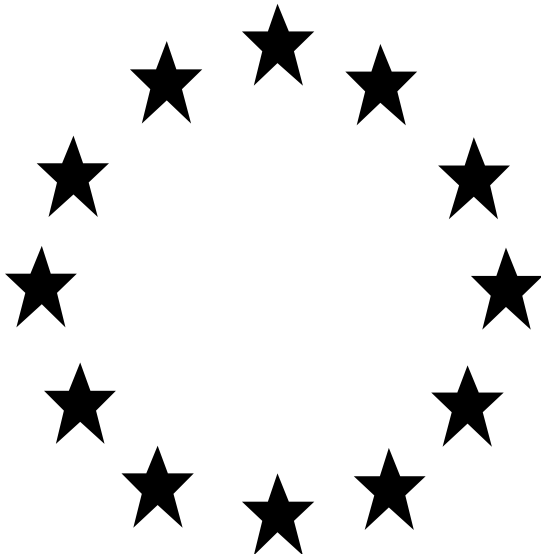

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Austria for the existing active substance

FLUAZINAM

of the third stage (part A) of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

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July 2006

Annex B

Fluazinam

B.9 Ecotoxicology

B.9 Ecotoxicology

B.9.1 Effects on birds (Annex II A 8.1, III A 10.1)

B.9.1.1 Acute oral toxicity (Annex II A 8.1.1)

active substance: Fluazinam

Reference: Hakin, B., Johnson, A. J., Anderson, A., Dawe, I. S. (1991): B-1216 Technical Acute Oral Toxicity (LD_{50}) to Bobwhite Quail, Report No. ISK 48/91161
Test guideline: U.S. EPA FIFRA, No. 71-1
GLP: Yes
Material and methods:
Technical fluazinam (Lot No. 8412-20, purity 95.3 %) was administered via oral intubation at dose levels of 0, 500, 1000 or 2000 mg/kg bw to bobwhite quail (*Colinus virginianus*). Forty quails were utilized for the study; the control group and each treatment group consisted of 5 male and 5 female birds. A control group was given the test vehicle alone, 1 % w/v methylcellulose. Observations including mortality, clinical signs, body weight and food consumption were made for 14 days. Ten birds from the two highest dose groups were subjected to a gross *post-mortem* examination.
Findings:
One mortality occurred at 1000 mg/kg and six at 2000 mg/kg. Birds dosed at 2000 mg/kg exhibited toxic signs including subdued behaviour and ruffled feathers prior to death. Over days 0 to 7, bodyweight gain and female food consumption were slightly reduced at 2000 mg/kg. At *post-mortem* examination, the intestines of all birds that died during the study were observed to be red in colour. The liver of one bird had pale blotches. No other abnormalities were detected in any birds at termination of the study.
Conclusion:
The acute oral LD_{50} of technical fluazinam to the bobwhite quail was determined to be 1782 mg/kg (95 % confidence limits of 1321–3631 mg/kg). The no effect level was considered to be 500 mg/kg, as no effects were observed on any of the measured parameters at this level.
Comment (RMS): Study considered acceptable.

Reference: Roberts, N. L., Anderson, A., Dawe, I. S. (1991a): The Acute Oral Toxicity of B1216 to the Mallard Duck, Report No. ISN 31BT/841207
Test guideline: U.S. EPA FIFRA No. 71-1
GLP: Yes
Material and methods:
Technical fluazinam (batch No. 8402-6, purity 95.3 %) was administered via oral intubation to young adult mallard ducks (*Anas platyrhynchos*) in corn oil at dose levels of 0, 484, 944, 2020, 3050 and 4190 mg/kg bw. Sixty ducks were utilized; the control group and each treatment group consisted of 5 male and 5 female birds. The dose levels were selected based on the results of a

range-finding study. A control group was given corn oil only. Observations including mortality, clinical signs, body weight and food consumption were made for 14 days. All birds were examined at termination of the study for gross pathological changes.

Findings:

No mortalities occurred at any dose level in the study. Female birds in the high-dose group (4190 mg/kg) were subdued on day 2. No other abnormalities were noted.

Conclusion:

The LD_{50} value was determined to be in excess of 4190 mg/kg bw.

Comment (RMS): Study considered acceptable.

Summary of acute toxicity

Table B.9.1.1-1 Acute oral toxicity of fluazinam technical in birds

Test organism	Number / Sex	NOED mg/kg bw	LD50 mg ai/kg bw	Guideline	Reference
bobwhite quail	5 m + 5 f	500	1782	EPA 71-1	Hakin et al. 1991
mallard duck	5 m + 5 f	3050	> 4190	EPA 71-1	Roberts et al 1991

B.9.1.2 Dietary toxicity (Annex II A 8.1.2)

Reference: Roberts, N. L., Anderson, A., Dawe, I. S. (1991b): The Subacute Dietary Toxicity of B1216 to the Bobwhite Quail, Report No. ISN 24BT/841206.

Test guideline: US EPA FIFRA No. 71-2

GLP: Yes

Material and methods:

Groups of northern bobwhite quail (*Colinus virginianus*), 10 days of age, were fed technical fluazinam (Lot No. 8402-6, purity 95.3 %) in the diet at analysed concentrations of 0, 313, 654, 1260, 2480, 5230 and 10500 ppm for 5 days. Each of the three control groups and each treatment group consisted of 10 birds of unknown sex. Dose levels were based on the results of a range-finding study. The birds were then maintained on untreated diet and observed for an additional 3-day period. All birds were observed for mortality, abnormal behaviour and signs of toxicity. Body weights and estimated food consumption were measured. A gross *post-mortem* examination was performed.

Findings:

No abnormalities were noted in any birds during the trial. Birds in the high-dose group of 10500 ppm (1244 mg/kg bw/d) showed a mean bodyweight decrease over the feeding period of fluazinam in the diet, followed by a large compensatory bodyweight increase over the three-day

observation period. Food consumption results were variable; however, food consumption in the control groups was similar to that of the test groups over the treatment and post-treatment periods. Therefore, the decrease in food consumption, observed during the test period, was not considered to be significant. Three mortalities occurred during testing, one each in the control, 2480- and 5230 ppm treatment groups. The death in the control group was due to an accident. Necropsies were performed on the animals which died on test; no treatment related abnormalities were observed.

Conclusion:

The dietary LC_{50} was determined to be higher than the highest level tested of 10500 ppm, corresponding to > 1244 mg/kg bw/d (calculated according to SANCO 4145/2000, mean bw 13 g, mean food consumption 1.6 g).

Comment (RMS): It is noted that in the range-finding study which is shortly reported in the study report 4 of 5 quails died at 10000 ppm in the diet. Nevertheless, the study is considered acceptable.

Reference: Roberts, N. L., Anderson, A., Dawe, I. S. (1991c): The Subacute Dietary Toxicity of B1216 to the Mallard Duck. Report No. ISN 25BT/841208

Test guideline: U.S. EPA FIFRA No. 71-2

GLP: Yes

Material and methods:

Groups of Mallard ducks (*Anas platyrhynchos*), 9 days of age, were fed technical fluazinam (Lot No. 8402-6, purity 95.3 %) in the diet at measured concentrations of 0, 333, 651, 1260, 2590, 5230 and 10600 ppm for 5 days. Each of the three control groups and each treatment group consisted of 10 birds of unknown sex. Dose levels were based on the results of a range-finding study. The birds were then maintained on untreated diet and observed for an additional 3-day period. All birds were observed for mortality, abnormal behaviour and signs of toxicity. Body weights and estimated food consumption were measured. A gross *post-mortem* examination was performed.

Findings:

One mortality occurred in the high-dose group (10600 ppm, 1147 mg/kg bw/d) on day 3. No abnormality was noted in any bird, even in the one that died. Birds in the high-dose group showed a reduced mean bodyweight gain over the five treatment days. The mean bodyweight for this group was lower than for all other groups at day 8. There was a slight treatment-related bodyweight gain decrease in the 5230 ppm dose group. Food consumption was reduced in the high dose group during and post-treatment. Macroscopic examination of the bird that died on test showed fluid beneath the skin of both legs. Pale orange/yellow coloured livers were noted in two birds of the 1260 ppm dose group and all birds from higher-dose groups (2590, 5230 and 10600 ppm) at *post-mortem* examination. No other abnormalities were noted.

Conclusion:

The dietary LC_{50} for technical fluazinam was determined to be > 10600 ppm, corresponding to > 1230 mg/kg bw/d (calculated according to SANCO 4145/2000, mean bw 87 g, mean food

consumption 10.1 g). The NOEC was 651 ppm.

Comment (RMS): Study considered acceptable.

Summary of short-term toxicity

Table B.9.1.2-1 Dietary toxicity of fluazinam technical to birds.

Test organism	Number	NOEC (ppm)	LC50 (ppm)	LD50 (mg/kg bw)	Guideline	Reference
bobwhite quail	10	1260	> 10500	> 1244	EPA 71-2	Roberts et al. 1991a
mallard duck	10	651	> 10600	> 1230	EPA 71-2	Roberts et al. 1991b

B 9.1.3 Reproductive toxicity (Annex IIA 8.1.3)

Pilot studies

Reference: Turck, P. A., Laveglia, J. (1995a): Technical Fluazinam: A Pilot Reproduction Study with the Northern Bobwhite (*Colinus virginianus*), Report No. 5511-92-0453-TX-003

Test guideline: US EPA FIFRA No. 71-4

GLP: Yes

Material and methods:

Test diets of 0, 250, 500, 1000 and 2000 ppm nominal concentration of fluazinam (Lot No. 1030/91, 96.8 % purity) were fed to 20 weeks old northern bobwhites for 6 weeks (5 pairs per group). The birds were observed daily for mortality, abnormal behaviour and signs of toxicity. The body weight, feed consumption and egg production were also measured.

Findings:

No overt signs of toxicity were observed at any of the concentrations tested. Three mortalities were noted at the 2000 ppm test concentration, two of which (one male, one female) were attributed to incidental injuries. While a necropsy of the third bird, a male, found dead revealed a pale and mottled liver, it could not be determined whether the third mortality was treatment related. There were no apparent treatment related effects on body weight or feed consumption at any of the concentrations tested. There appeared to be a treatment related effect on egg production at the 2000 ppm level. However, egg production was highly variable among all groups including the control. Based upon these results, the NOEC in this study was 1000 ppm (102 mg/kg bw/d). The results of this study were used to select the dietary concentrations in the subsequent main reproduction study.

Comment (RMS): Study considered acceptable. Study not essential.

Reference: Turck, P. A., Laveglia, J. (1995b): Technical Fluazinam: A Pilot Reproduction Study with the Mallard Duck(*Anas platyrhynchos*), Report No. 5511-92-0452-TX-003

Test guideline: US EPA FIFRA No. 71-4
GLP: Yes
Material and methods:
Test diets of 0, 250, 500, 1000 and 2000 ppm nominal concentration of Fluazinam (Lot No. 1030/91, 96.8 % purity) were fed to 22 weeks old mallard ducks for 6 weeks (5 pairs per group). The birds were observed daily for mortality, abnormal behaviour and signs of toxicity. The body weight, feed consumption and egg production were also measured.
Findings:
One mortality at 2000 ppm was considered not treatment related. No other mortalities or overt signs of toxicity were observed at any of the concentrations tested. There were no apparent treatment related effects on body weight, feed consumption or egg production. Based upon these results, the NOEC was 2000 ppm (308 mg/kg bw/d), the highest concentration tested. The results of this study were used to select the dietary concentrations in the subsequent main reproduction study.
Comment (RMS): Study considered acceptable. Study not essential.

Main reproduction studies

Reference: Turck, P. A., Laveglia, J. (1996a): Technical Fluazinam: A Reproduction Study with the Northern Bobwhite (*Colinus virginianus*) Phases I and II, Report No. 5512-92-0455-TX-003
Test guideline: U.S. EPA FIFRA 71-4 and OECD 206
GLP: Yes
Material and methods:
During Phase I of the study, groups of 16 pairs of bobwhite quails (*Colinus virginianus*) received technical fluazinam (Lot No. 1030/91, purity 97.0 %) at nominal dietary concentrations of 0, 750, 1500 and 5000 ppm for 21 weeks (only 6 weeks for the 5000 ppm group). Treatment related effects were seen at the lowest concentration tested. In order to determine the NOEC, a second test phase was conducted. During Phase II, bobwhite quails were exposed to fluazinam at dietary concentrations of 0, 50, 200, 350 and 500 ppm for 22 weeks. A control group, fed untreated diet, was maintained concurrently with each set of treatment groups. The quails were observed daily for mortality, abnormal behaviour and signs of toxicity. Necropsies were performed on all adults. The effects of fluazinam on the number of eggs laid, normal development of eggs, viability of the embryos, percent hatchability, offspring survival and egg shell thickness were evaluated.
Findings:
Analyses performed on diet samples revealed actual concentrations to range from 97 to 102 % of nominal at the day of preparation.
Due to excessive mortalities, profound clinical signs of toxicity, and effects on body weight, the 5000 ppm treatment group of Phase I was terminated at the end of week 6. There were no treatment-related mortalities, overt signs of toxicity or effects on body weight at test concentrations up to 1500 ppm. The 1500 ppm treatment group showed an increase in feed consumption from

week 7 to 13 that was statistically significant though the differences were very slight. This effect was not judged to be adverse. At 750 ppm there appeared to be slight effects upon egg production and offspring survival, with a more pronounced effect upon hatchability. There were profound effects upon the reproductive parameters measured at the 1500 ppm test concentration.
During Phase II, there were no treatment-related mortalities, overt signs of toxicity or treatment-related effects on body weight or feed consumption at any of the test concentrations. There were no apparent treatment-related effects upon reproductive performance. No changes in egg shell thickness were noted in any group. No statistical differences in hatchling and 14-d old survivor body weights were seen up to and including 750 ppm. There appeared to be a slight incidental increase in cracked eggs as a percentage of eggs laid at 50 ppm. The observed difference from the control group was primarily the result of an increase of the number of cracked eggs in three of the 16 pens. Since the difference was slight, not concentration responsive and confined to 3 pens, this effect was not considered to be treatment related. The effects on reproductive parameters are shown in the table below.

Table 9.1.3-1: Effects of Fluazinam on Reproduction of the Bobwhite Quail

Reproductive Parameter	Dietary Concentration (ppm)							
	Phase I			Phase II				
	0	750	1500	0	50	200	350	500
Number of Pairs	15	16	13	16	16	16	16	16
Total Eggs Laid	712	673	386	787	855	870	806	739
No. of eggs/female/day	0.51	0.45	0.32	0.51	0.55	0.56	0.52	0.48
Eggs cracked/eggs laid (%)	1	3	4	3	5*	1	2	3
Viable embryos/eggs set (%)	95	93	79**	95	95	93	98	93
Viable 3-wk embryos/viable embryos(%)	100	99	95**	99	99	99	99	98
Hatchlings/viable 3-wk embryos (%)	94	83*	75**	82	91	89	86	89
Hatchlings/eggs set (%)	88	76*	7**	77	86	82	83	81
14-day old survivors/hatchlings (%)	76	67	50**	88	86	88	79	84
No. of 14-day old survivors/female	30	18	8	32	32	35	30	26
14-day old survivors/eggs set (%)	68	51	31**	69	74	73	66	69

* Significantly different from control at p<0.05
** Significantly different from control at p<0.01

Conclusion:

Based on the overall results of this study (Phases I and II), the parental NOEC for fluazinam in bobwhite quail was 1500 ppm and the reproductive NOEC was 500 ppm, corresponding to 60.4 mg/kg bw (see comment). No adverse reproductive effects were noted at dietary levels up to 500 ppm.

Comment (RMS):

The corresponding daily dose was calculated according to SANCO 4145/2000, however, bodyweights of adult birds were not measured over the entire 22 week exposure period, but only during weeks 0, 2, 4, 6 and 8. As bodyweights can be assumed to be lower during the first period of the study, the calculation using bodyweights over these weeks gives a “worst case” NOEL and

this deviation from the rules in the guidance document is considered negligible (mean bw 211 g, mean food consumption 25.5 g). Study considered acceptable.

Reference: Turck, P. A., Laveglia, J. (1996b): Technical Fluazinam: A Reproduction Study with the Mallard (*Anas platyrhynchos*) Phases I and II, Report No. 5512-92-0454-TX-003
Test guideline: US EPA FIFRA No. 71-4 and OECD 206
GLP: Yes
Material and methods:
During Phase I of the study, groups of 16 pairs of mallard ducks (*Anas platyrhynchos*) received technical fluazinam (Lot No. 1030/91, purity 97.0 %) at nominal dietary concentrations of 0, 750, 1500 and 5000 ppm for 21 weeks. During Phase II, mallards were exposed to fluazinam at dietary concentrations of 0, 50, 200, 350 and 500 ppm for 21 weeks. A control group, fed untreated diet, was maintained concurrently with each set of treatment groups. The birds were observed daily for mortality, abnormal behaviour and signs of toxicity. Necropsies were performed on all adults. The effects of fluazinam on the number of eggs laid, normal development of eggs, viability of the embryos, percent hatchability, offspring survival and egg shell thickness were evaluated.
Findings:
Analyses performed on diet samples revealed actual concentrations to range from 97 to 102 % of nominal at the day of preparation.
Due to excessive mortalities, profound clinical signs of toxicity, and effects on body weight, the 5000 ppm treatment group of Phase I was terminated at the end of week 9. Two mortalities that were possibly treatment-related were noted at the 1500 ppm level. There were no treatment-related overt signs of toxicity or effects on body weight at 750 or 1500 ppm. There were treatment-related effects upon reproductive parameters at both the 750- and 1500 ppm treatment levels. In the 750 ppm treatment group, there were reductions in both egg production and embryo viability, while in the 1500 ppm group, there were reductions in egg production, embryo viability, hatchability and hatchling body weight. The effects resulted in an overall reduction in reproductive performance for both treatment groups.
During Phase II, there were no treatment-related mortalities, overt signs of toxicity or treatment-related effects on body weight or feed consumption at any of the test concentrations. Statistically significant reductions in egg shell thickness were noted in the 50 and 500 ppm groups when compared with controls. Since there appeared to be no dose response and no differences were seen in Phase I, the differences were not considered treatment related by the study authors. Statistically lower body weights were noted in 14-d-old survivors in the 500 ppm group. Since there appeared no dose response following comparison of data of phase I and II, this bodyweight reduction was not considered treatment related by the study authors. There were no other apparent treatment-related effects upon reproductive performance in Phase II. The effects of fluazinam on reproductive parameters measured in the study are shown in the table below.

Table 9.1.3-2: Effects of Fluazinam on Reproduction of the Mallard Duck

Reproductive Parameter	Dietary Concentration (ppm)							
	Phase I			Phase II				
	0	750	1500	0	50	200	350	500
Number of Pairs	16	16	14	16	16	16	16	16
Total Eggs Laid	811	635	565	876	692	767	803	719
No. of eggs/female/day	0.58	0.46	0.46	0.66	0.52	0.58	0.60	0.54
Mean egg shell thickness (mm)	0.385	0.372	0.388	0.379	0.358*	0.365	0.371	0.360*
Eggs cracked/eggs laid (%)	1	2	2	1	2	7	2	1
Eggs laid/max. laid (%)	78	61*	62	76	60	67	70	63
Viable embryos/eggs set (%)	95	80*	79	89	96	93	89	91
Hatchling bodyweight (g)	34	33	32**	36	36	35	35	35
14-d-old bodyweight (g)	291	278	279	287	278	274	284	257*
Viable 3-wk embryos/viable embryos (%)	99	98	99	100	98	97	99	99
Hatchlings/viable 3-wk embryos (%)	73	69	54*	70	60	72	69	67
14-day old survivors/hatchlings (%)	96	98	93	96	91	91	98	97
Hatchlings/eggs set (%)	69	55	44*	63	58	68	60	59
14-day old survivors/eggs set (%)	66	53	40*	61	55	60	59	58
Hatchlings/maximum set (%)	50	31**	25**	49	34	44	39	37
14-day old survivors/maximum set	49	31*	23**	47	32	43	38	36

*Significantly different from control at p<0.05
** Significantly different from control at p<0.01

Conclusion:

Based on the overall results of this study (Phases I and II), the parental NOEC for fluazinam in mallard ducks was 1500 ppm and the reproductive NOEC 500 ppm (61.4 mg/kg bw).

Comment (RMS):

The corresponding daily dose was calculated according to SANCO 4145/2000, however, bodyweights of adult birds were not measured over the entire 21 week exposure period, but only during weeks 0, 2, 4, 6 and 8. As bodyweights can be assumed to be lower during the first period of the study, the calculation using bodyweights over these weeks gives a “worst case” NOEL and this deviation from the rules in the guidance document is considered negligible (mean bw 1125 g, mean food consumption 138 g). Study considered acceptable.

Summary of reproductive toxicity

Table B.9.1.3-3 Reproductive toxicity of fluazinam in birds.

test organism	number / sex	NOEC (ppm)	NOEL (mg/kg bw)	guideline	reference
bobwhite quail - *range finding*	5 m + 5 f	1000	102	EPA 71-4	Turck & Laveglia 1995a
mallard duck - *range finding*	5 m + 5 f	2000	308	EPA 71-4	Turck & Laveglia 1995b
bobwhite quail	16 m + 16 f	500	60.4	EPA 71-4, OECD 206	Turck & Laveglia 1996a
mallard duck	16 m + 16 f	500	61.4	EPA 71-4, OECD 206	Turck & Laveglia 1996b

B.9.1.4 Risk assessment

Birds may be exposed to fluazinam by eating contaminated vegetation, seeds and fruits, invertebrate prey like arthropods (i.e. insects) or earthworms or vertebrate prey.

Tier-1 standard exposure scenarios were performed according to the guidance document SANCO/4145/2000 (September 2002).

The relevant toxicity figures were taken from laboratory studies with fluazinam. All of them were considered acceptable. These endpoints are listed in table 9.1.1-1, table 9.1.2-1 and table 9.1.3-3 for the different time scales.

The risk assessment is based on the following toxicity endpoints:

	species	ppm diet	mg ai/kg bw
acute oral LD_{50}	bobwhite quail	-	1782
short-term LC_{50} / LD_{50}	mallard duck	> 10600	> 1230
reproductive NOEC / NOEL	bobwhite quail	500	60.4

Assessment of exposure / Selection of scenarios:

The following relevant exposure scenario was considered:

potatoes: ten applications of 200 g ai/ha (interval 7 d)

Three exposure routes are considered:

1) Dietary exposure, standard scenarios

2) Exposure of birds via bioaccumulation and food chain behaviour

3) Exposure via drinking water

ad 1)

a) dietary exposure of a medium herbivorous bird feeding on contaminated potato foliage

b) dietary exposure of a small insectivorous bird feeding on contaminated arthropods

Exposure estimates were conducted according to the standard scenarios provided in Sanco/4145/2000. In order to calculate the estimated theoretical exposure the respective FIR/bw and RUD figures were chosen to estimate food intake rates and concentrations in the food of concern (see tables 9.1.4-1-3). As ten applications are intended, figures for MAF are chosen according to Sanco/4145/2000. For acute exposure, the RUD is the 90th percentile residue estimate, and for short- and long-term esposure mean residues on vegetation (RUD) are used. A default half-life of 10 d is assumed for estimating residue decline on leaves. In the first tier worst case assessment it is assumed that the birds feed on a single food type, which is not avoided and that they satisfy their entire food demand in the treated area (PD, PT = 1).
Possible other food items, for which no respective risk assessment was performed, are covered by the calculations presented because residues in these items are expected to be much lower.
Metabolites
In plant tissues fluazinam is converted to several metabolites; none of these was found in relevant amounts (10 % TRR). In animal metabolism studies the main metabolites were the same as in plants and no further ecotoxicological consideration of these compounds is required.

Table 9.1.4.-1: Estimation of the TERacute for birds, Tier 1.

scenario	FIR / bw	RUD	Rate [kg/ha]	MAF	ETE	Tox.figure [mg ai/kg bw]	**TER**
medium herbivorous bird	0.76	87	0.2	2	26.5	1782	**67**
insectivorous bird	1.04	52	0.2	-	10.8	1782	**165**

Table 9.1.4.-2: Estimation of the TERshort-term for birds, Tier 1.

scenario	FIR / bw	RUD	Rate [kg/ha]	MAF	ETE	Tox.figure [mg/kg bw]	**TER**
medium herbivorous bird	0.76	40	0.2	2.6	15.7	> 1230	**> 78**
insectivorous bird	1.04	29	0.2	-	6.0	> 1230	**> 205**

Table 9.1.4.-3: Estimation of the TERlong-term for birds, Tier 1.

scenario	FIR / bw	RUD	Rate [kg/ha]	f_{twa}	MAF	ETE	Tox.figure [mg/kg bw]	**TER**
medium herbivorous bird	0.76	40	0.2	0.53	2.6	8.4	60.4	**7.2**
insectivorous bird	1.04	29	0.2	-	-	6.0	60.4	**10**

All resulting TER figures are above the respective trigger values given in Annex VI of directive 91/414 and indicate that the risk for wild birds after application of fluazinam is low. (As potato foliage is unpalatable to birds, the herbivorous route moreover is considered as an unrealistic scenario).

2) Exposure of birds via bioaccumulation and food chain behaviour:

Because the $logP_{ow}$ > 3 a potential for bioaccumulation of fluazinam is indicated and this route of exposure is addressed according to SANCO/4145/2000 (Sept. 2002).

a) Food-chain from earthworm to earthworm-eating birds:

time-weighted PEC_{soil} for a period of 21 d after application: PEC_{soil} = 0.454 mg/kg soil

To estimate the $BCF_{earthworm}$ the following formula was used:

$BCF = (0.84 + 0.01\ K_{ow}) / f_{oc}\ K_{oc}$

The mean of the K_{oc} - figures from adsorption/desorption studies was used: K_{oc} = 1958; as f_{oc} the default-value of 0.02 was taken.

Log K_{ow} = 4.03, K_{ow} = 10715

BCF = (0.84 + 0.01 x 10715) / (0.02 x 1958) = 2.76

$PEC_{worm} = PEC_{soil}$ x BCF = 0.454 x 2.76 = 1.25 mg/kg

ETE_{birds}: 1.1 x 1.25 = 1.38 (factor 1.1 given in SANCO/4145/2000)

The TER-calculation is presented in table 9.1.4.-4.

Table 9.1.4.-4: TERlong-term for earthworm-eating birds under consideration of biomagnification in the food-chain.

ETE	NOEL [mg/kg bw]	**TER**
1.38	60.4	**43.8**

The resulting TER is above the trigger and indicates a low risk via this route.

b) Food chain from fish to fish-eating birds

highest PEC_{water} (twa, 3 weeks, FOCUS step 1, 1 m distance) = 40.4 µg/L

whole-body-BCF for fish = 1090

$PEC_{fish} = PEC_{water}$ * BCF = 40.4 * 1090 = 44036 µg/kg bw (fish) = 44 mg/kg bw (fish)

ETE_{birds}: 44 x 0.21 = 9.24 (figure 0.21 given in guidance document)

Table 9.1.4.-5: TERlong-term for fish-eating birds under consideration of biomagnification in the food-chain.

ETE	NOEL [mg/kg bw]	**TER**
9.24	60.4	**6.5**

The resulting TER is above the trigger and indicates an acceptable risk via this route.

c) Biomagnification in terrestrial food chains

According to the evaluation in the toxicology part of the DAR, the potential of bioaccumulation of fluazinam in vertebrates is low due to extensive metabolism of the substance. In lactating goats and laying hens the maximum level of radioactivity - including metabolites - found in total tissue after oral administration of fluazinam was < 2 % and < 3 % of the administered dose, respectively. It can be concluded that the risk of biomagnification in terrestrial food chains is low.

3) Exposure of birds via drinking water

The water intake of birds is calculated according to SANCO/4145/2000.

Total water ingestion rate (L / day) = $0.059W^{0.67}$

where W is the body weight in kg.

The exposure from puddles of spray liquid is assessed as a worst case. The dilution for spraying is 200 (-500) L water/ha containing 200 g ai. When a dilution factor of 5 is applied for the concentration in the puddle, a $PEC_{drinking\ water}$ of 200 mg ai/L is derived.

The total water ingestion rate (daily water intake DWI) of a small bird (10 g) is calculated as 2.7 mL/d. The acute toxicity (LD_{50}) for a 10 g bird is calculated as 17.8 mg ai/bird. The short-term LD_{50} is > 12.3 mg ai/bird.

The Toxicity Exposure Ratios (TER) for the acute and short-term time scales are calculated as LD_{50}/ETE. The ETE is calculated as $PEC_{drinking\ water}$ x DWI (daily water intake).

Table 9.1.4.-6: Acute and short-term TERs for birds drinking contaminated water from puddles

scenario		**ETE**	**TER**
acute	potatoes	0.54	33
short-term		0.54	> 23

The resulting TER figures indicate that the risk for wild birds after all representative uses of fluazinam is acceptable.

Conclusion:

After application of fluazinam under the intended representative use conditions the risk to wild birds is acceptable.

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

B.9.2.1 Acute toxicity of active substance, metabolites and formulations to aquatic organisms

Active substance

Acute toxicity to fish (IIA 8.2.1)

Reference: Gelin, M.D & J. Laveglia (1992): Technical Fluazinam (IKF-1216) – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions. Report No. 5099-91-0422-TX-002

Test guideline: FIFRA Guideline 72-1

GLP: yes

Test item: Fluazinam techn.: 96.8 % w/w, lot no. 1030/91

Material and methods:

A 96 hours acute toxicity test of fluazinam to rainbow trout was performed. 20 fish (10 per replicate) were exposed to nominal test concentrations of 0 (dilution water control), 0 (solvent control, acetone), 19, 27, 39, 56 and 80 µg/L, respectively, under flow through conditions. The fish were 5.1 cm (mean) in length and had an average weight of 2.0 g. Fish were exposed to test concentrations and controls under the following conditions: 16/8-hour light/dark photoperiod, 12 – 13 °C, pH 6.8 – 7.1, 68 – 98 % O_2 saturation, a total hardness of 30 mg/L as Ca CO_3 and a specific conductivity of 120 µmhos/cm. Analyses of test substance were conducted at the start and end of the test.

Findings:

Mean measured concentrations were 10, 15, 28, 33 and 56 µg/L, therefore the assessment is based on mean measured concentrations.

Behavioural or sublethal effects like changing of pigmentation (darkening), partial and complete loss of equilibrium and lethargy were observed at test concentrations of 28 and 33 µg/L, therefore the 96 hours “no effect” concentration (NOEC) was determined to be 15 µg/L. After 96 hours at 33 µg/L 35 % and at 56 µg/L 100 % mortality was noted. The 96 hours LC_{50} was estimated to be 36 µg/L (95% CL 33 – 56 µg/L).

Conclusion: LC_{50} (96 h): 36 µg/L and NOEC: 15 µg/L based on mean measured concentrations

Comment (RMS): Study considered acceptable.

Reference: Hill, R. W. (1985): PP192: Determination of Acute Toxicity to Rainbow Trout (*Salmo gairdneri*). Report No. BL/B/2560

Test guideline: US EPA § 72-1

GLP: yes

Test item: PP192 (technical fluazinam): 97.3% w/w, Lot no. 8303-4

Material and methods:

The acute toxicity of fluazinam to *Oncorhynchus mykiss* (formerly *Salmo gairdneri*) was studied in a 96 hours flow-through test. 20 fish per treatment (with a mean length of 34.6 mm and a mean

weight of 0.54 g) were exposed to test concentrations of 0.056, 0.075, 0.1, 0.18, 0.32, 0.56 mg/L, one solvent control (acetone and Tween 80) and one dilution water control. For chemical analysis of test substance samples were taken daily. During the study the following physical parameters were monitored in fish exposure vessels: 10.6 – 11.4 mg/L O_2, pH 7.6 – 7.8, 11.8 – 12.7 °C, total hardness 50 – 56 mg/l as $CaCO_3$ and conductivity 130 – 170 µS/cm.

Findings:

Mean measured concentrations of fluazinam were 0.057, 0.064, 0.091, 0.16, 0.27 and 0.46 mg/L (82.1 – 101.8 % of nominal concentrations), thus toxicity endpoints are based on mean measured concentrations. After 93 hours at all tested concentration behavioural or sublethal effects (loss of equilibrium, darkening in pigmentation, surfacing and rapid respiration) were observed, therefore the NOEC for sublethal effects was < 0.057 mg/L. Mortalities were observed at concentrations ≥ 0.091 mg/L. The 96 hours LC_{50} was calculated to be 0.11 mg/L (95 % CL 0.1 – 0.13 mg/L).

Conclusion: LC_{50} (96 h): 110 µg/L and NOEC ≤ 57 µg/L, based on mean measured concentrations

Comment (RMS): Study considered acceptable.

Reference: Gelin, M.D. & J. Laveglia (1993): Technical Fluazinam (IKF-1216) – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions. Report No. 5099-91-0421-TX-002

Test guideline: FIFRA Guideline 72-1

GLP: yes

Test item: Fluazinam techn.: 96.8 % w/w, lot no. 1030/91

Material and methods:

A 96 hours test on the acute toxicity of fluazinam to bluegill sunfish was performed under flow through conditions at five nominal test concentrations, one control and one solvent control (acetone). The nominal test concentrations were 31, 45, 64, 91 and 130 µg/L, respectively. Twenty fish (10 per replicate, fish had a mean length and weight of 36 mm and 1.1g) were exposed to each test concentration under the following test conditions: 16/8-hour light/dark photoperiod, temperature was maintained at 21 °C, pH 6.7 – 7.1, 76 – 102 % O_2 saturation and total alkalinity 20 – 24 mg/l $CaCO_3$.

Findings:

Mean measured exposure concentrations were 21, 34, 44, 66 and 93 µg/l, respectively. All toxicity endpoints are based on mean measured concentrations.

No mortalities and sublethal effects were observed in controls and at the lowest concentration of 21 µg/L, thus the NOEC was 21 µg/L. Behavioural or sublethal effects (loss of equilibrium, lethargy, and swimming at the surface) were noted at 66 µg/L. After 96 hours 10 % mortality was observed at 34 µg/L and at the highest concentration of 93 µg/l all fish were dead. The 96 hours EC_{50} was calculated to be 55 µg/L (95% CL 44 – 66 µg/L).

Conclusion: LC_{50} (96 h): 55 µg/L and NOEC: 21 µg/L based on mean measured concentrations

Comment (RMS): Study considered acceptable.

Reference: Peither, A. (2001a): Acute Toxicity of Fluazinam to Zebra Fish (*Brachydanio rerio*) in a 96-Hour Flow-Through Test. Report No. 813431

Test guideline: OECD 203

GLP: yes

Test item: Fluazinam tech.: 98.4 % w/w, batch no.: A629/1995

Material and methods:

The acute toxicity of fluazinam to zebra fish was assessed in a 96 hours test under flow-through conditions. Seven fish were exposed in replicates to 11, 25, 52, 110, 250 µg/L, one dilution water control and one solvent control (N,N-dimethylformamide). The following exposure conditions were measured during test period: pH 7.8 – 8.2, 22 – 23 °C, a total hardness of 216 mg/L as $CaCO_3$, 7.2 – 8.2 mg/L dissolved O_2 and a light intensity of 50 – 500 Lux (16/8 hours light/dark photoperiod). After 24 hours in concentrations ≥ 52 µg/L the test item was noted at the surface of water. The body weight and length of ten fish were measured at the start of the test: fish had an average weight of 0.18 ± 0.04 g and a mean length of 2.8 ± 0.2 cm. For the analysis of test concentrations, duplicate samples were taken at the start of the test, after 48 hours and at the end of the test.

Findings:

Mean measured concentrations were: not analysed, 19, 49, 79 and 208 µg/L, all reported results are based on mean measured concentrations. After 96 hours no mortalities or other symptoms of intoxication were noted in controls and concentration up to 19 µg/L. Thus the NOEC was 19 µg/L. After 72 hours at the next higher concentration level (49 µg/L) mortalities and sublethal effects (fish mainly at water surface) were observed. At the highest concentration level all fish died until 48 hours. The 96 hours EC_{50} was calculated to be 89 µg/L (95% CL 64 – 123 µg/L).

Conclusion: LC_{50} (96 h): 98 µg/L and NOEC: 19 µg/L based on mean measured concentrations

Comment (RMS): Study considered acceptable.

Reference: Peither, A. (2001b): Acute Toxicity of Fluazinam to Guppy (*Poecilia reticulata*) in a 96-Hour Flow-Through Test. Report No. 813453

Test guideline: OECD 203

GLP: yes

Test item: Fluazinam tech.: 98.4 % w/w, batch no.: A629/1995

Material and methods:

A 96 hours test on the acute toxicity of fluazinam to guppy, was performed under flow through conditions at five nominal test concentrations, one dilution water control and one solvent control (N,N-dimethylformamide). The nominal test concentrations were 2.4, 7.6, 24, 78, and 250 µg/L, respectively. The fish were 3.7 ± 0.3 cm (mean) in length and had an average weight of 0.48 ± 0.21 g (measured at start of the test from 10 fish). Two replicates with seven fish each were exposed to each test concentration under the following test conditions: 16/8-hour light/dark photoperiod, 22 – 23 °C, pH 7.7 – 8.0, ≥ 7.3 mg/L dissolved O_2 and a total hardness of 198 mg/l as

$CaCO_3$. Chemical analyses of the test item were performed at concentrations ≥ 24 µg/L and samples were taken at the start of the test (0 h), after 48 hours and at the end of the test (96 h).
Findings:
Mean measured concentrations were: not analysed, not analysed, 22, 68 and 234 µg/L. The reported results are related to mean measured concentrations. No mortalities and other symptoms of intoxication were observed at concentrations up to 22 µg/L, therefore the NOEC was determined to be 22 µg/L. After 48 hours mortalities and effects (staying at the bottom of the test vessels) were noted at 68 µg/L. At the highest concentration (234 µg/L) after 48 hours 100 % mortality was recorded. The 96 hours LC_{50} was calculated to be 109 µg/L (95% CL 52 – 226 µg/L).
Conclusion: LC_{50} (96 h): 109 µg/L and NOEC: 22 µg/L based on mean measured concentrations
Comment (RMS): Study considered acceptable.

Reference: Shults, S. K, A. W. Brock & L. Laveglia (1993): Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Conditions with Technical Fluazinam (IKF-1216). Report No. 5017-91-0415-TX-002
Test guideline: FIFRA Guideline 72-3
GLP:
Test item: Fluazinam techn.: 100 % ai, lot # 1030/91
Material and methods:
A 96 hours test on the acute toxicity of fluazinam to marine fish (*Cyprinodon variegatus*), was performed under flow-through conditions at five nominal test concentrations, one dilution water control and one solvent control (acetone). The nominal test concentrations were 0.13, 0.22, 0.36, 0.6, and 1.0 mg/L, respectively. A representative sample of fish were measured (N = 30) and fish had a mean length of 26 (24 – 35) mm and an average weight of 0.41 (0.25 – 0.7) g. Twenty organisms (ten per replicate) were exposed to each test concentration under the following test conditions: 16/8-hour light/dark photoperiod, 22 – 23 °C, pH 7.8 – 8.2, 64 – 94 % oxygen saturation and a salinity of 31 – 32 ‰. Chemical analysis of test item concentrations in test media was carried out at 0, 48 and 96 hours of the exposure period.
Findings:
Mean measured concentrations were 0.08, 0.14, 0.24, 0.33 and 0.52 mg/L. No effects were observed at control and lowest concentration (0.08 mg/L) tested. Therefore the NOEC was 0.08 mg/L and the LOEC 0.14 mg/L. At 0.24 mg/L after 24 hours the mortality was 100 %. The 96 hours LC_{50} was calculated to be 0.12 mg/L (95% CL 0.08 – 0.24 mg/L).
Conclusion: LC_{50} (96 h): 120 µg/L and NOEC: 80 µg/L based on mean measured concentrations
Comment (RMS): Study considered acceptable.

Acute toxicity to aquatic invertebrates (IIA 8.2.4)

Reference: Farrelly, E., Vincent, J., Hamer, M. J. & Hill, I. R (1984): B 1216 (PP192): Toxicity to

First Instar *Daphnia magna*. Report No. RJ0392B

Test guideline: OECD 202

GLP: yes

Test item: Fluazinam techn. (PP192): purity 98.5 %, Lot No. 8303-2

Material and methods:

The acute toxicity of fluazinam to *Daphnia magna* (first instar < 24 h old) was studied over a 48 h exposure period under static conditions. The daphnids were exposed to six nominal concentrations 0.625, 0.125, 0.25, 0.5, 1.0, 2.0 mg/L and one solvent control (0.01% methanol). Three replicates with 10 daphnids each were prepared for each concentration and control. As test medium served reconstituted hard water. The temperatures of the water bath containing the test vessels was 20 ± 1°C (direct measurements in test vessels were not performed). The pH was in the range of 8.0 – 8.5 and the oxygen content was ≥ 88 % saturation during the study. Chemical analyses of the test item were performed at each concentration and samples were taken at the start (0 h) and the end of the test (48 h).

Findings:

Mean measured concentrations were 0.05, 0.1, 0.19, 0.41, 0.76 and 1.67 mg/L. Already at lowest concentration the immobility of daphnids was 12 % and at 0.76 mg/L 100 % of daphnids were immobile. Therefore the NOEC was < 50 µg/L and the EC_{50} was calculated to be 0.19 mg/L (95 %CL: 0.16 – 0.22).

Conclusion: EC_{50} (48 h): 190 µg/L and NOEC < 50 µg/L based on mean measured concentrations

Comment (RMS): The total hardness (in mg/L $CaCO_3$) of test solutions was not stated, direct measurements of temperature in test media were not performed and no valid NOEC could be derived due to effects in the lowest tested concentration. Therefore the study was considered to be not acceptable.

Reference: Shults, S. K., Brock, A. W. & Laveglia, J. (1992): Acute Toxicity to Daphnids (*Daphnia magna*) Under Flow-Through Conditions with Technical Fluazinam (IKF-1216). Report No. 5108-91-0418-TX-002

Test guideline: OECD 202

GLP: yes

Test item: Fluazinam techn. (IKF-1216): purity 100 %, Lot# 1030/91

Material and methods:

The acute toxicity of fluazinam to the waterflea *Daphnia magna* was studied under flow through conditions over a 48 hours exposure period. Twenty daphnids (< 24 h old, 10 daphnids per replicate) were exposed to five nominal concentrations (39, 65, 110, 180 and 300 µg/L), a control and a solvent control. During the exposure period water quality parameters were measured: 20 – 21 °C, 78 – 93 % O_2 saturation, pH 8.1 – 8.3, 170 mg/L $CaCO_3$.

Chemical analysis of fluazinam was done at initiation (0 h) and termination (48 h) of the study.

Findings:

Mean measured concentrations were 34, 54, 94, 150 and 260 µg/L. After 48 hours at the lowest concentration the immobility of daphnids was 5 %, however at the next higher concentration level (54 µg/L) no effects were observed. The effects at the lowest concentration were not related to the presence of the toxicant, therefore the NOEC was estimated to be 54 µg/L. At the highest tested concentration the immobility was 65 % and a clear dose/response relationship could be noted. The slope of the concentration-response curve was calculated to be 2.8 and the EC_{50} was determined to be 220 µg/L (95%CL: 190 – 300 µg/L) by the moving average method.

Conclusion: EC_{50} (48 h): 220 µg/L and NOEC: 54 µg/L based on mean measured concentrations

Comment (RMS): Study considered acceptable.

Reference: Schmidt, T. (2003): Acute Toxicity of Fluazinam Technical to First-Instar Larvae of *Chironomus riparius* in a 48-Hour Test. Report No. 851182

Test guideline: OECD 202, OECD proposal 219

GLP: yes

Test item: Fluazinam techn., purity: 97.8 %, batch no.: A629/1995

Material and methods:

The acute toxicity of fluazinam to the midge *Chironomus riparius* (2 – 3 days old first instar larvae) was studied under static test conditions. Larvae were exposed in a water phase without sediment for 48 hours. Six nominal concentrations (10, 20, 40, 80, 160 and 320 µg/L), a water control and a solvent control (DMF) were tested. 20 larvae were exposed to each concentration level and control. At the start of the test larvae were fed (mixture of green algae and fish food) to ensure their survival. The water temperature, pH and dissolved oxygen concentrations of test media were measured at the start and end of the study and reported as follows: 20 – 22°C, pH 7.8 (start), pH 9.5 – 10.1 (end) and ≥ 8.1 mg O_2/L (> 60 % saturation). Additionally chemical analysis of test item concentrations was carried out for all concentration levels except the lowest concentration of 10 µg/L.

Findings:

Mean measured concentrations were: not analysed, 12, 33, 68, 120, 238 µg/L. All biological endpoints are based on mean measured concentrations.

After 48 hours no midge larvae were immobilized (dead) in both control and up to a concentration level of 12 µg/L. Therefore the NOEC was determined to be 12 µg/L and the LOEC was 33µg/L. The 48 hours EC50 was calculated to be 45 µg/l (95% CL: 43 – 48 µg/L).

Conclusion: EC_{50} (48 h): 45 µg/L and NOEC: 12 µg/L based on mean measured concentrations

Comment (RMS): Study not considered acceptable. The chironomid larvae were exposed without sediment. This exposure condition was caused a high stress situation for the normally sediment-dwelling organisms. Therefore the results of the study are not reliable and are not considered in the risk assessment. Furthermore a *Chironomus riparius* study according to the BBA proposal 1995 was performed (evaluated under point B.9.2.2) and was included into the risk assessment to cover the risk to sediment-dwelling organisms.

Effects on algal growth and growth rate (IIA 8.2.6)

Reference: Smyth, D. V. & Tapp, J. F. (1987): PP192 (B1216): Determination of Toxicity to the Green Alga *Selenastrum capricornutum*. Report No. BL/B/3056

Test guideline: OECD 201

GLP: Yes

Test item: Fluazinam techn. (PP162): purity 97 %, batch no: not stated

Material and methods:

A test on growth inhibition of *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) was performed with fluazinam under static conditions.

The algal cultures (1.0×10^4 cells/ml in culture media) were exposed to seven nominal concentrations: 0.01, 0.018, 0.032, 0.056, 0.1, 0.18 and 0.32 mg/L as well as to a dilution and a solvent control (acetone). The test samples were incubated for up to 96 hours under static conditions, at temperatures from 23.8 – 24 °C, pH 6.9 – 7.4, and continuous illumination (light intensity: 7200 Lux). Cell densities were determined after 24, 48, 76 and 96 hours by electronic particle counting using a Coulter Counter Model ZB. The calculation of test substance inhibiting the growth (biomass and growth rate) was done separately for each treatment in comparison to control. Chemical analyses of fluazinam were conducted for all treatment levels at the start and end of the testing.

Findings:

Mean measured concentrations were: 0.008, 0.015, 0.026, 0.048, 0.082, 0.15 and 0.2 mg/L. All biological endpoints are based on mean measured concentrations.

No significant inhibition of biomass and growth rate were observed in concentration up to 0.048 mg/L, therefore the NOEC was 0.048 mg/L for both endpoints. The 96 h E_bC_{50} was calculated to be 0.16 mg/L (95% CL 0.12 – 0.22 mg/L). The growth rate at each concentration was relatively constant and at the highest tested concentration the inhibition was 13 %, therefore the E_rC_{50} was estimated to be > 0.22 mg/L.

Conclusion: 96 hour E_bC_{50}: 160 µg/L, E_rC_{50}: > 220 µg/L, NOEC: 48 µg/L based on mean measured concentrations

Comment (RMS): Study considered acceptable.

Effects on aquatic plants (IIA 8.2.8)

Reference: Boeri, R. & T.J. Ward (2001): IKF-1216: Toxicity to the Duckweed, *Lemna gibba.* Report No. 2129-SK

Test guideline: ASTM 1991, EPA OPPTS 850.4400

GLP: yes

Test item: Fluazinam techn., purity: 98.4 %, batch no: A626/1995

Material and methods:

The toxicity of fluazinam to the duckweed *Lemna gibba* was assessed in a static renewal system (solution renewals on day 3 and 5) over a 7 days exposure period. Three replicates of aquatic

plants (12 fronds per replicate) in 20X-AAP media were exposed to seven nominal concentrations: 1.0, 2.0, 5.0, 10, 20, 40 and 80 µg/L as well as to a dilution control and a solvent control (DMF). Environmental conditions throughout the study were monitored: 23.8 – 25.7 °C, pH 7.5 – 7.6 (day 0), pH 9.3 – 10.2 (day 7) and continuous illumination with an intensity of 5030 – 5480 lux.
Total number of fronds and abnormal appearance of fronds was observed on day 0, 3, 5 and 7. Inhibition of frond growth (biomass and growth rate) was calculated by standard statistical methods relative to pooled control data. Chemical analyses of fluazinam were conducted on day 0, 3 and 5 of each freshly prepared test solution and old samples were analysed on day 3, 5 and 7.
Findings:
Mean measured concentrations of fresh solutions were 0.859, 1.73, 4.58, 7.96, 17.5, 35.9 and 69.1 µg/L test item corresponding to 80 to 92 % of the nominal concentrations. In old solutions fluazinam was found in amounts of 0.645, 1.25, 3.03, 5.82, 11.4, 21.3 and 37.5 µg/L corresponding to 46.9 – 64.5 % of nominal concentrations. Thus all biological endpoints were related to mean initial measured concentrations.
On day 7 no significant inhibition of frond growth (biomass: AUC) and fronds growth rate was observed at concentrations up to 35.9 µg/L compared to the pooled control data. At 69.1 µg/L the inhibition of growth rate was 14 % and the inhibition of biomass was 26 %, both values were significantly different when compared to control. Therefore the NOEC was 35.9 µg/L and LOEC was 69.1 µg/L. The E_bC_{50} and E_rC_{50} could not be calculated because inhibition of biomass and growth rate were < 50 % in all tested concentrations, thus the E_bC_{50} and E_rC_{50} were estimated to be > 69.1 µg/L, based on initial measured concentration.
Conclusion: 7 d E_bC_{50} and E_rC_{50}> 69.1 µg/L, NOEC = 35.9 µg/L based on initial measured concentrations
Comment (RMS): In order to obtain a clear concentration response curve and a reliable EC50 the inhibition at highest tested concentration should be at least 50 %. Thus the study is considered not acceptable. However, fluazinam is a fungicide and a study with a higher plant species is not necessary according to the directive 91/414/EEC. Therefore the results will be accepted as additional information and there is no need to perform a new study.

Metabolites

Acute toxicity to fish (IIA 8.2.1)

Reference: Hertl, A. (1997a): Acute Toxicity of AMPA to Zebra Fish (*Brachydanio rerio*) in a 96-Hour Static Test. Report No. 662512
Test guideline: OECD 203
GLP: yes
Test item: AMPA 98.7 % w/w, Lot No.: 9511
Material and methods:
The acute toxicity of the metabolite AMPA to zebra fish (*Brachydani rerio*) was assessed in a 96 hours static test. Due to the low solubility of the test substance (< 0.04 mg test substance/L) fish were exposed to undiluted filtrate of a supersaturated stock solution (100 mg/L continuously stirred

for up to 2 hours in the dark), dilutions of 1:2, 1:4, 1:8, and 1:16 and an untreated control. Seven fish were tested for each concentration. At the start of the test the fish had a mean body length of 3.0 ± 0.15 cm and a mean body wet weight of 0.26 ± 0.03 g. The water temperature, pH and dissolved oxygen concentrations of test media were measured daily and reported as follows: 23 – 24°C, pH 7.5 – 7.9 and ≥ 8.1 mg O_2/L. Chemical analysis of test item concentrations was carried out only for the undiluted stock solution at 0, 48 and 96 hours of the exposure period.

Findings:

The measured concentrations in filtrate (highest concentration level) were: 0.131 mg/L at 0 hr, 0.084 mg/L at 48 hr and 0.063 mg/L at 96 hr, respectively. The mean measured concentration was 0.09 mg/L (calculated as the average over all measured concentrations) and was well above the maximal water solubility of 0.04 mg/L.

In all concentrations (filtrate with 0.09 mg/L and 1:2, 1:4, 1:8, 1:16 dilutions) and the control no sublethal effects and mortalities were observed until the end of the study. Therefore the NOEC is ≥ 0.09 mg/L and the LC_{50} is > 0.09 mg/L.

Conclusion: LC_{50} (96 h): > 0.09 mg AMPA/L and NOEC: ≥ 0.09 mg AMPA/L based on mean measured concentrations of the filtrate of a supersaturated stock solution (solubility limit AMPA: 0.04 mg/L)

Comment (RMS): Study considered acceptable.

Acute toxicity to aquatic invertebrates (IIA 8.2.4)

Reference: Hertl, J. (1997b): Acute Toxicity of AMPA to *Daphnia magna* in a 48-Hour Immobilization Test. Report No. 662490

Test guideline: OECD 202

GLP: yes

Test item: AMPA, purity: 98.7 %, Lot No: 9511

Material and methods:

Young daphnids (less than 24 hours old) were exposed to AMPA (metabolite of fluazinam) under static conditions for 48 hours. Due to the low solubility of the test substance (< 0.04 mg test substance/L) daphnids were exposed to undiluted filtrate of a supersaturated stock solution (100 mg/L continuously stirred for up to 2 hours in the dark). The study was performed as a limit test with one concentration (using the filtrate) and a control. The test concentration was analytically determined in the test medium (undiluted filtrate) at the start and at the end of the test.

Test conditions: 20 ± 1 °C; 16 h light / 8 h dark photoperiod, pH 7.8 – 9.0; 7.8 – 8.9 mg/L dissolved O_2, a total hardness of 250 mg/L (as $CaCO_3$).

Findings:

At the initiation 0.28 mg/L and at termination 0.24 mg/L of test substance was measured. All biological endpoints were related to the mean measured concentration of 0.26 mg/L. During the exposure period no sublethal effects or immobility were observed in the control and at the tested concentration (0.26 mg/L). Therefore the NOEC was ≥ 0.26 mg/L and the EC50 > 0.26 mg/L:

Conclusion: EC_{50} (48 h): > 0.26 mg AMPA/L and NOEC: ≥ 0.26 mg AMPA/L based on mean

measured concentrations of the filtrate of a supersaturated stock solution (solubility limit AMPA: 0.04 mg/L)

Comment (RMS): Study considered acceptable.

Effects on algal growth and growth rate (IIA 8.2.6)

Reference: Hertl, J. (1997c): Toxicity of AMPA to *Scenedesmus subspicatus* in a 72-Hour Algal Growth Inhibition Test for Poorly Soluble Test Substances. Report No. 662477

Test guideline: OECD 201

GLP: yes

Test item: AMPA, purity: 98.7 %, Lot No: 9511

Material and methods:

The effects of AMPA on the growth of the unicellular green alga *Scenedesmus subspicatus* were assessed in a growth inhibition test (limit test). The algal cultures (1 x 10^4 cells/ml) were exposed to undiluted filtrate of a supersaturated stock solution (100 mg/L continuously stirred for up to 2 hours in the dark) and a control. Test samples (3 replicates of filtrate, 6 controls) were incubated for up to 72 hours under static conditions and continuous illumination. The temperature was 23 °C and the pH ranged from 7.9 – 8.1 at the start of the test. Cell densities were determined by electronic particle counter (Coulter Counter Model ZM). Chemical analysis of test item in the undiluted stock solution was carried out at the start and the end of the test.

Findings:

At the end of the test pH had increased to 10.7 due to the high CO_2 consumption of fast-growing algae.

At the initiation 0.25 mg/L and at termination 0.23 mg/L of test substance were measured. All biological endpoints were related to the mean measured concentration of 0.24 mg/L.

No toxic effects were observed in the highest tested concentration (filtrate with maximal dissolvable concentration of AMPA). Therefore the NOEC is ≥ 0.24 mg/L and the E_bC_{50}/E_rC_{50} (72 h) is > 0.24 mg/L.

Conclusion: E_bC_{50}/E_rC_{50} (72 h): > 0.24 mg AMPA/L and NOEC: ≥ 0.24 mg AMPA/L based on the mean measured concentration of the filtrate of a supersaturated stock solution (solubility limit of AMPA: 0.04 mg/L)

Comment (RMS): Study considered acceptable.

Formulation

Acute toxicity to fish (IIIA 10.2.1)

Reference: Sankey, S.A., Tapp, J.F., Caunter, J.E. & Penwell, A.J. (1991): Fluazinam: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) of a 500 g/L SC Formulation. Report No: BL4323/B

Test guideline: OECD 203

GLP: Yes

Test item: Fluazinam 500 SC: 38.2 % w/w fluazinam, equivalent to 476.4 g ai/L (specific gravity:

1.228), batch no: RS346/A

Material and methods:

The 96 hours acute toxicity of the formulation Fluazinam 500SC to rainbow trout was assessed under flow through conditions. Ten fish per treatment were exposed to nominal concentration of 0 (untreated control), 32, 56, 100, 180, 320 and 560 µg/L.

The fish were 3.3 cm (mean) in length and had an average weight of 4.7 g. The following environmental test conditions were given during testing: 16/8-hour light/dark photoperiod, 15.1 – 15.4 °C, pH 7.58 – 7.73, 9.4 – 10.0 mg/L O_2 content, a conductivity of 171.6 – 214 µS/cm and a total hardness of 42.3 – 43 mg/L $CaCO_3$.

Chemical analyses of the active substance were performed daily for all concentration levels.

Findings:

Measured concentrations of formulation were in the range of 77 – 95 % of nominal concentrations and reported as follows: 29, 53, 83, 140, 280 and 430 µg/L. All biological endpoints are based on mean measured concentrations. In the lowest concentration no sublethal effects and mortality were observed, thus the NOEC was determined to be 29 µg/L, expressed as fluazinam 11.1 µg ai/l. Fish exposed to ≥ 53 µg/L showed intoxication symptoms like surfacing, dark discoloured, abnormal respiration and loss of balance. At a concentration of 430 µg/L all fish were dead after 96 hours. The LC_{50} was calculated to be 160 µ/L (95% CL 130 – 210 µg/L), expressed as fluazinam 61.1 µg ai/L (95 % CL 49.7 – 80.2 µg ai/L).

Conclusion: 96 hours EC_{50}: 61.1 µg ai/l, NOEC: 11.1 µg ai/L, based on mean measured concentrations

Comment (RMS): Test fish were smaller than recommend in OECD guideline, however no adverse effects on results of the study were noticed. Study considered acceptable.

Acute toxicity to aquatic invertebrates (IIIA 10.2.1)

Reference: Farrelly, E., Navet, X. & Hamer, M. J. (1991): Fluazinam: Acute Toxicity of a 500 g/L SC Formulation to First Instar *Daphnia magna*. Report No: RJ1024B

Test guideline: OECD 202

GLP: Yes

Test item: Formulation YF7604B: 38.4 % w/w fluazinam, equivalent to 495 g ai/L (specific gravity: 1.29), batch no: not specified

Material and methods:

A 48 hours acute test to *Daphnia magn*a with Fluazinam 500SC under static conditions was performed. The effects of the formulation to first instar daphnids (< 24 h old) were observed at nine nominal test concentrations: 55, 91, 152, 254, 423, 704, 1170, 1960 and 3260 µg/L, equivalent to 21, 35, 58, 97, 162, 270, 451, 751 and 1250 µg ai/L. Twenty daphnids were exposed to each treatment and a control. At the start and the end of the test period the following parameters were measured: pH 8.0 – 8.2, temperature 20 ± 1°C, ≥ 91 % oxygen saturation and light intensity 800 Lux (16/8 hours light/dark photoperiod)

Samples for chemical analyses of the active substance were taken before adding the test

organisms and at test termination from each concentration.

Findings:

Mean measured concentrations of fluazinam in test solutions were < 40 (limit of detection), 44, 67, 113, 182, 268. 425, 628 and 1148 µg/L (84 – 126 % of nominal concentrations). Biological endpoints were related to nominal concentrations. Statistically significant effects (immobility) were reported for concentrations ≥ 254 µg/L, thus the NOEC was determined to be 152 µg/L (58.4 µg ai/L expressed as fluazinam). The EC_{50} was calculated to be 310 µg/L expressed as fluazinam 119 µg ai/L.

Conclusion: 48 hours EC_{50}: 119 µg ai/L; NOEC: 58.4 µg ai/L, based on nominal concentrations

Comment (RMS): Study considered acceptable.

Effects on algal growth and growth rate (IIIA 10.2.1)

Reference: Smyth, D. V., Sankey, S.A. & Stanley, R.D. (1991): Fluazinam: Toxicity to the Green Alga *Selenastrum capricornutum* of a 500 g/L SC Formulation. Report No: BL4362/B

Test guideline: OECD 201

GLP: Yes

Test item: Fluazinam 500 SC: 38.2 % w/w fluazinam, equivalent to 476.4 g ai/L (specific gravity: 1.228), batch no: RS346/A

Material and methods:

The growth inhibition effects of the formulation to the unicellular green alga *Pseudokirchneriella subcaptitata* (formerly *Selenastrum capricornutum*) were studied under static conditions. The algal cultures (initial cell density: 10^4 cells/ml) were exposed to nominal concentrations of 0.08, 0.25, 0.8, 2.5, 8.0, 25, 80 and 250 mg/L (three replicates for each concentration) in artificial algal culture medium.

The test samples were incubated for up to 96 hours under static conditions at a temperature ranging from 23.9 – 24.0°C, pH 7.2 – 7.4 (start), pH 7.7 – 10.3 (end), continuous “cool white” illumination and under continuous shaking on an orbital incubator. Algal cell densities were determined using an electronic particle counter (Coulter counter model ZB). The endpoint were inhibition of growth and biomass (AUC) compared to control. Chemical analysis of fluazinam was performed at the beginning and at the end of the test.

Findings:

The mean measured concentrations of the formulation were determined to be 0.057, 0.15, 0.41, 1.2, 5.7, 10, 68 and 180 mg/L (60 – 81 % of nominal concentration). All endpoints are based on mean measured concentrations. At the three highest exposure concentrations (10, 68 and 180 mg/L) the suspended particulate matter was very high. This resulted in high background counts and it was impossible to determine valid algal counts. Therefore for statistical analysis the three top concentrations were excluded. After 72 hours the biomass (AUC) and growth rate were significantly effected at a concentration of 0.41 mg/L and 1.2 mg/L, thus the NOEC was 0.15 mg/L for biomass and 0.41 mg/L for growth rate, expressed as fluazinam: 0.057 mg ai/L and 0.157 mg ai/L. The 72 h E_bC_{50} and E_rC_{50} were determined to be 1.4 mg/L and > 5.7 mg/L, expressed as fluazinam:

E_rC_{50} > 2.176 mg ai/L and E_bC_{50} = 0.534 mg ai/L.

Conclusion: 72 h E_bC_{50}: 0.534 mg ai/L, 72 h E_rC_{50} > 2.176 mg ai/L, NOEC (biomass): 0.057 mg ai/L, NOEC (growth rate): 0.157 mg ai/L, based on mean measured concentrations.

Comment (RMS): Study considered acceptable.

Table B. 9.2.1-1: Summary of acute toxicity data for aquatic organisms; values which are relevant for risk assessment are in bold

Test organism	test condition	time	endpoint	test conc.	NOEC [µg ai/L]	EC_{50}/LC_{50} [µg ai/L]	Reference/ study acceptable
fluazinam							
Oncorhynchus mykiss Rainbow trout	flow through	96 hr	mortality	m	15	**36**	Gelin & Laveglia 1992/ yes
Oncorhynchus mykiss Rainbow trout	flow through	96 hr	mortality	m	≤ 57	110	Hill 1985/yes
Lepomis macrochirus Bluegill sunfish	flow through	96 hr	mortality	m	21	55	Gelin & Laveglia 1993/ yes
Brachydanio rerio Zebra fish	flow through	96 hr	mortality	m	19	89	Peither 2001a/ yes
Poecilia reticulata Guppy	flow through	96 hr	mortality	m	22	109	Peither 2001b/ yes
Cyprinodon variegatus Sheepshead minnnow	flow through	96 hr	mortality	m	80	120	Shults et al 1993/ yes
Daphnia magna Waterflea	static	48 hr	immobility	m	< 50	190	Farrelly et al 1984/ no
Daphnia magna Waterflea	flow through	48 hr	immobility	m	54	**220**	Shults et al 1992/ yes
Chironomus riparius Midge	static	48 hr	mortality	m	12	45	Schmidt 2003/ no
Pseudokirchn. subcapitata Green alga	static	96 hr	biomass growth rate	m	48	**160** **> 220**	Smyth & Tapp 1987/ yes
Lemna gibba Duckweed	static renewal	7 d	biomass growth rate	im	35.9	> 69.1	Boeri & Ward 2001/ no
metabolite AMPA (water solubility limit: < 0.04 mg/L)							
Brachydanio rerio Zebra fish	static	96 hr	mortality	m	≥ 90	**> 90**	Hertl 1997a/ yes
Daphnia magna Waterflea	static	48 hr	immobility	m	≥ 260	**> 260**	Hertl 1997b/ yes
Scenedesmus subspicatus Green alga	static	72 hr	biomass growth rate	m	≥ 240	**> 240**	Hertl 1997c/ yes
Fluazinam 500SC in µg ai/L (results expressed as µg formulation/L)							
Oncorhynchus mykiss Rainbow trout	flow through	96 hr	mortality	m	11.1 (29)	**61.1** (160)	Sankey et al 1991/ yes
Daphnia magna Waterflea	static	48 hr	immobility	n	58.4 (152)	**119** (310)	Farelley et al 1991/ yes

Test organism	test condition	time	endpoint	test conc.	NOEC [µg ai/L]	EC_{50}/LC_{50} [µg ai/L]	Reference/ study acceptable
Pseudokirchn. subcapitata Green alga	static	72 hr	biomass growth	m	57 (150) 157 (410)	**534** (1400) >2176 (>5700)	Smyth et al 1991/ yes

Test conc.: test concentration based on mean measured (m), initial measured (im) or nominal (n) concentration

B.9.2.2 Chronic toxicity of active substance, metabolites and formulations to aquatic organisms

Active substance

Prolonged toxicity (21 day exposure) to fish (IIA 8.2.2.1)

Reference: Sankey, S. A., Tapp, J. F., Caunter, J. E. & Stanley, R. D. 1992 Fluazinam: The 28 Day LC50 to Rainbow Trout (*Oncorhynchus mykiss*). Report No: BL4167/B

Test guideline: OECD 204

GLP: yes

Test item: Fluazinam techn., purity: 98.1 %, batch no: not stated

Material and methods:

The prolonged toxicity of fluazinam to rainbow trout (*Oncorhynchus mykiss*) was assessed under flow through conditions over a 28 day exposure period. Fish were exposed to five nominal concentrations: 5.6, 10, 18, 32 and 56 µg/L, a dilution water control and a solvent control (DMF). Ten trout per treatment and control were incubated under a 16/8-hour light/dark photoperiod and were fed daily during the study. Environmental test conditions were determined daily for the first three days and then 3 times per week, mean values were 15.0 – 15.3°C, pH 7.5 – 7.86, 8.6 – 10.0 mg/L O_2 content, a conductivity of 176 – 207 µS/cm and a dilution flow-rate of 240 – 255 mL/min. The total hardness was determined by titration and was 40.3 mg/L as $CaCO_3$.
The mortality was recorded daily, behaviour and appearance of fish were checked on days 4, 7, 10, 14, 21 and 28 in each test vessel. At the end of the exposure period the length and weight of alive fish were measured. Chemical analyses of fluazinam were conducted on day 1, 2, 3, 8, 10, 13, 17, 20, 23 and 28 at each tested concentration.

Findings:

Mean measured concentrations were 4.0, 7.4, 12, 24 and 44 µg/L, all endpoints are based on mean measured concentrations. During the 28 days exposure period no sublethal effects and no mortalities were noted in the dilution water control and in concentrations up to 12 µg/L. At day 28 30 % of fish were dead at 24 µg/L and 100 % at 44 µg/L. At this two highest concentrations sublethal effects like reduced or no feeding, dark discoloration, quiescence, surfacing and rapid respiration were also observed. Additionally the growth (mean length and weight) was effected at concentrations of 24 and 44 µg/L. Thus the 28 days NOEC was 12 µg/L and the LC50 was calculated to be 26 µg/l (95 % CL 21 – 32 µg/L)

Conclusion: 28 d LC_{50} (mortality): 26 µg/L, 28 d NOEC and LOEC (mortality, sublethal effects, growth): 12 µg/L and 24 µg/L based on mean measured concentrations

Comment (RMS): Study considered acceptable.

Fish early life stage toxicity test (IIA 8.2.2.2)

Reference: Fillmore, G. E. & J. Laveglia (1993): Technical Fluazinam (IKF-1216) – The Toxicity to Fathead Minnow (*Pimephales promelas*) During Early Life-Stage Exposure. Report No: 5018-91-0425-TX-002

Test guideline: FIFRA Guideline 72-4

GLP: Yes

Test item: Fluazinam techn., purity: 96.8 %, batch no: 1030/91

Material and methods:

The chronic effects of fluazinam to early life stages of fathead minnow were performed in flow through exposure systems. Organisms (eggs and fry) were exposed to nominal concentrations of 1.6, 3.1, 6.3, 12 and 25 µg/L, a dilution control and a solvent control (DMF). At test initiation 2 x 60 eggs (≤ 24 hours old) per treatment and control were incubated in egg incubation cups for up to 4 days (hatch period), after hatching 2 x 40 fry per treatment and control were transferred into exposure aquaria and exposed for up to 30 days (posthatch period). Fry were fed with live brine shrimp nauplii three times daily (weekday) or two times daily (weekend). The following environmental test conditions were maintained: Dissolved oxygen: 7.9 – 8.6 mg O_2/L, pH 6.8 – 7.2, a total hardness of 25 – 26 mg $CaCO_3$/L, a specific conductivity of 140 µmhos/cm and a 16/8-hour light/dark photoperiod.

Observations for mortality and abnormal appearance or behaviour were made daily until complete swim up. At study termination weight and length were determined. The following endpoints were assessed: organism survival at hatch, larval survival and larval growth (wet weight and total length).

Samples for chemical analyses of fluazinam in test solutions were removed from both replicates of each tested concentration and the control on day 0, 5, 12, 19, 26 and 34.

Findings:

Mean measured exposure concentrations were 1.6, 2.7, 5.3, 10 and 23 µg/l, all endpoints are based on mean measured concentrations.

The effects in dilution and solvent control did not significantly differ, therefore controls were pooled for statistical analysis. After the hatching period (day 4) survival was significantly effected at 23 µg/L (50 % mortality). At test termination significant effects on larval survival were already observed at 10 µg/L (30 % mortality). The growth was not influenced in the control and all treatment levels up to 5.3 µg/L at the end of testing. The larval survival was significantly effected at the two highest concentration levels and these treatments were excluded from statistical analysis of growth. Based on these data the 34 d NOEC for survival of larvae and growth was 5.3 µg/L and the 4d NOEC for survival at hatching was 10 µg/L. The 34 d LOEC for survival of larvae and growth was 10 µg/L and the 4d LOEC for survival at hatching was 23 µg/L.

Conclusion: Survival and growth: 34 d NOEC = 5.3 µg/L, LOEC = 10 µg/L; hatchability: 4 d NOEC = 10 µg/L, LOEC = 23 µg/L

Comment (RMS): Study considered acceptable.

Fish life cycle test (IIA 8.2.2.3)

Reference: Shults, S. K., Brock, A. W. & Laveglia, J. (1995): Technical Fluazinam (IKF-1216)– The Chronic Toxicity to the Fathead Minnow (*Pimephales promelas*) During a Full Life-Cycle Exposure. Report No:5107-92-0035-TX-00

Test guideline: FIFRA Guideline 72-5

GLP: Yes

Test item: Fluazinam techn., purity: 96.8 %, batch no: 1030/91

Material and methods:

The chronic effects of fluazinam to fathead minnow (*Pimephales promelas*) were studied for a complete life-cycle over 278 days. Additionally the progeny (F_1) was exposed for 30 days post hatch. The following endpoints were observed during the study: Hatching success, survival, growth (wet weight and body length) of first generation fish (F_0) and hatching success survival, growth (wet weight and body length) of their progeny (F_1).

The organisms were exposed to five nominal concentrations (1.3, 2.5, 5.0, 10 and 20 mg/L), a dilution control and a solvent control under flow-through conditions.

The exposure system was a two-tiered system, consisting of an upper and a lower level waterbath. Each waterbath contained fourteen exposure aquaria. The exposure of embryos started in aquaria in the upper level water bath and 100 embryos (2 x 50) were exposed in egg incubation cups to each treatment and control for up to 5 days. After 5 days the hatching success was calculated based on the number of introduced embryos. Furthermore 50 (2 x 25) newly hatched larvae were selected for each tested concentration and controls, and transferred in larval growth chambers. These chambers were examined daily for dead larvae. After 30 and 61 days each larval group was photographed over a grid to determine total length. Additionally, percent larval survival was also noted. At day 37 (post hatch) fish were released from growth chambers to the corresponding aquarium and after 61 days (post-hatch) 25 larvae were randomly selected to remain in each exposure vessels. On day 151 all fish were examined to confirm the existence of reproductive males and females to isolate spawning groups.

On day 161 one male and two females (representing one spawning group) were transferred to spawning aquarium in the second lower level water bath. Remaining fish were also continued in exposure. Dead males in spawning groups were replaced by males from this remaining fish. Females were not replaced. Observations for the presence of eggs were made daily. 2 x 50 embryos from the first 10 spawns of ≥ 50 eggs in each aquarium were incubated and the percent hatch was determined. After hatching of the F1 embryos 2 x 25 newly hatched larvae groups were established in each aquarium as the spawning activity permitted. After 30 days post hatch exposure of F1 each larval group was terminated. The growth (individual length and wet weight) were measured and percent survival for each group recorded. The exposure of F_0 fish was terminated after 278 days. Each fish was measured (wet weight and length) and examined to verify sex and gonadal conditions. Additionally deformities or injuries were noted.

During the study newly hatched larvae were fed live brine shrimp nauplii three times daily, juvenile and adult fish were fed twice daily: frozen brine shrimp and “Ziegler® Brother Prime” flakes.

The following water quality parameters were monitored: Temperature, dissolved oxygen and pH were measured daily, and total hardness and specific conductivity were measured weekly. During the chronic study, samples for chemical analyses of fluazinam in test solutions the test solution in each aquarium on the upper level was sampled a minimum of once each week, until the spawning (lower) level of the system was activated. Subsequently, test solution samples were taken weekly (minimum) from one replicate aquaria of each treatment level from the corresponding upper and lower level.

Findings:

Mean measured exposure concentrations were 0.69, 1.4, 2.9, 6.4 and 14 µg/L, which averaged 61 % of nominal concentrations. All biological endpoints are based on mean measured concentrations. The results of water quality parameters were: 24 – 25 °C, 6.9 – 7.5 mg O_2/L, pH 6.7 – 7.6, 24 – 30 mg $CaCO_3$/L (total hardness) and a specific conductivity of 125 – 150 µmhos/cm.

Table 9.2.2-1: Survival, growth, and reproduction data after 278 days exposure to fluazinam

endpoints	Mean measured concentrations (µg/L)					
	control	**0.69**	**1.4**	**2.9**	**6.4**	**14**
F_0 generation						
Survival day 30 (%)	87	94	89	86	81	32*
Survival day 278 (%)[a)]	88	100	100	96	90	62*
Mean blotted wet weight (g) 61 d post hatch	0.588	0.569	0.584	0.664	0.608	NA
Mean standard length (mm) 61 d post hatch	41	40	40	41	40	41[b)]
Mean standard length male (g) day 278	86	84	87	85	83	81[b)]
Mean standard length female (mm) day 278	69	67	66	66	65	67[b)]
Mean blotted wet weight male (g) day 278	8.4	7.8	8.3	7.6	7.2*	6.9[b)]
Mean blotted wet weight female (g) day 278	3.7	3.2	3.2	3.1	3.1	3.5[b)]
Eggs /mature female (nº)	760	1056	475	539	84	422
Eggs / spawning (nº)	89	98	80	83	35*	75*
Hatching success (%)	88	85	80[c)]	85	83	63*
F_1 generation						
Survival %	94	89	76	95	92	80
Hatching success (%)	88[d)]	89	78**	76**	93[e)]	24**
Mean standard length (mm)	30	30	30	30	29 [e)]	26 [e)]
Mean blotted wet weight (g)	0.25	0.26	0.26	0.25	0.23 [e)]	0.17 [e)]

* significantly different when compared to pooled control
** significantly different when compared to solvent control.
NA not applicable due to reduced survival

[a)] Calculation is based on 25 fish per replicate, which continued in exposure after 61 day post-hatch exposure

[b)] Values not statistically analysed due to significantly reduced survival

[c)] Significant reduction is not considered to be toxicant related, as the test concentrations 2x and 4x higher did not produce an adverse effect

[d)] Results only from solvent control

[e)] Only results of replicate "A" were analysed

Additional information to the statistical analysis of hatching success of F1:
The statistical analysis of the F1 hatching success data was performed with a standard (chi-square) contingency table test. However the authors of the study have indicated that this analysis is not appropriate for the experimental design used in this study and the high variations in the raw-data between replicates for the mentioned endpoint. Therefore a revised statistical analysis was presented which intended to account for the complexity of the test design and the specific data. The revised statistical analysis of the hatching success of the F1 generation resulted in a NOEC of 2.9 µg/L and a NOEC of 6.4 µg/L.

Table 9.2.2-2: Fish Full-Life-Cycle study: Summary of all assessed endpoints

endpoints (time)	NOEC [µg/L]	LOEC [µg/L]
F0 generation		
embryo hatching success, larval survival and growth		
F_0 hatching success (5 d)	6.4	14
F_0 survival (30 day post hatch)	6.4	14
F_0 mean length (30 day post hatch)	2.9	6.4
F_0 mean weight (61 day post hatch)	no effects until 6.4 µg/L, the next higher treatment level (14 µg/L) could not be statistically analyzed due to significantly reduced survival	
F_0 mean length (61 day post hatch)	no effects until 6.4 µg/L, the next higher treatment level (14 µg/L) could not be statistically analyzed due to significantly reduced survival	
survival and growth of adults:		
F_0 survival (test termination)	6.4	14
F_0 mean male total length (test termination)[1)]	2.9	6.4
F_0 mean male wet weight (test termination) [1)]	2.9	6.4
reproductive success		
F_0 number egg/spawn	6.4	14
F_0 number spawns/females	2.9	6.4
F_0 number eggs/females	6.4	14
F1 generation		
embryo hatching success, larval survival and growth of F_1		
F_1 hatching success (5 d)	6.4	14
F_1 survival (30 day post hatch)	6.4	14
F_1 mean length (30 day post hatch)	14	> 14
F_1 mean weight (30 day post hatch)	14	> 14

[1)] for females no effects on growth until 6.4 µg/L treatment level were observed, the next higher treatment level (14 µg/L) could not be statistically analyzed due to significantly reduced survival.

Conclusion: The most sensitive endpoints of F_0 were mean length of larvae(30 days post hatch), mean total length and wet weight of males (test termination) and number spawns/female with a NOEC of 2.9 µg/L. The most sensitive endpoints of F_1 were the hatching success and survival with a NOEC of 6.4 µg/L.

Comment (RMS): Study considered acceptable.

Chronic toxicity to aquatic invertebrates (IIA 8.2.5)

Reference: van den Bogaaert, M., Farrelly, E., J. & Hamer, M. (1991): Fluazinam: Chronic Toxicity to *Daphnia magna*. Report No: RJ0974B

Test guideline: OECD 202

GLP: Yes

Test item: Fluazinam techn., purity: 98.1 %, batch no: not stated

Material and methods:

The chronic effects of fluazinam on the survival, reproduction and growth of *Daphnia magna* were determined. 10 replicates of one daphnid (< 24 hours old) per test concentration were incubated under static renewal conditions for 21 days with daily feeding (*Chlorella vulgaris* suspension) and observation. Test solutions were renewed every 2 days and samples of the freshly prepared and used test solutions were analysed for fluazinam. The nominal exposure concentrations were 0.0125, 0.025, 0.5, 0.1and 0.2 mg/L, additionally a water and a solvent (methanol) control were prepared. Following water quality parameters were recorded: The temperature was in the range of 18.5 – 20.5 °C, the pH was between 7.4 and 8.5, the dissolved oxygen was in the range of 8.2 – 10.1 mg/L in fresh solutions and in old solutions the lowest measured value was 2.4 and the highest 10.6 mg/L. The water hardness was in the range of 167 – 176 mg/L $CaCO_3$.

Findings:

The mean measured concentrations in freshly prepared solutions were 0.014, 0.029, 0.056, 0.098 and 0.202 mg/L (98 – 117 % of nominal) and in old solutions 0.007, 0.013, 0.026, 0.054 and 0.112 mg/L (52 – 57 % of nominal). Endpoints are based on nominal concentrations.

Low dissolved oxygen concentrations were measured in old test solutions and could be explained with increased microbial activity in older solutions, however there were no observable effects on daphnids. On day 21 at the highest tested concentration (0.2 mg/L) 50 % of adult daphnids had died. For controls and concentrations up to 0.05 mg/L 10 % mortality was recorded. At 0.1 mg/L 20 % of adult daphnids were dead, thus the NOEC (mortality) was 0.05 mg/L and LOEC (mortality) was 0.1 mg/L. The number of live young per daphnid was significantly affected at 0.1 mg/L, therefore, the 21 d NOEC was determined to be 0.05 mg/L. No effects on growth (length) were observed at 0.0125 mg/L, whereas at the next higher concentration level (0.025 mg/L) the growth was significantly influenced. Thus the 21 d NOEC for growth was 0.0125 mg/L.

Conclusion: 21 d NOEC (growth): 12.5 µg/L and LOEC: 25 µg/L; 21 d NOEC (mortality and reproduction): 50 µg/L and LOEC: 100 µg/L based on nominal concentrations.

Comment (RMS): Study considered acceptable.

Reference: Shults, S. K., Brock, A. W. & Laveglia, J. (1993): Chronic Toxicity to *Daphnia magna* Under Flow-Through Conditions with Technical Fluazinam (IKF-1216). Report No. 5109-91-0419-TX-002

Test guideline: FIFRA 72-4

GLP: Yes

Test item: Fluazinam techn., purity: 96.8 %, batch no: 1030/91

Material and methods:

The study was performed to assess the chronic effects of fluazinam on *Daphnia magna*. Replicates of 4 x 10 daphnids (< 24 hours old) per test concentration were incubated under flow-through conditions for 21 days. The nominal exposure concentrations were 9.4, 19, 38, 75 and 150 µg/L, additionally a water and a solvent (acetone) control were prepared. Observations on the survival, growth (mean total length and dry weight) and reproduction of adults as well as the number of immobilized young were recorded. The following water quality parameters were measured during the study: The temperature was in the range of 19 - 21°C, the pH was between 7.9 and 8.2, the dissolved oxygen was in the range of 7.2 – 8.4 mg/L, the water hardness was 170 mg/L as $CaCO_3$ and the specific conductivity was 500 µmhos/cm. Chemical analyses of fluazinam in exposure solutions were performed weekly.

Findings:

The mean measured concentrations of fluazinam in test solutions were 8.9, 16, 33, 68 and 140 µg/L. Biological endpoints are based on mean measured concentrations.

After 21 days survival of adults was significantly effected at the highest tested concentration (140 µg/L), thus NOEC (mortality) was 68 µg/L and LOEC was 140 µg/L. Since the survival was significantly influenced at 140 µg/L, reproduction and growth data for this treatment level was excluded from statistical analyses for treatment effects. At lowest concentration level no effects on reproduction and growth were observed, Therefore the NOEC was 68 µg/L as well.

Conclusion:

21 d NOEC (mortality, growth, reproduction): 68 µg/L and LOEC: 140 µg/L based on mean measured concentrations.

Comment (RMS): Study considered acceptable.

Chronic toxicity to sediment dwelling organisms (IIA 8.2.7)

Reference: Stewart, K.M. & Shillabeer, N. (1997): Fluazinam: Determination of the Effects on Emergence of *Chironomus riparius*. Report No. BL6115/B

Test guideline: Proposed BBA Guideline 1995

GLP: yes

Test item: Fluazinam techn., purity: 97.9 %, batch no: AD0408

Material and methods:

The toxicity of fluazinam to sediment dwelling larvae *Chironomus riparius* was investigated in a 28 day static sediment toxicity test. For each tested treatment (3.13, 6.25, 12.5, 25, 50 and 100 µg/l), 3 biological replicates and 1 sample for chemical analyses were prepared containing 245 g of an artificial sediment (2 cm depth) and 1700 mL overlying water (15.5 cm water layer). After a standing period of 7 days 25 first instar larvae (2 days post hatch) were applied to each test vessel. One day after the addition of the test organisms the test substance was applied in required quantities to the overlaying water and test media were carefully mixed without disturbing the

sediment. Observations were made daily for emergent adults and at test termination replicates without 100 % emergence were examined for number of live and dead larvae and pupae. During the test the following water quality parameter were reported: The temperature ranged from 19.4 to 20.1°C, the pH values were in the range of 7.6 – 8.1, the dissolved oxygen concentration ranged from 7.8 – 9.4 mg O_2/L, the water hardness was in the range of 82 – 102 mg $CaCO_3$/L and the conductivity increased from 368 µS/cm (day 0) to 482 µS/cm (day 28).

Findings:

Chemical Analysis: On day 0 mean measured concentrations of fluazinam in overlaying water were 3.27, 6.05, 13.1, 23.7, 44.9 and 88.2 µg/L (88 – 105 % of nominal concentrations). After 7 days exposure the mean measured concentrations ranged from 3 – 4 % of nominal (in three highest treatments) and at test termination the fluazinam concentrations were below the limit of detection. Thus all biological endpoints are based on nominal concentrations applied to overlaying water.

Biological data: Data for males and females were pooled for all evaluations, because no significant differences were found in the sex distribution of adults after 28 days. The time to first emergence and time to 50 % emergence were significantly influenced at the to highest concentration levels (50 and 100 µg/L). The total emergence after 28 days was not reduced at concentrations up to 6.25 µg/L. Thus the NOEC and LOEC for emergence are 6.25 µg/L and 12.5 µg/L. The 28 d EC50 (total emergence) was determined to be 77 (69 – 86) µg/L.

Conclusion:

28 d NOEC (emergence): 6.25 µg/L and LOEC: 12.5 µg/L, 28 d EC_{50} (emergence): 77 (69 – 86) µg/L, based on nominal concentrations

Comment (RMS): Study considered acceptable.

Metabolites

Chronic toxicity to fish (IIA 8.2.2.1, IIA 8.2.2.2, IIA 8.2.2.3)

No study submitted, not required.

Chronic toxicity to aquatic invertebrates (IIA 8.2.5)

No study submitted, not required.

Formulation

Chronic toxicity to fish (IIIA 10.2.4)

No study submitted, not required.

Chronic toxicity to aquatic invertebrates (IIIA 10.2.4)

No study submitted, not required.

Microcosmos or mesocosmos study (IIIA 10.2.2)

Reference: van Wijngaarden, R. & Boonsta, H. (2004): Fate and effects of the fungicide Shirlan® (active ingredient fluazinam) in indoor freshwater microcosms. Report No. WA2004

Test guideline: none

GLP: no

Test item: Shirlan®, SC-formulation, 38.5 % w/w fluazinam, batch no: not stated

Material and methods:

Test design: 19 indoor full-glass cylinder microcosms (diameter 25 cm, height 35 cm, total volume ca. 18 L) containing 2 cm sediment layer ad 30 cm water layer

Test organisms: plankton, macroinvertebrates (*Assellus aquaticus, Gammarus pulex,* snails, oligochaetes) and *Myriophyllum spicatum* (all species were collected from uncontaminated fresh water ditches)

Test concentrations: 0, 0.4, 2, 10, 50 and 250 µg fluazinam/L, treatments for "effect microcosms" performed in duplicates, treatments for "fate microcosms" performed in singular, four untreated replicates served as controls

Application interval: four times (day 0, 7, 14 and 21) at target concentration (see test concentration)

Test duration: 12 weeks

Test conditions: temperature: 23 ± 2°C (maintained in water bath), 14 hours daily photoperiod (artificial daylight by high pressure metal halide lamps), dissolved oxygen: 4.35 – 8.55 mg O_2/L, pH: 7.5 – 9, conductivity: 149 – 242 µS/cm

Sampling and analysis: Two hours after each application water samples of 100 mL were collected to determine the concentration of fluazinam by solid phase extraction and HPLC, for "fate microcosms" samples were analysed after first and fourth application at 0, 2, 4, 8, 24, 48 and 168 hours; zoo- and phytoplankton were sampled weekly for qualitative and quantitative analysis and chlorophyll a was determined; macroinvertebrates were sampled at day -2, 2, 28, 56 and 70 and were identified and counted; macrophytes (*M. spicatum*) were harvested at the last day of the study and dry weight was determined

Statistical analysis: all data were (ln)-transformed for univariate and multivariate analyses; NOEC calculation at taxon level and at community level: Williams test (Williams, 1972), community level effects: Principal response component analysis (PRC) (Van der Brink & Ter Braak, 1999), significance of PRC: Monte Carlo permutation test

Findings and conclusion:

Exposure concentration: measured two hours after each application: 55 – 88 % of nominal concentrations (mean over all applications),

DT50: 0.8 – 2 d (water phase)

Biological effects:

0.4 µg ai/L: No treatment related effects.

2 µg ai/L: No treatment related effects. $NOEC_{community}$ and $NOEC_{population}$ most sensitve species

10 µg ai/L: Slight transient effects on some copepod and some rotifer populations only.

50 µg ai/L: Clear short-term effects. Reduction in rotifers, copepods and *Asellus aquaticus*. Increased number on individual sampling dates in some phytoplankton populations.

250 µg ai/L: Clear effects. Reduction in macro-crustaceans, snails, oligochaeta, copepods and rotifers. Increase in some cladocerans. Increase in rotifers later in study, and by other species than the reduced ones. Indirect effects on phytoplankton and community metabolism. In many cases, changes lasted up to and including the end of the study.

The NOAEC was determined to be 10 µg ai/L, because the effects were only transient and no significant effects on important species (e.g. cladoceran *D. gr. galeata*, rotifer *K. quadrata*) were observed.

Comment (RMS):

No information was provided concerning the properties of the sediment used in test systems e.g. C_{org}-content, particle size distribution, microbial biomass. It is unclear if the test system represented worst case or best case conditions. In general a very low level of "field realism" was achieved in the indoor microcosms.

An indoor microcosm test design which was used in the presented study is appropriate to assess effects of phytoplankton and zooplankton communities. The test design is not suitable for the assessment of effects on larger organisms. However in the case of fluazinam the most sensitive organisms are fish and aquatic insects and these important groups were not tested. Additionally the long-term effects on species with complex life cycles (e.g. aquatic insects) were not determined. Therefore the study provides additional information related to acute and chronic effects of fluazinam on zooplankton community level, however it is not adequate for a higher tier risk assessment of aquatic insects and fish. Anyway the study is acceptable concerning a higher tier risk assessment for zooplankton communities, but due to the uncertainties regarding the test conditions, the safety factor should not be lower than five.

Reference: René P.A. van Wijngaarden, Jan G.M. Cuppen, Gertie H.P. Arts, Steven J.H. Crum, Martin W. van den Hoorn, Paul J. van den Brink, and Theo C.M. Brock (2004): Aquatic risk assessment of a realistic exposure to pesticides used in bulb crops: A microcosm study. Environmental Toxicology and Chemistry. Vol. 23 No. 6 pp. 1479 – 1498.

Abstract: The fungicide fluazinam, the insecticide lambda-cyhalothrin, and the herbicides asulam and metamitron were applied to indoor freshwater microcosms (water volume approximately 0.6 m^3). The treatment regime was based on a realistic application scenario in tulip cultivation. Concentrations of each pesticide were equal to 0%, 0.2%, 0.5%, 2%, and 5% spray drift emission of label-recommended rates. Contribution of compounds to the toxicity of the pesticide package was established by expressing their concentrations as fractions of toxic units. The fate of the compounds in the water, and responses of phytoplankton, zooplankton, periphyton, macroinvertebrates, macrophytes, decomposition, and water quality were followed for 13 weeks. The half-lives of lambda-cyhalothrin, metamitron, and fluazinam were 1 to 2 d; that of asulam was >30 d. No consistent effects could be demonstrated for the 0.2% treatment regime that was therefore considered the no-observed-effect concentration community (NOEC). The macroinvertebrate populations of *Gammarus pulex*, *Asellus aquaticus*, and *Proasellus meridianus* were the most sensitive end points, followed by species of copepods and cladocerans. Responses mainly were due to lambda-cyhalothrin. The 0.5% treatment regime resulted in short-term effects. Pronounced effects were observed at the 2% and 5% treatment levels. At the end of the experiment, the macrophyte biomass that consisted of *Elodea nuttallii*, showed a decline at the two

highest treatment levels, asulam being the causal factor (NOEC: 0.5% treatment level). Primary production was reduced at the 5% treatment level only. In our experiment, the first-tier risk assessment procedure for individual compounds was adequate for protecting sensitive populations exposed to realistic combinations of pesticides. Spray drift reduction measures seem to be efficient in protecting aquatic ecosystems in agricultural areas.

Findings and conclusion:

The half-live of fluazinam was 1 – 2 d; the $NOEC_{community}$ was considered at the 0.2 % treatment regime in tulip cultivation

Comments (RMS):

The publication provided only additional information, which was not relevant for the aquatic risk assessment.

Table B.9.2.2-3: Summary of chronic toxicity data for aquatic organisms; values which are relevant for risk assessment are in bold.

test organism	test condition	time	endpoint	test conc.	NOEC (µg ai/l)	LOEC (µg ai/l)	Reference/ study acceptable
fluazinam							
Oncorhynchus mykiss Rainbow trout	flow through	28 d	mortality weight	m	12	24	Sankey et al 1992/ yes
Pimephales promelas Fathead minnow (ELS)	flow through	34 d 34 d 4 d	survival growth hatchability	m	5.3 5.3 10	10 10 23	Fillmore & Laveglia 1993/ yes
Pimephales promelas Fathead minnow (Life cycle)	flow through	5 d >161 d 278 d 30 d 5 d	F_0 hatchability F_0 reproduction F_0 growth F_1 survival F_1 hatchability	m	6.4 **2.9** **2.9** 6.4 6.4	14 6.4 6.4 14 14	Shults et al. 1995/ yes
Daphnia magna Waterflea	static renewal	21 d	mortality reproduction growth	n	50 50 **12.5**	100 100 25	van den Bogaaert et al. 1991/ yes
Daphnia magna Waterflea	flow through	21 d	mortality reproduction growth	m	68	140	Shults et al. 1995/ yes
Chironomus riparius Midge	static	26 d	emergence	in	**6.25**	12.5	Stewart & Shillabeer 1997/ yes
formulation (Shirlan® 500 g fluazinam/L							
Indoor freshwater microcosm	static	12 w	zooplankton community	n	NOEAC: **10 µg ai/L**		van Wijngaarden & Boonstra 2004/ yes

Test conc.: test concentration based on mean measured (m), nominal (n) or initial nominal (in) concentration

B.9.2.3. Bioaccumulation (Annex IIA 8.2.3)

Reference: Lentz, N. R. & Huhtanen, K. L. (1994): Uptake, Depuration, and Bioconcentration and Metabolism of (Fluazinam) Carbon-^{14} IKF-1216 in Bluegill Sunfish (*Lepomis macrochirus*) Under Flow Through Test Conditions. Report No. 5311-93-0013-EF-001

Test guideline: EPA Guideline 165-4

GLP: Yes

Test item: ^{14}C-phenyl labelled Fluazinam (radiochemical purity > 98 %) and ^{14}C-pyridyl labelled Fluazinam (radiochemical purity > 98 %), Lot Numbers; T9002 and 0201

Material and methods:

Bluegill sunfish were exposed to ^{14}C-phenyl and ^{14}C-pyridyl labelled Fluazinam under flow-through conditions to assess the uptake, the depuration, the bioconcentration and metabolism of the active substance. For the 35-day exposure period mean measured water concentrations of 0.66 (±0.176) µg/L for the ^{14}C-phenyl label and 0.77 (±0.124) µg/L for the ^{14}C-pyridyl label were maintained. Observations of mortality and sublethal effects were made twice daily. After the exposure fish were placed in clean water for up to 21 days (depuration period). During the uptake and depuration phase radioanalyses (LSC) of fillet (edible portion), whole fish, viscera (non edible portion) and water samples were performed. Additionally HPLC analyses were performed for fish samples to evaluate the ^{14}C-distribution in tissues, the extraction-partitioning behaviour and the identification of metabolites. The BCF_{ss} was calculated as the ratio of concentration in fish (C_f) and in the water (C_w). Additionally the kinetic bioconcentration factor (BCF_k) at steady state as the ratio of the rate constants of uptake (k1) and depuration (k2) was determined. For the calculation of rate constants the BIOFAC computer program was used. Water quality parameters like temperature, dissolved oxygen and pH were recorded initially and at fixed intervals during the study. The fluazinam concentration in the water phase was also measured at three time points: 21, 28 and 35 days uptake phase.

Findings:

The fluazinam concentration (both labels) in water phase ranged between 0.591 and 0.862 µg/L, which corresponds to 56 – 70 % of the total radioactive residues (TRR).

Table B.9.2.3-1: Results of bioconcentration in bluegill sunfish after 35 days exposure to phenyl and pyridyl labelled fluazinam.

phenyl label: C_w = 0.66 µg/L			
	whole fish	viscera	fillet
Total ^{14}C tissue residues after 35 d [µg/kg]	720	1100	230
BCFss	1090	1670	348
k_1 [1/d]	117 ± 8	-	-
k_2[1/d]	0.11 ± 0.01	-	-
BCF_k	1018 ± 96	-	-
CT_{50}	6.0 ± 0.4 d	-	-
Time to reach 90 % steady state	20 ± 1 d	-	-
Elimination during 14 d (21 d) depuration	78 % (78 %)		

pyridyl label: C_w = 0.77 µg/L			
	whole fish	viscera	fillet
Total ^{14}C tissue residues after 35 d [µg/kg]	740	910	210
BCFss	960	1180	273
k_1 [1/d]	114 ± 5.1	-	-
k_2[1/d]	0.14 ± 0.01	-	-
BCF_k	827 ± 60	-	-
CT_{50} [d]	5.0 ± 0.3	-	-
Time to reach 90 % steady state [d]	17 ± 1	-	-
Elimination during 14 d (21 d) depuration	76 % (79 %)		

Analysis of residues (TRR) in tissues:

After the extraction with acetonitrile, hexane and acetonitrile:water the majority of extractable ^{14}C-residues was found in the acetonitrile fraction, for an average of 32.5 % TRR in fillet and 37.5% TRR in viscera. In hexane extracts an average of 6.7 %TRR (fillet) and 9.3 % TRR (viscera), and in acetonitrile:water extracts an average of 12.8 %TRR (fillet) and 9.3 % TRR (viscera) were analysed. Additionally in PES (postextraction solids) an average of 48 %TRR (fillet) and 29 %TRR (viscera) were found.

Table 9.2.3-2: Identified metabolites in fish fillet (acetonitrile extracts)

Compound	28 days exposure		35 days exposure	
	phenyl-label (mg/kg)	pyridyl-label (mg/kg)	phenyl-label (mg/kg)	pyridyl-label (mg/kg)
Fluazinam	ND	ND	NQ	NQ
AMPA	ND	0.019	0.009	0.012
MAPA	ND	0.006	NQ	0.001
DAPA	ND	ND	ND	0.002
unknown metabolite	0.018	0.011	0.003	0.006
Total Residue	0.199	0.209	0.232	0.224
Total metabolites [%TRR]	9.0 %	**17.2 %**	5.2 %	9.4 %

ND = not detected; NQ = not quantifiable

Table 9.2.3-3: Identified metabolites in fish viscera (acetonirile extracts)

Compound	28 days exposure	35 days exposure

	phenyl-label (mg/kg)	pyridyl-label (mg/kg)	phenyl-label (mg/kg)	pyridyl-label (mg/kg)
Fluazinam	0.021	0.008	0.007	0.010
AMPA	0.008	0.042	0.030	0.048
MAPA	ND	ND	0.007	0.018
DAPA	ND	ND	ND	0.006
unknown metabolite	0.032	0.047	0.024	0.030
Total Residue	1.226	1.193	1.122	0.966
Total metabolites [%TRR]	5.0 %	8.1 %	6.1 %	**11.6 %**

ND = not detected

Metabolism: The patterns of ^{14}C-residues obtained by HPLC analyses of both label positions were very similar, thus it can be concluded that in fish to a certain degree no cleavage of the amine linkage between the two ring system of fluazinam occurred. The ^{14}C-residues which were identified included fluazinam, AMPA, MAPA and DAPA. Each of the residues of total metabolites were accounted for max. 17.2 % of the fillet after 35 days and max. 11.6 % of the viscera after 28 days. Additionally numerous other ^{14}C-components were presented but none of the single compounds was found in amounts ≥ 10 %.

Conclusion:

Fluazinam accumulated in whole fish with BCF of values of 960 and 1090. In non-edible portions BCF values of 1670 and 1180 were determined. All BCF values are based on calculations with total ^{14}C-residues. The 90 % level of steady state was reached after 17 – 20 days. During the depuration period the ^{14}C-residues were incompletely eliminated after 14 days and 22 and 24 % of the TRR remained in the whole fish. The depuration half-life (CT_{50}) was estimated to be 5 – 6 days. In general the high BCF of 960 – 1090 (whole fish) and the incomplete elimination of radioactive residues (22 – 24 % remained in fish after 14 days) indicate a potential to bioaccumulation.

Comment (RMS): Study considered acceptable.

B.9.2.4 Risk assessment

The PEC values of the intended application scenario potatoes were calculated with FOCUS surface water model, Step 1and 2 for the active substance and the metabolite AMPA as well as $FOCUS_{sw}$ Step 3 and Step 4 for the active substance. TER calculations for Step 3 and Step 4 are based on the respective worst case PEC values of a single or multiple applications. For details see section B.8.6.2.

The results of $FOCUS_{sw}$ Step 4 calculations are only necessary to assess the risk of chronic effects on fish and aquatic invertebrates.

Acute risk:

Active substance, metabolites and formulation

The TER_a-values were estimated for the most sensitive species of fish, invertebrates and algae for

each test substance as the ratio of acute toxicity (EC/LC_{50}) to PEC_{max}

Results for FOCUS Step 1 & 2 are summarised in table B.9.2.4-1/1 and results for FOCUS Step 3 B.9.2.4-1/2.

Table B.9.2.4-1/1: Acute toxicity exposure ratio (TERa) for applications to potatoes (10 x 200 g ai/ha, spring) with FOCUS Step 1 & 2

test organism	test substance	EC_{50} [µg ai/l]	PEC_{max} [µg ai/l]		TER	
			Step 1	Step 2*	Step 1	Step 2
Onchorynchus mykiss	Fluazinam	36	203	9.02	0.18	3.99
Daphnia magna		220	203	9.02	1.08	21.06
Pseudokirchnerilla subcapitata		160[1]/>220[2]	203	9.02	0.8/>1.1	17/24
Onchorynchus mykiss	metabolite AMPA	>90	10.2	1.35	>8.82	>67
Daphnia magna		>260	10.2	1.35	>25.49	>193
Scenedesmus subspicatus		>240	10.2	1.35	>23.53	>178
Onchorynchus mykiss	Fluazinam 500 SC	61.1	203	9.02	0.30	6.77
Daphnia magna		119	203	9.02	0.59	13.19
Pseudokirchnerilla subcapitata		534[1]/>2176[2]	203	9.02	2.6/10.7	59/241

* PEC for South Europe (worst case)
[1] biomass
[2] growth rate

Table B.9.2.4-1/2: Acute exposure ratio (TERa) for applications to potatoes (10 x 200 g ai/ha, spring) with FOCUS Step 3

FOCUS scenario	water body	PEC_{max} [µg as/l]	*O. mykiss*		*D. magna*	
			EC_{50} [µg as/L]	TER_a [1)]	EC_{50} [µg as/L]	TER_a
D3	ditch	1.045	36	35	119	114
D4	pond	0.042		857		2833
	stream	0.866		42		137
D6	ditch	1.028		35		116
R1	pond	0.058		621		2052
	stream	0.726		50		164
R2	stream	0.958		38		124
R3	stream	1.023		35		116

[1)] since five different fish species were tested the TER was lowered to 10 according to the HARAP guidance document

Active substance and formulation:

The active substance fluazinam and the formulation (Fluazinam 500 SC containing 50 % active substance) are acutely very toxic to fish, aquatic invertebrates and algae. The risk assessment was performed assuming the intended application scenarios in potatoes (10 x 200 g ai/ha, spring) with concentrations calculated by FOCUS Step 1, 2 and 3. The results of calculations with FOCUS Step 1 indicate a high risk for all tested organisms because trigger values for the safety factors established in Annex VI to EEC Directive 91/414 were not met. For fish a TER of 10 was applied in accordance with the HARAP guidance document (1999), since five fish species were tested and

the data confirmed a very narrow range of sensitivities (EC50 ranged from 36 µg/L to 120 µg/L). The TER values calculated with FOCUS Step 2 resulted in acceptable safety factors for algae, however TER values for fish and dapnids were not sufficient. Thus FOCUS Step 3 calculations were performed for these two species. For all scenarios the calculated TER values were above the relevant trigger values. Therefore the acute risk to aquatic organisms regarding the application of the active substance fluazinam with the intended uses in potatoes is acceptable.

Metabolites:

The major metabolite in water phase was **AMPA**. This metabolite is rather insoluble in water and the solubility limit is < 40 µg/L. Acute test with the standard species were performed with a filtrate of a supernatant AMPA-stock solution and no effects were observed at concentrations higher than the solubility limit. TER values calculated with Focus Step 2 were provided a sufficient margin of safety and the risk to aquatic organisms is acceptable.

G-504 is a major metabolite in aqueous photolysis study, therefore this metabolite could be relevant for the aquatic ecosystem. Aquatic toxicity data of G-504 were calculated by "Syracus Research Cooperation ECOWIN software" for fish, daphnids and green algae. The EC50 were estimated to be 36.5 mg/l for fish, 3.6 mg/L for daphnids and 14.8 mg/L for green algae. These toxicity data are indicated that G-504 is much less toxic than the major metabolite AMPA. Therefore the risk can be concluded as very low.

The soil metabolite **HYPA** could reach surface water via run-off and drainage but no studies or estimations regarding the toxicity on aquatic organisms were submitted, therefore the risk for aquatic organisms has to be addressed.

Chronic risk:

Active substance and formulation

The chronic risk assessment is based on the calculation of the TER_{lt}-values as the ratio of chronic toxicity NOECs to PEC_{max} from FOCUS Step 1 – 4.

Results of this conservative chronic risk assessment are summarised in tables B.9.2.4-2/1 – B.9.2.4-2/4.

A refined exposure risk assessment for the most sensitive fish species fathead minnow (FLC-study) was performed by using the PECtwa from FOCUS Step 4 calculation. Results are presented in tables B.9.2.4-2/5 and B.9.2.4-2/6.

Table B.9.2.4-2/1: Chronic toxicity exposure ratio (TERlt) calculated with NOEC/PECmax (FOCUSsw Step 1 & 2) for application in potatoes

test organism	test substance	time[1)]	NOEC	PEC_i [µg/l]		TER	
			[µg ai/l]	Step 1	Step 2	Step 1	Step 2
Pimephales promelas	fluazinam	> 161 d	2.9	203	9.02	0.014	0.322
Daphnia magna		21 d	12.5	203	9.02	0.062	1.386
Chironomus riparius		26 d	6.25	203	9.02	0.031	0.693

test organism	test substance	time[1)]	NOEC	PEC$_i$ [µg/l]		TER	
			[µg ai/l]	Step 1	Step 2	Step 1	Step 2
Zooplankton (Indoor microcosm)	Shirlan (500 SC formulation)	12 w	10.0[2)]	203	9.02	0.049	1.109

[1)] Time where the mentioned NOEC was fixed and no endpoint related effects were observed
[2)] NOAEC

Table B.9.2.4-2/2: Chronic toxicity exposure ratio (TERlt) for fish (O.mykiss) and aquatic insects (C. riparius) with FOCUSsw Step 3

FOCUS scenario	water body	PEC$_{max}$ [µg as/L]	*O. mykiss*		*C. riparius*	
			NOEC [µg as/L]	TER$_{lt}$	NOEC [µg as/L]	TER$_{lt}$
D3	ditch	1.045	2.9	2.78	6.25	6.0
D4	pond	0.042		69.05		148.8
	stream	0.866		3.35		7.2
D6	ditch	1.028		2.82		6.1
R1	pond	0.058		50.00		107.8
	stream	0.726		3.99		8.6
R2	stream	0.958		3.03		6.5
R3	stream	1.023		2.83		6.1

Table B.9.2.4-2/3: Chronic toxicity exposure ratio (TERlt) for Daphnia magna and zooplankton community with FOCUSsw Step 3

FOCUS scenario	water body	PEC$_{max}$ [µg as/l]	*D. magna*		zooplankton (indoor microcosm)	
			NOEC [µg as/L]	TER$_{lt}$	NOAEC [µg as/L]	TER[1)]
D3	ditch	1.045	12.5	12	10.0	9.6
D4	pond	0.042		298		238.1
	stream	0.866		14		11.5
D6	ditch	1.028		12		9.7
R1	pond	0.058		216		172.4
	stream	0.726		17		13.8
R2	stream	0.958		13		10.4
R3	stream	1.023		12		9.8

[1)] Regarding the uncertainties of the test conditions and a low level of realistic field conditions, the safety factor for the effects on zooplankton in microcosm should not be lower than 5.

Table B.9.2.4-2/4: Chronic toxicity exposure ratio (TERlt) for fish (O.mykiss) and aquatic insects (C. riparius) with FOCUSsw Step 4 (5 m buffer zone)

FOCUS scenario	water body	PEC$_{max}$ [µg as/L]	*O. mykiss*		*C. riparius*	
			NOEC [µg as/L]	TER$_{lt}$	NOEC [µg as/L]	TER$_{lt}$
D3	ditch	0.342	2.9	8.48	6.25	18
D4	pond	0.038		76.32		165
	stream	0.364		7.97		17

FOCUS scenario	water body	PEC_{max} [µg as/L]	*O. mykiss*		*C. riparius*	
			NOEC [µg as/L]	TER_{lt}	NOEC [µg as/L]	TER_{lt}
D6	ditch	0.337		8.61		19
R1	pond	0.038		76.32		165
	stream	0.305		9.51		21
R2	stream	0.403		7.20		16
R3	stream	0.431		6.73		15

Table B.9.2.4-2/5: Chronic toxicity exposure ratio (TERlt) for fish (O.mykiss) with FOCUSsw Step 4 (5 m buffer zone) with PECtwa from single application

FOCUS scenario	water body	*O. mykiss*	PEC [µg as/L] single application		TER	
		NOEC [µg as/L]	1 d TWA	28 d TWA	1 d TWA	28 d TWA
D3	ditch	2.9	0.112	0.004	26	725
D4	pond		0.027	0.002	107	1450
	stream		0.022	0.001	132	2900
D6	ditch		0.067	0.002	43	1450
R1	pond		0.026	0.002	112	1450
	stream		0.067	0.006	43	483
R2	stream		0.033	0.002	88	1450
R3	stream		0.098	0.005	30	580

Table B.9.2.4-2/6: Chronic toxicity exposure ratio (TERlt) for fish (O.mykiss) with FOCUSsw Step 4 (5 m buffer zone) with PECtwa from multiple application

FOCUS scenario	water body	*O. mykiss*	PEC [µg as/L] single application		TER	
		NOEC [µg as/L]	1 d TWA	28 d TWA	1 d TWA	28 d TWA
D3	ditch	2.9	0.062	0.009	47	322
D4	pond		0.015	0.003	193	967
	stream		0.021	0.002	138	1450
D6	ditch		0.062	0.007	47	414
R1	pond		0.027	0.006	107	483
	stream		0.155	0.021	19	138
R2	stream		0.080	0.007	36	414
R3	stream		0.060	0.012	48	242

Fluazinam is very toxic for all tested organisms (fish and aquatic invertebrates) in chronic studies. In general the chronic NOEC was ≤ 0.0125 mg/L. The most sensitive organisms were fish (fathead minnow) with a NOEC of 2.9 µg/L from a full life cycle test.

TER calculations with FOCUS Step 1 and 2 were indicated a high chronic risk to the aquatic biocenosis, since no TER value met the relevant safety factor. Therefore for all organisms calculations with FOCUS Step 3 were performed: TER values calculated for daphnids and zooplankton (microcosm) met the trigger values of 10 (aquatic invertebrates) and 5 (microcosm) in

all scenarios. However the TER values for the most sensitive fish species as well as for the sediment dwelling midge larvae were still below the relevant trigger value of 10 for all scenarios and therefore FOCUS Step 4 calculations were done considering a 5 m buffer zone. With Step 4 PECs the TER values were sufficient for chironomus for all scenarios and for fish for the two Pond "sub scenarios" D4 and R1, whereas the TER values related to stream and ditch scenarios were insufficient with values between 6.7 and 9.5. However, this TER-calculation did not consider the contrast between the unrealistic exposure under flow-through test conditions with constant concentrations and the realistic exposure profiles after the application of fluazinam in potatoes according to GAP, which were considered in the FOCUS Models Step 3 and 4. Therefore the notifier submitted a refined exposure risk assessment:

Refined chronic risk assessment from the notifier:

Reference: Gurney, A. (2005): Fluazinam Statement: Aquatic risk assessment for chronic effects on fish using FOCUS Step 4 predicted concentrations in surface water with a 5 m unsprayed buffer.
Report No. A44280-B.

1. Basically the risk assessment is based on the most sensitive NOEC of 2.9 µg/L from a FLC study with fathead minnow and $FOCUS_{sw}$ Step 4 PEC calculations. Furthermore a target TER of 10 was set according to the European commission in order to ensure a conservative risk assessment.

2. As a first approach a simple comparison of the reproductive NOEC of 2.9 µg/L from the FLC study with the maximum initial surface water concentration 0.43 µg/L from the $FOCUS_{sw}$ Step 4 calculations with a 5 m buffer resulted in TER values above 6.7. This already demonstrates a large margin of safety, but is nevertheless below the target value of 10.

3. The next step reflected a refined exposure assessment by using the PEC_{twa}.
Since the flow-through exposure conditions of the FLC study with a constant level of exposure concentrations during the 278-day test duration significantly differed from the exposure scenarios derived with the $FOCUS_{sw}$ Model. Thus the relevant $FOCUS_{sw}$ scenarios consisted of a small number of very short pulses. The TER calculated on the basis of the maximum 1-day TWA PEC_{SW} of 0.16 µg/L was 19 and clearly exceeding the target of 10. By using the maximum 28-day TWA PEC_{sw} of 0.02 µg/L the TER was > 100.

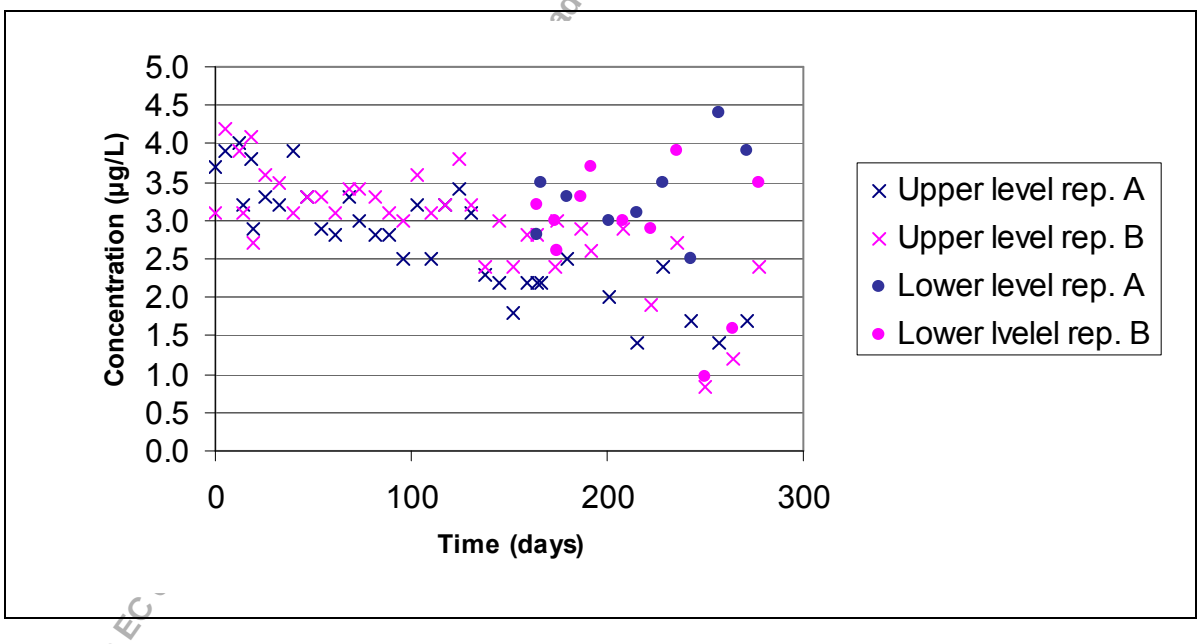
4. At last as an alternative approach a comparison of the exposure regime of the FLC study to FOCUS scenarios was performed. This was suggested by the EFSA Scientific Panel on Plant health, Plant protection and their Residues (EFSA, 2005)[1].
For this purpose the actual measured test concentrations in the FLC-study over the 278 days of exposure were plotted (figure B.9.2.4-1). These values correspond to the F0 reproduction NOEC

concentration (nominal concentration 5 µg/L, overall mean measured concentration 2.9 µg/L). The upper and lower level replicates are resulted from the two-tiered system exposure system consisting of an upper and a lower level water-bath. Aquaria in upper level water bath contained the different development stage from embryos until fertile adults. Spawning groups were kept in aquaria in lower level water-bath and all F1 endpoints were observed there.

The concentrations measured in the lower chamber during the FLC test are used for this comparison since the lower chamber was used for the spawning phase of the study and is therefore most relevant to an assessment of effects on reproduction. Since concentrations in replicates A and B from the lower chambers were generally measured at different time points, and no significant differences are expected between the replicates, the data are treated in this analysis not as replicates but as a single sequence. Since it was the intention to maintain a constant concentration during the FLC study, it was additionally assumed that the concentration between sample points can be adequately approximated by linear interpolation.

In order to obtain an 'ecotoxicological trigger concentration' (ETC)[2] the measured concentrations from the FLC study were divided by the safety factor of 10.

Figure B.9.2.4-1: Exposure variation during the fish full life-cycle test - mean concentration 2.9 µg/L



At the next step these ETC values were compared graphically to the predicted concentration profile from the FOCUS scenario. The graph for the R3 scenario (scenario with the highest calculated PEC_{max}) is shown in figure B.9.2.4-2 and figure B.9.2.4-3. Graphical comparisons for single and multiple applications for all FOCUS scenarios at Step 4 with a 5 m buffer were also done and presented in the Appendix III of the statement.

If the ETC represents an upper bound to the predicted concentration profiles, it can be concluded

[1] The EFSA Journal (2005), 178 1 – 45
[2] Term introduced by the PPR Panel

that the risk is acceptable, even if the observed toxicological effects could be caused by exposures of short duration.

Figure B.9.2.4-2: Comparison of ETC and PEC profiles for the R3-stream scenario, 5 m buffer, after single application

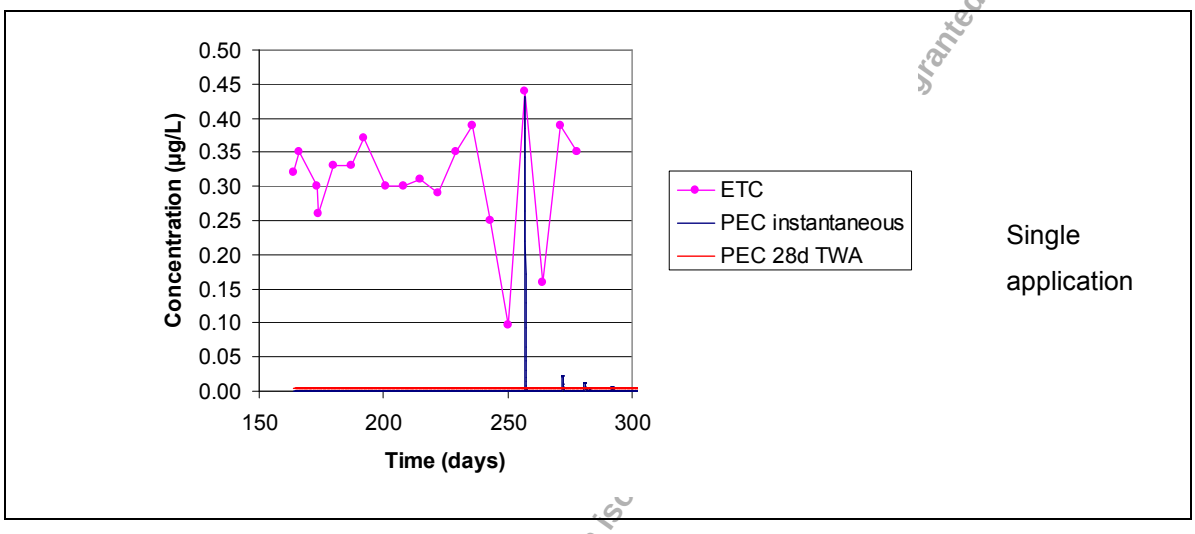
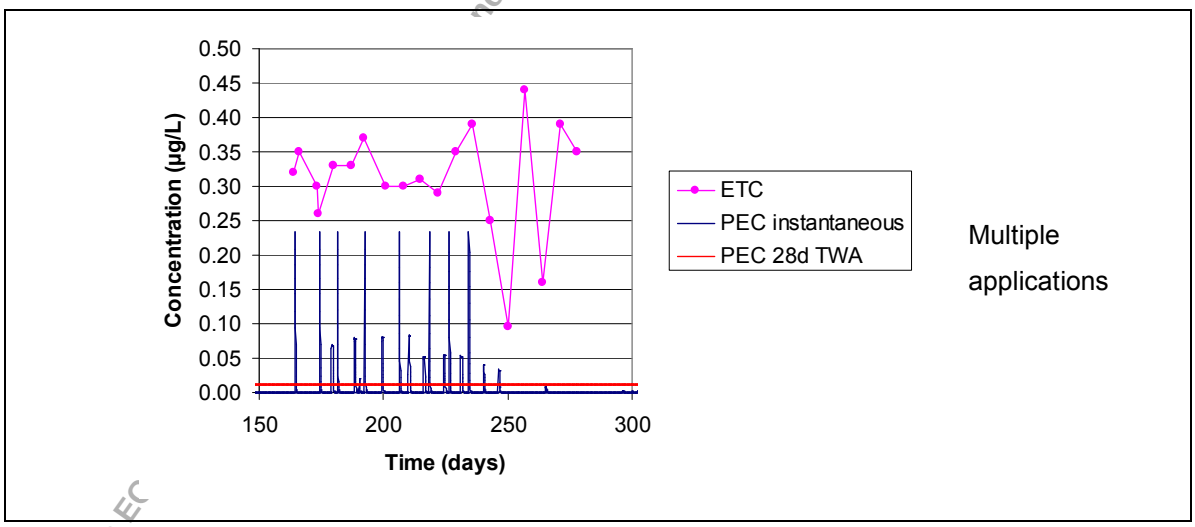


Figure B.9.2.4-3: Comparison of ETC and PEC profiles for the R3-stream scenario, 5 m buffer, after multiple application



Conclusion:

Since the ETC represents an upper bound to the predicted concentration profiles for all FOCUS scenarios, no adverse chronic effects on fish are expected from the use of fluazinam according to Good Agricultural Practice with a 5 m unsprayed buffer zone along riparian margins.

Comment (RMS):

RMS agreed with the assumptions mentioned under point 1 and 2.

To point 3: In general a refined chronic risk assessment by using the PEC_{twa} may lead to underestimations by overlooking effects which are induced after short term exposure. However in

the case of fluazinam the calculation with the 1-day PEC_{twa} already results in acceptable TER values (≥ 19) and the risk to overlook early instating effects is low.
To point 4: RMS accepted the assessment according to the alternative approach of the EFSA Scientific Panel on Plant health by the comparison between ETC and PECs from $FOCUS_{sw}$ scenarios.

Overall conclusions:
Considering the refined chronic risk for fish assessed with the FOCUS Model Step 4 and appropriate risk mitigation measures, the risk for all scenarios is acceptable when fluazinam is applied according to GAP with a buffer zone of at least 5 m.
Thus it can be concluded that the chronic risk for aquatic organisms exposed to fluazinam is acceptable. However in either case, adequate risk mitigation measures (e.g. buffer zones) have to be considered at MS-Level to ensure an acceptable risk to aquatic organisms.

Bioaccumulation
The high BCF values of 960 and 1090 of fluazinam in whole fish and the incomplete depuration (after 14 days 22 – 24 % of total ^{14}C-tissue residues remained in fish) indicate a risk of bioaccumulation. However, fluazinam was extensively metabolised in fish organisms to AMPA, DAPA, MAPA and numerous non identified degradation products. Neither the active substance nor the identified or non-identified metabolites were found in amounts > 10 %. Since the determination of the BCF values and the residues are based on the total ^{14}C-residues, the respective values could not only be attributed to the active substance alone. Furthermore in metabolism studies with birds and mammals the same extensive metabolism with a similar pattern of metabolites was found (see sections B.6.1 and B.7.2). For these test organisms no potential for bioaccumulation of fluazinam could be identified. Thus, the potential of bioaccumulation in fish is considered to be low.
Additionally the risk of secondary poisoning to fish eating-birds and mammals was already considered in the long term risk assessment for birds and mammals (see chapter B.9.1 and B.9.3) and no unacceptable risk was identified.
Apart from these conclusions for the active substance the potential risk of the bioaccumulation of the major metabolite found in surface water AMPA has to be addressed.

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

B.9.3.1 Toxicity to mammals

A detailed assessment of all mammalian toxicity data on fluazinam and Fluazinam 500SC is provided in the “Annex B.6 Toxicology and Metabolism“ part of this monograph.

B.9.3.1.1 Acute oral toxicity

The acute oral toxicity (LD_{50}) of the ai in rats is 4100 / 4500 mg/kg bw in female / male rats (Liggett

1988). The acute oral toxicity (LD_{50}) of the 50 % suspension formulation in rats is > 2000 mg/kg bw in rats (Barber 1985).

B.9.3.1.2 Subchronic toxicity

and

B.9.3.1.3 Chronic (long-term) toxicity

and

B.9.3.1.4 Reproductive toxicity

It is referred to the B.6 part of the monograph.

B.9.3.2 Risk assessment

Wild mammals may be exposed to fluazinam by eating contaminated vegetation, seeds and fruits, invertebrate prey like arthropods (i.e. insects) or earthworms or vertebrate prey.
Exposure estimates were conducted according to the guidance document SANCO/4145/2000 (September 2002). The risk assessment is based on toxicity figures of the active substance and, for the acute scenario, also of the formulation.

Selection of exposure scenarios:

The following relevant exposure scenarios were considered:

potatoes: ten applications of 200 g ai/ha (interval 7 d)

Three routes of exposure are considered:

1) Dietary exposure, standard scenarios

2) Exposure of mammals via bioaccumulation and food chain behaviour

3) Exposure via drinking water

ad 1)

a) dietary exposure of a medium herbivorous mammal feeding on contaminated potato foliage

b) dietary exposure of an insectivorous mammal feeding on contaminated arthropods

The following endpoints were considered as relevant for ecotoxicological risk assessment:

acute oral toxicity LD_{50}	4100 mg ai/kg bw (ai, female rat) > 2000 mg form./kg bw (Fluazinam 500 SC)
reproductive toxicity NOAEL	5 / 6.7 mg/kg bw (m / f, 2-generation rat)

Choice of long-term/reproductive NOEL:

The NOEC of the rat multigeneration study is reported as 20 ppm (1 mg/kg bw) in the toxicology section. However, effects observed at the next higher dosage level of 100 ppm (5 – 6.7 mg/kg bw

for males/females) were slightly reduced bodyweights of females and slightly reduced conception rates and fertility indices only in F_1. These slight effects are considered as not ecologically relevant at population level. The NOAELs of two rabbit developmental toxicity studies with respect to fetal toxicity are 1 and 2 mg/kg bw as reported in the toxicology section. However, effects at the next dosages are again only slightly pronounced and are not considered to be of ecotoxicological relevance. Significant skeletal abnormalities are observed at a dosage of 12 mg/kg bw and above. From test design and relevance of effects, the 2-generation study and the NOAEL of 5 mg/kg bw (males) is considered most appropriate for ecological risk assessment.

In order to calculate the estimated theoretical exposure the respective figures were chosen according to the guidance document to estimate food intake rates and concentrations in the food of concern. As 10 applications are intended, figures for MAF are chosen according to SANCO/4145/2000. A default half-life of 10 d is assumed for estimating residue decline on vegetation. For acute exposure, the 90th percentile and for long-term exposure (averaging time 21 d) mean initial residue levels in vegetation were applied. In the first tier worst case assessment it is assumed that the mammals feed on a single food type, which is not avoided and that they satisfy their entire food demand in the treated area.
Possible other food items, for which no respective risk assessment was performed, are covered by the calculations presented because residues in these items are expected to be much lower.

To give a broad picture of the exposure of wild mammals, and because potato foliage is not a preferred feed type of mammals, insectivorous species are also considered for TER estimations. These mammals are assumed to feed on large arthropods on the ground.

Metabolites, impurities:

In plant tissues fluazinam is converted to several metabolites; none of these was found in relevant amounts (10 % TRR). In animal metabolism studies the main metabolites were the same as in plants and no further ecotoxicological consideration of these compounds is required. Metabolite HYPA was found to be more toxic than the parent after oral administration, however, it was not detected in plant metabolism and its potential occurrence in other foodstuffs is supposed to be of minor quantity.
Data on impurities of technical fluazinam are evaluated under B.6.8.2.. There is no indication that these impurities are more toxic than fluazinam and their hazard potential is covered with the toxicity studies with the technical substance.

In the following tables the TER calculations for fluazinam are presented.

Table 9.3.2.-1: Estimation of the TER_{acute} for mammals, Tier 1.

scenario	FIR / bw	RUD	Rate [kg/ha]	MAF	ETE	Tox.figure [mg ai/kg bw]	**TER**
fluazinam techn.							
medium herbiv`s mammal	0.28	87	0.2	2	9.7	4100	**421**

scenario	FIR / bw	RUD	Rate [kg/ha]	MAF	ETE	Tox.figure [mg ai/kg bw]	**TER**
insectivorous mammal	0.63	14	0.2	-	1.8		**2324**
Fluazinam 500 SC							
medium herbiv`s mammal	0.28	87	0.2	2	9.7	> 1000	**> 103**
insectivorous mammal	0.63	14	0.2	-	1.8		**> 556**

Table 9.3.2.-2: Estimation of the $TER_{long-term}$ for mammals, Tier 1.

scenario	FIR / bw	RUD	Rate [kg/ha]	f_{twa}	MAF	ETE	Tox.figure [mg ai/kg bw]	**TER**
medium herbiv`s mammal	0.28	40	0.2	0.53	2.6	3.06	5	**1.6**
insectivorous mammal	0.63	5.1	0.2	-	-	0.64		**7.8**

The acute TER values for both the active substance and Fluazinam 500SC are above the Annex VI trigger, indicating that fluazinam presents a low acute risk to wild mammals.
The long-term TER for insectivorous mammals does also meet the trigger.
The long-term TER for herbivores is below the trigger of 5, which indicates a potential long-term risk following the use of fluazinam. In the calculation it is assumed that a medium herbivore feeds exclusively on potato foliage, which is a worst-case scenario. However, it is well known that potato (a member of the family Solanaceae) foliage is not palatable to mammals and very unlikely to be eaten by mammals. In common agricultural practice it is moreover unlikely that weeds are present in significant amounts beneath the potato plants. Hence, the scenario of herbivorous mammals is considered as not relevant for the use of a fungicide in potatoes. The risk assessment should focus on insectivorous and earthworm-eating mammals instead.

2) <u>Exposure of mammals via bioaccumulation and food chain behaviour:</u>
Because the $logP_{ow}$ is > 3 a potential for bioaccumulation of fluazinam is indicated and this route of exposure is addressed according to SANCO/4145/2000 (Sept. 2002).

a) <u>Food-chain from earthworm to earthworm-eating mammals</u>
time-weighted PEC_{soil} for a period of 21 d after application: PEC_{soil} = 0.454 mg/kg soil
To estimate the $BCF_{earthworm}$ the following formula was used:
$BCF = (0.84 + 0.01\ K_{ow}) / f_{oc}\ K_{oc}$
The mean of the K_{oc} - figures from adsorption/desorption studies was used: K_{oc} = 1958;
as f_{oc} the default-value of 0.02 was taken.
Log K_{ow} = 4.03, K_{ow} = 10715
BCF = (0.84 + 0.01 x 10715) / (0.02 x 1958) = 2.76

PEC_{worm} = PEC_{soil} x BCF = 0.454 x 2.76 = 1.25 mg/kg

$ETE_{mammals}$: 1.4 x 1.25 = 1.75 (factor 1.4 given in SANCO/4145/2000)

The TER-calculation is presented in table 9.3.2.-3.

Table 9.3.2.-3: $TER_{long-term}$ for earthworm-eating mammals under consideration of biomagnification in the food-chain, Tier 1.

ETE	NOEL [mg/kg bw]	**TER**
1.75	5	**2.9**

Tier 2: Refinement: :

Refinement of Notifier:

"The refinement for worm-eating mammals uses a revised BCF factor instead of relying on Tier I estimates. Tier I estimates are not appropriate for compounds that react and metabolize rapidly, as with fluazinam. (In all animals tested, (fish, rats, goats and chicken) fluazinam represents a maximum of about 2 % of total residues).

Tier I BCF factors are based on a model developed by Jager (1998). However, the paper admits that some nitrogen-containing compounds may not fit the model, especially if they are extensively metabolized. A refined BCF for fluazinam is derived by multiplying the Tier I BCF by a factor to account for metabolism, F_{Met} = 0.02, where F_{Met} is equal to the maximum percentage of fluazinam in tissues. Since use of the BCF is designed to assess the potential amount of an active ingredient in food items, it is appropriate to make a correction when extensive metabolism reduces the level of that compound."

When this factor of 0.02 is included in the calculation of ETE, it is obvious that the resulting TER will be significantly above 5.

RMS opinion and Refinement:

We agree that the Tier 1 calculation is an overestimation of the exposure in the case of fluazinam, because of the rapid metabolism and excretion of the substance (in lactating goats and laying hens the maximum level of radioactivity - including metabolites - found in total tissue after oral administration of fluazinam was < 2 % and < 3 % of the administered dose, respectively). However, the inclusion of a factor of 0.02 in the ETE calculation is considered not appropriate in the case of earthworm-eaters. The levels of fluazinam in the tissue of earthworms might be different than in vertebrates, because earthworms live in the contaminated soil and might also take up fluazinam via the skin. Also a factor derived from vertebrate metabolism studies is considered not appropriate for invertebrates.

However, in the RMS view the risk to earthworm-eating mammals is considered low based on a weight-of-evidence approach: As explained above, the standard tier 1 calculation will overestimate the level of fluazinam + metabolites because of the rapid metabolism and excretion. Furthermore potato fields are not an attractive habitat for mammals and it can be strongly supposed that the proportion of time spent in the treated area (PT) will be < 1.

Based on more realistic assumptions the TER will be clearly above 5 and indicate an acceptable

risk.

b) Food chain from fish to fish-eating mammals:

Tier 1: PEC from FOCUS step 1:

highest PEC_{water} (twa, 3 weeks, FOCUS step 1; 1 m distance) = 40.4 µg/L

whole-body-BCF for fish = 1090

PECfish = PEC_{water} * BCF = 40.4 * 1090 = 44036 µg/kg bw (fish) = 44 mg/kg bw (fish)

$ETE_{mammals}$: 44 x 0.13 = 5.72

The TER-calculation is presented in table 9.3.2.-4.

Table 9.3.2-4: TERlong-term for fish-eating mammals under consideration of biomagnification in the food-chain, Tier 1.

ETE	NOEL [mg/kg bw]	**TER**
5.72	5	**0.87**

Tier 2: Refinement: PEC from FOCUS step 2:

highest PECwater (twa, 3 weeks, FOCUS step 2; 1 m distance, worst case: S-EU) = 0.96 µg/L

whole-body-BCF for fish = 1090

PECfish = PECwater * BCF = 0.96 * 1090 = 1064 µg/kg bw (fish) = 1.1 mg/kg bw (fish)

$ETE_{mammals}$: 1.1 x 0.13 = 0.14

Table 9.3.2-5: TERlong-term for fish-eating mammals under consideration of biomagnification in the food-chain, Tier 2.

ETE	NOEL [mg/kg bw]	**TER**
0.14	5	**35**

The resulting TER is above the trigger and indicates an acceptable risk for fish-eating mammals.

c) Biomagnification in terrestrial food chains

According to the evaluation in the toxicology part of the DAR, the potential of bioaccumulation of fluazinam in vertebrates is low due to extensive metabolism of the substance. In lactating goats and laying hens the maximum level of radioactivity - including metabolites - found in total tissue after oral administration of fluazinam was < 2 % and < 3 % of the administered dose, respectively. It can be concluded that the risk of biomagnification in terrestrial food chains is low.

3) Exposure of mammals via drinking water

The water intake of mammals is calculated according to SANCO/4145/2000.

Total water ingestion rate (L/day) = $0.099W^{0.90}$

where W is the body weight in kg.

The exposure from puddles of spray liquid is assessed as a worst case. The dilution for spraying is 200 (-500) L water/ha containing 200 g ai. When a dilution factor of 5 is applied for the

concentration in the puddle, a $PEC_{drinking\ water}$ of 200 mg ai/L is derived.

The total water ingestion rate (daily water intake DWI) of a small mammal (pygmy shrew, 6 g) is calculated as 1 mL/d. The acute toxicity (LD_{50}) for a 6 g shrew is calculated as 24.6 mg ai/shrew and > 6 mg ai/shrew for the ai and the formulation, respectively.

The Toxicity Exposure Ratio (TER) for the acute time scale is calculated as LD_{50}/ETE. The ETE_{acute} is calculated as $PEC_{drinking\ water}$ x DWI.

Table 9.3.2.-6: Acute TER for small mammals drinking contaminated water from puddles

scenario		ETE	TER (ai)	TER (form.)
acute	potatoes	0.2	**123**	**> 30**

It appears that the risk via contaminated drinking water for wild mammals is low.

Conclusion:

The application of fluazinam under conditions of the intended representative does not pose an unacceptable risk to wild mammals.

B.9.4 Effects on bees (Annex IIA 8.3.1, Annex IIIA 10.4)

B.9.4.1 Acute Toxicity

Active Substance: Fluazinam

Reference: Collins, I. G., Gough, H. J., Wilkinson, W. (1984): B 1216 (PP192): Acute Contact and Oral Toxicity to Honey Bees (*Apis mellifera*). Document No. RJ 0401B

Test guideline: UK MAFF Pesticides Safety Precautions Scheme, Document D3 (1979)

GLP: Yes

Material and methods:

The toxicity of technical fluazinam (B 1216, Lot No. 8303-4, purity 98.8 %) and a 50 % WP formulation of fluazinam (JF 9545, Lot No. 3059-2W) to honey bees was investigated by both the oral and contact exposure routes. Each test was run in duplicate using three replicate cages of 10 bees per dose rate (60 bees tested per dose). The number of bees affected, or found dead, were counted at 1, 4, 24 and 48 h after treatment. Technical dimethoate was used as toxic standard for both contact and oral toxicity tests.

In the contact tests, anesthetized bees were given a 1 µL drop of material applied to the thorax. The concentration range was 0, 20, 50, 100 and 200 µg ai/bee for the technical substance and 0, 1, 2 and 4 µg ai/bee for the formulated substance. The substance was solved in acetone (technical ai) or in water (formulation). Because of problems of low solubility the range of concentrations that could be prepared with the formulated compound was limited. The dose of 4 µg ai/bee had to be applied in two drops. In the oral tests, bees were fed 0.2 mL of a given concentration per cage via feeding tube. The concentration range for both the technical and formulated material was 0, 10, 20, 50 and 100 µg ai/bee.

Findings:

The mortality in the highest dose in the contact tests for the technical substance (200 µg ai/bee) was only slightly greater than in the controls (see table below).

Because of low solubility, only a limited concentration range of the formulated material could be tested. The contact toxicity of the high dose (4 µg ai/bee) was comparable to the control after 48 h (4 dead bees out of 60).

In the oral toxicity tests, the solubility of fluazinam in 50 % sucrose solution limited the upper range of doses that could be validly tested. However, few bees died in either the technical or formulation material tests at any doses.

Table 9.4.1.-1: Mortality in the contact and oral toxicity tests with fluazinam technical (number of dead bees out of 60).

contact	control	20 µg ai/bee	50 µg ai/bee	100 µg ai/bee	200 µg ai/bee
	2	1	6	7	5
oral	control	10 µg ai/bee	20 µg ai/bee	50 µg ai/bee	100 µg ai/bee
	3	6	5	5	6

The 24- and 48 h LD_{50} values for dimethoate for both the contact and oral toxicity tests were within the expected range.

For technical fluazinam the LD_{50} (48 h) for contact toxicity is > 200 µg ai/bee and the oral LD_{50} > 100 µg ai/bee. For the WP formulation the LD_{50} (48 h) contact is > 4 µg ai/bee and oral > 100 µg ai/bee. In all cases, the highest dose tested was below the estimated LD_{50} value. It can be concluded that fluazinam is of low toxicity to honey bees by either the oral or contact route.

Comments (RMS):

The formulation tested in this study is not similar to the lead formulation (wettable powder vs. suspension concentrate) and the results referring to the WP formulation are therefore not relevant for the risk assessment.

The study was not declared to be performed according to EPPO 170 or OECD 213 or 214 as required in SANCO/10329/2002 (terrestrial guidance document). However, the test design is similar to those guidelines and the study is considered acceptable.

Formulation: Fluazinam 500 SC

Reference: Kleiner, R. (1992): Testing Toxicity to Honey Bee - *Apis mellifera* L. (laboratory) According to BBA Guideline VI, 23-1. Report No. 921048038

Test guideline: BBA Guideline VI, 23-1 (1991)

GLP: Yes

Material and methods:

The toxicity of Fluazinam 500SC, Lot No. RS 048/B, fluazinam concentration 509 g/kg, to honey bees was investigated by the respiratory, direct and indirect contact and feeding routes of exposure. The dosage used in the test was 0.8 L/ha in 400 L/ha (0.2 % v/v). Each test was run using three replicate cages of 10 bees per dose rate. Two repetitions were run for each test. In

each experiment, bees were observed continuously for 30 min immediately following application, after 1 h, in the evening of the day of application, in the morning immediately following application day and 24 h after application (test conclusion). An EC formulation of parathion-methyl was used as a toxic standard control for all tests along with a deionized water control.

For the respiratory effect test, test cages were placed over Petri dishes which were half-filled with the test solution, exposing bees to the vapour phase of the substance. In the direct contact test, the test substance (1 mL/cage) was sprayed by hand-sprayer through the wire mesh, wetting the bees completely and uniformly. In the indirect contact test, prefolded filter paper were drenched with the test solution and put into the cages after air-drying. In the feeding test, following a one hour fasting period, the bees were fed a 50 % aqueous sugar or honey solution containing 1 % of the test substance. The treated sugar water solution was offered for a maximum of 3 h, after which it was replaced by untreated sugar solution. Bees were observed for mortality as well as symptoms of poisoning and anomalous behaviour in comparison to untreated bees.

Findings:

The mortality of the untreated controls in each test was 0 %. Fluazinam 500SC at 0.8 L/ha exhibited no significant mortality on the honeybees in the direct contact test (1 bee died in one of the two repetitions), the indirect contact test (no mortalities) or the respiratory effect test (no mortalities). At a feeding concentration of 1 %, there were no fluazinam associated mortalities in the feeding test. Therefore the LD_{50} value was > 198 µg formulation/bee (101 µg ai/bee), the concentration tested. The reference substance application resulted in 100 % mortality in each test following signs of irritation by the bees.

Conclusion:

Fluazinam 500SC can be classified as harmless to honeybees.

Comment (RMS): Study considered acceptable.

B.9.4.2 Cage tests

No data provided.

Summary of toxicity data on bees

Table B.9.4.2-1 Acute oral and contact toxicity of fluazinam to honey-bees, lab tests

compound	test type	number of bees, test duration	dosage (µg ai/bee)	mortality (%)	LD_{50} (µg ai/bee)	reference
fluazinam techn.	contact toxicity	60, 48 h	20, 50, 100, 200	8 - 10	> 200	Collins et al. (1984)
	oral toxicity	60, 48 h	10, 20, 50, 100	2 - 12	> 100	
Fluazinam 500SC	contact toxicity	60, 24 h	direct spray 0.2 %	2	/	Kleiner (1992)
	oral toxicity	60, 24 h	101	0	> 101	

B.9.4.3 Risk assessment for honey bees

Honey-bees may be exposed to formulated fluazinam by direct spraying of the plant protection product while bees are foraging on the crop or flowers and weeds present in or adjacent to the crop treated. They may also be exposed through contact with fresh or dry residues or by oral uptake of contaminated pollen, nectar and honey dew.

To account for the multiple application scenario, the single rate is multiplied with a multiple application factor (MAF) according to Escort 2.

Table B.9.4.3-1 Bee hazard quotients (QHC and QHO) for fluazinam

test substance	crop	max. field rate x MAF [g ai/ha]	route of exposure	LD50 [µg ai/bee]	hazard quotient
fluazinam	potatoes	200 x 3.5	contact	> 200	**< 3.5**
			oral	> 100	**< 7**

The resulting hazard quotients for oral and contact toxicity (Q_{HO} and Q_{HC}) are below 50. Values below the trigger value of 50 indicate an acceptable risk to bees.

Conclusion:

After application of fluazinam under the intended representative use conditions the risk to honeybees is low.

B.9.5 Effects on other arthropod species (Annex IIA 8.3.2, IIIA 10.5)

B.9.5.1 Acute toxicity

Plant Production Product

Reference: Canning, L., Lloyd, E.J., Lewis, G.B. (1992): Fluazinam – Investigation of the Toxicity of a 50 % w/v SC Formulation to the Carabid Beetle *Pterostichus melanarius* and a Lycosid Spider, Report No. RJ1070B

Test guideline: Hassan et al., OEPP/EPPO Bulletin 15, (1985)

GLP: Yes

Materials and methods:

The Fluazinam 500SC formulation, Lot YF7604B, with an ai content of 38.4 % (w/w), was tested on two beneficial arthropods, the carabid beetle *Pterostichus melanarius* and the lycosid spider (family Lycosidae*)* at two field rates. Dimethoate 40, applied at 0.7 kg/ha, served as toxic standard and deionized water containing 0.05 % Agral (alkylphenylethoxylate wetting agent) as control substance.

Test animals were individually caged in containers filled with 4 cm of sieved sandy loam soil. The test substance was applied to the arthropods and the soil surface at a field application rate of 200 g ai/ha. A second application rate of 2000 g ai/ha was applied to the soil only, to simulate the effects from successive applications over a season. Each spray replicate consisted of thirty individuals.

Mortality and behavioural assessments were carried out on each species at 4 hours, 1, 2 and 6 days after dosing. Only animals on the soil surface were assessed in the intermediate sampling periods. Buried animals were removed from the soil at the 6-day assessment and their condition recorded. Feeding activity was monitored by feeding dead *Drosophila melanogaster* to carabids and lycosids on days 0 and 4 and assessing feeding activity on days 2 and 6 respectively.
Statistical analysis: Percentage mortality, percentage affected and percentage feeding were calculated for the treated and the control groups. A normal approximation to the binomial distribution was made and a t-test conducted to test for a difference between the treated and control group.

Findings:

No significant mortality was observed following exposure of the carabid beetles or lycosid spiders to Fluazinam 500SC applied at either the maximum single application rate of 200 g ai/ha or the maximum annual application rate of 2000 g ai/ha. The results were not significantly different for the numbers of animals affected, with the exception of beetles directly exposed to the chemical at the lower application rate. The results of the testing are shown in the table below.

Table 9.5.1-1: Effects of Fluazinam 500SC on Carabid Beetles and Lycosid Spiders after 6 d

Treatment	Carabid Beetle		Lycosid Spider	
	% mortality	% affected	% mortality	% affected
Control	0	0	3.4	3.4
Fluazinam 50 % SC (200 g ai/ha)	10	16.7*	3.3	3.3
Fluazinam 50 % SC (2000 g ai/ha)	0	0	0	0
Dimethoate Control	100	100	90	90

* significantly different from control (P<0.05)

In addition, there was no significant effect on feeding activity for either the carabid beetles or the lycosid spiders at either of the application rates.
According to the IOBC Classification Scheme the fluazinam 500SC formulation is classified as "Harmless" to carabid beetles (*P. melanarius*) or lycosid spiders
Comment (RMS): Study considered acceptable.

Reference: Coulson, J.M., Lavender, K.H. (1996): Investigation into the Toxicity of a 500 g ai/L Suspension Concentrate Formulation to the Cereal Aphid Parasitoid *Aphidius rhopalosiphi*, Report No. RJ2109B
Test guideline: Bigler, 1988
GLP: Yes
Material and methods:

Parasitic wasps (*Aphidius rhopalosiphi*) were exposed to Fluazinam 500SC (lot number YF8053, containing 39.13 % fluazinam (w/w)) at a rate of 200 g ai/ha. Dimethoate 400 g/L EC was used as a positive control and deionized water as control. The materials were applied to glass plates using a hydraulic track sprayer. There were four replicates per treatment, each containing five adult female wasps which were exposed for two days. Honey and water on cotton wool was provided as

food. The parasitoids were observed for lethal and sublethal effects. Ten randomly selected surviving females from the fluazinam and control treatments were introduced into barley plants infected with the aphid species *Metopolophium dirhodum* for 24 hours to assess fecundity.

Findings:

Mortality and behaviour: No behavioural abnormalities were observed in the treatment or control groups. 50 % mortality (statistically significant when compared to the control, P = 5 %) following exposure to the Fluazinam formulation occurred. All wasps in the toxic standard (dimethoate) group died by the 24h observation. The results are summarized in the table below.

Parasitation: 9.4 mummies were produced per female in the fluazinam treatment and 7.5 per female in the control.

Table 9.5.1-2: Mortality and sublethal effects (%) of Fluazinam on female Parasitoid Wasps (*Aphidius rhopalosiphi*)

Time After Exposure	Control			Fluazinam			Dimethoate		
	H	A	D	H	A	D	H	A	D
1 hour	100	0	0	100	0	0	100	0	0
3 hours	100	0	0	100	0	0	95	5	0
7 hours	100	0	0	100	0	0	15	55	30
1 day	95	0	5	90	0	10	0	0	100
2 days	90	0	10	50	0	50	0	0	100

H = Healthy, A = Affected, D = Dead

The corrected mortality is 44 %. The effect on fecundity following exposure to fluazinam (R = - 27 %) was not statistically significant.

Comment (RMS): Study considered acceptable.

Reference: Thompson, B. (1996): A Laboratory Evaluation of the Side-Effects of the Fungicide Fluazinam on Larvae of the Lacewing *Chrysoperla carnea*. Report No. ZEN-95-3/C

Test guideline: Bigler, (1988)

GLP: Yes

Material and methods:

Lacewing larvae (*Chrysoperla carnea*), were exposed to Fluazinam 500SC (lot no. YF8053, 39.13 % fluazinam (w/w)), at a rate of 200 g ai/ha. Atlas Dimethoate 40 EC (400 g/L) was used as a positive control and tap water as control treatment.

Each material was applied to glass plates and allowed to dry. Once the glass plates were dry, lacewing larvae (2-3 days old, 30 larvae per treatment) were confined individually on the fresh residues and provided daily with untreated aphids for food. The condition of the larvae was monitored through to pupation and the number of emerging adults was recorded. To determine any sublethal treatment effects on the fecundity of the resultant adult lacewings, the number and viability of eggs produced by mated females was recorded over a three-week period.

Findings:

In the fluazinam treatment, 26 out of 29 larvae (90 %) pupated (one larva escaped from its arena) and 24 of these developed into adults. This gave an overall success rate for larval to adult development of 83 %. In the control, 25 out of 30 larvae (83 %) pupated and 20 of these developed into adults. The overall success rate was therefore 67 %. This is equivalent to a pre-imaginal mortality rate of 33 %, which exceeds the validity criteria of 15 % mortality (Vogt et al. 2000).

Four adults in the control and two adults in the fluazinam treatment died during hatching. All of the larvae in the toxic standard died within two days. The time taken for most of the larvae to pupate was similar for both treatments, 8-13 days after the test began.

In the fecundity assessments, the mean number of eggs/female/day was similar for both treatments, being 9.0 for fluazinam and 9.4 for the control. The viability of the eggs set aside was also similar, being 78 % and 81 % for fluazinam and control sets, respectively. These results do not indicate any differences between the treatments. The data are summarized in the table below.

Table 9.5.1-3: Summary of Results – Fluazinam Toxicity to Lacewing (Chrysoperla carnea)

	Control (water)	Fluazinam SC (500 g/L)	Toxic standard
Number of Larvae Pupating (N = 30)	25	26	0
Number of Adults Emerging	20	24	-
Mean larval hatch rate (%)	67	83	-
Number of Eggs Produced Per Female/Day	9.4	9.0	-
Proportion of Eggs Giving Rise to Larvae	0.81	0.78	-

When fluazinam 500SC formulation was applied to glass plates at the maximum recommended rate of 200 g ai/ha, the fresh residues were not harmful to larval *Chrysoperla carnea* and did not affect the fecundity of the adult insects that subsequently developed.

Comment (RMS):

Because of high pre-imaginal mortality in the control the study is not valid. Study considered not acceptable and not essential.

Reference: Jansen, J-P. (2000a): Side Effects of Fluazinam (IKF-1216) 500 g/L SC Formulation on the Parasitic Wasp *Aphidius rhopalosiphi* (Hym.; Aphidiidae) in the Laboratory on Potato Detached Leaves Treated in the Field. Report No. AE.01/2000

Test guideline: Mead-Briggs, M. (1992) “A laboratory method for evaluating the side-effects of pesticides on the cereal aphid parasitoid *Aphidius rhopalosiphi* (Destefani-Perez) Asp. Appl. Biol. 31:179-189.

GLP: Yes

Material and methods:

The effects of Fluazinam 500SC (Batch No. 0/0001036, ai concentration 39.49 % w/w) on adult *Aphidius rhopalosiphi* (2 - 48 hours post-emergence) were determined. The toxic control used was

Perfekthion S, a 500 g/L EC formulation of dimethoate.

The wasps were placed in exposure units covered (floor and ceiling of cage) with potato leaves taken from plots that had been sprayed at a rate of 200 g ai/ha over ten applications at 7-10 day intervals. The potato seeds planted in the plots had been previously treated with 200 g ai/ton of fluazinam 500SC. The leaves were taken from the field about one hour after the last application, brought in the laboratory in a cooled box and placed into the test units either upperside or underside facing the insects (3 test units each). An additional series of test units with control leaves was set up in similar manner (3 replicates with leaf upperside up, 3 with underside up). Three units contained leaves which were sprayed in the laboratory with the toxic standard at an application rate of 2 mg/cm^2 and used approximately 1 to 2 h after spraying. The leaves were placed on wetted filter paper and put on glass plates. Two plates, held apart by a shallow untreated frame, comprised a test unit. Wasps were provided with food (water and water/honey) and a peristaltic pump was used to renew the air one to two times per minute to avoid build-up of pesticide vapours. Within 1-2 h after field collection for fluazinam-treated leaves, ten wasps (5 male, 5 female) were placed in each unit. The units were placed in a climate controlled chamber at 19°C and 50-80 % relative humidity. After 48 hours, surviving adult wasps were counted, harvested and sexed. Mortalities were counted at this time.

The surviving females were individually confined for 24 h in fertility assessment units containing barley seedlings infested with *S. avenae* aphids. At the end of the 24h period, the females were removed and the fertility assessment units kept for an additional 10 to 12 days to allow development of aphid mummies. Mummies developing after this time period were recorded for each wasp and mean production was calculated for each treatment.

Mortality in each test treatment was corrected for control mortality. The reproductive (R) ratio was calculated according to Schneider-Orelli.

Findings:

The results of the test are shown in the table below. After 48 h exposure, mortalities in the control units were 3.3 % and 6.7 % for units having leaves turned underside up and upperside up, respectively. The corresponding fluazinam treated leaf unit mortalities were 3.0 % and 78.1 %, after correcting for the control mortalities. When underside and upperside leaf surface mortalities are combined, the overall fluazinam mortality was 40.6 %. The study author reports that one week prior to fluazinam application the potato field had also been treated with mancozeb. The possible effects of this treatment on the wasps is unknown.

All wasps died during the initial exposure phase in the toxic control (dimethoate) units.

In the fertility assessment, there was a slight increase in the number of aphid mummies produced by the fluazinam treated wasps vs. the control wasps. Therefore, fluazinam did not negatively affect the reproduction of surviving wasps.

Table 9.5.1-4: Effects of Fluazinam 500SC Formulation on *A. rhopalosiphi*

Treatment	Observed Mortality (%)	Corrected Mortality (%)	Mummies/ Female	R (%)	E (%)
Control –underside	3.3	-	11.1	-	
Fluazinam – underside	6.7	3.0	15.3	- 38	-33.7
Control –upperside	6.7	-	10.7	-	
Fluazinam – upperside	80.0	78.1	17.8*	- 66	63.6
Control – both sides	5.0	-	10.9	-	
Fluazinam – both sides	43.3	40.6	16.5	- 51	10.1
Dimethoate control	100.0	100.0	-	-	100

* only 4 surviving females

E = combined effect

Under a realistic worst-case exposure scenario, Fluazinam 500SC caused a mortality of 40.6 % and a total effect (including reproduction) of 10.1 % to the parasitic wasp *A. rhopalosiphi* following 10 applications to a treated potato field in laboratory testing.

Comment (RMS):

It is questionable why the potato leaves had also been treated with mancozeb shortly before the onset of fluazinam treatments. For the assessment, all effects are assumed to be caused by fluazinam treatment. Study considered acceptable.

Reference: Jansen, J-P. (2000b): Side Effects of Fluazinam (IKF-1216) 500 g/L SC Formulation on the Predacious Mite *Typhlodromus pyri* Scheuten (Acari; Phytoseiidae) in the Laboratory on Potato Detached Leaves Treated in the Field. Report No. TE.01/2000

Test guidelines: Overmeer, W. P. J. (1988): Laboratory method for testing side-effects of pesticides on the predacious mites *Typhlodromus pyri* and *Amblyseius potentillae*. IOBC/WPRS Bulletin 1988/XI/4:65-70. Samsoe-Petersen, L. (1983): Laboratory method for testing side-effects of pesticides on juvenile stages of the predatory mite, *Phytoseiulus persimilis* (Acarina, Phytoseiidae). Entomophaga, 28(2): 167-178.

GLP: Yes

Material and methods:

The protonymphs of *Typhlodromus pyri* (2-3 days old) were placed on potato leaves taken from plots that had been sprayed with Fluazinam 500SC (Batch No. 0/0001036, ai concentration of 39.49 % w/w) at a rate of 200 g ai/ha over ten applications at 7-10 day intervals. The potato seeds planted in the plots had been previously treated with 200 g ai/ton of fluazinam 500SC. The toxic control used was an EC formulation of parathion-ethyl, with a nominal content of 250 g ai/L.

The leaves were taken from the field about one hour after the last application, brought in the laboratory in a cooled box and placed into the test units either upperside or underside up (3 test

units each). An additional series of test units with control leaves were set up in similar manner (3 replicates with leaf upperside up, 3 with underside up). Three leaves were sprayed in the laboratory with the toxic standard (parathion-ethyl) at an application rate of 2 mg/cm^2. The leaves were placed on wetted cotton pads in the centre of plastic Petri dishes. The leaves were placed in close contact with the glass wool around the sides to keep the mites from escaping.

Within 1-2 h of field collection for fluazinam-treated leaves or 1-2 h after laboratory spraying of the toxic control leaves, twenty protonymphs were placed on each leaf (test unit). *Pinus negra* pollen was provided as food. At the end of the one week exposure, surviving adult mites were counted and transferred to fertility assessment units. Mortalities were calculated based on surviving mites collected at day 7. Mites not found were considered dead. For the test substance, observed mortalities were corrected by the value of the corresponding control (underside, upperside, underside and upperside together).

Fertility assessments were made on the basis of whether protonymphs had been placed on underside or upperside of leaves. Males and females from each treatment type (underside or upperside of leaf) were kept together, with the exception that some males of the same treatment were redistributed to obtain an approximately 1 : 3 male : female ratio. The total number of viable eggs/female were calculated per unit for each count and added. The reproductive ratio (R) was calculated according to Schneider-Orelli.

Findings:

The results of the test are shown in the table below. No protonymphs survived the toxic control to calculate a reproductive effect. The mortality observed as reported in the table is the combination of mites found dead or missing.

Table 9.5.1-5: Effects of Fluazinam 500SC Formulation on *T. pyri*

Treatment	Mortality (%)	corrected mortality (%)	Eggs/Female	R (%)	E (%)
Control –underside	11.7	-	4.24	-	-
Fluazinam – underside	6.7	-5.5	4.12	2.8	-2.5
Control –upperside	8.3	-	3.71	-	-
Fluazinam – upperside	11.7	6.1	3.87	-4.3	2.1
Control – both sides	10.0	-	3.98	-	-
Fluazinam – both sides	9.2	0.3	4.00	- 1	-0.2
Parathion-ethyl control	100.0	100.0	-	-	100

E = combined effect

Under a realistic worst-case exposure scenario, Fluazinam 500SC formulation can be considered as harmless to *T. pyri* according to IOBC criteria.

Comment (RMS): Study considered acceptable.

Reference: Jansen, J-P. (2000c): Side Effects of Fluazinam (IKF-1216) 500 g/L SC Formulation on Larvae of the Green Lacewing *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae) in the Laboratory on Potato Detached Leaves Treated in the Field. Report No. CCE.01/2000

Guidance: SETAC Europe "Guidance Document on Regulatory Testing Procedures for Pesticides with Non-Target Arthropods" (1994).

GLP: Yes

Material and methods:

The lacewing (*Chrysoperla carnea*) larvae were placed on potato leaves taken from plots that had been sprayed with Fluazinam 500SC (Batch No. 0/0001036, ai concentration 39.49 % w/w) at a rate of 200 g ai/ha over ten applications at 7-10 day intervals. Larvae were fed aphids (*A. pisum*) *ad libitum* and replaced daily. The toxic control used was Perfekthion S, a 500g/L EC formulation of dimethoate.

The potato seeds planted in the plots had been previously treated with 200 g ai/ton of fluazinam 500SC. The leaves were taken from the field about one hour after the last application, brought in the laboratory in a cooled box and placed into the test units either upperside or underside up (20 test units each). An additional series of test units with control leaves were set up in similar manner (20 replicates with leaf upperside up, 20 with underside up). Twenty leaves were sprayed in the laboratory with the toxic standard (dimethoate) at an application rate of 2 mg/cm^2. The leaves were placed on moistened cotton pads covering an untreated glass plate. A 5 cm diameter Perspex ring was put on the leaf and the inner wall coated with Fluon GP1 to prevent escape of the larvae. One *C. carnea* larva was released in each unit.

Mortality was checked daily and pupae carefully removed when formed and placed in Petri dishes to check adult emergence. When they emerged, adults were released into fertility units for assessment. Egg counting began one week after the mean adult emergence date for each unit and lasted four weeks. Eggs were counted twice a week. The viability of approximately 200 eggs per count was assessed. At the end of the fifth week, all living adults were harvested and sexed. The total number of viable eggs/female were calculated per cage for each count and added. The reproductive ratio (R) was calculated according to Schneider-Orelli.

Findings:

The results of the test are shown in the table below. No larvae survived the toxic control to calculate a reproductive effect. The mortality observed as reported in the table is the combination of larvae mortality and preimaginal mortality.

Table 9.5.1-6: Effects of Fluazinam 500SC on Lacewings (*C. carnea*)

Treatment	Mortality (%)	Corrected Mortality (%)	Eggs/Female	R (%)	E (%)
Control –underside	20.0	-	180.3	-	
Fluazinam – underside	35.0	18.8	171.5	4.9	22.8
Control –upperside	15.0	-	141.5	-	
Fluazinam – upperside	25.0	11.8	168.4	- 19	-5.0

Treatment	Mortality (%)	Corrected Mortality (%)	Eggs/Female	R (%)	E (%)
Control – both sides	17.5	-	160.9	-	
Fluazinam – both sides	30.0	15.2	170.0	- 5.7	10.4
Dimethoate control	100.0	100.0	-	-	100

E = combined effect

Under a realistic worst-case exposure scenario, Fluazinam 500SC can be considered as harmless to the green lacewing, *C. carnea*.

Comment (RMS): Study considered acceptable.

B.9.5.2 Semi-field or field tests

None performed.

B.9.5.3 Summary

Table B.9.5.3-1: Summarized effects of Fluazinam 500 EC on non-target arthropods, lab and extended lab tests

Species & stage	type of study	Dose rate [g ai/ha	No. of individuals	Kind of exposure	Parameters observed[1]	Effects [%]	Reference
Parasitoids							
Aphidius rhopalosiphi adults	lab	200	20 ♀	contact with dried residues	mortality / fecundity	44 / - 27 LR50 > 200 g ai/ha	Coulson et al. 1996
Aphidius rhopalosiphi adults	ext lab potato leaves	10 x 200 (7-10 d)	30	contact with dried residues	mortality fecundity	3 / 78.1 / 40.6[3] - 38 / - 66 / - 51[3]	Jansen 2000a
Predatory mites							
Typhlodromus pyri proto-nymphs	ext lab potato leaves	10 x 200 (7-10 d)	60	contact with dried residues	mortality fecundity	- 5.5 / 6.1/ 0.3[3] 2.8 / - 4.3 / - 1[3] LR50 > 200 g ai/ha	Jansen 2000b
Foliage dwelling predators							
Chrysoperla carnea larvae until adult	ext lab potato leaves	10 x 200 (7-10 d)	200	contact with dried residues	mortality fecundity	18.8 / 11.8 / 15.2[3] 4.9 / - 19 / - 5.7[3]	Jansen 2000c
Ground dwelling predators							
Pterostichus melanarius adults	lab	200 2000[2]	30 30	overspray and soil appl.	mortality / feeding activity	10 / 0 0 / 0	Canning et al. 1992
lycosid spider	lab	200 2000[2]	30 30	overspray and soil appl.	mortality / feeding activity	3.3 / 0 0 / 0	Canning et al. 1992

[1] corrected figures for mortality are reported
[2] only soil application
[3] underside / upperside / both sides of leaves

B.9.5.4 Risk assessment for non-target arthropods

Non-target arthropods may be exposed to formulated fluazinam by direct spraying, contact with fresh or dry residues or by oral uptake of contaminated prey, nectar and pollen or via host organisms.

In the following a risk assessment is performed for the four functional groups of arthropods:

Parasitoids:

The laboratory study described above provides evidence that the LR_{50} for *A. rhopalosiphi* is > 200 g ai/ha.

Based on this value the hazard quotients (HQs) for the in- and off-field area are calculated as follows for *A. rhopalosiphi* (according to ESCORT 2 procedure, MAF 3.5, drift 1.52 % at 1 m, vdf 10):

Table 9.5.4-1: HQ estimations for *A. rhopalosiphi* after exposure to fluazinam

species	application rate x MAF (g ai/ha)	LR_{50} [g ai/ha]	HQ in-field	HQ off-field
A. rhopalosiphi	200 x 3.5	> 200	< 3.5	< 0.11

The HQ in-field possibly exceeds the critical value of 2.

In an extended lab study with detached potato leaves mortality rates of 3 % and 78.1 % were obtained on the underside and upperside of the leaves, respectively. When taken together the mortality rate is 40.6 %. As aphids which are assumed to be parasitised by *Aphidius* are primarily found on the underside of leaves, it seems reasonable to regard the combined figure of 40.6 % mortality as a realistic worst case. As this figure is below the Escort 2 trigger of 50 % mortality and as parasitoids due to their mobility have a high potential of recolonisation, the risk to parasitoids is considered acceptable.

Predatory mites:

T. pyri were not significantly affected by Fluazinam 500 SC in an extended laboratory study when applied according to GAP. Hence, the product does not pose an unacceptable risk to predatory mites.

Foliage dwelling arthropods:

C. carnea were not significantly affected by Fluazinam 500 SC in the extended laboratory. Hence, the product does not pose an unacceptable risk to foliage dwelling arthropods.

Ground dwelling arthropods:

P. melanarius and lycosid spiders were not affected by Fluazinam 500 SC in the laboratory. Hence, the product does not pose an unacceptable risk to ground dwelling arthropods.

Conclusion:

The risk for terrestrial non-target arthropod populations posed by fluazinam when applied according to GAP is low.

B.9.6 Effects on earthworms

B.9.6.1 Acute toxicity (Annex IIA 8.4, Annex IIIA 10.6.1)

Active substance

Reference: Edwards, P. J., Coulson, J. M. 1985 B 1216 (PP192): Toxicity of Technical Material to the Earthworm (*Eisenia fetida*). Report No. RJ0409B

Guideline: OECD Guideline 207

GLP: Yes

Test item: Fluazinam techn., purity: 97.3 %, lot no: 8303-4

Material and methods:

The effect of fluazinam on earthworm (*Eisenia fetida*) has been determined in a laboratory test using artificial soil. The soil consisted of 70 % fine silica sand, 20 % kaolinite clay, 10 % sedge peat with an organic matter content of 79.5 % and 10 mg/kg $CaCO_3$, the pH was 7.0 ± 0.2. Worms were adults > 2 month old with a mean weight of 0.37 ± 0.12 g. Following a range-finding test, batches of artificial soil were prepared with three concentrations of fluazinam: 10, 100 and 1000 mg/kg dry soil. Fluazinam was mixed into the soil. Additionally, the toxic standard 2-chloroacetamide was tested at four rates: 18, 32, 56 and 100 mg/kg. Three replicates of each compound and concentration level and three untreated controls were prepared. Thirty earthworms were exposed to each concentration. Throughout the study the moisture content of the soils was maintained at 35 % by weight. The cultures were kept under a 16 hours light / 8 hours dark photoperiod and a temperature of 20°C ± 2°C. Mortality and behavioural effects were assessed after 7, 14 and 28 days exposure. The weight of live earthworms was determined at the beginning and the end of the study. The statistical analysis of LC50 was performed by logit analysis and percent of weight losses were analysed by an one way analysis of variance.

Findings:

After 7, 14 and 28 days no mortalities were observed in untreated control (validity criterion was met) and at the concentration level of 10 mg/kg. At 100 mg/kg 0 %, 10 % and 13 % and at 1000 mg/kg 10 %, 10 % and 23 % mortality was observed after 7, 14 and 28 days. Furthermore at these concentration levels earthworms were found to congregate together. After 28 days a significant reduction in weight was reported at 100 and 1000 mg/kg. Thus, the NOEC (behaviour and weight) and NOLC were 10 mg/kg. The 14 day and 28 day LC_{50} was estimated to be > 1000 mg/kg. For the reference substance 2-chloroacetamide the 14 day LC_{50} was calculated to be 90 mg/kg

Conclusion: 14 day LC_{50} > 1000 mg/kg

Comments (RMS):

Deviations to the OECD guideline: Only three replicates per treatment were tested. Earthworms were exposed under a 16 hours light / 8 hours dark photoperiod. However, the study is considered acceptable.

Formulation

Reference: Yearsdon, H. A., Burgess, A. J., Coulson, J. M.(1991): Fluazinam: Toxicity to the

Earthworm (*Eisenia fetida*) of a 500g/litre Suspension Concentrate Formulation. Report No. RJ1087B

Guideline: OECD Guideline 207

GLP: Yes

Test item: Fluazinam 500 g/L SC, purity: 38.4 % w/w equivalent to 495 g ai/L (specific gravity: 1.29), batch no: not stated

Material and methods:

The influence of the formulation to earthworms of the species *Eisenia fetida* was examined in an artificial soil test. The test soil was a mixture of 70 % fine silica sand, 20% kaolin clay, 10 % peat and 5 g $CaCO_3$/kg. The following nominal test concentrations were studied: 138 and 1376 mg formulation/kg artificial dry soil beside an untreated control. The test item was mixed directly to the soil and deionised water was added to reach the moisture content up to 50 %. Two tests were run, each consisting of two replicates of each treatment and control. Ten worms were placed to each test soil, before each batch of 10 individuals was weighed. The following test conditions were maintained during the study: The pH was within the range of 6.0 ± 0.2, the soil moisture contents were from 45 to 50 %, the temperature was 20 ± 2°C and the cultures were continuously illuminated. The effects of a toxic standard (2-chloroacetamide) were studied under identical test conditions. After 7 and 14 days the mortality and abnormal behaviour of earthworms were assessed. Furthermore the bodyweight of each batch of 10 individuals were determined at the end of the test. The statistical analysis is based on the obtained data from both tests. For mortality of fluazinam a one-way analysis of variance and for the change of final bodyweight a one-way analysis of covariance were performed.

Findings:

The LC_{50} of the toxic standard 2-chloroacetamide was determined to be 38 mg/kg.

After 14 days the mortality in control was 5 %. At 138 mg product/kg 8 % mortality and at 1376 mg product/kg 23 % mortality was noted, which was not significantly different (at 5 % level) when compared to control. The LC_{50} was determined to be > 1376 mg product/kg. The bodyweight was significantly effected at both concentration levels, therefore a NOEC could not be determined.

Conclusion: LC_{50} > 1376 mg product/kg (dry weight), corresponding to LC_{50} > 682 mg ai/kg (dry weight)

Comment (RMS): Study considered acceptable.

Metabolites

Reference: Lührs, U (2000): Acute Toxicity (14 Days) of HYPA to the Earthworm *Eisenia fetida* (Savigny 1826) in Artificial Soil. No. 8691021

Guideline: OECD Guideline 207

GLP: Yes

Test item: HYPA, purity 99.7 %, Lot No: 0006

Material and methods:

The effect of HYPA, metabolite of fluazinam, on the earthworm *Eisenia fetida* has been determined

in a laboratory test using artificial soil (10 % sphagnum peat, 20 % kaolinite clay, 69.5 % quartz sand, 0.5 % $CaCO_3$ to adjust pH to 6.0 ± 0.5). Following a range finding test batches of artificial soil were treated with six concentrations: 269, 350, 455, 592, 769 and 1000 mg/kg. Four replicates with ten earthworms were prepared for each concentration level and control. First the test substance was mixed with 10 g quartz sand and then into 2050 g dry artificial soil. After mixing the soil was moistened and the water content was maintained at ca. 28 % during the study. The pH were in the range of 6.3 – 6.5. The cultures were exposed under continuous light at a temperature of 20 – 21°C.

Findings:

The control mortality was 0 %. After 14 day no mortality was observed up to concentrations of 769 mg/kg. At highest tested concentration (1000 mg/kg) 2.5 % of earthworms were dead, this was not significant differed when compared to control. Thus the NOLC was 769 mg/kg and the EC_{50} was > 1000 mg/kg. The weight of surviving earthworms decreased in all treatment groups after 14 days. A significant decrease was found at a concentration of 350 mg/kg and higher concentrations (Dunnett-test). Furthermore at the two highest concentrations worms were lethargic and flabby. The NOEC (weight) was determined to be 269 mg/kg and LOEC was 350 mg/kg.

Conclusion: 14 day LC_{50} >1000 mg/kg

Comment (RMS): Study considered acceptable

B.9.6.2 Sublethal effects (Annex IIA 8.4, Annex IIIA 10.6.1)

Formulation

Reference: Römbke, J., Moser, Th. (1999): A Study on the Reproduction Toxicity of Fluazinam 500 g/L SC to the Earthworm *Eisenia andrei* (Bouche). Report No. F3RR (Reference: WINo. 45373)

Guideline: BBA Guideline VI, 2-2

GLP: Yes

Test item: Fluazinam 500 g/L SC, purity: 39.4 % w/w, formulation no: YF8053, batch no: H 692 A

Material and methods:

The effects of fluazinam 500 g/L SC formulation on the survival, growth and reproduction of *Eisenia andrei* was carried out in an artificial soil system over a 8 week period. Earthworms (adult worms with clitellum, 250 – 600 mg fresh weight) were exposed to nominal concentrations of 0.35, 1.75, 3.5, 17.5 and 35 mg ai/kg, beside a toxic reference (0.665 mg benomyl/kg) and a control. The test substance was applied by thoroughly mixing into the artificial soil. The soil was composed by 10 % Sphagnum peat, 20 % kaolin-clay, 68 – 69 % quartz sand and 1 % $CaCO_3$. Deionized water was added to achieve a water content of 40 – 60 % of MHC. 650g moist artificial soil (corresponding to 500 g dry weight) was filled into the test vessels to obtain a 5 cm depth of the soil, additionally 5 g dried cow manure were mixed into the soil. For each concentration and control four replicates were prepared. At the start of the test 10 individually weighted worms were placed into each test vessel. During the test earthworms were fed with different amounts of cow manure. After four week

exposure adult worms were removed and surviving worms were weighted. Further morphological and behavioural changes were recorded. The cocoons were kept another four weeks in the treated test soil. At the end of the test (week 8) juvenile worms were sampled using water bath method. Numbers of juveniles per test vessels, morphological and behavioural changes were noted. The test conditions in the course of the study were as follows: Temperature: 19 – 21.2 °C, 16/8 hours light/dark cycle, pH: 5.7 – 6.4 and soil moisture: 40.6 – 52.6 % (dw). Results were statistically analysed by ANOVA and Dunett t-test.

Findings:

Validity criteria: After four weeks the mortality of adults in the control was 5 % and the biomass of adult test animals increased with 20.4 %. After eight weeks the mean number of juveniles per replicate in the control was 14 ± 10.2 (mean number of offspring: 47 ± 10.2) with an coefficient of variation of 21.7 %.

Mortality: After four weeks the mortality was low with 0 – 7.5 % in all treatment levels and not significantly different when compared to control. Thus the LC50 was > 35 mg ai/kg dw.

Biomass (bodyweight): An increase of biomass was noted for adult animals within the test period of four weeks (19 – 45.2 % of initial weight). This increase was in the same range as recorded for the untreated control. Thus the NOEC for biomass was 35 mg ai/kg dw.

Feeding rate: The feeding rate was not affected.

Reproduction: After eight weeks the number of juvenile was significantly reduced at all tested concentrations, therefore it was not possible to determine a NOEC. The LOEC (reproduction) was ≤ 0.35 mg/kg.

Morphological and behavioural changes: At all concentrations juveniles were relatively small when compared to control. However, behavioural changes were not recorded.

Conclusion: NOEC (biomass, adults): ≥ 35 mg ai/kg dw, NOEC (reproduction): < 0.35 mg ai/kg

Comment (RMS): Study considered acceptable.

B.9.6.3 Field studies (Annex IIA 8.4, Annex IIIA 10.6.1)

Formulation

Reference: Mills, H. (2001): Field Study to Determine the Effects of a 500 g/L SC Formulation of Fluazinam on Earthworms. Report No. 1883/001-D2143

Guideline: BBA Guideline VI, 2-3

GLP: Yes

Test item: Fluazinam 500 g/L SC, purity: 39.7 ± 2.5 % equivalent to 516.1 ± 3.3 g/L, batch no: O/0001036

Material and methods:

The toxicity of the formulation Fluazinam 500 g/L SC to earthworms was assessed in a field study over a twelve month period. The effects of the formulation were tested in two application rates: 10 x 200 g ai/ha at 7 day intervals and ten gradated applications (140, 126, 6 x 58, 128 and 128 g ai/ha. This gradated application procedure should simulated the ground exposure of an application to growing potato crops with interception. The product was applied by spraying to

simulate normal using conditions. The study site was a commercial grassland located in North Yorkshire, UK. The soil was a clay loam soil type, for soil characteristics see table B.9.6.3-1. At the test site three predominant earthworm species were identified: *Lumbricus terrestris*, *Allolobophora rosea* and *Satchellius mammalis*. The site comprised four plots, which were replicated four times in a randomised block design: One plot served as untreated control, two plots were treated with the formulation and one plot was treated with the toxic reference (Benalate 50 % WP, active ingredient: benomyl, application rate: 4 kg ai/ha). Each replicate plot was 10 m x 10 m, separated by a distance of 1.5 m on each side. Five weeks before the first application of test item the test side was treated with a total herbicide (Gramoxone 100, active ingredient: paraquat) to kill all present vegetation. Then the grass was cut and the site was cultivated using a harrow. Until the first application of test item there were no visible living vegetation. The trial site was re-seeded six weeks after the first application of test item. An irrigation program was necessary due to untypical rain free periods during the course of the study. The irrigation was adapted to local conditions. Meteorological data were recorded at each time of application. Earthworms were collected at following sample intervals: Five days prior to application (T1-5d), five days after 4th application (T4+5d), four days after 10th application (T10+4d) and 5, 6 and 12 month after first application (T1+5month, T1+6month, T1+12month). The worms were collected using a 0.49 m² quadrat (0.7 m x 0.7 m) after drenching the soil area with 0.2 % formaldehyde solution. Animals were stored in 5 % formaldehyde solution. Adult individuals were identified up to species level (immature earthworms were recorded as tanylobus or non-tanylobus) and number of species per m² was determined. For the determination of the biomass the preserved worms were put onto filter paper to remove adhering liquid and then weighted. All results were tested for normality (Shapiro-Wilk test) and for homogeneity of variance (Levene's test). Further treated groups were compared with control using one-way of covariance (ANOVA) and pair wise comparisons using Dunett's test.

Table B.9.6.3-1: Soil characteristics

Soil	% sand	% silt	% clay	% OC	% OM	pH (KCl)	% WHC (at pF 0.0001.bar)
clay loam	41	32	27	4.9	8.4	6.7	102.1

Findings:

Five days prior to first application an average of 188 earthworms/m² were found. Predominant species were *Lumbricus terrestris* (33 % of total adult) *Allolobophora rosea* (13 % of total adult) and *Satchellius mammalis* (6 % of total adult).

Effects of the toxic standard (benomyl):

At collection time T10+4days and T10+5month the reduction of population was noted with 34 % and 27 %. The reduction of total biomass was 46 % and 54 % at the mentioned collection time. Additionally the appearance of the earthworms was clearly different when compared to earthworms collected from control plots and plots which were treated with fluazinam: The worms were much more lethargic, darker in colour and smaller in size.

Effects of fluazinam:

At no collection time significant effects on population and on total biomass of earthworms collected from plots which were treated with fluazinam were found.

Conclusion:

Application of fluazinam with a rate of 10 x 200 g ai/ha at 7 day intervals and ten gradated applications (140, 126, 6 x 58, 128 and 128 g ai/ha) had no significant effects on the earthworm population and on the total biomass under tested field conditions.

Comment (RMS): Study considered acceptable.

B.9.6.4 Risk assessment for earthworms

The acute and long term TER values for earthworms were calculated as the ratio between the acute and the chronic effect concentrations (LC50 and NOEC), respectively and the initial PECs. Worst case PECs values of active substance and HYPA after the 10th application in potatoes were calculated to be 0.54 mg/kg (fluazinam) and 0.114 mg/kg (HYPA); details for calculations see section B.8.3.

Table B.9.6.4-1: TER acute for earthworms

Test substance	14 days LC_{50} (mg as/kg soil)	initial PECs (mg/kg)	TER acute	TER acute corrected*	Trigger value
Fluazinam	> 1000	0.54	> 1852	> 926	10
Fluazinam 500 SC	> 682	0.54	> 1263	> 632	10
HYPA	> 1000	0.114	> 8772	> 4386	10

* corrected by a factor of 2 due to the high $\log_{POW}$ of 4.03 of fluazinam

Table B.9.6.4-2: TER long term for earthworms

Test substance	NOEC (mg as/kg soil)	initial PECs (mg/kg)	TER lt	TER lt corrected*	Trigger value
Fluazinam 500 SC	< 0.35	0.54	< 0.65	0.325	5

* corrected by a factor of 2 due to the high $\log_{POW}$ of 4.03 of fluazinam

Active substance and formulation:

The calculated acute TER value for fluazinam (tested as active substance and formulation) is well above the trigger value of 10, thus the acute risk to earthworms after the application can be considered as low. Due to the high number of applications in potatoes (10 x 200 g as/ha) a reproduction test was required. The effects of the formulation on the reproduction of earthworms were studied. The calculated TER value for this long term exposure is < 1 and does not meet the relevant trigger, thus a higher tier earthworm field study was performed. Under realistic field exposure conditions the active substance fluazinam (applied at a rate of 10 x 200 g as/kg in potatoes) had no significant effects on the earthworm population and on the total biomass. Therefore the long term risk for earthworms after the application of fluazinam is considered

acceptable.

Metabolite:

The acute TER value for the metabolite HYPA is also far above the trigger value of 10.The long term risk will be covered by the field study with fluazinam. Thus, it can be assumed that HYPA poses no unacceptable risk to earthworms.

B.9.7 Effects on soil non-target macro-organisms (Annex IIA 8.6, IIIA 10.6.2)

B.9.7.1 Effects on collembola

Formulation

Reference: Klein, S. & Meister, A. (2002): Effects of IKF-1216 500 SC (Fluazinam 500SC) on Reproduction of the Collembola *Folsomia candida* in Artificial Soil Report No. 13781016

Guideline: ISO 11267, 1999

GLP: Yes

Test item: Fluazinam 500 SC, purity: 39.4 % ± 0.07 w/w equivalent to 500.7 ± 0.96 g/L (specific density 1.272), batch no: 31 111/01

Material and methods:

The study was conducted to determine the effects of the formulation Fluazinam 500 SC on the reproduction of springtails (collembola) *Folsomia candida* in artificial soil. Springtails (10 – 12 days old) were exposed to 3.12, 6.25, 12.5, 25, 50 and 100 mg formulation/kg artificial soil dw and control (treated with deionised water) for 28 days. Ten individuals were transferred to each test unit. Vessels were closed by plastic–lids, but were ventilated by opening twice a week. For each treatment group and control 5 replicates and one additional test vessel for measurements of pH and water content were prepared and were incubated under following test conditions: 19 – 21 °C in a controlled environment room, a 16 hours light/8 hours dark photoperiod with a light intensity of 400 – 700 lux. The pH values and humidity were checked at the start and the end of the study. Additionally the water content was controlled at day 14 and if necessary water losses were compensated. Collembola were fed with 2 mg of granulated dry yeast at the beginning of the test and after 14 day dry yeast was added *ad libidum*.

After 28 days exposure test substrate of each test unit were added into another container and carefully mixed with tap water and black ink. Living adults and juvenile collembola were drifted to the surface and were counted under binocular microscopes.

Mortality data were statistically analysed by Fisher's Exact test and EC50 were calculated by Logit-analysis. For the analysis of the reproduction first the data were checked for normal distribution and homogeneity (Kolmogoroff-Smirnov-Test and Cochran –Test) and than analysed by Dunnett-Test.

A toxic standard (active ingredient: phenmedipham) is tested at least once a year to ensure that laboratory conditions are adequate and to verify the response of test organisms.

Findings:

The water content was 32 – 33 % (corresponding 52 – 53 % WHC) at the start of the test and 31 – 33 % (corresponding 50 – 53 % WHC) at the end of the test. The pH was 5.8 at day 0 and 5.6 after

28 days.

Table B.9.7.1-1: Effects of the formulation on mortality of adult collembola and reproduction after 28 days.

endpoints	control	Fluazinam 500 SC [mg/kg]					
		3.13	6.25	12.5	25.0	50.0	100
surviving adult (mean)	9.4	7.8	6.4	7.4	7.4	6.8	4.2
mortality [%]	6	22*	36*	26*	26*	32*	58*
reproduction rate (mean)	903.2[a)]	833.9	672.6	699.8	536.8	193.9	0.2
% of control	-	92.3	74.5**	77.5**	59.4**	21.5**	0**

*significantly different when compared to control (Fisher exact test, α = 0.05)

**significantly different when compared to control (Dunett test, α = 0.05)

[a)] the coefficient of variation of control reproduction was 4.8 %

At all tested concentration the mortality of adult collembola was significantly effected when compared to control, therefore a NOEC and a LOEC could not be estimated. The LC50 was calculated to be 80.3 mg formulation/kg artificial soil dw (CL95% 44.6 – 144.5 mg formulation/kg artificial soil), corresponding to 40.2 mg fluazinam/kg artificial soil. The reproduction rate was significantly effected at 6.25 mg formulation/kg (3.13 mg fluazinam/kg), thus the NOEC was estimated to be 3.13 mg formulation/kg (1.57 mg fluazinam/kg) and the LOEC = 6.25 mg formulation/kg (3.13 mg fluazinam/kg). The EC50 was determined to be 27.9 mg formulation/kg artificial soil, corresponding to 14 mg fluazinam/kg artificial soil

Conclusion: Mortality: NOEC < 1.57 mg ai/kg, LOEC ≤ 1.57 mg ai/kg, Reproduction: NOEC = 1.57 mg ai/kg, LOEC = 3.13 mg ai/kg

Comment (RMS): Study considered acceptable.

Metabolite

Reference: Lührs (2004): Effects of HYPA on Reproduction of the Collembola *Folsomia candida* in Artificial Soil, Report No. 19161016

Guideline: ISO 11267, 1999

GLP: Yes

Test item: HYPA, purity: 99.4 %, lot no: 0205

Material and methods:

The effects of metabolite HYPA on reproduction of collembola *Folsomia candida* were studied in artificial soil over 28 days. Springtails were exposed to following concentrations of HYPA: 0.38, 0.76, 1.52, 3.04, 6.08 mg/kg artificial soil dw, an untreated control and a solvent control (acetone). Test units were prepared by filling 30 g ± 1.5 g (wet weight) artificial soil treated with test item or deionised water (control) in glass vessels (100 mL, 5 cm diameter). The artificial soil was prepared according to OECD 207: 10 % sphagnum peat, 20 % kaolin clay, 69.5 % quartz sand, 0.5 % $CaCO_3$. Ten test organisms (10 – 12 days old) were placed to each vessel and than these test

units were closed by plastic-lids. For each concentration and the control five replicates and one additional test vessel for measurements of pH and water content were prepared. All test units were incubated in a controlled environment room with 20 – 21 °C and a 16 hours light (light intensity of 400 – 560 lux) and 8 hours dark photoperiod. Twice a week the plastic-lids were opened to allow ventilation. The pH values and humidity were checked at the start and the end of the study. Additionally the water content was controlled at day 13 and if necessary water losses were compensated. Collembola were fed with 2 mg of granulated dry yeast at the beginning of the test and after 13 day dry yeast was added *ad libidum*.

After 28 days of exposure the content of test units were suspended in water and tinted with dark ink. The number of living adult and juvenile collembola were recorded by counting under binocular microscope.

Mortality data were statistically analysed by Fisher's Exact test. For the analysis of the reproduction first the data were checked for normal distribution and homogeneity (Kolmogoroff-Smirnov-Test and Cochran –Test) and than the treatment groups were compared with the pooled controls using Dunnett-Test.

A toxic standard (active ingredient: phenmedipham) is tested at least once a year to ensure that laboratory conditions are adequate and to verify the response of test organisms.

Findings:

The water content was 35 % equivalent to 50 – 51 % of MWHC at the start of the test and 31 – 33 % (corresponding to 46 – 48 % of max. WHC) at the end of the test. The pH was in the range of 6.0 – 6.5 at day 0 and ranged from 6.2 to 6.5 after 28 days.

Table B.9.7.1-2: Effects of the metabolite HYPA on mortality of adult collembola and reproduction after 28 days.

endpoints	control	solvent control	HYPA [mg/kg]				
			0.38	0.76	1.52	3.04	6.08
surviving adult (mean)	8	10	9	9	9	9	9
mortality [%]	18±4	2±4	12±18	12±13	8±8	12±8	10±14
reproduction rate (mean)	524	464	536	536	514	465	430
% of pooled control	89[2]		108	109	104	94	87

[1] Between untreated and solvent control were no significant statistical differences, therefore the controls were pooled.

[2] The coefficient of variation of untreated control reproduction was 16.6 % and of solvent control 8.6 %.

No significant effects of HYPA on the mortality of adults and on the reproduction rate were recorded. Thus the NOEC for both endpoints was estimated to be 6.08 mg/kg artificial soil dw.

Conclusion: Mortality and reproduction: NOEC = 6.08 mg HYPA/kg artificial soil

Comment (RMS): Study considered acceptable.

B.9.7.2 Effects on the degradation in litter bags

No study submitted, however study is required due to significant effects in collembola reproduction

test. The Notifier has started the required study in July 2005.

B.9.7.3 Risk assessment of other soil non-target macro-organisms

Studies to assess the risk for collembola were performed with the metabolite HYPA and the formulation Fluazinam 500 SC.

The TER values for collembola were calculated as the ratio between the NOEC and the initial PECs. Worst case PECs values of active substance and HYPA after the 10th application in potatoes were calculated to be 0.54 mg/kg (fluazinam) and 0.114 mg/kg (HYPA); details for calculations see section B.8.3.

Table B.9.7.3-1: TER long term for collembola

Test substance	NOEC (mg as/kg soil)	initial PECs (mg/kg)	TER lt	TER lt corrected*	Trigger value
Fluazinam 500 SC	< 1.57	0.54	< 2.9	< 1.45	5
HYPA	6.08	0.114	53	26.5	5

* corrected by a factor of 2 due to the high log POW of 4.03 of fluazinam

The calculated TER value for fluazinam (tested as the formulated product) of < 1.45 is indicated a high risk for soil non-target macro-organisms. Therefore a litter bag test under field conditions is required to evaluate the risk of the active substance. The notifier has started a litterbag study in July 2005.

For the metabolite HYPA the TER value is well above the required safety factor and the risk can be concluded as acceptable.

B.9.8 Effects on soil non-target micro-organisms (Annex IIA 8.5, IIIA 10.7)

Formulation

Reference: Reis, K-H. (2002): Effects of Fluazinam 500 SC on the Activity of the Soil Microflora in the Laboratory. Report No. 9321080

Guideline: OECD 216 and 217

GLP: Yes

Test item: Fluazinam 500 SC purity: 39.49 ± 0.25 % w/w corresponding to 516.1 ± 3.3 g/L, batch no: O/0001036

Material and methods:

The effects of the formulation Fluazinam 500 SC on the nitrogen and carbon transformation by soil micro-organisms were studied in a loamy sand soil in laboratory. Two doses of the formulation were tested: 0.684 mg formulation/kg soil dw (corresponding to 0.27 mg fluazinam/kg, which is equivalent to a single foliar application with 200 g ai/ha) and 5.748 mg formulation/kg soil dw (corresponding to 2.27 mg fluazinam/kg, which is representing the PEC plateau value after ten years, each year with application rates of 800 g ai/ha by seed treatment and 10 x 200 g ai/ha by foliar application). The test item was solved in deionised water and was mixed into the soil (soil characteristics in detail: see table B.9.8-4) using a laboratory mixer. For the toxic standard, dinoterb

was dissolved in acetone and applied onto quartz sand, after the acetone was evaporated the quartz sand was mixed into the soil. The treated soil was filled into test vessels (plastic boxes): 1000 g soil for carbon test and 300 g soil for nitrogen test. Additionally for the nitrogen transformation test 0.5 % lucerne meal (41.7 % C-content, 2.86 % N-content, C/N ratio: 14.6/1) was added to the soil. Controls were prepared in the same way, but without the treatment of test item.

Table B.9.8-1: Soil characteristics

Soil	% sand	% silt	% clay	% OC	pH	total N (mg/100g soil dw)	Biomass (mg C/kg soil dw)	WHC (ml H_2O/100 g soil dw)
loamy sand	52.2	37.5	10.3	1.34	7.4	1.84	268 ± 71.1	48

All test units (3 replicates per treatment group, control and toxic standard) were incubated in an air conditioned room with temperature in the range of 20 – 22 °C into the dark. The moisture was controlled at day 0 and afterwards once a week and if necessary evaporated water was replaced. The pH values were checked at each sampling date.

Soil samples were taken after 0 (within 6 hours), 7, 14 and 28 days:

For the determination of C-transformation glucose induced respiration rate was measured. The soil samples (100 g) were taken and mixed with 5 g/kg (moist soil) of glucose (2 mL of a solution of 250 g glucose/L), then samples were incubated at 20 ± 2°C. The oxygen consumptions were measured up to 24 hours. For calculation of short term respiration the linear part of the curve (2 hours up to 14 hours) was used.

For the determination of N-content soil samples were extracted with 0.1 M KCL-solution. After centrifugation and filtration the extracts were analysed for ammonium, nitrate and nitrite. However ammonium content was not determined at days 0 and 7 due to technical problems.

For statistical analysis all obtained data were tested for normality and homogeneity of variance by R/S test and Bartlett's test. The variations between the replicates of the controls and the variations between treatments groups and control were analysed by Student-t-test.

Findings:

The pH values for both transformation tests were in the range of 7.3 – 7.4. The mean soil water content for the control and both treatment groups was in the range of 42.3 – 43.5 % in C-transformation test and 46.3 – 47.5 % in N-transformation test. The variations between replicate control samples on day 28 were 2.89 % (C-transformation) and 13.2 % (N-transformation).

Table B.9.8-2: Summary of Fluazinam 500 SC formulation effect on microbial respiration rate (mg CO2/100 g soil dry wt/h)

Day	Control		Fluazinam 500 SC (0.684 mg/kg soil)		Fluazinam 500SC (5.748 mg/kg soil)		Dinoterb (75 mg/kg soil)	
	respiration rate	replicate variation [%]	respiration rate	dev. [%]	respiration rate	dev. %	respiration rate	dev. [%]
0	2.083	7.97	2.121	1.82	2.175	4.42	1.189	-42.9

7	2.572	2.73	2.377	-7.58	2.196	-14.6	2.404	-6.53
14	1.834	7.62	1.775	-3.22	1.496	-18.4	1.653	-9.87
28	1.867	2.89	1.754	-6.05	1.813	-2.89	1.385	-25.8

Table B.9.8-3: Effects of formulation Fluazinam 500 SC on soil nitrogen content (mean values)

Treatment group	N-species	mg N/100g dry weight				% deviations from control on day 28
		0 d	7 d	14 d	28 d	
control	NH_4-N	nd	nd	-	-	---
	NO_2-N	-	-	-	-	---
	NO_3-N	1.84	0.75	1.44	2.52	---
	N_{min}	1.84	0.75	1.44	2.52	---
Formulation 0.684 mg/kg	NH_4-N	nd	nd	-	-	---
	NO_2-N	-	-	-	-	---
	NO_3-N	0.9	0.5	1.22	1.96	-22.2
	N_{min}	0.9	0.5	1.22	1.96	-22.2 *
formulation 5.748 mg/kg	NH_4-N	nd	nd	-	-	---
	NO_2-N	-	-	-	-	---
	NO_3-N	1.05	0.61	0.92	2.49	-1.19
	N_{min}	1.05	0.61	0.92	2.49	-1.19
dinoterb	NH_4-N	nd	nd	2.93	4.18	---
	NO_2-N	0.86	-	-	-	---
	NO_3-N	0.4	0.84	0.96	1.58	-37.3 *
	N_{min}	1.26	0.84	3.89	5.76	129 *

nd : not detected
-: below the limit of quatification
*: significant according Student-t-test, two sided, α = 0.05

Conclusion:
For nitrogen and carbon transformation the differences of the transformation rate between control soil and soil treated with the formulation Fluazinam 500 SC, applied with dose rates of 0.684 mg/kg soil and 5.748 mg/kg soil (corresponding to 0.27 mg fluazinam/kg and 2.27 mg fluazinam/kg) were less than 25 % after 28 days. Therefore the formulation had no unacceptable long-term adverse effects on the soil microflora under test conditions.
Comment (RMS): Study considered acceptable.

Metabolite

Reference: Reis, K. (2002): Effect of HYPA on the Activity of the Soil Microflora in the Laboratory. Report No. 8692080

Guideline: OECD 216 and 217

GLP: Yes

Test item: HYPA, purity: 99.7 %, Lot no. 0006

Material and methods:

The effects of the metabolite HYPA on the nitrogen and carbon transformation by soil micro-organisms were studied in a loamy sand soil in laboratory. Two doses of HYPA were tested: 0.03 mg/kg (equivalent to a single foliar application with 200 g ai/ha) and 0.38 mg/kg (corresponding to PEC plateau value of HYPA after ten years, each year with application rates of 600 g ai/ha by seed treatment and 10 x 200 g ai/ha by foliar application). The test item was solved in acetone and applied onto quartz sand. After evaporation of the solvent the quartz sand was mixed into the soil (soil characteristics in detail: see table B.9.8-1) and filled into test vessels (plastic boxes). Controls were prepared in the same way, but without the treatment of test substance. For the nitrogen transformation test 0.5 % lucerne meal (41.7 % C-content, 2.86 % N-content, C/N ratio: 14.6/1) was added to the soil.

Table B.9.8-4: Soil characteristics

Soil	% sand	% silt	% clay	% OC	pH	total N (mg/100g soil dw)	Biomass (mg C/kg soil dw)	WHC (ml H_2O/100 g soil dw)
loamy sand	52.2	37.5	10.3	1.34	6.1-7.5	1.1	268	48 - 52

All test units (3 or 4 replicates per treatment, control and toxic standard) were incubated in an air conditioned room with temperature in the range of 20 – 22 °C in the dark. The moisture was regularly controlled and if necessary adjusted to ca. 40 – 50 % by adding deionised water. Soil samples were taken within 6 hours after application and after day 7, 14 and 28. For the determination of C-transformation, glucose induced respiration rate was measured. Appropriate soil samples (100 g) were taken and mixed with 5 g/kg (moist soil) of glucose and incubated at 20 ± 2°C. The oxygen consumptions were measured up to 24 hours using BSB Sensomat system. For the determination of N-content soil samples were extracted with 0.1 M KCl-solution. After centrifugation and filtration the extracts were analysed for ammonium, nitrate and nitrite.

Findings:

For C-transformation test pH values ranged from 7.4 to 7.5 and for N-transformation test pH ranged from 6.0 to 6.1. The WHC was in the range 41 – 47 % in the soil of test units for control, treatment and toxic standard of both transformation tests.

The variation between replicate control samples on day 28 was 14 % (soil respiration) and 8.41 % (N-transformation).

Table B.9.8-5: Effects of HYPA on soil respiration

	soil respiration (mg CO_2/100 g soil dry weight/hr)			
Day	**Control**	**HYPA (0.03 mg/kg soil)**	**HYPA (0.38 mg/kg soil**	**Dinoterb (75 mg/kg soil)**

	Respiration Rate	Replicate variation (%)	Respiration Rate	Dev. %	Respiration Rate	Dev. %	Respiration Rate	Dev. %
0	1.587	15.1	1.206	-24.0	1.529	-3.65	1.439	-9.33
7	0.991	5.76	0.993	+0.20	0.986	-0.50	1.018	+2.72
14	1.135	19.2	1.113	-1.94	1.124	-0.97	1.045	-7.93
28	1.164	14.0	1.213	+4.21	1.230	+5.67	0.773	-33.6

Table B.9.8-6: Effects of HYPA on soil nitrogen content (mean values)

Treatment group	N-species	mg N/100g dry weight 0 d	7 d	14 d	28 d	% deviations from control on day 28
control	NH_4-N	0.70	0.21	-	-	--
	NO_2-N	0.09	0.03	-	-	--
	NO_3-N	1.19	0.95	2.1	3.18	--
	N_{min}	1.98	1.19	2.1	3.18	--
HYPA 0.03 mg/kg	NH_4-N	0.59	-	-	-	
	NO_2-N	0.12	0.08	-	-	
	NO_3-N	1.23	0.95	1.7	2.81	-11.6*
	N_{min}	1.94	1.03	1.7	2.81	-11.6*
HYPA 0.38 mg/kg	NH_4-N	0.53	-	-	-	--
	NO_2-N	0.10	0.07	-	-	--
	NO_3-N	1.32	1.11	1.96	3.03	-4.72
	N_{min}	1.95	1.18	1.96	3.03	-4.72

-: below the limit of quantification
*: significant according Student-t-test, two sided, α = 0.05

Conclusion:
After 28 days of incubation the deviations between control soil and soil treated with HYPA (applied with dose rates of 0.03 mg/kg soil and 0.38 mg/kg soil) was < 25 %, thus HYPA had no adverse effects on soil respiration and N-transformation under test conditions.
Comment (RMS): Study considered acceptable.

Reference: Reis, K. (2004): Effect of HYPA on the Growth of Pure Cultures in Soil Fungi in the Laboratory. Report No. 19164083
Guideline: none
GLP: Yes
Test item: HYPA, purity: 99.4 %, batch no. 0205

Material and methods:

The effects of metabolite HYPA on the growth of fungi on agar plates containing sterilized soil were assessed in laboratory. Pure active cultures of four species of soil fungi were exposed to following concentrations of HYPA: *Paecilomyces marquandii*: 21.4, 49.3, 113.4, 260.9 and 600 mg/kg soil dw, *Fusarium oxysporum*: 3.7, 11.1, 33.3, 100 and 300 mg/kg soil dw, *Rhizoctonia solani*: 15.6, 31.3, 62.5, 125, 250 mg/kg soil dw and *Mucor circinelloides*: 21.4, 49.3, 113.4, 260.9 and 600 mg/kg soil dw. The test item was mixed into sterilized soil and 10 g were weighted into petri dishes and mixed with 20 mL liquid nutrient agar (malt extract agar) . Five replicates were prepared for each treatment, control and solvent (acetone) control. Agar plates were inoculated using platelets of agar containing the respectively growing mycelium. Inoculated test units were incubated at room temperature (20 – 22°C) in the dark up to 14 days. From day 3 mycelium measurements were performed until the control plates were full of mycelium. The average colony diameter of treated test units and untreated controls were compared and statisically analysed. ECx were calculated by LOGIT-analysis.

Findings and conclusions:

Table B.9.8-7: Effects of HYPA on the growth of soil fungi

Species	Incubation period	Measurement day EC_{10}/EC_{50}/NOEC	EC_{10}	EC_{50}	NOEC
			mg/kg soil dw		
Paecilomyces marquandii	14 d	7/10/14	36.8	1067.5	21.4
Fusarium oxysporum	5 d	3/7/5	42.9	439.2	11.1
Rhizoctonia solani	7 d	3/4/5	76.8	305.5	62.5
Mucor circinelloides	5 d	3/3/3	113.8	495.2	21.4

Comment (RMS): Study considered acceptable. However study is not necessary according 91/414/EWG and provided additional information only.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be a risk (Annex IIA 8.6)

B.9.9.1 Effects on terrestrial non-target plants

Active Substance: Fluazinam technical

Reference: Backus, P. (1993a): Effect of IKF-1216 (Fluazinam) on Seed Germination/Seedling Emergence (Tier I). Document No. 5549-92-0483-BE-001

Test guideline: Pesticide Assessment Guidelines, Subdiv. J Hazard Evaluation: Non-target Plants and Hazard Evaluation Division Standard Evaluation Procedure Non-target Plants.

GLP: Yes

Material and methods:

This study was performed to determine the effects of technical fluazinam (Lot No. 1030/91, purity 97.3 %) on seed germination/seedling emergence for ten non-target species of plants representing

dicotyledons (six species) and monocotyledons (four species). The plants evaluated were buckwheat (*Fagopyrum esculentum*), corn (*Zea mays*), cucumber (*Cucumis sativus*), mustard (*Brassica kaber*), oat (*Avena sativa*), onion (*Allium cepa*), radish (*Raphanus sativus*), sorghum (*Sorghum bicolor*), soybean (*Glycine max*) and tomato (*Lycopersicon esculentum*). A single dose of 1.5 kg ai/ha was applied to two test systems, dosing solutions were adjusted for purity. In the seed germination bioassay, seeds on filter papers in Petri dishes were placed in direct contact with the test material. The Petri dishes were held in darkness for 6 days and, at the end of this period, percent of seed germination was recorded. A solvent control (acetone) and untreated control were also maintained. In the seedling emergence from soil bioassay, seeds were planted in soil, and the test material was applied directly to the soil surface. The bioassay was held for 14 d in an environmentally controlled greenhouse and then percent seedling emergence and fresh weights were recorded.

Findings:

The effects of technical fluazinam on seed germination, seedling emergence and fresh weight of seedlings compared to untreated controls are shown in the table below.

Table 9.9.1-1: Effects of technical fluazinam on seedling germination and emergence

Plant species	germination vs. controls (%)	emergence vs. controls (%)	effect on fresh weight vs. controls (%)
Buckwheat	111.43	100.00	112.77
Corn	102.56	100.00	99.17
Cucumber	100.00	94.12	100.44
Mustard	97.50	95.00	94.07
Oats	97.44	97.37	104.08
Onion	111.76	103.33	122.67
Radish	100.00	95.00	105.81
Sorghum	102.86	89.19	92.67
Soybean	100.00	105.41	109.92
Tomato	112.50	91.18	129.91

In conclusion, fluazinam shows no detrimental effect towards non-target crop seedling germination and emergence.

Comment (RMS): Study considered acceptable.

Reference: Backus, P. (1993b): Effect of IKF-1216 (Fluazinam) on Vegetative Vigor of Plants (Tier I). Report No. 5549-92-0484-BE-001

Test guideline: Pesticide Assessment Guidelines, Subdivision J Hazard Evaluation: Non-target Plants and Hazard Evaluation Division Standard Evaluation Procedure Non-Target Plants, Guideline 122-1.

GLP: Yes

Material and methods:

The effects of a single dose of fluazinam on plant foliage to ten non-target species of plants representing dicotyledons (six species) and monocotyledons (four species) were evaluated. The test material was IKF-1216 (technical fluazinam), Lot No. 1030/91, purity 97.3 %. The plants tested were buckwheat (*Fagopyrum esculentum*), corn (*Zea mays*), cucumber (*Cucumis sativus*), mustard (*Brassica kaber*), oat (*Avena sativa*), onion (*Allium cepa*), radish (*Raphanus sativus*), sorghum (*Sorghum bicolor*), soybean (*Glycine max*) and tomato (*Lycopersicon esculentum*). A single dose of 1.5 kg ai/ha was applied directly to plant foliage. After spraying, they were held in an environmentally controlled greenhouse for 14 days. Observations were recorded twice per week. Growth parameters or unusual growth, as compared to untreated controls, were noted. Phytotoxicity symptoms recorded included visible symptoms of injury, such as discoloration, malformation, desiccation, defoliation, or death. At the conclusion of the experimental phase of the study, all above-ground parts of the plants were cut off and fresh weights were recorded.

Findings:

The effects of technical fluazinam on vegetative vigour based upon effects on fresh weight, compared to untreated controls, are shown in the table below.

Table 9.9.1-2: Effects of Technical Fluazinam on Vegetative Vigour

Species Tested	% fresh weight vs. untreated control
Buckwheat	81.42
Corn	127.86
Cucumber	72.27
Mustard	82.48
Oats	91.83
Onion	93.01
Radish	89.26
Sorghum	109.15
Soybean	96.09
Tomato	77.84

The only species tested that exhibited inhibition greater than 25 % was cucumber. This species was re-tested at the multiple rate Tier II level.

Comment (RMS): Study considered acceptable.

Reference: Crosby, K. (1995): Effect of IKF-1216 on Vegetative Vigor of Plants (Tier II). Report No. 6363-95-0019-BE-001

Test guideline: Pesticide Assessment Guidelines, Subdivision J Hazard Evaluation: Non-target Plants and Hazard Evaluation Division Standard Evaluation Procedure Non-Target Plants, Guideline 123-1.

GLP: Yes

Material and methods:

This study was performed to determine the effects of a range of doses of fluazinam (IKF-1216) on plant foliage for one non-target species of crop plant, cucumber. The test material used was technical fluazinam, Lot No. 1030/91, purity 97.3 %. Doses of 0.05, 0.09, 0.19, 0.38, 0.75, and 1.5 kg ai/ha were applied directly to plant foliage. Three controls were used in the test: an untreated control, a water-treated control and a solvent-treated (acetone) control. Four replicates of each dosage and each control were applied. After the plants were sprayed with the treatment solution, they were held in an environmentally controlled greenhouse for 14 days. Observations were recorded weekly and were rated according to a rating system of phytotoxicity scores from 0 to 100 where 0 = no effect, 10-30 = slight effect, 40-60 = moderate effect, 70-90 = severe effect, and 100 = complete crop destruction. At the conclusion of the experimental phase of the study, all above-ground parts of the plants were cut off and fresh weights were recorded.

Findings:

The phytotoxicity as indicated by fresh weight of plants following 14 days of exposure, is shown in the table below.

Table 9.9.1-3: Effects of Fluazinam on fresh weight of cucumber plants

Rate (kg/ha)	Fresh Weight (g)
0 (pooled controls)	96.1
0.05	106.3
0.09	97.5
0.19	104.2
0.38	117.4
0.75	121.2
1.5	118.3

Fluazinam showed slight damage to cucumber foliage when applied as a series of doses including the highest use rate of 1.5 kg ai/ha. These effects were rated according to phytotoxicity scores of 4-12 (up to 30 = slight effect). For comparison, effects to the pooled controls ranged from 2-6 in this system. The effects were not systemic, and no detectable effect on plant growth, as expressed by fresh weight differences compared to controls, was seen at the end of the experiment. Thus, foliar doses of fluazinam up to rates of 1.5 kg ai/ha are unlikely to have significant, long-lasting effects on non-target plants.

Comment (RMS): Study considered acceptable.

Metabolite HYPA

Reference: Sugimoto, K., Hayashi, H. (2004): Terrestrial Plants, Growth Test assessing HYPA, metabolite of fluazinam, Report No. AL-0404-F-01

Test guideline: OECD 208

GLP: No

Material and methods:

The objective of this study is to determine possible toxic effects of soil-incorporated HYPA, a soil degradation metabolite of fluazinam, on the emergence of seedlings and the early stages of growth of a variety of terrestrial plants after a single application. The species oat (*Avena sativa*), turnip (*Brassica rapa*) and lettuce (*Lactuca sativa*) were selected. The soil (carbon content 1.09 %, pH 5.9) was collected from field (Japan). HYPA was mixed into the soil to achieve concentrations of 1, 10 and 100 mg HYPA/kg dry soil. Five seeds of each plant species were planted in one pot with 3.5 kg soil; 4 pots per concentration as well as an untreated control were tested in the glasshouse. 7 and 14 d after sowing the evaluation of the emergence percentage of seedlings was performed. Evaluation of the growth was done by comparison of dry weight between control and treated.

Findings:

HYPA incorporated into the soil did not significantly affect the seedling emergence and growth of the tested species. No phytotoxicity was observed on oat at any of the tested concentrations. The high rate (100 mg HYPA/kg dry soil) slightly retarded initial growth of turnip and lettuce. Thereafter growth became equivalent to control and dry weight of shoots was not significantly different from the control. The LC_{50} / EC_{50} values for all three species were considered to be > 100 mg HYPA/kg dry soil.

Comment (RMS): Study considered acceptable.

B.9.9.2 Risk assessment for terrestrial non-target plants

Terrestrial non-target plants may be exposed to fluazinam by spray drift in the vicinity of the treated area.

Fluazinam is mainly taken up via the leaves. It is applied 10 times at 200 g ai/ha in potatoes.

The most sensitive of the species and parameters tested was biomass fresh weight of cucumber (*Cucumis sativus*) determined in the vegetative vigour test. However, this effect of a 28 % reduction of fresh weight was not confirmed in a subsequent multi-dose test on cucumber. The EC_{50} for all species tested is therefore > 1.5 kg ai/ha. As the maximum single rate is 0.2 kg ai/ha, no significant effects of fluazinam or its soil metabolite HYPA on non-target terrestrial plants in- or off-field are expected.

B.9.10 Effects on biological methods of sewage treatment (Annex IIA 8.7)

Active substance

Reference: Sankey, S.A., Coleman, C.A., Tapp, J.F. (1992): Fluazinam: Toxicity to *Pseudomonas putida*. Report No. BL4364/B

Guideline: no
GLP: Yes
Test item: Fluazinam techn., purity: 98.1 %,
Material and methods:
The influence of fluazinam to the inhibition of growth of unique soil microorganism *Pseudomonas putida* was assessed according to the method described by Bringman & Kuhn (1980) and Slabbert (1986). Following nominal concentrations of fluazinam were tested: 0.56, 1.0, 1.18, 1.32 and 1.53 mg/L, additionally an untreated control and the toxic reference substance 3,5-dichlorophenol. All test flasks were incubated at 25 °C on a shaker (150 rpm) for six hours. After this incubation the degree of inhibition of a pure culture of the bacteria in a growth medium were measured when the cells are in logarithmic growth phase. The optical density was measured at 600 nm on a spectrometer (Uvikon 930).
Findings:
At highest tested concentration (1.53 mg/L) 42 % inhibition was noted, thus the EC_{50} was > 1.53 mg/L. The EC_{10} was calculated to be 0.73 mg/L.
Conclusion: EC_{50} > 1.53 mg/L, EC_{10}: 0.73 mg/L.
Comment (RMS): Study considered acceptable.

Reference: Grützner, I. (2000): Toxicity of Fluazinam to Activated Sludge in a Respiration Inhibition Test. Report No. 774887
Guideline: OECD 209
GLP: Yes
Test item: Fluazinam, techn., purity: 98.4 %, batch no A629/1995
Material and methods:
The influence of fluazinam on the inhibition of the respiration rate of aerobic wastewater micro-organisms was investigated. Activated sludge from a wastewater treatment plant was washed and suspended in tap water to obtain a concentration equivalent to 3 g dry material/L. The pH of activated sludge was adjusted to 7.4. Two inoculum controls, three test media with reference substance (3,5-dichlorophenol) and test media of 10, 32, 100, 320 and 1000 mg fluazinam/L were prepared and the test was started by adding 200 mL inoculum to each test unit. Test vessels were incubated for exactly 3 hours. During this incubation period the media were continuously aerated (oxygen content: at least 6.9 mg O_2/L) and the temperature was 20 °C. At the end of the test the respiration rate was measured by determining the oxygen consumption with an oxygen electrode. The inhibitory effect of fluazinam was expressed as a percentage of the mean respiration rate of two controls.
Findings:
The oxygen consumption rates of the two controls at the start and the end of the test differ only by 1.0 %. The EC_{50} of 3,5-dichlorophenol was 28.3 mg/L. The inhibitory effect of fluazinam at lowest concentration was 21.5 % and at the highest concentration a inhibition of 64.9 % was found. The

EC_{20} and EC_{50} were calculated to be 3.0 mg/L (95 % CL 0 – 19.9 mg/L) and 118.1 mg/L (95 % CL 14.9 – 1751 mg/L).

Conclusion:

Disturbance in the biodegradation process of activated sludge is not expected if the test substance is applied according to GAP.

Comment (RMS): Study considered acceptable.

B.9.11 References relied on

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Annex II Data and Information					
AII, 8.1.1	Hakin, B., Johnson, A. J., Anderson, A., Dawe, I. S.	1991	B-1216 Technical Acute Oral Toxicity (LD50) to Bobwhite Quail. [redacted] Report No. ISK 48/91161 GLP Yes, not published	N	ISK
AII, 8.1.1	Roberts, N. L., Anderson, A., Dawe, I. S.	1991 a	The Acute Oral Toxicity of B1216 to the Mallard Duck. [redacted] Report No. ISN 31BT/841207 GLP Yes, not published	N	ISK
AII, 8.1.2	Roberts, N. L., Anderson, A., Dawe, I. S.	1991 b	The Subacute Dietary Toxicity of B1216 to the Bobwhite Quail. [redacted] Report No. ISN 24BT/841206 GLP Yes, not published	N	ISK
AII, 8.1.2	Roberts, N. L., Anderson, A., Dawe, I. S.	1991 c	The Subacute Dietary Toxicity of B1216 to the Mallard Duck. [redacted] Report No. ISN 25BT/841208 GLP Yes, not published	N	ISK
AII, 8.1.3	Turck, P. A., Laveglia, J.	1996 a	Technical Fluazinam: A Reproduction Study with the Northern Bobwhite (*Colinus virginianus*) Phases I and II. [redacted] Report No. 5512-92-0455-TX-003 GLP Yes, not published	N	ISK
AII, 8.1.3	Turck, P. A., Laveglia, J.	1996 b	Technical Fluazinam: A Reproduction Study with the Mallard (*Anas platyrhynchos*) Phases I and II. [redacted] Report No. 5512-92-0454-TX-003 GLP Yes, not published	N	ISK
All 8.2.1	Gelin, M.D, Laveglia, J.	1992	Technical Fluazinam (IKF-1216) – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions. Generated by: [redacted] Report No: 5099-91-0422-TX-002 GLP / GEP: yes unpublished	N	ISK
All 8.2.1	Hill, R. W.	1985	PP192: Determination of Acute Toxicity to Rainbow Trout (*Salmo gairdneri*). Generated by: [redacted] Report No: BL/B/2560 GLP / GEP: yes unpublished	N	ISK

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
AII 8.2.1	Gelin, M.D, Laveglia, J.	1993	Technical Fluazinam (IKF-1216) – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions. Generated by: [redacted] Report No: 5099-91-0421-TX-002 GLP / GEP: yes unpublished	N	ISK
AII 8.2.1	Peither, A.	2001 a	Acute Toxicity of Fluazinam to Zebra Fish (*Brachydanio rerio*) in a 96-Hour Flow-Through Test. Generated by: [redacted] Report No: 813431 GLP / GEP: yes unpublished	Y	ISK
AII 8.2.1	Peither, A.	2001 b	Acute Toxicity of Fluazinam to Guppy (*Poecilia reticulata*) in a 96-Hour Flow-Through Test. Generated by: [redacted] Report No: 813453 GLP / GEP: yes unpublished	Y	ISK
AII 8.2.1	Shults, S. K, A. W. Brock & L. Laveglia	1993	Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Conditions with Technical Fluazinam (IKF-1216). Generated by: [redacted] Report No: 5017-91-0415-TX-002 GLP / GEP: yes unpublished	N	ISK
AII 8.2.1	Hertl, A.	1997 a	Acute Toxicity of AMPA to Zebra Fish (*Brachydanio rerio*) in a 96-Hour Static Test. Generated by: [redacted] Report No: 662512 GLP / GEP: yes unpublished	N	ISK
AII, 8.2.2.1	Sankey, S. A., Tapp, J. F., Caunter, J. E., Stanley, R. D.	1992	Fluazinam: The 28 Day LC50 to Rainbow Trout (*Oncorhynchus mykiss*). Generated by: [redacted] Report No. BL4167/B GLP / GEP: yes unpublished	N	ISK

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
All, 8.2.2.2	Fillmore, G. E. & J. Laveglia	1993	Technical Fluazinam (IKF-1216) – The Toxicity to Fathead Minnow (*Pimephales promelas*) During Early Life-Stage Exposure. Generated by: [redacted] Report No. 5018-91-0425-TX-002 GLP / GEP: yes unpublished	N	ISK
All, 8.2.2.3	Shults, S. K., Brock, A. W., Laveglia, J.	1995	Technical Fluazinam (IKF-1216)– The Chronic Toxicity to the Fathead Minnow (*Pimephales promelas*) During a Full Life-Cycle Exposure. Generated by: [redacted] Report No. 5107-92-0035-TX-00 GLP / GEP: yes unpublished	N	ISK
All, 8.2.3	Lentz, N. R., Huhtanen, K. L.	1994	Uptake, Depuration, and Bioconcentration and Metabolism of (Fluazinam) Carbon-14 IKF-1216 in Bluegill Sunfish (*Lepomis macrochirus*) Under Flow Through Test Conditions. Generated by: [redacted] Report No. 5311-93-0013-EF-001 GLP / GEP: yes unpublished	N	ISK
All 8.2.4	Shults, S. K., Brock, A. W., Laveglia, J.	1992	Acute Toxicity to Daphnids (*Daphnia magna*) Under Flow-Through Conditions with Technical Fluazinam (IKF-1216). Generated by: Springborn Laboratories Report No. 5108-91-0418-TX-002 GLP / GEP: yes unpublished	N	ISK
All 8.2.4	Hertl, J.	1997 b	Acute Toxicity of AMPA to *Daphnia magna* in a 48-Hour Immobilization Test. Generated by: RCC Umweltchemie AG Report No. 662490 GLP / GEP: yes unpublished	N	ISK
All, 8.2.5	van den Bogaaert, M., Farrelly, E., J., Hamer, M.	1991	Fluazinam: Chronic Toxicity to *Daphnia magna*. Generated by: ICI Plant Protection Division Report No. RJ0974B GLP / GEP: yes unpublished	N	ISK

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
AII, 8.2.5	Shults, S. K., Brock, A. W., Laveglia, J.	1993	Chronic Toxicity to *Daphnia magna* Under Flow-Through Conditions with Technical Fluazinam (IKF-1216). Generated by: Springborn Laboratories, Report No. 5109-91-0419-TX-002 GLP / GEP: yes unpublished	N	ISK
AII 8.2.6	Smyth, D. V., Tapp, J. F.	1987	PP192 (B1216): Determination of Toxicity to the Green Alga *Selenastrum capricornutum*. Generated by: Imperial Chemical Industries PLC Report No: BL/B/3056 GLP / GEP: yes unpublished	N	ISK
AII 8.2.6	Hertl, J.	1997 c	Toxicity of AMPA to *Scenedesmus subspicatu*s in a 72-Hour Algal Growth Inhibition Test for Poorly Soluble Test Substances. RCC Umweltchemie AG Report No. 662477 GLP / GEP: yes unpublished	N	ISK
AII, 8.2.7	Stewart, K.M., Shillabeer, N.	1997	Fluazinam: Determination of the Effects on Emergence of *Chironomus riparius*. Generated by: Zeneca Limited Brixham Environmental Laboratory Report No. BL6115/B GLP / GEP: yes unpublished	N	ISK
AII, 8.3.1.1	Collins, I. G., Gough, H. J., Wilkinson, W.	1984	B 1216 (PP192): Acute Contact and Oral Toxicity to Honey Bees (*Apis mellifera*). ICI Plant Protection Division, Report No. RJ0401B GLP Yes, not published	N	ISK
AII, 8.3.2			See Annex III, Point 10.5.1/03		
AII, 8.4.1	Edwards, P. J., Coulson, J. M.	1985	B 1216 (PP192): Toxicity of Technical Material to the Earthworm (*Eisenia foetida*). Generated by: ICI Plant Protection Division, Report No. RJ0409B GLP / GEP: yes unpublished	N	ISK

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
AII, 8.4.1	Lührs, U.	2000	Acute Toxicity (14 Days) of HYPA to the Earthworm *Eisenia fetida* (Savigny 1826) in Artificial Soil. Generated by: Institut für Biologische Analytik und Consulting IBACON GmbH Report No. 8691021 GLP / GEP: yes unpublished	Y	ISK
AII, 8.5	Reis, K.	2002	Effect of HYPA on the Activity of the Soil Microflora in the Laboratory. Generated by: Institut für Biologische Analytik und Consulting IBACON GmbH Report No. 8692080 GLP / GEP: yes unpublished	Y	ISK
AII, 8.6	Lührs, U.	2004	Effects of HYPA on Reproduction of the Collembola *Folsomia candida* in Artificial Soil Generated by: Institut für Biologische Analytik und Consulting IBACON GmbH Report No. 19161016 GLP / GEP: yes unpublished	Y	ISK
AII, 8.6	Backus, P.	1993 a	Effect of IKF-1216 (Fluazinam) on Seed Germination/Seedling Emergence (Tier I). Ricerca, Inc., Report No. 5549-92-0483-BE-001 GLP Yes, not published	N	ISK
AII, 8.6	Backus, P.	1993 b	Effect of IKF-1216 (Fluazinam) on Vegetative Vigor of Plants (Tier I). Ricerca, Inc., Report No. 5549-92-0484-BE-001 GLP Yes, not published	N	ISK
AII, 8.6	Crosby, K.	1995	Effect of IKF-1216 on Vegetative Vigor of Plants (Tier II). Ricerca, Inc., Report No. 6363-95-0019-BE-001 GLP Yes, not published	N	ISK
AII, 8.6	Sugimoto, K., Hayashi, H.	2004	Terrestrial Plants, Growth Test assessing HYPA, metabolite of fluazinam ISK Central Research Laboratory, Japan, Report No. AL-0404-F-01 GLP No, not published	Y	ISK
AII, 8.7	Grützner, I.	2000	Toxicity of Fluazinam to Activated Sludge in a Respiration Inhibition Test. Generated by: RCC Ltd. Report No. 774887 GLP / GEP: yes unpublished	Y	ISK

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
AII, 8.7	Sankey, S.A., Coleman, C.A., Tapp, J.F.	1992	Fluazinam: Toxicity to *Pseudomonas putida*. Generated by: Imperial Chemical Industries PLC Group Environmental Laboratory Report No. BL4364/B GLP / GEP: yes unpublished	N	ISK
Annex III Data and Information					
AIII, 10.2	Gurney, A	2005	Fluazinam Statement: Aquatic risk assessment for chronic effects on fish using FOCUS Step 4 predicted concentrations in surface water with a 5 m unsprayed buffer. Generated by: RCC Ltd. Report No. A44280-B GLP / GEP: not applicable unpublished	Y	ISK
AIII, 10.2.1	Sankey, S.A., Tapp, J.F., Caunter, J.E., Penwell, A.J.	1991	Fluazinam: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) of a 500 g/L SC Formulation. Generated by: [redacted] PLC, Group Environmental Laboratory Report No: BL4323/B GLP / GEP: yes unpublished	N	ISK
AIII, 10.2.1	Farrelly, E., Navet, X., Hamer, M. J.	1991	Fluazinam: Acute Toxicity of a 500 g/L SC Formulation to First Instar *Daphnia magna*. Generated by: ICI Agrochemicals, UK Report No: RJ1024B GLP / GEP: yes unpublished	N	ISK
AIII, 10.2.1	Smyth, D. V., Sankey, S.A., Stanley, R.D.	1991	Fluazinam: Toxicity to the Green Alga *Selenastrum capricornutum* of a 500 g/L SC Formulation. Generated by: Imperial Chemical Industries PLC, Group Environmental Laboratory, UK Report No: BL4362/B GLP / GEP: yes unpublished	N	ISK
IIIA, 10.2.2	van Wijngaarden R., Boonstra H.	2004	Fate and effects of the fungicide Shirlan (active ingredient fluazinam) in indoor freshwater microcosms Generated by: Aquatic Ecology and Water Quality Management Group, Wageningen University, Wageningen, NL Report No: WA2004 GLP / GEP: no unpublished	Y	ISK

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AIII, 10.4.1	Kleiner, R..	1992	Testing Toxicity to Honey Bee - *Apis mellifera* L. (laboratory) According to BBA Guideline VI, 23-1. BioChem GmbH Karlsruhe Labor Cunnersdorf, Germany, Report No. 921048038 GLP Yes, not published	N	ISK
AIII, 10.5.1	Canning, L., Lloyd, E.J., Lewis, G.B.	1992	Fluazinam – Investigation of the Toxicity of a 50% w/v SC Formulation to the Carabid Beetle *Pterostichus melanarius* and a Lycosid Spider. ICI Agrochemicals, UK, Report No. RJ1070B GLP Yes, not published	N	ISK
AIII, 10.5.1	Coulson, J.M., Lavender, K.H.	1996	Investigation into the Toxicity of a 500 g a.i./L Suspension Concentrate Formulation to the Cereal Aphid Parasitoid *Aphidius rhopalosiph*i. Zeneca Agrochemicals, UK, Report No. RJ2109B GLP Yes, not published	N	ISK
AIII, 10.5.1	Thompson, B.	1996	A Laboratory Evaluation of the Side-Effects of the Fungicide Fluazinam on Larvae of the Lacewing *Chrysoperla carnea*. Agrochemical Evaluation Unit, Department of Biology, The University, Southampton, UK, Report No. ZEN-95-3/C GLP Yes, not published	N	ISK
AIII, 10.5.1	Jansen, J-P.	2000 a	Side Effects of Fluazinam (IKF-1216) 500 g/L SC Formulation on the Parasitic Wasp *Aphidius rhopalosiphi* (Hym.; Aphidiidae) in the Laboratory on Potato Detached Leaves Treated in the Field. Laboratoire d'Ecotoxicologie, Ministry of Agriculture, Belgium, Report No. AE.01/2000 GLP Yes, not published	N	ISK
AIII, 10.5.1	Jansen, J-P.	2000 b	Side Effects of Fluazinam (IKF-1216) 500 g/L SC Formulation on the Predacious Mite *Typhlodromus pyri* Scheuten (Acari; Phytoseiidae) in the Laboratory on Potato Detached Leaves Treated in the Field. Laboratoire d'Ecotoxicologie, Ministry of Agriculture, Belgium, Report No. TE.01/2000 GLP Yes, not published	Y	ISK

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AIII, 10.5.1	Jansen, J-P.	2000 c	Side Effects of Fluazinam (IKF-1216) 500 g/L SC Formulation on Larvae of the Green Lacewing *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae) in the Laboratory on Potato Detached Leaves Treated in the Field. Laboratoire d'Ecotoxicologie, Ministry of Agriculture, Belgium, Report No. CCE.01/2000 GLP Yes, not published	Y	ISK
AIII, 10.6.1.1	Yearsdon, H. A., Burgess, A. J., Coulson, J. M.	1991	Fluazinam: Toxicity to the Earthworm (*Eisenia fetida*) of a 500g/litre Suspension Concentrate Formulation. Generated by:ICI Agrochemicals, UK Report No. RJ1087B GLP / GEP: yes unpublished	N	ISK
AIII, 10.6.1.2	Römbke, J., Moser, Th.	1999	A Study on the Reproduction Toxicity of Fluazinam 500 g/L SC to the Earthworm *Eisenia andrei* (Bouche). Generated by: ECT Oekotoxikologie GmbH Report No. F3RR (Reference: WINo. 45373) GLP / GEP: yes unpublished	N	ISK
AIII, 10.6.1.3	Mills, H.	2001	Field Study to Determine the Effects of a 500 g/L SC Formulation of Fluazinam on Earthworms. Generated by: Covance Laboratories Ltd, UK Report No. 1883/001-D2143 GLP / GEP: yes unpublished	Y	ISK
AIII, 10.6.2	Klein, S., Meister, A.	2002	Effects of IKF-1216 500 SC (Fluazinam 500SC) on Reproduction of the Collembola *Folsomia candida* in Artificial Soil. Generated by: Institut für Biologische Analytik und Consulting IBACON GmbH, Germany Report No. 13781016 GLP / GEP: yes unpublished	Y	ISK
AIII, 10.7.1	Reis, K-H.	2002	Effects of Fluazinam 500 SC on the Activity of the Soil Microflora in the Laboratory. Generated by: Institut für Biologische Analytik und Consulting IBACON GmbH, Germany, Report No. 9321080 GLP / GEP: yes unpublished	Y	ISK