₀	>51.41
48-h Oral NOEL	51.41
48-h Contact LD ₅₀	>100
48-h Contact NOEL	100

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

B.9.4.2 Bees brood feeding test (Annex IIA 8.3.1.2)

Bensulfuron methyl is not an insect growth regulator. Therefore a bee brood feeding test is not required.

B.9.4.3 Acute toxicity of the preparation to bees (Annex IIIA 10.4.1)

Laboratory oral and contact toxicity tests with the formulation product Bensulfuron methyl 60WG on honey bees are covered by the test on active substance. Further testing is not necessary because, the toxicity of Bensulfuron methyl to bees is very low (Table B.9. 101) and the product contains only one active substance.

Table B.9. 101: Summary of honeybee acute oral and contact toxicity end-points for Bensulfuron methyl

Test substance	Test organism	Exposure type	Mortality at highest test concentration (%)	LD ₅₀ (µg as/bee)	Cross reference
Bensulfuron methyl technical	<i>Apis mellifera</i>	Oral	1.7	>51.41 ^a	IIA, 8.3.1.1.1/01
Bensulfuron methyl technical	<i>Apis mellifera</i>	Contact	5.0	>100	IIA, 8.3.1.1.1/01

^a Due to avoidance of the diet, the reported LD₅₀ is based on the maximum amount of diet consumed.

B.9.4.4 Residue test (Annex IIIA 10.4.2)

The Q_{HO} and Q_{HC} are <1.2 and <0.6, respectively. Therefore, residues of Bensulfuron methyl 60WG will pose a low risk to honey bees and no further testing is needed.

B.9.4.5 Cage test (Annex IIIA 10.4.3)

The Q_{HO} and Q_{HC} are <1.2 and <0.6, respectively. Therefore, Bensulfuron methyl 60WG will pose a low risk to honey bees and no further testing is needed.

B.9.4.6 Field tests (Annex IIIA 10.4.4)

The Q_{HO} and Q_{HC} are <1.2 and <0.6, respectively. Therefore, Bensulfuron methyl 60WG will pose a low risk to honey bees and no further testing is needed.

B.9.4.7 Tunnel test (Annex IIIA 10.4.5)

The Q_{HO} and Q_{HC} are <1.2 and <0.6, respectively. Therefore, Bensulfuron methyl 60WG will pose a low risk to honey bees and no further testing is needed.

B.9.4.8 Summary of effects, exposure and hazard assessment for bees

Based on the maximum application rate specified on the GAP (60 g a.i./ha) and the endpoints from acute oral and contact toxicity tests, the hazard quotients were calculated (Table B.9. 102).

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9. 102: Summary of hazard quotients for honey bees and Bensulfuron methyl

Test substance	Exposure route	Max. application rate of Bensulfuron methyl 60WG (g a.i./ha)	LD ₅₀ (µg a.i./bee)	HQ	Cross reference
Bensulfuron methyl	oral	60	>51.41	<1.2	IIA, 8.3.1.1.1/01
Bensulfuron methyl	contact	60	>100	<0.6	IIA, 8.3.1.1.1/01

The Q_{HO} and Q_{HC} for Bensulfuron methyl are below the Annex VI decision making criterion of 50. In addition, the very low toxicity of Bensulfuron methyl formulated product, Bensulfuron methyl 60WG, to non-target arthropods suggests that the formulation would be no more toxic to bees than the active substance. Therefore, it may be concluded that Bensulfuron methyl 60WG poses a low risk to honey bees.

B.9.5 Effects on other arthropod species (Annex IIA 8.3.2; IIIA 10.5)

B.9.5.1 Effects of the active substance (Annex IIA 8.3.2)

50) *Bensulfuron methyl (DPX-F5384) 60WG: A multi-rate toxicity test with the predatory mite, Typhlodromus pyri Scheuten (Acari: Phytoseiidae). Nienstedt, K.M., Kollmann, S.I., 2001.*

Cross reference IIA, 8.3.2./01

Guidelines:

Directive 91/414/EEC; Louis and Ufer (1995); Blümel *et al.* (2000)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 60WG

Batch: F5384-322

Purity: 600 g a.i./kg

Predatory mite mortality and reproductive effects (mean cumulative eggs per female) were evaluated in a multi-rate test during a 14-day exposure to fresh-dried spray residues of Bensulfuron methyl 60WG on treated glass plates. The test comprised 5 replicates of 20 protonymphs for each treatment rate, control, and toxic reference (dimethoate). The test item was applied at 100, 500, and 1000 g Bensulfuron methyl 60WG/ha in a volume equivalent to 200 L water/ha. These rates were equivalent to 60, 300, and 600 g Bensulfuron methyl a.i./ha (nominal). The toxic reference was applied at 12 mL Perfekthion/ha. Mortality was assessed on after 7 days of exposure; reproduction (fecundity) was assessed on Days 7, 9, 11, and 14.

Findings:

A summary of the effects of Bensulfuron methyl 60WG on mortality and reproduction (fecundity) are shown in Table B.9. 103. Mortality in all Bensulfuron methyl 60WG treatment groups was not statistically different from controls. Adult mortality after 7 days of exposure in the control and toxic standard groups was 5 and 98%,

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

respectively. All validation criteria were met or exceeded. The LR_{50} was >1000 g Bensulfuron methyl 60WG/ha in the highest treatment group. The differences of reproduction between the control and the 100 g product/ha test item treatment were statistically significant, but this significance was not confirmed in the higher test item rates, which did not differ statistically significantly.

Table B.9. 103: Effects on mortality and reproduction of the predatory mite, *Typhlodromus pyri*, exposed to fresh-dried spray deposits of Bensulfuron methyl 60WG in the laboratory

Test item	Rate (g product/ha)	Mortality (%)	Corrected mortality (%) ^a	Mean cumulative reproduction	Reduction in reproduction (%)
Untreated control	-	5.0 ± 7.1	-	9.1 ± 1.5	-
Toxic Standard (dimethoate)	12 (mL product/ha)	98.0 ± 2.7	97.9	-	-
Bensulfuron methyl 60WG	100	4.0 ± 5.5 ^b	-1.1	6.2 ± 2.2 ^c	32.5
Bensulfuron methyl 60WG	500	12.0 ± 10.4 ^b	7.4	8.0 ± 0.5 ^d	12.6
Bensulfuron methyl 60WG	1000	6.0 ± 6.5 ^b	1.1	6.7 ± 1.7 ^d	26.7

- Not assessed

^a Corrected mortality according to Schneider-Orelli

^b Not statistically significantly different from the control group (Fisher exact test: $p > 0.05$)

^c Statistically significantly different from the control (ANOVA: $p = 0.043$; Dunnet test: $p = 0.026$)

^d Statistically significantly different from the control (ANOVA: $p = 0.043$; Dunnet test: $p > 0.05$)

Conclusions:

The LR_{50} for *Typhlodromus pyri* was >1000 g Bensulfuron methyl 60WG/ha (equivalent to >600 g Bensulfuron methyl a.i./ha).

51) *Bensulfuron methyl (DPX-F5384) 60WG: A multi-dose toxicity test with the aphid parasitoid, Aphidius rhopalosiphii (Hymenoptera: Braconidae). Nienstedt, K.M., Novent, O., 2001.*

Cross reference IIA, 8.3.2./02

Guidelines:

Directive 91/414/EEC; Mead-Briggs (1992); Mead Briggs *et al.* (2000)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 60WG

Batch: F5384-332

Purity: 600 g a.i./kg

Adult mortality and reproductive effects (parasitism rate) were evaluated in a multi rate test following a 48-hour exposure period to fresh-dried spray residues of the test item on treated glass plates. The test comprised 4 replicates of 10 individuals for each treatment rate, control, and toxic reference (0.5 mL Perfekthion (dimethoate)/ha). The test

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

item was applied at 100, 500, and 1000 g Bensulfuron methyl 60WG/ha in a volume equivalent to 200 L water/ha. These rates are equivalent to 60, 300, and 600 g Bensulfuron methyl a.i./ha (nominal).

Mortality of the adult wasps was assessed after 48 hours exposure to fresh residues. Fecundity was measured after the mortality assessment by counting the number of mummies produced with 24 hours by 15 females (15 replicates, 1 female/replicate).

Findings:

No adult mortality was observed in the control or any of the Bensulfuron methyl 60WG treatment groups. The toxic reference resulted in 100% mortality. All study validation criteria were met or exceeded. Results of the multi rate testing are given below in Table B.9. 104.

Table B.9. 104: The effects on mortality of the aphid parasitoid, *Aphidius rhopalosiphi*, exposed to fresh-dried spray deposits of Bensulfuron methyl 60WG on glass plates in the laboratory

Test item	Rate (g a.i./ha)	Healthy (%)	Affected ^a (%)	Moribund (%)	Dead (%)	Moribund + dead (%)
Untreated control	-	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Toxic standard (dimethoate)	0.50 (mL product/ha)	0 ± 0	0 ± 0	0 ± 0	100 ± 0	100 ± 0
Bensulfuron methyl 60WG	100 ^b	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0.0
Bensulfuron methyl 60WG	500	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0.0
Bensulfuron methyl 60WG	1000	100 ± 0	0 ± 0	0 ± 0	0 ± 0	0.0

^a Wasps showing uncoordinated movements

^b Only 3 replicates were used for the assessment at this rate due to a problem with the test unit that allowed all the wasps to escape.

The mean numbers of mummies per surviving female were 28.1, 27.7, 22.9, and 29.8 for the control and 100, 500, and 1000 g formulated Bensulfuron-methyl 60WG/ha, respectively. There were no statistically significant differences between the test item treatments and the control (ANOVA: $p = 0.271$) (Table B.9. 105).

Table B.9. 105: The effects on reproduction of the aphid parasitoid, *Aphidius rhopalosiphi*, exposed to fresh-dried spray deposits of Bensulfuron methyl 60WG on glass plates in the laboratory

Test Item	Rate (g product/ha)	Aphid mummies/ surviving female (mean ± SD) ^a	% Reduction	Female mortality (mean ± SD)
Untreated control	-	28.1 ± 10.8	0	0 ± 0
Toxic Standard (dimethoate)	n.a.	n.a.	n.a.	n.a.
Bensulfuron methyl 60WG	100	27.7 ± 7.6	1.2	0 ± 0
Bensulfuron methyl 60WG	500	22.9 ± 9.2	18.5	0 ± 0
Bensulfuron methyl 60WG	1000	29.8 ± 11.5	-6.1	0 ± 0

^a No statistically significant differences between test item and control treatments were observed (ANOVA: $p = 0.271$)

Conclusions:

The LR₅₀ for *Aphidius rhopalosiphi* was >1000 g Bensulfuron methyl 60WG/ha, which is equivalent to >600 g Bensulfuron methyl a.i./ha.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

B.9.5.2 Effects of the formulation (Annex IIIA, 10.5)

Since no significant effects on the organisms in comparison with the control are reported in the laboratory test at the maximum recommended dose (see B.9.5.1), other testing are not required.

B.9.5.3 Summary of effects, exposure and hazard assessment for other arthropods species

B.9.5.3.1 Toxicity

Worst-case (Tier 1) laboratory studies bioassays with Bensulfuron methyl 60WG were conducted on the predatory mite, *Typhlodromus pyri*, and the aphid parasitoid, *Aphidius rhopalosiphi*. A summary of the endpoints is presented in Table B.9. 106.

Table B.9. 106: Summary of effects of Bensulfuron methyl on non target arthropods

Species	Test	Measurement endpoint	Endpoint value	Cross reference
<i>Aphidius rhopalosiphi</i>	Tier 1	LR ₅₀	> 600 g a.i./ha	IIA, 8.3.2/02
<i>Typhlodromus pyri</i>	Tier 1	LR ₅₀	> 600 g a.i./ha	IIA, 8.3.2/01

B.9.5.3.2 Risk assessment

Risk assessment methods for non-target arthropods are based on recommendations from the Guidance Document on Regulatory Testing and Risk Assessment Procedures for Plant Protection Products with Non-target Arthropods, ESCORT 2 (Candolfi *et al.* 2001).

The Tier 1 risk assessment for non-target arthropods is conducted by comparing calculated in-field and off-field hazard quotients (HQs) to a proposed regulatory decision making criterion of 2. If the HQs fall below 2, risk to the test species is considered to be low.

The following equations were used to calculate the HQs:

$$\text{In-field HQ} = \frac{\text{Application rate} \times \text{MAF}}{\text{LR}_{50}}$$

$$\text{Off-field HQ} = \frac{\text{Application rate} \times \text{MAF} \times (\text{drift factor/veg. distr. factor}) \times \text{correction factor}}{\text{LR}_{50}}$$

Where,

- Application rate = maximum proposed GAP application rate (g/ha)
- MAF = multiple application factor (ratio of vegetative DT₅₀: Spray application interval plus the number of applications)
- LR₅₀ (lethal rate causing 50% mortality in g/ha)
- Drift factor = crop-specific and growth-stage specific drift at a specified distance (%) (2.77%)
- Vegetation distribution factor = correction factor used to take into consideration the effect of the field boundary and vegetated filter strip on reducing drift; default value of 10
- Correction factor (uncertainty factor): this factor takes into consideration the uncertainty with the extrapolation from *T. pyri* and *A. rhopalosiphi* to all off-field non-target arthropods; default value = 10

Input parameters for the non-target arthropod worst-case Tier 1 risk assessment, for Bensulfuron methyl 60WG use in rice, 1 application, were the following:

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

- Application rate: 60 g Bensulfuron methyl a.i./ha
- MAF: 1 (for one application)
- *Aphidius rhopalosiphi* LR₅₀ = 600 g a.i./ha and *Typhlodromus pyri* LR₅₀ = 600 g a.i./ha
- Drift factor = 2.77% (basic 90th percentile drift value for one application, 1 m distance, in field crops; see ESCORT 2 Appendix VI)
- Vegetation distribution factor = 10 (default value)
- Correction factor (uncertainty factor) = 10 (default value)

Table B.9. 107: Tier 1 summary of non-target arthropod in-field and off-field hazard quotients (HQ) for Bensulfuron methyl 60WG use in rice at 1 x 60 g a.i./ha

Species	LR ₅₀ (g a.i./ha)	In-field HQ	Off-field HQ	Trigger
<i>T. pyri</i>	>600	<0.1	<0.0028	2
<i>A. rhopalosiphi</i>	>600	<0.1	<0.0028	2

Based on the Tier 1 risk assessment for the two sensitive indicator species, *Aphidius rhopalosiphi* and *Typhlodromus pyri*, Bensulfuron methyl 60WG poses a very low risk to in-field and off-field non-target arthropod populations (all HQ values are much less than 2), when used according to Good Agricultural Practice. This conclusion is supported by the low toxicity and hazard quotients calculated for honeybees.

B.9.6 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.6.1)

B.9.6.1 Acute toxicity (Annex IIA 8.4.1)

B.9.6.1.1 Acute toxicity – Bensulfuron methyl

52) *Bensulfuron methyl technical: acute toxicity to the earthworm, Eisenia foetida (Savigny) in artificial soil. Lührs, U., 2001.*

Cross reference IIA, 8.4.1/01

Guidelines:

OECD 207 (1984); ISO 11268, part 1 (1993)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 technical

Batch: F5384-333

Purity: 98.66%

Acute toxicity of Bensulfuron methyl to earthworms, *Eisenia foetida* Savigny, was determined in a 14-day soil exposure laboratory study conducted under OECD Guideline 207. Four replicates of 10 clitellated adult earthworms were each exposed to Bensulfuron methyl at nominal concentrations of 198, 296, 444, 667, and 1000 mg/kg soil dry weight. Control was replicated 4 times, with 10 earthworms in each replicate. The toxic reference standard, 2-

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Chloroacetamide, is tested at least once a year at 5 rates. Worms were assessed for mortality and sublethal effects after 7 and 14 days of exposure and earthworm body weights were assessed at days 0 and 14.

Findings:

Cumulative mortality results at 7 and 14 days and weight loss at 14 days are reported in the summary Table B.9. 108. The body weight decreases observed 198 and 296 mg a.i./kg soil dry weight, although statistically significant, are not considered to be treatment related because significant effects were not seen at higher concentrations. The LD₅₀ for the toxic reference standard was 21.5 mg a.i./kg soil dry weight; this is within accepted limits, indicating the validity of this test.

Table B.9. 108: Acute toxicity of Bensulfuron methyl to earthworms

Bensulfuron methyl concentration (mg a.i./kg soil wt)	Cumulative mortality (%)		Weight loss (%)
	7 days	14 days	14 days
0	0	0	-12.4
198	0	0	-6.9 ^a
296	0	0	-6.4 ^a
444	0	0	-8.6
667	0	0	-10.3
1000	0	0	-8.5

^a Statistically different from controls (Dunnett's test, $\alpha = 0.05$)

Conclusions:

The acute earthworm LC₅₀ of Bensulfuron methyl was determined to be higher than 1000 mg/kg soil dry weight.

B.9.6.1.2 Acute toxicity – metabolites of Bensulfuron methyl

53) IN-J0290: Acute toxicity to the earthworm, *Eisenia foetida* in artificial soil. Lührs, U., 2002a.

Cross reference IIA, 8.4.1/02

Guidelines:

OECD 207; ISO 11268-1

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-J0290 technical metabolite

Batch: J0290-17

Purity: 99.6%

Acute toxicity of IN-J0290 to earthworms, *Eisenia foetida* Savigny, was determined in a 14-day soil exposure laboratory study conducted under OECD Guideline 207 and ISO 11268-1 (1993). Four replicates of 10 clitellated adult earthworms were each exposed to nominal concentrations of 198, 296, 444, 667, and 1000 mg IN-J0290/kg soil dry weight. Control was replicated 4 times, with 10 earthworms in each replicate. The toxic reference standard, 2-

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Chloroacetamide, is tested at least once a year at 5 rates. Worms were assessed for mortality and sublethal effects after 7 and 14 days of exposure and earthworm body weights were assessed at days 0 and 14.

Findings:

Cumulative mortality results at 7 and 14 days and weight loss at 14 days are reported in the summary Table B.9. 109. No mortality was observed up to 1000 mg IN-J0290/kg soil dry weight. Body weight changes were significantly different compared to the control in the concentration of 1000 mg IN-J0290/kg soil dry weight. No behavioural effects were observed at either 7 or 14 days after exposure in any of the treatment groups.

The LD₅₀ for the toxic reference standard was 21.1 mg 2-Chloroacetamide/kg soil dry wt.; this is within accepted limits, indicating the validity of this test.

Table B.9. 109: Acute toxicity of IN-J0290 to earthworms

IN-J0290 concentration (mg a.i./kg soil dry wt)	Cumulative mortality (%)		Mean cumulative weight change (%)
	7 days	14 days	14 days
0	0	0	-5.3
198	0	0	2.4
296	0	0	-2.4
444	0	0	5.5
667	0	0	-7.7
1000	0	0	-1.7

None of the treatment effects were statistically different when compared to control (Dunnett's test, $\alpha = 0.05$)

Conclusions:

The 14-d acute earthworm LC₅₀ of IN-J0290 was determined to be higher than 1000 mg a.i./kg soil dry wt.

54) *IN-D1R84: Acute toxicity to the earthworm, Eisenia foetida in artificial soil. Lührs, U., 2002b.*

Cross reference IIA, 8.4.1./03

Guidelines:

OECD 207 (1984); ISO 11268, part 1 (1993)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-D1R84 technical metabolite

Batch: D1R84-001

Purity: 98.9%

Acute toxicity of IN-D1R84 or 2-[(aminosulfonyl)methyl]benzoic acid (uninverted CAS name), a metabolite of Bensulfuron methyl, to earthworms, *Eisenia foetida* Savigny, was determined in a 14-day soil exposure laboratory study conducted under OECD Guideline 207. Four replicates of 10 clitellated adult earthworms were each exposed to nominal concentrations of 95, 171, 309, 556, and 1000 mg of IN-D1R84/kg soil dry weight. Control was replicated 4 times, with 10 earthworms in each replicate. The toxic reference standard, 2-Chloroacetamide, is tested at least once

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

a year at 5 rates. Worms were assessed for mortality and sublethal effects after 7 and 14 days of exposure and earthworm body weights were assessed at days 0 and 14.

Findings:

Cumulative mortality results at 7 and 14 days and weight loss at 14 days are reported in the summary Table B.9. 110. No mortality, no effect on body weight changes and no adverse behavioural effects were observed at either 7 or 14 days after exposure in any of the treatment groups.

The LD₅₀ for the toxic reference standard was 21.1 mg a.i./kg soil dry wt; this is within accepted limits, indicating the validity of this test.

Table B.9. 110: Acute toxicity of IN-D1R84 to earthworms

IN-D1R84 concentration (mg a.i./kg soil dry wt)	Cumulative mortality (%)		Cumulative weight loss (%)
	7 days	14 days	14 days
0	0	0	7.9
95	0	0	8.3
171	0	0	6.6
309	0	0	4.6
556	0	0	4.4
1000	0	0	7.4

No effects were significantly different from the controls (Dunnnett test, $\alpha = 0.05$)

Conclusions:

The 14-d acute earthworm LC₅₀ of IN-D1R84 was determined to be higher than 1000 mg/kg soil dry wt.

55) *IN-R9419: Acute toxicity to the earthworm, Eisenia foetida in artificial soil. Lührs, U., 2002c.*

Cross reference IIA, 8.4.1./04

Guidelines:

OECD 207 (1984); ISO 11268, part 1 (1993)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-R9419 technical metabolite

Batch: R9419-002

Purity: 98.5%

Acute toxicity of IN-R9419 to earthworms, *Eisenia foetida* Savigny, was determined in a 14-day soil exposure laboratory study conducted under OECD Guideline 207. Four replicates of 10 clitellated adult earthworms were each exposed to nominal concentrations of 198, 296, 444, 667, and 1000 mg of IN-R9419/kg soil dry weight. Control was replicated 4 times, with 10 earthworms in each replicate.

The toxic reference standard, 2-Chloroacetamide, is tested at least once a year at 5 rates. Worms were assessed for mortality and sublethal effects after 7 and 14 days of exposure and earthworm body weights were assessed at days 0 and 14.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Findings:

Cumulative mortality results at 7 and 14 days and weight loss at 14 days are reported in the summary Table B.9. 111. No adverse behavioural effects were observed at either 7 or 14 days after exposure in any treatment group. The LD₅₀ for the toxic reference standard was 21.1 mg a.i./kg soil dry wt; this is within accepted limits, indicating the validity of this test.

Table B.9. 111: Acute toxicity of IN-R9419 to earthworms

IN-R9419 concentration (mg a.i./kg soil dry wt)	Cumulative mortality (%)		Cumulative weight loss (%)
	7 days	14 days	14 days
0	0	0	-3.3
198	0	0	-1.6
296	0	0	-3.1
444	0	0	-0.2
667	0	2.5	3.5
1000	0	0	3.6

No effects were significantly different from the controls (Dunnett test, $\alpha = 0.05$)

Conclusions:

The 14-d acute earthworm LC₅₀ of IN-R9419 was determined to be higher than 1000 mg/kg soil dry wt.

56) *IN-N5297: Acute toxicity to the earthworm, Eisenia foetida in artificial soil. Lührs, U., 2002d.*

Cross reference IIA, 8.4.1./05

Guidelines:

OECD 207 (1984); ISO 11268, part 1 (1993)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-N5297 technical metabolite

Batch: N5297-004

Purity: 99.5%

Acute toxicity of IN-N5297 to earthworms, *Eisenia foetida* Savigny, was determined in a 14-day soil exposure laboratory study conducted under OECD Guideline 207. Four replicates of 10 clitellated adult earthworms were each exposed to nominal concentrations of 63, 125, 250, 500, and 1000 mg IN-N5297/kg soil dry weight. Control was replicated 4 times, with 10 earthworms in each replicate. The toxic reference standard, 2-Chloroacetamide, is tested at least once a year at 5 rates. Worms were assessed for mortality and sublethal effects after 7 and 14 days of exposure and earthworm body weights were assessed at days 0 and 14.

Findings:

Cumulative mortality results at 7 and 14 days and weight loss at 14 days are reported in the summary Table B.9. 112. No mortality was observed up to 1000 mg IN-N5297/kg soil dry weight. Body weight changes were significantly

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

different compared to the control in the concentration of 1000 mg IN-N5297/kg soil dry weight. No significant sublethal behavioural effects were observed.

The LD₅₀ for the toxic reference standard was 30.4 mg a.i./kg soil dry wt; this is within accepted limits, indicating the validity of this test.

Table B.9. 112: Acute toxicity of IN-N5297 to earthworms

IN-N5297 concentration (mg a.i./kg soil dry wt)	Cumulative mortality (%)		Cumulative weight change (%)
	7 days	14 days	14 days
0	0	0	1.8
62.5	0	0	2.2
125	0	0	-2.2
250	0	0	-1.4
500	0	0	-1.5
1000	0	0	-5.8 ^a

^a Statistically different from the controls (Dunnett test, $\alpha = 0.05$)

Conclusions:

The 14-d acute earthworm LC₅₀ of IN-N5297 was determined to be higher than 1000 mg/kg soil dry wt.

57) *IN-F7880: Acute toxicity to the earthworm, Eisenia foetida in artificial soil. Lührs, U., 2002e.*

Cross reference IIA, 8.4.1./06

Guidelines:

OECD 207 (1984); ISO 11268, Part 1 (1993)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-F7880 technical metabolite

Batch: F7880-004

Purity: 97.3%

Acute toxicity of IN-F7880 to earthworms, *Eisenia foetida* Savigny, was determined in a 14-day soil exposure laboratory study conducted under OECD Guideline 207. Four replicates of 10 clitellated adult earthworms were each exposed to nominal concentrations of 198, 296, 444, 667, and 1000 mg IN-F7880/kg soil dry weight. Control was replicated 4 times, with 10 earthworms in each replicate. The toxic reference standard, 2-Chloroacetamide, is tested at least once a year at 5 rates. Worms were assessed for mortality and sublethal effects after 7 and 14 days of exposure and earthworm body weights were assessed at days 0 and 14.

Findings:

Cumulative mortality results at 7 and 14 days and weight loss at 14 days are reported in the summary Table B.9. 113. After 7 days of exposure one worm was observed with cut off body parts in the treatment group of 198 mg/kg soil dry weight. No other adverse effects were observed at either 7 or 14 days in any other treatment group.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

No significant sublethal behavioural effects were observed. The LD₅₀ for the toxic reference standard was 30.4 mg/kg soil dry wt; this is within accepted limits, indicating the validity of this test.

Table B.9. 113: Acute toxicity of IN-F7880 to earthworms

IN-F7880 concentration (mg a.i./kg soil dry wt)	Cumulative Mortality (%)		Cumulative Weight Change (%)
	7 days	14 days	14 days
0	0	0	7.3
198	0	0	0.1
296	0	0	2.3
444	0	0	5.7
667	0	0	4.9
1000	0	0	-4.3

No effects were significantly different from the controls (Student-t test, $\alpha = 0.05$)

Conclusions:

The 14-d acute earthworm LC₅₀ of IN-F7880 was determined to be higher than 1000 mg/kg soil dry weight.

B.9.6.2 Sublethal effects (Annex IIA 8.4.2)

This study was not conducted based on the low toxicity of Bensulfuron methyl to earthworms (no significant mortality or sublethal effects at up to 1000 ppm) tested for 14 days in artificial soil in which Bensulfuron methyl would not be degraded to a significant extent for the duration of the test. Bensulfuron methyl may be applied once per season, and its relatively rapid degradation under field conditions indicates that sublethal effects on earthworms are very unlikely.

B.9.6.3 Acute toxicity of the formulation (Annex IIIA 10.6.1.1)

A toxicity test on earthworms with the formulated product Bensulfuron methyl 60WG was not conducted for the following reasons:

- The product contains only one active substance,
- Testing was performed on the active substance Bensulfuron methyl,
- Toxicity of Bensulfuron methyl technical to earthworms is very low (Table B.9. 114), and
- Testing on other non-target organisms has indicated that the toxicity of the formulated product can be predicted from the toxicity of the active substance.

B.9.6.4 Sublethal effects of the formulation (Annex IIIA 10.6.1.2)

A sublethal earthworm test was not conducted for the following reasons:

- The product contains only one active substance,
- Testing the formulated product Bensulfuron methyl 60WG on non-target arthropods, aquatic species and mammals suggests that the toxicity of the formulation may be predicted based on the toxicity of the active substance,
- The acute toxicity of Bensulfuron methyl technical to earthworms is very low (Table B.9. 114). Bensulfuron methyl is not expected to persist in the soil. The average DT₅₀ measured in paddy soil is 8 days (Bensulfuron methyl EU Dossier, Annex IIA, Document M-II, Section 5, DuPont-6185 EU, Point 7.1.1.2.2, Report No AMR 4162-96).

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

B.9.6.5 Field studies of the formulation (Annex IIIA 10.6.1.3)

The earthworm TER_a value for Bensulfuron methyl 60WG was much greater than the Annex VI criterion of 10. Therefore field testing is not necessary.

B.9.6.6 Summary of effects, exposure and hazard assessment for earthworms**B.9.6.6.1 Toxicity**

Bensulfuron methyl and its soil metabolites have low toxicity to earthworms.

Table B.9. 114: Summary of the acute toxicity endpoint values for Bensulfuron methyl and soil metabolites

Test substance	Test organisms	Test/duration	Endpoint	Endpoint value (mg a.i./kg soil dry weight)
Bensulfuron methyl	<i>Eisenia foetida</i>	Acute (14d)	LC ₅₀	>1000
IN-J0290	<i>Eisenia foetida</i>	Acute (14d)	LC ₅₀	>1000
IN-N5297	<i>Eisenia foetida</i>	Acute (14d)	LC ₅₀	>1000
IN-D1R84	<i>Eisenia foetida</i>	Acute (14d)	LC ₅₀	>1000
IN-R9419	<i>Eisenia foetida</i>	Acute (14d)	LC ₅₀	>1000
IN-F7880	<i>Eisenia foetida</i>	Acute (14d)	LC ₅₀	>1000

The TER values were determined for Bensulfuron methyl and Bensulfuron methyl soil metabolites based on the ratio of the LC₅₀ values to the maximum initial PEC_{soil}. Worst-case, Tier 1 PEC_{soil} values were determined using the methods given in Med-Rice (2003) for both sand and clay soil scenarios. It was assumed that Bensulfuron methyl was applied at 60 g a.i./ha and initial PEC_{soil} for the metabolites was equivalent to the maximum observed in any soil degradation study. Because the clay scenario PECs were higher than the sand scenario values, they were chosen for use in the risk assessment.

B.9.6.6.2 Exposure

The Tier 1 predicted environmental concentrations of Bensulfuron methyl and metabolites IN-J0290, IN-F7880, IN-R9419, IN-N5297, and IN-D1R84 were determined for soil (PEC_{soil}) using the equations and guidance provided by the Med-Rice working group (Med-Rice, 2003).

For the Tier 1 assessment, a single application of 60 g a.i./ha to flooded rice was simulated per proposed Good Agricultural Practices (GAP). Foliar interception was conservatively assumed not to occur (100% reaching the flooded paddy surface). Partitioning from paddy water to paddy soil was treated as an instantaneous, irreversible process. The amount of partitioning was determined by the compound distribution coefficient ($K_{\text{foc}} \times \text{fraction organic carbon of soil}$), the depth of the paddy water (10 cm), depth of paddy soil active in the sorption process (5 cm), and the paddy soil bulk density (1.5 g/cm³).

Where available, the longest total system first-order DT₅₀ values from the water/sediment study were used to represent degradation in paddy water and paddy soil. Alternatively, the geomean DT₅₀ from the aerobic soil degradation study was selected. The average K_{foc} was used to represent sorption.

Tier 1 PEC_{soil} values for metabolites were calculated in the same manner as for Bensulfuron methyl, but with the dose rate determined using the maximum percentage formed in any environmental fate study and the molecular weight conversion. The actual and time-weighted average PEC_{soil} values over time were calculated using standard first-order expressions provided in the Med-Rice (2003) Guidance Document.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

B.9.6.6.3 Risk assessment

A summary of the calculated acute TERs for Bensulfuron methyl and its potential soil degradates is presented in Table B.9. 115.

Table B.9. 115: Acute TERs for earthworms exposed to maximum potential predicted environmental concentrations of Bensulfuron methyl and its degradates in soil for the use of Bensulfuron methyl 60WG in rice

Test substance ^c	LC ₅₀ (mg a.i./kg soil) ^a	Maximum initial PEC _{soil} (mg a.i./kg soil) ^b	TER _a	Annex VI Trigger
Bensulfuron methyl	>1000	0.0782	>12788	10
IN-F7880	>1000	0.00723	>138313	10
IN-R9419	>1000	0.0208	>48077	10
IN-D1R84	>1000	0.00418	>239234	10
IN-J0290	>1000	0.0136	>73529	10
IN-N5297	>1000	0.0117	>85470	10

^a The LC₅₀ was not adjusted for Log P because the Log K_{ow} for Bensulfuron methyl is 0.79 at pH 7.0.

^b Maximum Tier 1 PEC_{soil} derived from the clay scenario for Bensulfuron methyl and degradates determined using the methods given in Med-Rice (2003)

The TER_a values far exceed the relevant Annex VI decision-making criterion of 10 for earthworms. Therefore, it can be concluded that acute risk to earthworms from the use of Bensulfuron methyl 60WG in rice will be low.

Chronic earthworm testing with Bensulfuron methyl or its soil degradates was not considered necessary due to low persistence in field, extremely low acute earthworm toxicity and low PEC_{soil} for both the parent and its soil degradates.

B.9.7 Effects on other soil non-target macro-organisms (Annex IIIA 10.6.2)

The field DT₉₀ of Bensulfuron methyl in soil is much less than 100 days. In a field soil dissipation study conducted in rice paddies in Italy, the DT₅₀ was 8 days (average) and 12 days (longest). Based on the longest DT₅₀ value from the field dissipation study, the field DT₉₀ is estimated to be approximately 40 days.

Other non-target macro-organisms are not expected to be at risk from exposure to residues Bensulfuron methyl 60WG. Evidence from earthworms, honeybees, and beneficial arthropods toxicity tests suggest low toxicity of Bensulfuron methyl 60WG residues to non-target invertebrate fauna. Therefore, Bensulfuron methyl 60WG, when applied according to Good Agricultural Practice, is expected to pose little acute or long-term risk to soil non-target macro-organisms and no further testing is warranted.

Bensulfuron methyl 60WG is not expected to affect organic matter breakdown in the soil (DT₉₀ <365 days) and no further testing is warranted.

B.9.8 Effects on soil non-target micro-organisms (Annex IIA 8.5; Annex IIIA 10.7)

B.9.8.1 Effects of active substance

58) Bensulfuron methyl (DPX-F5384) 60WG: Assessment of the effects on soil microflora. Kolzer, U., 2002.

Cross reference IIA, 8.5/04

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Guidelines:

OECD 216 (2000), OECD 217 (2000), & BBA Part VI 1-1 (2nd Edition, 1990)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: DPX-F5384 60WG

Batch: F5384-332

Purity: 600 g a.i./kg

The effects of Bensulfuron methyl 60WG on soil nitrogen transformation and microbial respiration were examined in a loamy sand soil in a laboratory study. Soil used for nitrogen transformation was supplemented with Lucerne meal as a nitrogen source prior to application of the test item. For both nitrogen transformation and respiration tests, soil was treated with water (control), 0.134, or 0.672 mg Bensulfuron methyl 60WG/kg soil dry weight (equivalent to 1 and 5x the maximum field application rate of 100 g Bensulfuron methyl 60WG/ha). Three replicates were prepared for each treatment group and control. Nitrate, ammonium, and nitrite levels in soil supplemented with Lucerne meal were determined periodically up to 28 days exposure. The rate of short-term, glucose-induced respiration was measured periodically up to 28 days in soil treated with Bensulfuron methyl 60WG without Lucerne meal. The moisture and temperature were maintained throughout the test at 45% of maximum WHC and 20°C, respectively.

Findings:

A summary of the findings for nitrogen turnover in soil and soil respiration is presented in Table B.9. 116 and Table B.9. 117. The level of nitrate in soil treated with 0.672 mg product/kg soil dry weight was statistically different from control at the end of the test; however, the percent deviation in nitrate formation between soil treated with either concentration of Bensulfuron methyl 60WG/kg soil dry weight and control soil was <25%. Deviations in the levels of N_{min} (sum of NH_4^+-N , NO_3^-N , and NO_2^-N) compared to control were <25%, indicating that nitrogen transformation was not significantly affected at either concentration of Bensulfuron methyl 60WG. The short-term respiration rate in soil treated with either 0.134 or 0.672 mg Bensulfuron methyl 60WG/kg soil dry weight was not significantly different from control after 28 days exposure.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9. 116: Summary of the analysis of nitrogen turnover in soil treated with Bensulfuron methyl 60WG (1x, 5x the maximum field application rate)

Parameter (mg/100 g dry weight)	Treatment	Days after application			
		0	7	14	28
Ammonium	Control	b.q.	0.290	0.203	0.121
	Bensulfuron methyl 60WG 1x	b.q.	0.307	0.210	0.127
	% deviation from control	-	5.86	3.94	4.96
	Bensulfuron methyl 60WG 5x	b.q.	0.275	0.211	0.145
	% deviation from control ^a	-	-5.17	3.45	19.8
	Toxic	0.177	1.36	1.20	0.162
	% deviation from control	-	369	491	33.9
Nitrite	Control	b.q.	b.q.	b.q.	b.q.
	Bensulfuron methyl 60WG 1x	b.q.	b.q.	b.q.	b.q.
	% deviation from control	-	-	-	-
	Bensulfuron methyl 60WG 5x	b.q.	b.q.	b.q.	b.q.
	% deviation from control	-	-	-	-
	Toxic	b.q.	b.q.	1.06	b.q.
	% deviation from control	-	-	-	-
Nitrate	Control	1.30	0.838	0.998	3.88
	Bensulfuron methyl 60WG 1x	1.25	1.01	1.15	3.96
	% deviation from control ^a	-3.85	20.5	15.2	2.06
	Bensulfuron methyl 60WG 5x	1.30	1.32	1.53	4.77
	% deviation from control	0.00	57.5	53.3	22.9
	Toxic	1.33	0.675	1.15	7.48
	% deviation from control ^a	2.31	-19.5	15.2	92.8
Total nitrogen	Control	1.30	1.13	1.20	4.00
	Bensulfuron methyl 60WG 1x	1.25	1.32	1.36	4.09
	% deviation from control ^a	-3.85	16.8	13.3	2.25
	Bensulfuron methyl 60WG 5x	1.30	1.60	1.74	4.92
	% deviation from control	0.00	41.6	45.0	23.0
	Toxic	1.51	2.13	3.41	7.64
	% deviation from control	16.2	88.5	184.2	91.0

b.q. = below the limit of quantitation

- = no deviation from the control was calculated because the measured values were below the limit of quantitation

^a Negative value = % inhibition

Table B.9. 117: Summary of effects on short-term respiration in soil of Bensulfuron methyl 60WG (1x, 5x the maximum field application rate)

	Treatment	Days after application			
		0	7	14	28
Respiration rate (mg CO ₂ /h/100 g dry weight)	Control	0.596	0.540	0.527	0.424
	Bensulfuron methyl 60WG 1x	0.643	0.586	0.551	0.453
	% deviation from control	7.89	8.52	4.55	6.84
	Bensulfuron methyl 60WG 5x	0.611	0.570	0.486	0.441
	% deviation from control ^a	2.52	5.56	-7.78	4.01
	Toxic	0.606	0.641	0.586	0.305
	% deviation from control ^a	1.68	18.7	11.2	-28.1

^a Negative value = % inhibition

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Conclusions:

The effects on both nitrogen transformation and soil respiration in soil treated with up to 0.672 mg product/kg soil dry weight were <25% at the end of the study and, therefore, Bensulfuron methyl 60WG can be categorised as having low risk to soil microflora.

59) *Microbial response to Bensulfuron-methyl treatment in soil. El-Ghamry, A.M., Xu, J.M., Huang, C.Y., Gan, J., 2002.*

Cross reference IIA, 8.5./01

Guidelines:

Not applicable publication

GLP:

No

The study is acceptable as additional information (conditions not clearly explained)

Materials and methods:

Test Substance: Bensulfuron methyl technical

Batch: Not applicable publication

Purity: >99%

A laboratory study was conducted to evaluate the effect of Bensulfuron methyl treatment on soil microbial biomass and N-mineralisation in a loamy sand soil. Bensulfuron methyl concentrations were 0 (control), 0.01, 0.1 and 1.0 µg a.i./g soil dry weight. Soil microbial biomass carbon, soil microbial biomass nitrogen, and nitrogen mineralisation rate were measured at different times after applying the test item for up to a total of 45 days.

The loamy sand soil used in the test was collected from the 0-20 cm horizon of an agricultural field, sieved through a 2-mm sieve, and homogenised. Soil properties were: 8% clay, 10% silt, 82% sand, 1.76% total organic carbon, 0.16% total organic nitrogen, 10.6 meq/100g soil CEC, and pH 6.3.

Stock Bensulfuron methyl solutions were made in methanol at 0.5, 5, and 50 µg/mL. To apply the test item to soil, 48 mL of each stock was added to 120 g soil dry weight, mixed thoroughly, the methanol allowed to evaporate and then 5 g of the treated dry soil was mixed into 95 g (soil dry weight equivalent) of fresh, moist soil. Soil moisture was maintained at 22.4% (w/w) (= 60% of maximum water holding capacity) and incubated in the dark at 25±1°C. Samples were taken at 1, 3, 5, 7, 10, 15, 25 and 45 days after applying Bensulfuron methyl.

Microbial biomass characterisation: Soil samples were extracted by the chloroform-fumigation extraction method and then subjected to total organic carbon analysis or Kjeldahl digestion followed by total soil N analyses.

Determination of nitrogen mineralisation: The rate of nitrogen mineralisation was measured in samples taken at the same times as microbial biomass samples. The soil samples were incubated under anaerobic/flooded conditions for 7 days and the rates were expressed as the difference in ammonium content of soil before and after anaerobic incubation (N released per g of soil per day).

Findings:

Summaries of the effects of Bensulfuron methyl at concentrations of 0.01, 0.1, and 1.0 µg/g soil dry weight on microbial biomass carbon and nitrogen and nitrogen mineralisation rates are presented below. There was a small, but

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

statistically significant ($p \leq 0.05$) reduction in microbial biomass carbon content and nitrogen content when compared to the control at 0.1 and 1.0 μg Bensulfuron methyl/g soil dry weight. The effects appeared to be transient except at the highest tested concentration. The rate of nitrogen mineralisation was affected at the 2 highest treatment concentrations, however the effect lasted only a few days.

Table B.9. 118: Effect of Bensulfuron methyl treatment on microbial biomass carbon content ($\mu\text{g/g}$ soil dry weight) in a loamy sand soil

Days after treatment	Bensulfuron methyl concentration ($\mu\text{g/g}$ soil dry weight)			
	0	0.01	0.1	1.0
1	263	244 ^a	221 ^a	205 ^a
3	255	236 ^a	209 ^a	190 ^a
5	236	225	194 ^a	167 ^a
7	232	221	198 ^a	186 ^a
10	224	221	205	190 ^a
15	225	225	210	194 ^a
25	223	220	212	204 ^a
45	220	217 ^a	213	210 ^a

^a Statistically different from controls (ANOVA, Duncan's multiple range test, $\alpha=0.05$)

Table B.9. 119: Effect of Bensulfuron methyl treatment on microbial biomass nitrogen content ($\mu\text{g/g}$ soil dry weight) in a loamy sand soil

Days after treatment	Bensulfuron methyl concentration ($\mu\text{g/g}$ soil dry weight)			
	0	0.01	0.1	1.0
1	47	45	36 ^a	29 ^a
3	38	33 ^a	25 ^a	21 ^a
5	35	30 ^a	26 ^a	20 ^a
7	34	29 ^a	24 ^a	21 ^a
10	30	29	26 ^a	24 ^a
15	32	31	28 ^a	25 ^a
25	34	33	32	29 ^a
45	33	33	32	29 ^a

^a Statistically different from controls (ANOVA, Duncan's multiple range test, $\alpha=0.05$)

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.120: Effect of Bensulfuron methyl treatment on nitrogen mineralisation rate ($\mu\text{g/g}$ dry weight/day) in loamy sand soil

Days after treatment	Bensulfuron methyl concentration ($\mu\text{g/g}$ soil dry weight)			
	0	0.01	0.1	1.0
1	2.67	2.60	2.33	2.13 ^a
3	2.93	2.80	2.47 ^a	2.33 ^a
5	3.40	3.20	2.73 ^a	2.60 ^a
7	4.00	3.93	3.73	3.53
10	4.73	4.67	4.52	4.47
15	5.13	5.07	4.93	4.73
25	5.27	5.20	5.13	5.07
45	5.4	5.33	5.20	5.06

^a Statistically different from controls (ANOVA, Duncan's multiple range test, $\alpha=0.05$)

Conclusions:

Bensulfuron methyl may affect microbial biomass carbon and nitrogen content and nitrogen mineralisation in soil; however, the effects are temporary and only occur at relatively high concentrations.

- 60) *Microbial ecotoxicity and persistence in soil of the herbicide Bensulfuron-methyl. Gigliotti, C., Allievi, L., Salardi, C., Ferrari, F., Farini, A., 1998.*

Cross reference IIA, 8.5/02

Guidelines:

Not applicable publication

GLP:

No

The study is acceptable as additional information (conditions not clearly explained)

Materials and methods:

Test Substance: Not applicable publication

Batch: Not applicable publication

Purity: Not applicable publication

The effects of Bensulfuron methyl on 6 general and functional groups of microorganisms (number of culturable aerobic and anaerobic bacteria, aerobic N_2 fixers, autotrophic nitrifiers, and aerobic and anaerobic cellulolytic microorganisms) and 2 activities (respiration and nitrification) were examined in two loamy sand soils. In addition, the rate of degradation of Bensulfuron methyl in the 2 soils was determined.

Soil A was taken from an ecologically managed farm and had been used for the cultivation of vegetables. No pesticides had been applied to the soil for 10 years. The soil had a pH of 7.7 and a %OC of 2.3%. Soil B was a paddy soil, which was not flooded at the time of sampling. The soil had been treated with a commercial formulation containing Bensulfuron methyl for several years prior to its use in this study. The soil had a pH of 5.5 and a %OC of 0.7. For each soil, 5 vegetation-free samples were collected from the 0-20cm layer and pooled before use. The soils

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

were sieved (4 mm) brought to 50% WHC and stored at 4°C. Prior to test initiation, the soils were incubated at 25°C for 10 days.

Soils were adjusted for moisture content by adjusting them to 60% of WHC and then treated with 16 or 160 µg Bensulfuron methyl/kg air-dried soil. Control soil was treated with water. Soils were incubated. Samples were obtained from each of the 9 aliquots of each soil (3 treatments x 3 replicates/treatment) after 1 and 4 weeks incubation at 25°C in the dark. Microbial numbers, respiration and nitrification were measured. Degradation of Bensulfuron methyl was evaluated by extracting duplicate soil samples after 0, 1, and 4 weeks of incubation and analysing by HPLC.

Findings:

Summaries of microbial numbers are presented in Table B.9. 121 and Table B.9. 122. The difference in microbial numbers in relation to the herbicide treatment (20 to 21% reduction) was statistically significant only for cellulolytic microorganisms in soil A.

The rate of respiration (CO₂ production) was not statistically different between controls and soil incubated for 1 or 4 weeks with either 16 or 160 µg Bensulfuron methyl/kg air dried soil.

For nitrification, soil A showed a higher activity than soil B: the ammonium in soil A was almost completely consumed within the first 12 days of reincubation, whereas in soil B less than one third of the substrate was utilized over the entire 3-week reincubation period. A relationship between nitrification and the number nitrifiers was found: the quantity of nitrifiers in soil A was an order of magnitude (statistically significant) higher than in soil B. Differences in nitrifier activity attributable to an inhibition by Bensulfuron-methyl were always statistically significant. The authors noted that there was a discrepancy between the reduction in nitrification and the absence of an effect on the numbers of nitrifying bacteria, but did not have a clear explanation.

The half-lives for Bensulfuron methyl in the 2 soils was between <1 (soil A) week and about 3 weeks (soil B).

Table B.9. 121: Microbial numbers incubated in soil A for 1 and 4 weeks with 0, 16, or 160 µg Bensulfuron methyl/kg air dried soil

Organisms	1 week			4 weeks		
	0 ^a	16 ^a	160 ^a	0 ^a	16 ^a	160 ^a
Aerobic bacteria	7.88	7.91	8.01	8.17	8.24	8.07
Anaerobic bacteria	6.06	5.93	5.81	6.12	5.77	6.01
Aerobic N ₂ fixers	2.09	1.85	2.09	1.61	1.82	1.87
Nitrifiers	5.11	5.19	4.87	4.85	5.15	4.84
Aerobic cellulolytic	3.83 ^b	3.65 ^b	3.06 ^c	3.91 ^b	3.25 ^c	3.07 ^c
Anaerobic cellulolytic	3.40	3.65	3.91	3.65 ^b	3.56 ^b	3.10 ^c

^a Expressed as log₁₀ number of cells (CFU or MPN)/g dry weight

^{b, c} The results of Duncan's test is reported only in the case of statistical significance: the same letter indicates numbers not significantly difference at 5% level, for each time group.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.122: Microbial numbers incubated in soil B for 1 and 4 weeks with 0, 16, or 160 µg Bensulfuron methyl/kg air dried soil

Organisms	1 week			4 weeks		
	0 ^a	16 ^a	160 ^a	0 ^a	16 ^a	160 ^a
Aerobic bacteria	7.31	7.36	7.35	7.34	7.29	7.21
Anaerobic bacteria	6.06	6.20	5.95	6.11	6.17	5.96
Aerobic N ₂ fixers	nd	nd	nd	nd	nd	nd
Nitrifiers	4.21	4.48	4.23	3.69	3.82	3.87
Aerobic cellulolytic	2.57	2.14	2.53	2.98	3.03	2.90
Anaerobic cellulolytic	2.41	2.02	2.08	1.74	1.40	1.74

^a Expressed as log10 number of cells (CFU or MPN)/g dry weight.

nd = not detectable (<1 cell/g)

Conclusions:

Of the 6 general and functional groups of microorganisms and nitrification and respiration, Bensulfuron methyl was found to only have a negative effect on aerobic cellulolytic bacteria and nitrification at 160 µg/kg soil dry weight.

B.9.8.2 Effects of metabolites

61) *IN-J0290: Assessment of the effects on soil microflora. Kolzer, U., 2002.*

Cross reference IIA, 8.5/03

Guidelines:

OECD 216 (2000), OECD 217 (2000), & BBA Part VI 1-1 (2nd Edition, 1990)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-J0290 technical metabolite

Batch: J0290-17

Purity: 99.6%

The effects of IN-J0290 on soil nitrogen transformation and microbial respiration were examined in a loamy sand soil in a laboratory study. Soil used for nitrogen transformation was supplemented with Lucerne meal as a nitrogen source prior to application of the test item. For both nitrogen transformation and respiration tests, soil was treated with quartz sand (control), 0.0303, or 0.151 mg IN-J0290/kg soil dry weight (equivalent to 1 and 5x the maximum PEC_{soil}). A toxic reference treatment group was tested to validate the test methods. Three replicates were prepared for each treatment group, control and toxic. Nitrate, ammonium, and nitrite levels in soil supplemented with Lucerne meal were determined periodically up to 28 days exposure. The rate of short-term, glucose-induced respiration was measured periodically up to 28 days in soil treated with IN-J0290 without Lucerne meal. The moisture and temperature were maintained throughout the test at 45% of maximum WHC and 20°C, respectively.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Findings:

A summary of the findings for nitrate and soil respiration is presented in Table B.9. 123 and Table B.9. 124. The percent deviation in nitrate formation between soil treated with either concentration of IN-J0290/kg soil dry weight and control soil after 28 days was <25%. Deviations in the levels of N_{min} (sum of NH_4^+-N , NO_3^-N , and NO_2^-N) compared to control were <25% for either concentration of IN-J0290, indicating that nitrogen transformation was not significantly affected. The short-term respiration rate in soil treated with either 0.0303 or 0.151 mg IN-J0290/kg soil dry weight was not significantly different from control after 28 days.

Table B.9. 123: Summary of the analysis of nitrogen turnover in soil treated with IN-J0290 (1x, 5x the maximum field application rate)

Parameter (mg/100 g dry weight)	Treatment	Days after application			
		0	7	14	28
Ammonium	Control	b.q.	0.290	0.203	0.121
	IN-J0290 1x	b.q.	0.301	0.209	0.148
	% deviation from control	-	3.79	2.96	22.3
	IN-J0290 5x	b.q.	0.288	0.232	0.159
	% deviation from control ^a	-	-0.69	14.3	31.4
	Toxic	0.177	1.45	1.20	0.162
	% deviation from control	-	400	491	33.9
Nitrite	Control	b.q.	b.q.	b.q.	b.q.
	IN-J0290 1x	b.q.	b.q.	b.q.	b.q.
	% deviation from control	-	-	-	-
	IN-J0290 5x	b.q.	b.q.	b.q.	b.q.
	% deviation from control	-	-	-	-
	Toxic	b.q.	b.q.	1.06	b.q.
	% deviation from control	-	-	-	-
Nitrate	Control	1.30	0.838	1	3.88
	IN-J0290 1x	1.31	0.862	1.05	3.78
	% deviation from control ^a	0.769	2.86	5.00	-2.58
	IN-J0290 5x	1.32	0.839	1.15	3.55
	% deviation from control ^a	1.54	0.119	15.0	-8.51
	Toxic	1.33	0.675	1.15	7.48
	% deviation from control ^a	2.31	-19.5	15.0	92.8
Total nitrogen	Control	1.30	1.13	1.20	4.00
	IN-J0290 1x	1.31	1.16	1.26	3.93
	% deviation from control ^a	0.769	2.65	5.00	-1.75
	IN-J0290 5x	1.32	1.13	1.38	3.71
	% deviation from control ^a	1.54	b.q.	15.0	-7.25
	Toxic	1.51	2.13	3.41	7.64
	% deviation from control	16.2	88.5	184.0	91.0

b.q. = below the limit of quantitation

- = no deviation from the control was calculated because the measured values were below the limit of quantitation

^a Negative value = % inhibition

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9. 124: Summary of effects on short-term respiration in soil of IN-J0290 (1x, 5x the maximum field application rate)

	Treatment	Days after application			
		0	7	14	28
Respiration rate (mg CO ₂ /h/100 g dry weight)	Control	0.596	0.540	0.527	0.424
	IN-J0290 1x	0.613	0.591	0.493	0.480
	% deviation from control ^a	2.85	9.44	-6.45	13.20
	IN-J0290 5x	0.612	0.605	0.537	0.431
	% deviation from control	2.68	12.00	1.90	1.65
	Toxic	0.606	0.641	0.586	0.305
	% deviation from control ^a	1.68	18.7	11.2	-28.1

^a Negative value = % inhibition

Conclusions:

The effects of IN-J0290 at up to 0.151 mg/kg soil dry weight on both nitrogen transformation and soil respiration were <25% at the end of the study and, therefore, IN-J0290 can be categorised as having low risk to soil microflora.

62) *IN-R9419: Assessment of the effects on soil microflora, Reis, K.H., 2002.*

Cross reference IIA, 8.5/05

Guidelines:

OECD 216 (2000), OECD 217 (2000), & BBA Part VI 1-1 (2nd Edition, 1990)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-R9419 technical metabolite

Batch: R9419-002

Purity: 98.5%

The effects of IN-R9419 on soil nitrogen transformation and microbial respiration were examined in a loamy sand soil in a laboratory study. Soil used for nitrogen transformation was supplemented with Lucerne meal as a nitrogen source prior to application of the test item. For both nitrogen transformation and respiration tests, soil was treated with quartz sand (control), 0.078, or 0.388 mg IN-R9419/kg soil dry weight (equivalent to 1 and 5x the maximum PEC_{soil}). Three replicates were prepared for each treatment group and control. Nitrate, ammonium, and nitrite levels in soil supplemented with Lucerne meal were determined periodically up to 28 days exposure. The rate of short-term, glucose-induced respiration was measured periodically up to 30 days exposure. As at day 28 sampling, two pressure sensors were fault (test item 0.078 mg IN-R9419/kg soil dry weight), the measurement for two replicates was repeated at day 30. The moisture and temperature were maintained throughout the test at 45% of maximum WHC and 20°C, respectively.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Findings:

A summary of the findings for nitrate and soil respiration is presented in Table B.9. 125 and Table B.9. 126. The percent deviation in nitrate formation between soil treated with either concentration of IN-R9419/kg soil dry weight and control soil was <25% at the end of the test. Deviations in the levels of N_{min} (sum of NH_4^+-N , NO_3^-N , and NO_2^-N) compared to control were <25% for either concentration of IN-R9219, indicating that nitrogen transformation was not significantly affected. The short-term respiration rates in soil treated with both 0.078 and 0.388 mg IN-R9419/kg soil dry weight were not statistically significantly different from control after 30 days.

Table B.9. 125: Summary of the analysis of nitrogen turnover in soil treated with IN-R9419 (1x, 5x the maximum field application rate)

Parameter (mg/100 g dry weight)	Treatment	Days after application			
		0	7	14	28
Ammonium	Control	0.663	b.q.	b.q.	b.q.
	IN-R9419 1x	0.758	b.q.	b.q.	b.q.
	% deviation from control at day 28				-
	IN-R9419 5x	0.704	b.q.	b.q.	b.q.
	% deviation from control at day 28				-
Nitrite	Control	0.149	0.045	0.014	0.040
	IN-R9419 1x	0.166	0.043	0.022	0.029
	% deviation from control at day 28 ^a				-27.5
	IN-R9419 5x	0.149	0.045	0.014	0.040
	% deviation from control at day 28				0.00
Nitrate	Control	1.210	0.378	0.763	2.321
	IN-R9419 1x	1.195	0.436	0.768	2.244
	% deviation from control at day 28 ^a				-3.32
	IN-R9419 5x	1.260	0.649	1.082	2.512
	% deviation from control at day 28				8.23
Total nitrogen	Control	2.022	0.423	0.777	2.361
	IN-R9419 1x	2.119	0.479	0.790	2.273
	% deviation from control at day 28 ^a				-3.73
	IN-R9419 5x	2.113	0.694	1.096	2.552
	% deviation from control at day 28				8.09

b.q. = below the limit of quantitation

- = no deviation from the control was calculated because the measured values were below the limit of quantitation

^a Negative value = % inhibition

Table B.9. 126: Summary of effects on short-term respiration in soil of IN-R9419 (1x, 5x the maximum field application rate)

	Treatment	Days after application				
		0	7	14	28	30
Respiration rate (mg CO ₂ /h/100 g dry weight)	Control	0.915	0.939	0.963	0.887	0.789
	IN-R9419 1x	0.968	0.876	0.711	0.768*	0.808
	% deviation from control ^a	5.79	-6.71	-26.2	-13.4	2.41
	IN-R9419 5x	1.000	0.937	0.909	0.855	0.794
	% deviation from control ^a	9.29	-0.21	-5.61	-3.61	0.63

^a Negative value = % inhibition

*only one replicate

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Conclusions:

The effects of IN-R9419 at up to 0.388 mg/kg soil dry weight on both nitrogen transformation and soil respiration were <25% at the end of the study and, therefore, IN-R9419 can be categorised as having low risk to soil microflora.

63) *IN-N5297: Assessment of the effects on soil microflora. Reis, K.H., 2002.*

Cross reference IIA, 8.5/06

Guidelines:

OECD 216 (2000), OECD 217 (2000), & BBA Part VI 1-1 (2nd Edition, 1990)

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-N5297 technical metabolite

Batch: N5297-004

Purity: 99.5%

The effects of IN-N5297 on soil nitrogen transformation and microbial respiration were examined in a loamy sand soil in a laboratory study. Soil used for nitrogen transformation was supplemented with Lucerne meal as a nitrogen source prior to application of the test item. For both nitrogen transformation and respiration tests, soil was treated with quartz sand (control), 0.045, or 0.224 mg IN-N5297/kg soil dry weight (equivalent to 1 and 5x the maximum PEC_{soil}). Three replicates were prepared for each treatment group and control. Nitrate, ammonium, and nitrite levels in soil supplemented with Lucerne meal were determined periodically up to 28 days exposure. The rate of short-term, glucose-induced respiration was measured periodically up to 30 days exposure. As at day 28 sampling, two pressure sensors were fault (test item rate 0.045 and 0.224 mg IN-N5297/kg soil dry weight), the measurement for two replicates was repeated at day 30. The moisture and temperature were maintained throughout the test at 45% of maximum WHC and 20°C, respectively.

Findings:

A summary of the findings for nitrate and soil respiration is presented in Table B.9. 127 and Table B.9. 128. The levels of nitrate in soil treated with both 0.045 and 0.224 mg IN-N5297/kg soil dry weight were statistically different from control after 28 days exposure. However, the effects seen were not concentration dependent and the percent deviation in nitrate formation between soil treated with either concentration of IN-N5297/kg soil dry weight and control soil was <25%. Deviations in the levels of N_{min} (sum of NH₄⁺-N, NO₃⁻-N, and NO₂⁻-N) compared to control were <25%, for either concentration of IN-N5297 indicating that nitrogen transformation was not significantly affected. The short-term respiration rates in soil treated with both 0.045 and 0.224 mg IN-N5297/kg soil dry weight were not significantly different from control after 30 days.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9. 127: Summary of the analysis of nitrogen turnover in soil treated with IN-N5297 (1x, 5x the maximum field application rate)

Parameter (mg/100 g dry weight)	Treatment	Days after application			
		0	7	14	28
Ammonium	Control	0.663	b.q.	b.q.	b.q.
	IN-N5297 1x	0.689	b.q.	b.q.	b.q.
	% deviation from control at day 28				-
	IN-N5297 5x	0.727	b.q.	b.q.	b.q.
	% deviation from control at day 28				-
Nitrite	Control	0.149	0.045	0.014	0.040
	IN-N5297 1x	0.172	0.042	0.022	0.029
	% deviation from control at day 28 ^a				-27.5
	IN-N5297 5x	0.168	0.045	0.015	0.032
	% deviation from control at day 28 ^a				-20.2*
Nitrate	Control	1.210	0.378	0.763	2.321
	IN-N5297 1x	1.224	0.356	0.733	1.782
	% deviation from control at day 28 ^a				-23.2*
	IN-N5297 5x	1.249	0.427	0.786	1.841
	% deviation from control at day 28 ^a				-20.7*
Total nitrogen	Control	2.022	0.423	0.777	2.361
	IN-N5297 1x	2.085	0.398	0.755	1.811
	% deviation from control at day 28 ^a				-23.3*
	IN-N5297 5x	2.144	0.472	0.801	1.873
	% deviation from control at day 28 ^a				-20.7*

b.q. = below the limit of quantitation

- = no deviation from the control was calculated because the measured values were below the limit of quantitation

^a Negative value = % inhibition

*Statistically different from control (Student t-test, two sided, $\alpha = 0.05$)

Table B.9. 128: Summary of effects on short-term respiration in soil of IN-N5297 (1x, 5x the maximum field application rate)

	Treatment	Days after application			
		0	7	14	30
Respiration rate (mg CO ₂ /h/100 g dry weight)	Control	0.915	0.939	0.963	0.789
	IN-N5297 1x	0.932	0.971	0.901	0.780
	% deviation from control ^a	1.86	3.41	-6.44	-1.14
	IN-N5297 5x	0.921	0.896	0.791	0.750
	% deviation from control ^a	0.66	-4.58	-17.9	-4.94

^a Negative value = % inhibition

Conclusions:

The effects of IN-5297 at up to 0.224 mg/kg soil dry weight on both nitrogen transformation and soil respiration were <25% at the end of the study and, therefore, IN-N5297 can be categorised as having low risk to soil microflora.

64) *IN-D1R84: Assessment of the effects on soil microflora. Reis, K.H., 2002.*

Cross reference IIA, 8.5/07

Guidelines:

OECD 216 (2000), OECD 217 (2000), & BBA Part VI 1-1 (2nd Edition, 1990)

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-D1R84 technical metabolite

Batch: D1R84-001

Purity: 98.9%

The effects of IN-D1R84 on soil nitrogen transformation and microbial respiration were examined in a loamy sand soil in a laboratory study. Soil used for nitrogen transformation was supplemented with Lucerne meal as a nitrogen source prior to application of the test item. For both nitrogen transformation and respiration tests, soil was treated with quartz sand (control), 0.042, or 0.208 mg IN-D1R84/kg soil dry weight (equivalent to 1 and 5x the maximum PEC_{soil}). Three replicates were prepared for each treatment group and control. Nitrate, ammonium, and nitrite levels in soil supplemented with Lucerne meal were determined periodically up to 28 days exposure. The rate of short-term, glucose-induced respiration was measured periodically up to 28 days in soil treated with IN-D1R84 without Lucerne meal. The moisture and temperature were maintained throughout the test at 45% of maximum WHC and 20°C, respectively.

Findings:

A summary of the findings for nitrate and soil respiration is presented in Table B.9. 129 and Table B.9. 130. The levels of nitrate in soil treated with 0.208 mg IN-D1R84/kg soil dry weight were statistically different from control after 28 days exposure. However, the percent deviation in nitrate formation between soil treated with either concentration of IN-D1R84/kg soil dry weight and control soil was <25%. Deviations in the levels of N_{min} (sum of NH₄⁺-N, NO₃⁻-N, and NO₂⁻-N) compared to control were <25%, for either concentration of IN-D1R84 indicating that nitrogen transformation was not significantly affected. The short-term respiration rates in soil treated with both 0.042 and 0.208 mg IN-D1R84/kg soil dry weight were not significantly different from control after 28 days.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9. 129: Summary of the analysis of nitrogen turnover in soil treated with IN-D1R84 (1x, 5x the maximum field application rate)

Parameter (mg/100 g dry weight)	Treatment	Days after application			
		0	7	14	28
Ammonium	Control	0.663	b.q.	b.q.	b.q.
	IN-D1R84 1x	0.709	b.q.	b.q.	b.q.
	% deviation from control at day 28				-
	IN-D1R84 5x	0.716	b.q.	b.q.	b.q.
	% deviation from control at day 28				-
Nitrite	Control	0.149	0.045	0.014	0.040
	IN-D1R84 1x	0.205	0.035	0.013	0.042
	% deviation from control at day 28				5
	IN-D1R84 5x	0.217	0.040	0.015	0.042
	% deviation from control at day 28				5
Nitrate	Control	1.210	0.378	0.763	2.321
	IN-D1R84 1x	1.188	0.486	0.942	2.349
	% deviation from control at day 28				1.21
	IN-D1R84 5x	1.145	0.374	0.741	2.095
	% deviation from control at day 28 ^a				-9.74*
Total nitrogen	Control	2.022	0.423	0.777	2.361
	IN-D1R84 1x	2.102	0.521	0.955	2.391
	% deviation from control at day 28				1.27
	IN-D1R84 5x	2.078	0.414	0.756	2.137
	% deviation from control at day 28 ^a				-9.49*

b.q. = below the limit of quantitation

- = no deviation from the control was calculated because the measured values were below the limit of quantitation

^a Negative value = % inhibition

*Statistically different from control (Student t-test, two sided, $\alpha = 0.05$)

Table B.9. 130: Summary of effects on short-term respiration in soil of IN-D1R84 (1x, 5x the maximum field application rate)

	Treatment	Days after application			
		0	7	14	28
Respiration rate (mg CO ₂ /h/100 g dry weight)	Control	0.915	0.939	0.963	0.887
	IN-D1R84 1x	0.950	0.892	0.853	0.961
	% deviation from control ^a	3.83	-5.01	-11.4	8.34
	IN-D1R84 5x	1.033	0.877	0.997	0.853
	% deviation from control ^a	12.9	-6.60	3.53	-3.83

^a Negative value = % inhibition

Conclusions:

The effects of IN-D1R84 at up to 0.208 mg/kg soil dry weight on both nitrogen transformation and soil respiration were <25% at the end of the study and, therefore, IN-D1R84 can be categorised as having low risk to soil microflora.

65) *IN-F7880: Assessment of the effects on soil microflora. Reis, K.H., 2002.*

Cross reference IIA, 8.5/08

Guidelines:

OECD 216 (2000), OECD 217 (2000), & BBA Part VI 1-1 (2nd Edition, 1990)

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

GLP:

Yes

The study is acceptable

Materials and methods:

Test Substance: IN-F7880 technical metabolite

Batch: F7880-004

Purity: 97.3%

The effects of IN-F7880 on soil nitrogen transformation and microbial respiration were examined in a loamy sand soil in a laboratory study. Soil used for nitrogen transformation was supplemented with Lucerne meal as a nitrogen source prior to application of the test item. For both nitrogen transformation and respiration tests, soil was treated with quartz sand (control), 0.078, or 0.388 mg IN-F7880/kg soil dry weight (equivalent to 1 and 5x the maximum PEC_{soil}). Three replicates were prepared for each treatment group and control. Nitrate, ammonium, and nitrite levels in soil supplemented with Lucerne meal were determined periodically up to 28 days exposure. The rate of short-term, glucose-induced respiration was measured periodically up to 28 days in soil treated with IN-F7880 without Lucerne meal. The moisture and temperature were maintained throughout the test at 45% of maximum WHC and 20°C, respectively.

Findings:

A summary of the findings for nitrate and soil respiration is presented in Table B.9. 131 and Table B.9. 132. The levels of nitrate in soil treated with both 0.078 mg IN-F7880/kg soil dry weight were statistically different from control after 28 days exposure. However, the effects seen were not concentration dependent and the percent deviation in nitrate formation between soil treated with either concentration of IN-F7880/kg soil dry weight and control soil was <25%. Deviations in the levels of N_{min} (sum of NH₄⁺-N, NO₃⁻-N, and NO₂⁻-N) compared to control were <25% for either concentration of IN-F7880, indicating that nitrogen transformation was not significantly affected. The short-term respiration rates in soil treated with both 0.078 and 0.388 mg IN-F7880/kg soil dry weight were not statistically significantly different from control after 28 days.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.131: Summary of the analysis of nitrogen turnover in soil treated with IN-F7880 (1x, 5x the maximum field application rate)

Parameter (mg/100 g dry weight)	Treatment	Days after application			
		0	7	14	28
Ammonium	Control	0.753	b.q.	0.268	b.q.
	IN-D1R84 1x	0.815	b.q.	0.275	b.q.
	% deviation from control	8.23	-	2.61	-
	IN-D1R84 5x	0.788	b.q.	0.274	b.q.
	% deviation from control	4.65	-	2.24	-
Nitrite	Control	0.195	0.040	0.113	0.079
	IN-D1R84 1x	0.196	0.031	0.114	0.084
	% deviation from control ^a	0.51	-22.5	0.88	6.33
	IN-D1R84 5x	0.227	0.029	0.121	0.088
	% deviation from control ^a	16.4	-27.5	7.08	11.4
Nitrate	Control	2.444	1.672	2.060	3.491
	IN-D1R84 1x	2.419	1.345	1.680	3.134
	% deviation from control ^a	-1.02	-19.6	-18.5	-10.2*
	IN-D1R84 5x	2.464	1.511	1.823	3.283
	% deviation from control ^a	0.82	-9.6	-11.5	-5.96
Total nitrogen	Control	3.392	1.712	2.441	3.570
	IN-D1R84 1x	3.430	1.376	2.069	3.218
	% deviation from control ^a	1.12	-19.6	-15.2	-9.86*
	IN-D1R84 5x	3.479	1.540	2.218	3.371
	% deviation from control ^a	2.56	-10.1	-9.14	-5.57

b.q. = below the limit of quantitation

- = no deviation from the control was calculated because the measured values were below the limit of quantitation

^a Negative value = % inhibition

*Statistically different from control (Student t-test, two sided, $\alpha = 0.05$)

Table B.9.132: Summary of effects on short-term respiration in soil of IN-F7880 (1x, 5x the maximum field application rate)

	Treatment	Days after application			
		0	7	14	28
Respiration rate (mg CO ₂ /h/100 g dry weight)	Control	1.235	1.158	1.213	1.074
	IN-F7880 1x	1.200	1.137	1.189	1.112
	% deviation from control ^a	-2.83	-1.81	-1.98	3.54
	IN-F7880 5x	1.205	1.122	1.213	1.117
	% deviation from control ^a	-2.43	-3.11	0.00	4.00

^a Negative value = % inhibition

Conclusions:

The effects of IN-F7880 at up to 0.388 mg/kg soil dry weight on both nitrogen transformation and soil respiration were <25% at the end of the study and, therefore, IN-F7880 can be categorised as having low risk to soil microflora.

B.9.8.3 Effects of formulations: laboratory testing (Annex IIIA 10.7.1)

Laboratory testing following OECD 216/217 test guidelines was conducted with Bensulfuron methyl 60WG and detailed descriptions of the study are contained in B.9.8.1.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

B.9.8.4 Effects of formulations: additional testing (Annex IIIA 10.7.2)

No additional testing is required since Bensulfuron methyl 60WG will have no significant effect (>25%) on soil microflora (see B.9.8.1).

B.9.8.5 Summary of effects, exposure and hazard assessment for non-target micro-organisms

B.9.8.5.1 Toxicity

A summary of the endpoints from tests on Bensulfuron methyl 60WG and its metabolites is provided below.

Table B.9. 133: Summary of the effects of Bensulfuron methyl 60WG and its soil degradates on soil nitrogen transformation and carbon mineralisation

Test substance	Concentrations tested (mg test substance/kg dry soil wt) corresponding to 1x and 5x PEC _{soil}	Effect ^a (% deviation between treated and control soil) ^b			
		Nitrogen transformation		Carbon mineralization ^b	
		1x PEC _{soil}	5x PEC _{soil}	1x PEC _{soil}	5x PEC _{soil}
Bensulfuron methyl 60WG	0.134 and 0.672	2.25	23	6.84	4.01
IN-N5297	0.045 and 0.224	-23.3	-20.7	-1.14	-4.94
IN-F7880	0.078 and 0.388	-9.86	-5.57	3.54	4.00
IN-D1R84	0.042 and 0.208	-1.27	-9.49	8.34	-3.83
IN-R9419	0.078 and 0.388	-3.73	8.09	2.41	0.63
IN-J0290	0.030 and 0.151	-1.75	-7.25	13.20	1.65

^a Negative numbers indicate inhibition relative to control

^b % deviation from control at end of the study

B.9.8.5.2 Risk assessment

Laboratory testing of Bensulfuron methyl 60WG, Bensulfuron methyl and the major soil degradates suggests an acceptable risk to soil microorganisms. Deviations between treatments and controls were <25% for both nitrogen transformation and carbon mineralisation by the end of the studies, even at concentrations far exceeding the PEC_{soil}. Studies reported in the literature (Cross reference IIA, 8.5/01-02) on the effects of Bensulfuron methyl on soil organisms and processes suggest that minor transient effects may occur, but only in some soils and only at concentrations that exceed the PEC_{soil}. Therefore the likelihood of long-term impact on soil microbial communities and soil function under field conditions is anticipated to be low.

Bensulfuron methyl 60WG is applied once per season at 60 g a.i./ha and has an average field soil DT₅₀ of 8 days. The maximum PEC_{soil} for Bensulfuron methyl, IN-F7880, IN-R9419, IN-D1R84, IN-J0290 and IN-N5297 were 0.0782, 0.00723, 0.0208, 0.00418, 0.0136 and 0.0117 mg/kg dry weight, respectively.

It is anticipated that soil microbial communities will be exposed to low and transient concentrations of Bensulfuron methyl and its metabolites when used according to Good Agricultural Practices and therefore the use of Bensulfuron methyl 60WG in rice poses an acceptable risk to soil microflora.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex IIA 8.6)

- 66) *Bensulfuron methyl (DPX-F5384) 60WG: A greenhouse study to investigate the effects on seedling emergence and growth of ten terrestrial plant species following soil exposure. Porch, J.R., Martin, K.H., 2004a.*

Cross reference IIA, 8.6./01

Guidelines:

USEPA 122-1 (1986), 123-1 (1986), OECD 208, USEPA 850.4100, 4225 (1996)

GLP:

Yes

The study is acceptable

Materials and Methods:

Test Substance: DPX-F5384 60WG

Batch: F5384-348

Purity: 600 g a.i./kg

Non-target terrestrial plant response to Bensulfuron methyl 60WG was evaluated on ten common plant species. Effects on seedling emergence and early growth following soil surface application to planted seeds prior to emergence were assessed. Testing was conducted with six to ten rates, ranging up to 100 g product/ha or 60 g active substance/ha, which is the maximum labeled use rate. Rates were selected based on the results of preliminary range-finding tests. Tests were conducted in a natural soil under greenhouse conditions. The test system comprised four replicates of ten plants per test rate and control. Applications were made with a track sprayer calibrated to apply approximately 400 L/ha. Test duration was 21 days. The number of plants emerged, plant shoot height, visual rating of plant condition, and the number of plants surviving was recorded on Days 7, 14, and 21. Dry weight was determined from plant shoots collected at test termination on Day 21. The rates producing the NOEL and the ER₅₀ were determined when possible.

Findings:

The results for the most sensitive parameter for each species, based on the ER₅₀ at 21 days after pre-emergence exposure to Bensulfuron methyl 60WG are summarised in the table that follows.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9. 134: Effects of Bensulfuron methyl 60WG on ten terrestrial plant species following soil surface (pre-emergent) application

Plant	Family	Genus/species	ER ₅₀ (g a.i./ha)		Parameter
			Rate	95% Conf. Interval ^a	
Monocots					
Corn	Gramineae	<i>Zea mays</i>	>60	n/a	All parameters
Oat	Gramineae	<i>Avena sativa</i>	>60	n/a	All parameters
Onion	Liliaceae	<i>Allium cepa</i>	>60	n/a	All parameters
Ryegrass	Gramineae	<i>Lolium perenne</i>	>60	n/a	All parameters
Dicots					
Cucumber	Cucurbitaceae	<i>Cucumis sativus</i>	>60	n/a	All parameters
Oilseed rape	Brassicaceae	<i>Brassica napus</i>	2.41	(<1.875–15.24)	Shoot height
Pea	Leguminosae	<i>Pisum sativum</i>	>60	n/a	All parameters
Soybean	Leguminosae	<i>Glycine max</i>	>60	n/a	All parameters
Sugar beet	Chenopodiaceae	<i>Beta vulgaris</i>	>60	n/a	All parameters
Tomato	Solanaceae	<i>Lycopersicon esculentum</i>	>60	n/a	All parameters

^a“n/a” indicates ‘not applicable’ as the endpoint (the ER₅₀) was greater than the highest rate tested in the statistical analysis.

Conclusions:

The ER₅₀ for the most sensitive dicot species, rape, is 2.41 g a.i./ha, based on shoot height.

There were no effects greater than 50% in magnitude for any of the four monocot species tested. Therefore, for all monocots tested, the ER₅₀ was determined to be greater than 60 g a.i./ha, the highest rate tested.

67) *Bensulfuron methyl (DPX-F5438) 60WG: A greenhouse study to investigate the effects on vegetative vigor of ten terrestrial plant species following foliar exposure. Porch, J.R., Martin, K.H., 2004b.*

Cross reference IIA, 8.6./02

Guidelines:

USEPA 850.4250 (1996)

GLP:

Yes

The study is acceptable

Materials and Methods:

Test Substance: DPX-F5384 60WG

Batch: F5384-348

Purity: 600 a.i. g/kg

Non-target terrestrial plant response to Bensulfuron methyl 60WG was evaluated on ten common plant species. Effects on vegetative vigor following foliar application to seedling plants were assessed. Testing was conducted with seven to ten rates, ranging up to 100 g product/ha or 60 g active substance/ha, which is the maximum labeled use rate. Rates were selected based on the results of preliminary range-finding tests. Tests were conducted in a natural soil under greenhouse conditions. For each species, the test system was composed of five replicates of four plants each (20 plants) per test rate and control. Applications were made with a track sprayer calibrated to apply approximately 400 L/ha. Test duration was 21 days. Shoot height and numeric visual response of plant condition

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

were recorded on Days 7, 14, and 21. The number of plants surviving was recorded on Days 7, 14, and 21. Dry weight was determined from plant shoots collected at test termination on Day 21. The rates producing the NOEL and ER₅₀ were determined when possible.

Findings:

The results for the most sensitive parameter for each species, based on the ER₅₀ at 21 days following foliar exposure to Bensulfuron methyl 60WG are summarised in the Table B.9. 135.

Table B.9. 135: Effects of Bensulfuron methyl 60WG on ten terrestrial plant species following foliar (postemergent) application

Plant	Family	Genus/species	ER ₅₀ (g a.i./ha)		Parameter
			Rate	95% Conf. Interval ^a	
Monocots					
Corn	Gramineae	<i>Zea mays</i>	>60	n/a	All parameters
Oat	Gramineae	<i>Avena sativa</i>	>60	n/a	All parameters
Onion	Liliaceae	<i>Allium cepa</i>	>60	n/a	All parameters
Ryegrass	Gramineae	<i>Lolium perenne</i>	>60	n/a	All parameters
Dicots					
Cucumber	Cucurbitaceae	<i>Cucumis sativus</i>	6.84	(3.08–15.18)	Shoot dry weight
Oilseed rape	Brassicaceae	<i>Brassica napus</i>	2.83	(2.02–3.99)	Shoot dry weight
Pea	Leguminosae	<i>Pisum sativum</i>	>60	n/a	All parameters
Soybean	Leguminosae	<i>Glycine max</i>	2.08	(1.06–4.09)	Shoot dry weight
Sugar beet	Chenopodiaceae	<i>Beta vulgaris</i>	>60	n/a	Shoot height
Tomato	Solanaceae	<i>Lycopersicon esculentum</i>	2.24	(0.22–22.57)	Shoot dry weight

^a 'n/a' indicates 'not applicable' as the endpoint (the ER₅₀) was greater than the highest rate tested in the statistical analysis.

Conclusions:

The ER₅₀ estimates for all monocot species tested (corn, oat, onion, and perennial ryegrass) were greater than 60 g a.i./ha for all parameters.

The ER₅₀ for the most sensitive dicot species, soybean, is 2.08 g a.i./ha, based on shoot dry weight.

B.9.10 Summary of effects, exposure and hazard assessment for other non-target organisms (flora and fauna)

A quantitative risk assessment approach following recommendations outlined in SANCO/10329/2002 17 Oct 2002 was taken to estimate potential risk to non-target terrestrial plants. TERs were determined based on drift deposition at 1m, and 5m from the edge of the paddy and the ER₅₀ values from the seedling emergence and vegetative vigour tests. The results are summarized in Table B.9. 136 and Table B.9. 137.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Table B.9.136: Summary of acute TERs for non-target terrestrial plants exposed by soil application to Bensulfuron methyl

Plant species	ER ₅₀ (g a.i./ha)	TER _a for PEC _{soil} at 1m ^a	TER _a for PEC _{soil} at 5m ^b	Trigger
Corn	>60	>36	175	5
Oat	>60	>36	175	5
Onion	>60	>36	175	5
Ryegrass	>60	>36	175	5
Cucumber	>60	>36	175	5
Oilseed rape	2.41	1.45	7.05	5
Pea	>60	>36	175	5
Soybean	>60	>36	175	5
Sugar beet	>60	>36	175	5
Tomato	>60	>36	175	5

^a PEC_{soil} at 1m (minimum distance for arable crops), based on 2.77% drift rate is 1.66 g a.i./ha (Rautmann *et al.*, for arable crops, 2001)

^b PEC_{soil} at 5m, based on 0.57% drift rate is 0.342 g a.i./ha. (Rautmann *et al.*, for arable crops, 2001)

For soil application, 9 of the 10 species tested exceeded the regulatory trigger at 1m, the trigger was not exceeded for the most sensitive species, oilseed rape; all species passed the trigger at 5m.

Table B.9.137: Summary of acute TERs for non-target terrestrial plants exposed by foliar application to Bensulfuron methyl

Plant species	ER ₅₀ (g a.i./ha)	TER _a for PEC _{foliage} at 1m ^a	TER _a for PEC _{foliage} at 5m ^b	Trigger
Corn	>60	>36	>175	5
Oat	>60	>36	>175	5
Onion	>60	>36	>175	5
Ryegrass	>60	>36	>175	5
Cucumber	6.84	4.1	20	5
Oilseed rape	2.83	1.7	8.3	5
Pea	>60	>36	>175	5
Soybean	2.08	1.3	6.1	5
Sugar beet	>60	>36	>175	5
Tomato	2.24	1.4	6.6	5

^a PEC_{foliage} at 1m (minimum distance for arable crops), based on 2.77% drift rate is 1.66 g a.i./ha. (Rautmann, *et al.*, for arable crops, 2001)

^b PEC_{foliage} at 5m, based on 0.57% drift rate is 0.342 g a.i./ha. (Rautmann, *et al.*, for arable crops, 2001)

For foliar exposure, 6 of the 10 species tested exceeded the regulatory trigger at 1m, and all species passed the trigger at 5m.

The risk of Bensulfuron methyl to sensitive off-crop non-target terrestrial plants when exposed to spray drift via soil or foliar exposure is considered acceptable with a buffer strip of 5m.

B.9.11 Effects on biological methods of sewage treatment (Annex IIA 8.7)

- 68) *Bensulfuron methyl technical (DPX-F5384): Activated sludge, respiration inhibition test. Hertl, J., 2001.*

Cross reference IIA, 8.7./01

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Guidelines:

OECD 209; Directive 87/302/EEC; Official Journal of the European Communities No. L 122/118-122 (May 30, 1988)

GLP:

Yes

*The study is acceptable*Materials and Methods:

Test Substance: DPX-F5384 technical

Batch: F5384-333

Purity: 98.66%

Effects of Bensulfuron methyl technical on biological methods for sewage treatment were determined in a 3-hours exposure laboratory study conducted under OECD Guideline 209 and Commission Directive 87/302/EEC. The influence of the test item Bensulfuron methyl technical on the activity of activated sludge was evaluated by measuring the respiration rate under defined conditions. Test concentrations: 1.0, 3.2, 10, 32, and 100 mg Bensulfuron methyl technical/L; 3.2, 10, and 32 mg 3,5-dichlorophenol/L; and two inoculum controls.

Findings:

The respiration rate in the control was determined to be 0.470 mg O₂/L-min (standard deviation: 4.1%). Bensulfuron methyl technical, at a concentration up to 100 mg/L, exhibited no acute toxic effects towards the microbiological inoculum. The sensitivity of the activated sludge to the toxic reference 3,5-dichlorophenol was in the range of 5-30 mg/L (EC₅₀ for 3 hours incubation period was 9.9 mg/L); this is within accepted limits, indicating the validity of this test.

Table B.9. 138: Effects of Bensulfuron methyl technical on biological methods for sewage treatment

Bensulfuron methyl technical (mg/L)	Mean respiration rate (mg O ₂ /L min)	Inhibition of respiration rate ^a (%)
0	0.470	-
1.0	0.463	1.5
3.2	0.506	-7.7 ^a
10.0	0.495	-5.3 ^a
32.0	0.440	6.4
100.0	0.440	6.4

^a negative values indicate an activation of the respiration rate

Conclusions:

Bensulfuron methyl technical exhibited no acute toxic effects to activated sludge up to 100 mg/L, the highest concentration tested. EC₂₀ and EC₅₀ values could not be determined due to the lack of any inhibitory effect of the test item. Therefore, it can be concluded that Bensulfuron methyl technical poses a very low risk to the activated sludge of a sewage treatment plant.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Risk assessment

Basing on the results that the maximum effect of Bensulfuron methyl on activated sludge corresponds to 6.4 % inhibition of the respiration at the tested concentrations of 100 mg/L, the risk for the biological methods of sewage treatment is considered to be low.

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

B.9.12 Reference relied on

Annex No., EU Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
IIA, 8.1.1./01 (IIA 8.1)	Beavers, J.B.	1984	An acute oral toxicity study in the mallard with H #15,190 [REDACTED] HLO 154-84 GLP: No Published: No	N	DuPont
IIA, 8.1.2./01 (IIA 8.1)	Beavers, J.B.	1984a	A dietary LC ₅₀ study in the mallard with H # 15,190 [REDACTED] HLO 34-84 GLP: No Published: No	N	DuPont
IIA, 8.1.2./02 (IIA 8.1)	Beavers, J.B.	1984b	A dietary LC ₅₀ study in the bobwhite with H # 15,190 [REDACTED] HLO 35-84 GLP: No Published: No	N	DuPont
IIA, 8.1.3./01 (IIA 8.1)	Gallagher, S.P., Beavers, J.B., Jaber, M.	2002	Bensulfuron methyl (DPX-F5384) technical: A one generation reproduction study with the mallard [REDACTED] DuPont-5081 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.1./01 (IIA 8.2)	Boeri, R.L.	1988	Static acute toxicity of Haskell sample number 16,985 to the sheepshead minnow, <i>Cyprinodon variegatus</i> [REDACTED] HLO 243-88 GLP: Yes Published: No	N	DuPont
IIA, 8.2.1./02	Gries, T.	2002	IN-N5297: Static, acute, 96-hour, limit test to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] DuPont-9791 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.1./03 (IIA 8.2)	Hall, C.L.	1986	Static acute 96-hour LC ₅₀ of INF-5384-52 to channel catfish (<i>Ictalurus punctatus</i>) [REDACTED] HLR 526-86 GLP: Yes Published: No	N	DuPont

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Annex No., EU Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
IIA, 8.2.1./04 (IIA 8.2)	Hoke, R.A.	2002	IN-J0290: Static, acute, 96-hour LC ₅₀ to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] DuPont-7358 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.1./05 (IIA 8.2)	Samel, A.	2001a	Bensulfuron methyl (DPX-F5384) technical: Static, acute, 96-hour, limit test to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] DuPont-5444 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.1./06 (IIA 8.2)	Samel, A.	2001b	Bensulfuron methyl (DPX-F5384) technical: Static, acute, 96-hour, limit test to bluegill sunfish, <i>Lepomis Macrochirus</i> [REDACTED] DuPont-5445 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.2.2./01 (IIA 8.2)	Hutton, D.G.	1988	Early life-stage toxicity of IN F5384-122 to fathead minnows, [REDACTED] [REDACTED] HLR 530-88, Supplement No. 1 GLP: Yes Published: No	N	DuPont
IIA, 8.2.2.2./02 (IIA 8.2)	Samel, A.	2003	Bensulfuron methyl (DPX-F5384) technical: early life-stage toxicity to rainbow trout, <i>Oncorhynchus mykiss</i> [REDACTED] DuPont-5446, Revision No.1 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.3./01 (IIA 8.2)	Larkin, J.C.	1984a	DPX-F5384 [¹⁴ C-phenyl(U)] flowthrough bioconcentration study with carp, <i>Cyprinus carpio</i> [REDACTED] AMR 180-84 GLP: No Published: No	N	DuPont
IIA, 8.2.3./02 (IIA 8.2)	Larkin, J.C.	1984b	DPX-F5384 [¹⁴ C-phenyl(U)] flow-through bioconcentration study with rainbow trout, <i>Salmo gairdneri</i> [REDACTED] AMR 181-84 GLP: No Published: No	N	DuPont

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Annex No., EU Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
IIA, 8.2.3./04 (IIA, 8.2)	Romaire, R.P., Kleinow, K.M.	1989	Flow-through crayfish bioconcentration study with ¹⁴ C-Bensulfuron methyl [REDACTED] AMR 965-87 GLP: Yes Published: No	N	DuPont
IIA, 8.2.4./01 (IIA, 8.2)	Boeri, R.L.	1988	Static acute toxicity of Haskell sample number 16,985 to embryos and larvae of the eastern oyster <i>Crassostrea virginica</i> Enseco Incorporated HLO 172-88 GLP: Yes Published: No	N	DuPont
IIA, 8.2.4./02 (IIA, 8.2)	Boeri, R.L.	1988	Static acute toxicity of Haskell sample number 16,985 to the <i>Mysid</i> , <i>Mysidopsis</i> <i>bahia</i> Enseco Incorporated HLO 242-88 GLP: Yes Published: No	N	DuPont
IIA, 8.2.4./03 (IIA, 8.2)	Gries, T.	2002	IN-N5297: Static, acute, 48-hour EC ₅₀ to <i>Daphnia magna</i> Springborn Laboratories (Europe) AG DuPont-9790 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.4./04 (IIA, 8.2)	Hoke, R.A.	2002	IN-J0290: Static, acute, 48-hour EC ₅₀ to <i>Daphnia magna</i> DuPont Haskell Laboratory DuPont-7357 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.4./05 (IIA, 8.2)	Johnson, I.C., Drotter, K.R.	1987	Acute toxicity of H-16,441 to the crayfish (<i>Procambarus clarki</i>) Environmental Science and Engineering, Inc. HLO 57-87 GLP: Yes Published: No	N	DuPont
IIA, 8.2.4./06 (IIA, 8.2)	Samel, A.	2001	Bensulfuron methyl (DPX-F5384) technical: Static, acute 48-hour toxicity test to <i>Daphnia magna</i> . DuPont Haskell Laboratory DuPont-5443 GLP: Yes Published: No	Y	DuPont

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Annex No., EU Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
IIA, 8.2.5./01 (IIA, 8.2)	Samel, A.	2002	Bensulfuron methyl (DPX-F5384) technical: 21 day chronic, static-renewal toxicity to <i>Daphnia magna</i> . DuPont Haskell Laboratory DuPont-9066 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.5./02	Samel, A.	2002	IN-J0290: 21-day chronic toxicity to <i>Daphnia magna</i> DuPont Haskell Laboratory DuPont-9340 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.5./03	Samel, A.	2002	IN-N5297: 21-day chronic toxicity to <i>Daphnia magna</i> DuPont Haskell Laboratory DuPont-10245 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.6./01 (IIA, 8.2)	Boeri, R.L., Wyskiel, D.C., Ward, T.J.	2002	IN-DIR84: Influence on growth and growth rate of the green algal <i>Selenastrum capricornutum</i> T.R. Wilbury Laboratories DuPont-9393, Revision No. 1 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.6./02 (IIA 8.2)	Gries, T.	2002	IN-N5297: Influence on growth and growth rate of the green alga <i>Selenastrum capricornutum</i> Springborn Laboratories (Europe) AG DuPont-9792 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.6./03 (IIA 8.2)	Sloman, T.L.	2001a	Bensulfuron methyl (DPX-F5384) technical: influence on growth and growth rate of the green algal <i>Selenastrum capricornutum</i> DuPont Haskell Laboratory DuPont-5440 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.6./04 (IIA 8.2)	Sloman, T.L.	2001b	Bensulfuron methyl (DPX-F5384) technical: Influence on growth and growth rate of the blue-green alga <i>Anabaena flos-aquae</i> DuPont Haskell Laboratory DuPont-5441, Revision No. 1 GLP: Yes Published: No	Y	DuPont

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Annex No., EU Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
IIA, 8.2.6./05	Sloman, T.L.	2002a	IN-J0290: Influence on growth and growth rate of the green alga <i>Selenastrum capricornutum</i> DuPont Haskell Laboratory DuPont-7359 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.6./06 (IIA, 8.2)	Sloman, T.L.	2002b	IN-R9419: Influence on growth and growth rate of the green algal <i>Selenastrum capricornutum</i> DuPont Haskell Laboratory DuPont-7360 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.6./07 (IIA 8.2)	Sloman, T. L.	2002c	IN-T5831: Influence on growth and growth rate of the green algal <i>Selenastrum capricornutum</i> DuPont Haskell Laboratory DuPont-7841 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.6./08 (IIA 8.2)	Sloman, T.L.	2002d	IN-F7880: Influence on growth and growth rate of the green alga <i>Selenastrum capricornutum</i> Dupont Haskell Laboratory DuPont-7362 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.6./09 (IIA 8.2)	Sloman, T.L.	2003	IN-DAT97: Influence on growth and growth rate of the green algal <i>Selenastrum capricornutum</i> DuPont Haskell Laboratory for health and environmental sciences DuPont-12200 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.7./01 (IIA 8.2)	Hoke, R.A.	1997	Bensulfuron methyl/metsulfuron methyl (DPX-JM206) 60WG(DF) (29:1): Static, acute, 96-hour test to the chironomid, <i>Chironomus tentans</i> DuPont Haskell Laboratory HL-1997-01207 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.8./01 (IIA 8.2)	Boeri, R.L., Wyskiel, D.C., Ward, T.J.	2002	IN-DIR84: Influence on growth and reproduction of <i>Lemna gibba</i> G3 T.R. Wilbury Laboratories DuPont-9437 GLP: Yes Published: No	Y	DuPont

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Annex No., EU Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
IIA, 8.2.8./02 (IIA 8.2)	Gries, T.	2002	IN-N5297: Influence on growth and reproduction of <i>Lemna gibba</i> G3 Springborn Laboratories (Europe) AG DuPont-9793 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.8./03	Sloman, T.L.	2001	Bensulfuron methyl (DPX-F5384) technical: Influence on growth and reproduction of <i>Lemna gibba</i> G3 DuPont Haskell Laboratory DuPont-5442 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.8./04 (IIA, 8.2)	Sloman, T.L.	2002a	IN-R9419: Influence on growth and reproduction of <i>Lemna gibba</i> G3 DuPont Haskell Laboratory DuPont-7361 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.8./05 (IIA 8.2)	Sloman, T.L.	2002	IN-T5831: Influence on growth and reproduction of <i>Lemna gibba</i> G3 DuPont Haskell Laboratory DuPont-7842 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.8./06 (IIA 8.2)	Sloman, T.L.	2002b	IN-F7880: Influence on growth and reproduction of <i>Lemna gibba</i> G3 DuPont Haskell Laboratory DuPont-7363 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.8./07 (IIA 8.2)	Sloman, T.L.	2002	IN-J0290: Influence on growth and reproduction of <i>Lemna gibba</i> G3 DuPont Haskell Laboratory DuPont-9339 GLP: Yes Published: No	Y	DuPont
IIA, 8.2.8./08 (IIA 8.2)	Leva, S.	2003	IN-DAT97: Influence on growth and reproduction of <i>Lemna gibba</i> G3 DuPont Haskell Laboratory for Health and Environmental sciences DuPont-12201 GLP: Yes Published: No	Y	DuPont
IIA, 8.3.1.1./01 (IIA 8.3)	Wainwright, M.J.	2002	Bensulfuron methyl (DPX-F5384): Acute oral and contact toxicity to the honey bee, <i>Apis mellifera</i> L. (Hymenoptera, Apidae) Huntingdon Life Sciences Limited DuPont-5088 GLP: Yes Published: No	Y	DuPont

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Annex No., EU Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
IIA, 8.3.2./01 (IIA 8.3)	Nienstedt, K.M., Kollmann, S.I.	2001	Bensulfuron methyl (DPX-F5384) 60WG: A multi-dose toxicity test with the predatory mite, <i>Typhlodromus pyri</i> Scheuten (Acari: Phytoseiidae) Springborn Laboratories (Europe) AG DuPont-5179 GLP: Yes Published: No	Y	DuPont
IIA, 8.3.2./02 (IIA 8.3)	Nienstedt, K.M., Novent, O.	2001	Bensulfuron Methyl (DPX-F5384) 60WG: A multi-dose toxicity test with the aphid parasitoid, <i>Aphidius</i> <i>rhopalosiphi</i> (Hymenoptera: Braconidae) Springborn Laboratories (Europe) AG DuPont-5178 GLP: Yes Published: No	Y	DuPont
IIA, 8.4.1./01 (IIA 8.4)	Luhrs, U.	2001	Bensulfuron methyl technical: Acute toxicity to the earthworm, <i>Eisenia foetida</i> (Savigny) in artificial soil Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-4591 GLP: Yes Published: No	Y	DuPont
IIA, 8.4.1./02 (IIA 8.4)	Luhrs, U.	2002a	IN-J0290: Acute toxicity of the earthworm, <i>Eisenia foetida</i> in artificial soil IBACON DuPont-7274 GLP: Yes Published: No	Y	DuPont
IIA, 8.4.1./03 (IIA 8.4)	Luhrs, U.	2002b	IN-D1R84: Acute toxicity to the earthworm, <i>Eisenia foetida</i> in artificial soil Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-7277 GLP: Yes Published: No	Y	DuPont
IIA, 8.4.1./04 (IIA 8.4)	Luhrs, U.	2002c	IN-R9419: Acute toxicity to the earthworm, <i>Eisenia foetida</i> in artificial soil Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-7272 GLP: Yes Published: No	Y	DuPont

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Annex No., EU Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
IIA, 8.4.1./05 (IIA 8.4)	Luhrs, U.	2002d	IN-N5297: Acute toxicity of the earthworm, <i>Eisenia foetida</i> in artificial soil Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-7273 GLP: Yes Published: No	Y	DuPont
IIA, 8.4.1./06 (IIA 8.4)	Luhrs, U.	2002e	IN-F7880: Acute toxicity of the earthworm, <i>Eisenia foetida</i> in artificial soil Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-7276 GLP: Yes Published: No	Y	DuPont
IIA, 8.5./01 (IIA 8.5)	El-Ghamry, A.M., Xu, J.M., Huang, C.Y., Gan, J.	2002	Microbial response to Bensulfuron-methyl treatment in soil Journal of Agricultural and Food Chemistry 50, pages 136-139 Not applicable – Publication GLP: No Published: Yes	N	Authors
IIA, 8.5./02 (IIA 8.5)	Gigliotti, C., Allievi, L., Salardi, C., Ferrari, F., Farini, A.	1998	Microbial ecotoxicity and persistence in soil of the herbicide Bensulfuron-methyl J. Environmental Science Health, Volume B33(4), pages 381-398 Not applicable - Publication GLP: No Published: Yes	N	Authors
IIA, 8.5./03 (IIA 8.5)	Kolzer, U.	2002	IN-J0290: Assessment of the effects on soil microflora Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH DuPont-9199 GLP: Yes Published: No	Y	DuPont
IIA, 8.5./04 (IIA 8.5)	Kolzer, U.	2002	Bensulfuron methyl (DPX-F5384) 60WG: Assessment of the effects on soil microflora Arbeitsgemeinschaft GAB Biotechnologie GmbH & IFU Umweltanalytik GmbH DuPont-9198 GLP: Yes Published: No	Y	DuPont

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Annex No., EU Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
IIA, 8.5./05 (IIA 8.5)	Reis, K.H.	2002	IN-R9419: Assessment of the effects on soil microflora Institut fur Biologische Analytik und Consulting IBACON GmbH DuPont-8050 Revision No. 1 GLP: Yes Published: No	Y	DuPont
IIA, 8.5./06 (IIA 8.5)	Reis, K.H.	2002	IN-N5297: Assessment of the effects on soil microflora Institut fur Biologische Analytik und Consulting IBACON GmbH DuPont-8051 GLP: Yes Published: No	Y	DuPont
IIA, 8.5./07 (IIA 8.5)	Reis, K.H.	2002	IN-D1R84: Assessment of the effects on soil microflora Institut fur Biologische Analytik und Consulting IBACON GmbH DuPont-8053 GLP: Yes Published: No	Y	DuPont
IIA, 8.5./08 (IIA 8.5)	Reis, K.H.	2002	IN-F7880: Assessment of the effects on soil microflora Institut fur Biologische Analytik und Consulting IBACON GmbH DuPont-8052 GLP: Yes Published: No	Y	DuPont
IIA, 8.6./01 (IIA 8.6)	Porch, J.R., Martin, K.H.	2004a	Bensulfuron methyl (DPX-F5384) 60WG: A greenhouse study to investigate the effects on seedling emergence and growth of ten terrestrial plant species following soil exposure Wildlife Int'l Ltd. DuPont-12416 GLP: Yes Published: No	Y	DuPont
IIA, 8.6./02 (IIA 8.6)	Porch, J.R.	2004b	Bensulfuron methyl (DPX-F5438) 60WG: A greenhouse study to investigate the effects on vegetative vigor of ten terrestrial plant species following foliar exposure Wildlife Int'l Ltd. DuPont-12417 GLP: Yes Published: No	Y	DuPont

Rapporteur Member State	Month and year	Active Substance (Name)
Italy	May 2006	Bensulfuron methyl

Ecotoxicology

Annex No., EU Data Requirement No., Reference No.	Author(s)	Year	Title Source Company Report No. GLP or GEP Status (where relevant) Published or not	EU Data Protection Claimed (Y/N)	Owner
IIA, 8.7./01 (IIA 8.7)	Hertl, J.	2001	Bensulfuron methyl technical (DPX-F5384): Activated sludge, respiration inhibition test Institut für Biologische Analytik und Consulting IBACON GmbH DuPont-5163, Revision No 1 GLP: Yes Published: No	Y	DuPont
IIIA, 10.2.1./01 (IIIA 10.2)	Hutton, D.G.	1988a	Static acute 48-hour EC ₅₀ of IN-F5384-85 to <i>Daphnia magna</i> DuPont Haskell Laboratory HLR 625-88 GLP: Yes Published: No	Y	DuPont
IIIA, 10.2.1./02 (IIIA 10.2)	Hutton, D.G.	1988b	Static acute 96-hour LC ₅₀ of IN-F5384-85 to rainbow trout (<i>Salmo gairdneri</i>) [REDACTED] HLR 654-88 GLP: Yes Published: No	Y	DuPont
IIIA, 10.2.1./03 (IIIA 10.2)	Sloman, T.	2001a	Bensulfuron methyl (DPX-F5384) 60WG: Influence on growth and growth rate of green alga <i>Selenastrum capricornutum</i> DuPont Haskell Laboratory DuPont-5437 GLP: Yes Published: No	Y	DuPont
IIIA, 10.2.1./04 (IIIA 10.2)	Sloman, T.	2001b	Bensulfuron methyl (DPX-F5384) 60WG: Influence on growth and growth rate of blue-green alga <i>Anabaena flos-aquae</i> DuPont Haskell Laboratory DuPont-5438 GLP: Yes Published: No	Y	DuPont
IIIA, 10.2.1./05 (IIIA 10.2)	Sloman, T.	2001c	Bensulfuron methyl (DPX-F5384) 60WG: Influence on growth and reproduction of <i>Lemna gibba</i> G3 DuPont Haskell Laboratory DuPont-5439 GLP: Yes Published: No	Y	DuPont