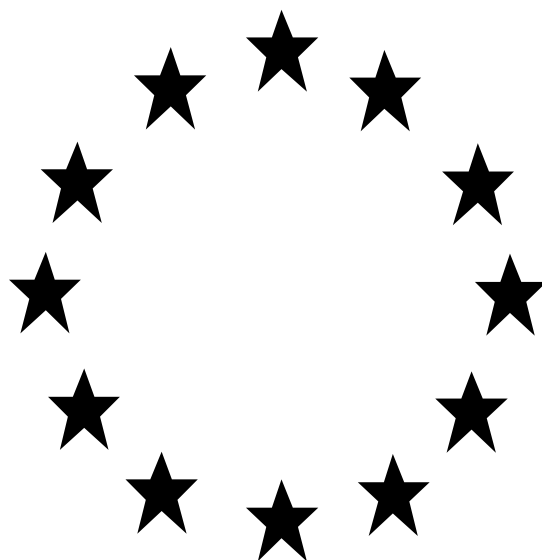




Draft Assessment Report (DAR)

- public version -

**Initial risk assessment provided by the rapporteur Member State
The Netherlands for the existing active substance**

PYRIDABEN

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

Volume 3, Annex B, part 5, B.9

August 2007

B.9 Ecotoxicology

Background information

Pyridaben is an existing insecticide/acaricide proposed for use on citrus in Southern Europe (one application at 0.3 kg a.s./ha) and tomato in Northern and Southern Europe (one application at 0.25 kg a.s./ha). The proposed formulated product, Nexter Pro, also referred to as BAS 300 11 I, pyridaben WP, Nexter and Pyridaben 75 WP, is a wettable powder formulation containing nominally 750 g/kg pyridaben. Pyridaben is also referred to as BAS 300 I (technical), BASF Pyridaben Technical, NC-129 (technical) and NC-129 TGA I.

Data were presented on the technical active substance (a.s.) and on the proposed Nexter Pro formulation, as well as on major metabolites and on 9 formulations with different names (BAS 9078 0 I (A), BAS 9078 4 I (A), BAS 9078 6 A, BAS 9078 6 I, formulated NC-129 (JF 10708C), Sanmite, Sanmite 15EC, Pyridaben 20 WP and Sanmite (Protex)). Table B.9-01 gives an overview of the products of which study results were used in the risk assessment. Composition of these products is given in Annex C of Volume 4.

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Table B.9-01 Overview of different formulations used for risk assessment

Formulated Product (Code)	Trade Name	Nominal a.i. content (g/kg or g/l)	Batch numbers	DAR References
Pyridaben 75WP in water soluble bag (BAS 300 19 I)	Nexter Pro	750		
Pyridaben 75WP (BAS 300 11 I)	Nexter Sanmite 75WP Note : Both of these products are used in the U.S.	750	AF137-50 OAJ 030	B.9.2.1.3 B.9.2.2.1.4 B.9.4.2 B.9.5.1 B.9.5.2 B.9.6.1.3 B.9.6.2 B.9.8.1.3 B.9.9.2
Pyridaben 20WP (BAS 9078 0 I (A))	Sanmite 20WP Note : This product is used in Central Europe	200	01B009	B.9.4.1.2 B.9.4.3 B.9.4.5
Pyridaben 20EC Pyridaben 200 g/l EC (BAS 9078 6 I, BAS 300 06 I)	Nexter EC Note : This product is used in Greece	200	89-1 (APE/CF), PCF 00030	B.9.4.4 B.9.8.1.3
Pyridaben 20EC BAS 9078 4 I (A) composition practically similar to BAS 9078 6 I (A)	Nexter Note : This product is used in Italy and Portugal	200		B.9.4.3

According to the notifier, study results of all products mentioned in Table B.9-01 can be used in support of Nexter Pro. Looking at coformulants, 20EC is expected to be more toxic than 20WP. Therefore, using data of the 20EC formulation for the 20WP formulation is acceptable. Similarly, coformulants of the 20% formulations are not expected to change the toxicity of pyridaben as compared to the 75% formulations. However, more information is considered necessary for the extrapolation of 20% formulation data to Nexter Pro (75%).

In table B.9-02 a comparison is made of available endpoints of the different formulations and the active substance. It is shown that in general, the active substance is at least as toxic as the formulations. The 20% formulations have only been used in tests with bees. No 75% formulation tests with bees are available. However, the notifier has announced to send in an acute toxicity test with *Daphnia*, which is reasonably relevant to discuss comparability for bees (both invertebrates). The preliminary endpoint (provided by the notifier), included in Table B.9-02, is in the same range as those from studies with the 75% formulation and the active substance. The acute toxicity study for *Daphnia magna* with Pyridaben 20WP will be evaluated for the addendum, to resolve the comparison issue. For now, it will be assumed that all formulations mentioned in Table B.9-01 can be used in support of Nexter Pro.

Table B.9-02 Toxicity testing outcomes for Pyridaben 20WP, Pyridaben 75WP, Pyridaben 20EC and technical pyridaben

Species	Pyridaben 20WP (BAS 9078 0 I (A)) LC/EC ₅₀ (µg as/l)	Pyridaben 20EC (BAS 9078 4 I (A)) LC/EC ₅₀ (µg as/l)	Pyridaben 75WP (BAS 300 11 I) LC/EC ₅₀ (µg as/l)	Pyridaben technical (BAS 300 I) LC/EC ₅₀ (µg as/l)
Rainbow trout	96-hour LC ₅₀ : 9.43 NAS402/023943, Jenkins (2002a) 96-hour LC ₅₀ : >100 < 220 E26, Munk (1988)	96-hour LC ₅₀ : >22 < 46 E27, Munk (1988)	96-hour LC ₅₀ : 2.2 semi-static B.9.2.1.3/02	96-hour LC₅₀: 0.73 flow-through B.9.2.1.1/02
Bluegill sunfish	-	-	96-hour LC ₅₀ : 64 static, outdoor B.9.2.1.3/01	96-hour LC₅₀: 3.47 flow-through B.9.2.1.1/04
<i>Daphnia magna</i>	<i>48-hour EC₅₀: 0.861 study will be submitted for addendum</i>	-	48-hour EC₅₀: 0.99 static B.9.2.1.3/03	48-hour EC ₅₀ : 1.0 flow-through B.9.2.1.1/07
Mysid	-	-	96-hour LC ₅₀ : 16 static B.9.2.1.3/04	96-hour LC₅₀: 0.67 flow-through B.9.2.1.1/08
<i>Selanastrum capricornutum</i>	-	-	72-hour E _r C ₅₀ : 68000 static B.9.2.1.3/05	96-hour E_rC₅₀: >1000 static B.9.2.1.1/10
Bee (contact)	48-hour LD ₅₀ : 3.68 µg as/bee B.9.4.1.2/02	-	-	44-hour LD₅₀: 0.024 µg as/bee B.9.4.1.1/01
Bee (oral)	24-hour LD ₅₀ : 2.38 µg as/bee B.9.4.1.2/02	-	-	48-hour LD₅₀: 0.535 µg as/bee B.9.4.1.1/02
Bee (Tunnel study)	Minimal mortality, some initial effects on foraging behaviour at 1.2 kg as/ha B.9.4.3/01, /03	Minimal mortality, some initial effects on foraging behaviour at 1.2 kg as/ha B.9.4.3/02	-	-
Earthworm	-	-	14 day LC ₅₀ : 84 mg/kg B.9.6.1.3/01	14 day LC₅₀: 38 mg/kg B.9.6.1.1/01

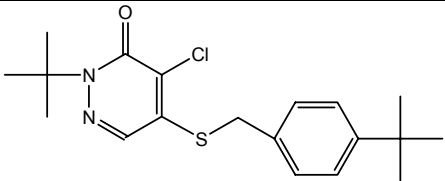
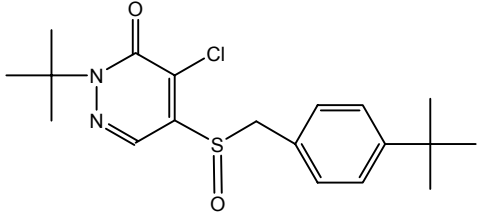
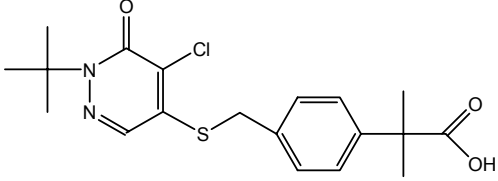
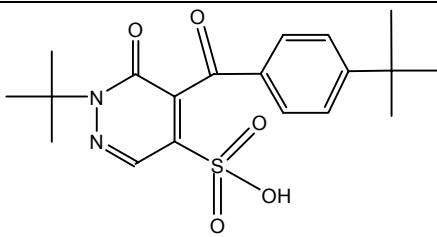
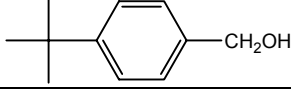
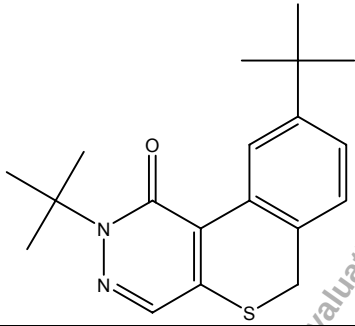
Bold indicates the lowest (most toxic) outcome for a given species

The products Sanmite (Protex) and NC-129 SC formulation (section B.9.4.1.2), BAS 9078 6 (A) (B.9.4.4), Pyridaben (Sanmite 15 EC) (section B.9.5.1) and BAS 300 I (sections B.9.5.1 and B.9.5.2) are not mentioned in Table B.9-01. The results of these studies were not taken to the List of Endpoints (studies considered not acceptable, only useful as supplementary information, or considered not representative for Pyridaben 75 WP), and further information on these products is not required.

Table B.9-03 below gives an overview of the major metabolites of pyridaben in the matrices water, sediment, water/sediment and soil. The maximum formation rates in the different matrices are included.

Studies summarised below under point B.9.1 to B.9.10 are acceptable unless noted.

Table B.9-03 Overview of metabolites of pyridaben

Structure	Name	Chemical name	Metabolite in	MW	LogPow
	Pyridaben	2-tert-butyl-5-(4-tert-butylbenzylthio)-4-chloropyridazin-3(2H)-one	-	364.94	>6.37 ^(A) 5.47 ^(B) 5.78 ^(C)
	PB-4	2-tert-butyl-5-(4-tert-butylbenzylsulfinyl)-4-chloropyridazin-3(2H)-one	Soil (7.6% AR) Water/sediment (2.1% AR)	380.94	3.02 ^(B) 3.83 ^(C)
	PB-7	2-tert-butyl-5-(4-(1-carboxy-1-methylethyl)-4-tert-butylbenzylthio)-4-chloropyridazin-3(2H)-one	Soil (8.5% AR) Water (17.2% AR) Sediment (14.1% AR)	394.92	3.86 ^(B) 3.67 ^(C)
	PB-22	2-tert-butyl-4-(4-tert-butylbenzoyl)-pyridazin-3(2H)-one-5-sulfonic acid	Soil (1.2% AR) Water/sediment (0.4% AR)	392.48	1.53 ^(B) 2.34 ^(C)
	B-3	Not available	Water (photolysis) (29.2% AR)	164.25	2.99 ^(B) 2.94 ^(C)
	W-1	Not available	Water (photolysis) (27.9% AR)	328.48	4.29 ^(B) 4.96 ^(C)

(A) Experimental value

(B) Estimated by RMS using EPA EPI Suite software

(C) Estimated by RMS using Pallas 3.0 software

B.9.1 Effects on birds (IIA8.1; IIIA 10.1)**B.9.1.1 Acute oral toxicity (IIA 8.1.1; IIIA 10.1.1)****Study 1****Characteristics of the study**

reference	:	Grimes J. & Jaber M. (1987a)	duration of	:	14 days
type of study	:	Acute oral toxicity study	observations	:	
year of execution	:	1986	nominal	:	0, 292, 486, 810, 1350 and 2250 mg
GLP statement	:	Yes	concentrations	:	a.s./kg bw
guideline	:	EPA 71-1	dosing method	:	Gavage of suspension in
test substance	:	NC-129 Technical (pyridaben), Lot No. 129T8605	acceptability	:	carboxymethyl cellulose
purity	:	98.0%	LD50	:	Acceptable
species	:	Bobwhite quail (<i>Colinus virginianus</i>)		:	>2250 mg a.s./kg bw

Methods

In a study on the acute oral toxicity of NC-129 Technical (pyridaben; 98.0% purity) to young adult bobwhite quail (*Colinus virginianus*), five male and five females per dose level (fasted overnight) were orally dosed with NC-129 at dose levels of 0, 292, 486, 810, 1350 and 2250 mg a.s./kg bw, followed by a fourteen day observation period.

Results

No birds died in the control or treated groups. No signs of toxicity were observed at any dose level. Body weight gain was reduced at 486, 810, 1350 and 2250 mg a.s./kg bw during days 1-3. Food consumption was slightly reduced at 1350 and 2250 mg a.s./kg bw.

Table B.9.1.1-01 Summary of acute oral toxicity to birds (data for pyridaben)

Purity	Species	Acute oral LD50 (mg a.s./kg bw)
98.0%	Bobwhite quail <i>Colinus virginianus</i>	>2250

Conclusion

The acute oral LD50 for bobwhite quail is >2250 mg a.s./kg bw.

Guidelines and limitations

The study was conducted according to EPA 71-1 and GLP.

Study 2

Characteristics of the study

reference	:	Grimes J. & Jaber M. (1987b)	species	:	Mallard duck (<i>Anas platyrhynchos</i>)
type of study	:	Acute oral toxicity study	duration of	:	14 days
year of execution	:	1986	observations	:	
GLP statement	:	Yes	nominal	:	0, 50, 100, 200, 400, 800, 1600 and
guideline	:	EPA 71-1	concentrations	:	2500 mg a.s./kg bw
test substance	:	NC-129 Technical (pyridaben), Lot No. 129T8605	dosing method	:	Gavage of suspension in corn oil
purity	:	98.0%	acceptability	:	Acceptable
			LD50	:	>2500 mg a.s./kg bw

Methods

In a study on the acute oral toxicity of NC-129 Technical (pyridaben; 98.0% purity) to young adult mallard duck (*Anas platyrhynchos*), five male and five females per dose level (fasted overnight) were orally dosed with NC-129 at dose levels of 0, 50, 100, 200, 400, 800, 1600 and 2500 mg a.s./kg bw, followed by a fourteen day observation period.

Results

No birds died in the control or treated groups. No signs of toxicity were observed and body weight gain and food consumption were not affected at any dose level.

Table B.9.1.1-02 Summary of acute oral toxicity to birds (data for pyridaben)

Purity	Species	Acute oral LD50 (mg a.s./kg bw)
98.0%	Mallard duck <i>Anas platyrhynchos</i>	>2500

Conclusion

The acute oral LD50 for mallard duck is >2500 mg a.s./kg bw.

Guidelines and limitations

The study was conducted according to EPA 71-1 and GLP.

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B.9.1.2 Dietary toxicity (IIA 8.1.2)**Study 1****Characteristics of the study**

reference	:	Grimes J. <i>et al.</i> (1987)	species	:	Bobwhite quail (<i>Colinus virginianus</i>)
type of study	:	Dietary LC50 study	exposure duration	:	5 days
year of execution	:	1986	nominal	:	0, 562, 1000, 1780, 3160 and 5620 mg
GLP statement	:	Yes	concentrations	:	a.s./kg diet
guideline	:	EPA 71-2	dosing method	:	Test substance was added to the diet as a mixture in corn oil (2% corn oil)
test substance	:	NC-129 Technical, Lot No. 129T8605	acceptability	:	Acceptable
purity	:	98.0%	LC50	:	>5620 mg a.s./kg diet ⇔ >4298 mg a.s./kg bw/day

Methods

In a study on the dietary toxicity of NC-129 (pyridaben) Technical (purity 98.0%), five groups of 10-day old Bobwhite quails of indeterminate sex (10/group) were fed NC-129 in the diet at analytically confirmed concentrations of 562, 1000, 1780, 3160 and 5620 mg a.s./kg diet, respectively, for a period of 5 days followed by a 3-day recovery period. Five control groups of ten 10-day old Bobwhite quails received feed treated with the vehicle (corn oil).

Results

No birds died in the control groups. One bird died at 5620 mg a.s./kg diet. Wing droop and lower limb weakness were observed at 3160 and 5620 mg a.s./kg diet between days 3 and 5. Body weight gain was reduced at 3160 and 5620 mg a.s./kg diet during days 0-5. Feed consumption was not affected at any dose level. The results are summarised in the table below.

Table B.9.1.2-01 Summary of short-term dietary toxicity to birds (data for pyridaben)

Diet concentration (mg a.s./kg diet)	Average body weight (g)		Feed consumption (g/bird/day), days 0-5	5day dietary LC50
	Day 0	Day 5		
0	18	29	9	>5620 mg a.s./kg diet ⇔ >4298 mg a.s./kg bw/day
562	17	28	9	
1000	19	28	10	
1780	20	29	11	
3160	18	24	14	
5620	16	18	13	

Conclusion

The 5-day dietary LC50 was >5620 mg a.s./kg diet, equivalent to >4298 mg a.s./kg bw/day. The 5-day NOEC was 1780 mg a.s./kg diet, equivalent to 799 mg a.s./kg bw/day.

Guidelines and limitations

1. The study was conducted according to EPA 71-2, and in compliance with GLP.

2. The 5-day dietary LC50 and NOEC were expressed by the reviewer in mg a.s./kg bw/day based on the reported feed consumption and the mean body weight (day 0-5).

Study 2

Characteristics of the study

reference	:	Grimes J. & Jaber M. (1987c)	exposure duration	:	5 days
type of study	:	Dietary LC50 study	nominal	:	0, 562, 1000, 1780, 3160 and
year of execution	:	1987	concentrations	:	5620 mg a.s./kg diet
GLP statement	:	Yes	dosing method	:	Test substance was added to the
guideline	:	EPA 71-2			diet as a mixture in corn oil (2% corn oil)
test substance	:	NC-129 Technical, Lot No. 129T8605	acceptability	:	Acceptable
purity	:	98.0%	LC50	:	4688 mg a.s./kg diet ⇔
species	:	Mallard duck (<i>Anas platyrhynchos</i>)			calculation to mg a.s./kg bw/day not possible

Methods

In a study on the dietary toxicity of NC-129 (pyridaben) Technical (purity 98.0%), five groups of 10-day old mallard ducks of indeterminate sex (10/group) were fed NC-129 in the diet at analytically confirmed concentrations of 562, 1000, 1780, 3160 and 5620 mg a.s./kg diet, respectively, for a period of 5 days followed by a 3-day recovery period. Four control groups of ten 10-day old mallard ducks received feed treated with the vehicle (corn oil).

Results

No birds died in the control groups or the treatment groups up to 3160 mg a.s./kg diet. Eight birds died at 5620 mg a.s./kg diet. Lethargy, reduced reaction to external stimuli, loss of coordination, lower limb weakness, depression, wing droop or ruffled appearance were observed at 1000 (day 4), 1780 (days 4-6), 3160 (days 2-7) and 5620 mg a.s./kg diet (days 1-7). Body weight gain and feed consumption were reduced at all dose levels during days 0-5. The results are shown in the table below. The 5-day dietary LC50 was 4688 mg a.s./kg diet. According to the Guidance Document on risk assessment for birds and mammals (Sanco/4145/2000, final, September 2002), this dose cannot be recalculated into mg a.s./kg bw/day, due to the marked effects on feed consumption, in particular at the highest two concentrations and the large difference in survival (100% and 20% respectively at 3160 and 5620 mg a.s./kg diet). Therefore, the LC50 for risk assessment is replaced by the NOEC for mortality (in accordance with the recommendations in Sanco/4145/2000), being 3160 mg a.s./kg diet, equivalent to 659 mg a.s./kg bw/day.

Table B.9.1.2-02 Summary of short-term dietary toxicity to birds (data for pyridaben)

Diet concentration (mg a.s./kg diet)	Average body weight (g)		Feed consumption (g/bird/day), days 0-5	5-day dietary LC50
	Day 0	Day 5		
0	139	256	67	4688 mg a.s./kg diet
562	133	189	52	NOEC (mortality) 3160 mg a.s./kg diet ⇔ 659 mg a.s./kg bw/day
1000	143	162	44	
1780	145	129	30	
3160	133	97	24	
5620	142	83	9	

Conclusion

The 5-day dietary LC50 was 4688 mg a.s./kg diet. The 5-day dietary NOEC for mortality was 3160 mg a.s./kg diet, equivalent to 659 mg a.s./kg bw/day (value to be used in risk assessment instead of LC50). The 5-day NOEC for sublethal effects was <562 mg a.s./kg diet, equivalent to <182 mg a.s./kg bw/day.

Guidelines and limitations

1. The study was conducted according to EPA 71-2, and in compliance with GLP.
2. The 5-day dietary NOEC values were expressed by the reviewer in mg a.s./kg bw/day based on the reported feed consumption and the mean body weight (day 0-5).

B.9.1.3 Long term/Reproductive toxicity (IIA 8.1.3)

Study 1

Characteristics

reference	: Beavers J.B. <i>et al.</i> (1994a)	species	: Bobwhite quail (<i>Colinus virginianus</i>)
type of study	: Dietary reproductive toxicity study	exposure duration	: 22 weeks
year of execution	: 1993	nominal concentrations	: 0, 100, 500 and 1000 mg/kg diet
GLP statement	: Yes	dosing method	: Pyridaben was mixed in a premix
guideline	: EPA 71-4	acceptability	: Acceptable
test substance	: Pyridaben (NC-129) TGAI, Lot No. 900304	NOEC	: 1000 mg/kg diet ⇔ 135 and 130 mg a.s./kg bw/day for males and females, respectively
purity	: 99.7%		

Methods

In a reproductive toxicity study, 17-weeks-old bobwhite quails were exposed to pyridaben (99.7%) in the diet for 22 weeks. Nominal diet concentrations were 0 (control), 100, 500 and 1000 mg/kg diet. There were 16 pairs per dose level, one pair per cage. The birds were maintained in a controlled environment test room. Eggs were collected daily from the onset of egg production (week 12). Hatchlings were leg banded and maintained for 14 days in cages according to pen. Reproduction endpoints were determined with eggs produced during the last 11 weeks of the study. Reproduction endpoints were: number of eggs laid, number of eggs cracked, eggshell thickness, number of fertile eggs, number of live 21-day-old embryos, number of hatchlings, hatchling weight, number of 14-day-old survivors, and chick weights at day 14 of age. For eggshell thickness determination, eggs were collected weekly throughout the egg laying period.

Results

No treatment-related effects were observed in the adult birds. Two birds died before and one after onset of egg production. The distribution of deaths among the treatment groups was one (control), one (500 mg/kg diet) and one (1000 mg/kg diet). There were no treatment-related effects on body weight, body weight change, feed consumption, survival and behavioural endpoints. No substance related abnormalities were noted at necropsy. Results on reproductive performance are shown in the table below. Differences between treated groups and the control were not statistically significant unless indicated.

Table B.9.1.3-01 Results of a reproductive study on bobwhite quail (22-week exposure period)

Nominal concentration (mg/kg diet)	0	100	500	1000
Mean measured concentration (mg a.s./kg diet)	- ^(A)	98	514	1011
Mean parental food consumption (g/bird/day)	27	26	28	28
Mean body weight change males (%)	+11	+11	+10	+8.1
Mean body weight males, week 0-week 22 (g)	208	213	211	207
Mean body weight change females (%)	+20	+16	+15	+20
Mean body weight females, week 0-week 22 (g)	218	216	211	214
Mean egg production (eggs/cage)	58	45	48	53
Total number of eggs set	748	626	603	695
Mean eggshell thickness (mm)	0.23	0.23	0.22	0.22
Percent of eggs laid that were cracked (%)	4	6	4	3
Percent of eggs set that were fertile (%)	87	94	89	89
21-day embryos as percentage of fertile eggs (%)	98	98	99	97
21-day embryos as percentage of eggs set (%)	85	93	87	89
Number hatchlings as percent of 21-day embryos (%)	94	94	94	90
Number hatchlings as percent of fertile eggs (%)	93	92	93	90
Number hatchlings as percent of eggs set (%)	81	87	83	79
Number 14-day chicks as percent of hatchlings (%)	89	82	90	78
Number 14-day chicks as percent of fertile eggs (%)	83	76	84	72
Number 14-day chicks as percent of eggs set (%)	72	71	75	63
Number 14-day chicks per hen	36	28	29	30
Mean body weight (g) of hatchlings	6	6	6	6
Mean body weight (g) of 14-day chicks	23	22	22	22

^(A) Not measured

There were no treatment-related effects on any reproductive parameter. There appeared to be a slight decrease in hatchling survival at 1000 mg/kg diet. The authors of the report attributed this to toe picking in one group of hatchlings, and concluded that this slight decrease was therefore not related to treatment. Since moreover the difference from the control was not statistically significant, this is acceptable. Mean body weights of hatchlings and 14-day old chicks were not affected at any dose level.

Conclusion

The NOEC in bobwhite quail exposed to pyridaben in the diet was 1000 mg/kg diet (mean measured concentration 1011 mg a.s./kg diet, equivalent to 135 and 130 mg a.s./kg bw/day for males and females, respectively).

Guidelines and limitations

- The following results were calculated from the reported raw data by the reviewer:
 - Mean food consumption for adult birds
 - Percentage body weight change for adult birds
 - Number of 21-day embryos as percentage of eggs set
 - Number of hatchlings and 14-day old chicks as percentage of fertile eggs
 - NOEC at 1000 mg/kg diet nominal (1011 mg a.s./kg diet mean measured), expressed as mg a.s./kg bw/day, based on mean measured pyridaben concentration, mean feed consumption for the whole group and mean body weights of males and females.
- The study was conducted according to EPA 71-4 (1982), in compliance with GLP, and is acceptable.

Study 2

Characteristics

reference	: Beavers J.B. <i>et al.</i> (1994b)	species	: Mallard duck (<i>Anas platyrhynchos</i>)
type of study	: Dietary reproductive toxicity study	exposure duration	: 21 weeks
year of execution	: 1993	nominal concentrations	: 0, 100, 500 and 1000 mg/kg diet
GLP statement	: Yes	dosing method	: Pyridaben was mixed in a premix
guideline	: EPA 71-4	acceptability	: Acceptable
test substance	: Pyridaben (NC-129) TGAI, Lot No. 900304	NOEC	: 100 mg/kg diet $\Leftrightarrow$ 10 and 11 mg a.s./kg bw/day for males and females, respectively
purity	: 99.7%		

Methods

In a reproductive toxicity study, 19-weeks-old mallard ducks were exposed to pyridaben (99.7%) in the diet for 21 weeks. Nominal diet concentrations were 0 (control), 100, 500 and 1000 mg/kg diet. There were 16 pairs per dose level, one pair per cage. The birds were maintained in a controlled environment test room. Eggs were collected daily from the onset of egg production (week 10). Hatchlings were wing banded and maintained for 14 days in cages according to pen. Reproduction endpoints were determined with eggs produced during the last 11 weeks of the study. Reproduction endpoints were: number of eggs laid, number of eggs cracked, eggshell thickness, number of fertile eggs, number of live 21-day-old embryos, number of hatchlings, hatchling weight, number of 14-day-old survivors, and chick weights at day 14 of age. For eggshell thickness determination, eggs were collected weekly throughout the egg laying period.

Results

No clinical toxic signs were observed at any of the dose levels. No birds died before or after onset of egg production. There were no treatment-related effects on feed consumption at any of the dose levels. At 500 and 1000 mg/kg diet, body weight gain was reduced, and the terminal body weights of drakes and hens were significantly lower ($p < 0.05$) than those of the control. At 500 and 1000 mg/kg diet, treatment related increases of the number of hens and drakes with regressed ovaries and small testes, respectively, were observed at necropsy. No treatment-related effects were observed in the adult birds at 100 mg/kg diet. Results on reproductive performance are shown in the table below. Differences between treated groups and the control were not statistically significant unless indicated.

Table B.9.1.3-02 Results of a reproductive study on mallard duck (21-week exposure period)

Nominal concentration (mg/kg diet)	0	100	500	1000
Mean measured concentration (mg a.s./kg diet)	- ^(A)	98	514	1011
Mean parental food consumption (g/bird/day)	141	126	146	134
Mean body weight change males (%)	+4.2	+2.9	-3.3	-8.5
Mean body weight males, week 0-week 22 (g)	1199	1207	1146	1145
Mean body weight change females (%)	+6.2	+9.3	-4.1	-12
Mean body weight females, week 0-week 22 (g)	1110	1120	1065	1025
Mean egg production (eggs/cage)	45	39	30	14*
Total number of eggs set	621	525	395	178
Mean eggshell thickness (mm)	0.38	0.39	0.38	0.38
Percent of eggs laid that were cracked (%)	2	2	4	4
Percent of eggs set that were fertile (%)	95	96	88	84
21-day embryos as percentage of fertile eggs (%)	98	99	98	97
21-day embryos as percentage of eggs set (%)	93	95	88	82
Number hatchlings as percent of 21-day embryos (%)	79	83	66	64
Number hatchlings as percent of fertile eggs (%)	79	82	71	61
Number hatchlings as percent of eggs set (%)	74	79	57	55
Number 14-day chicks as percent of hatchlings (%)	97	97	98	99
Number 14-day chicks as percent of fertile eggs (%)	76	79	69	61
Number 14-day chicks as percent of eggs set (%)	72	76	56	55
Number 14-day chicks per hen	28	25	15	6
Mean body weight (g) of hatchlings	35	32	35	33
Mean body weight (g) of 14-day chicks	262	255	254	240

* Significantly different from control at 5% level

(A) Not measured

Reproduction was adversely affected at 500 and 1000 mg/kg diet. At 500 and 1000 mg/kg diet, the number of eggs laid, fertile eggs as percentage of eggs set and hatchlings as percentage of 21-day embryos was reduced, resulting in decreased numbers of hatchlings and 14-day chicks as percentage of eggs set. These changes were not statistically significant, except the number of eggs laid at the highest test dose. Mean body weights of hatchlings and 14-day old chicks were not affected at any dose level.

Conclusion

The NOEC in mallard duck exposed to pyridaben in the diet was 100 mg/kg diet (mean measured concentration 98 mg a.s./kg diet, equivalent to 10 and 11 mg a.s./kg bw/day for males and females, respectively).

Guidelines and limitations

1. The following results were calculated from the reported raw data by the reviewer:
 - Mean food consumption for adult birds
 - Percentage body weight change for adult birds
 - Number of 21-day embryos as percentage of eggs set
 - Number of hatchlings and 14-day old chicks as percentage of fertile eggs
 - NOEC at 100 mg/kg diet nominal (98 mg a.s./kg diet mean measured), expressed as mg a.s./kg bw/day, based on mean measured pyridaben concentration in feed, mean feed consumption for the whole group and mean body weights of males and females.
2. The study was conducted according to EPA 71-4 (1982), in compliance with GLP, and is acceptable.

B.9.1.4 Summary

Acute oral toxicity studies on two species were supplied. The acute LD₅₀-values of pyridaben for bobwhite quail and mallard duck were >2250 and >2500 mg a.s./kg body weight, respectively.

Short-term dietary toxicity studies with pyridaben on two species were supplied. For bobwhite quail and mallard duck, the dietary LC₅₀-values were >5620 and 4688 mg a.s./kg diet. The dietary LC₅₀-value for bobwhite quail was equivalent to >4298 mg a.s./kg bw/day, while an LC₅₀-value expressed as mg a.s./kg bw/day could not be determined for mallard duck. For the latter species, the NOEC for mortality was 3160 mg a.s./kg diet, equivalent to 659 mg a.s./kg bw/day (value to be used in risk assessment).

Reproductive toxicity studies with pyridaben in bobwhite quail and mallard duck were submitted, in which there were no effects on reproductive parameters at 1000 and 100 mg a.s./kg diet, equivalent to 135 and 10 mg a.s./kg bw/day (males) and 130 and 11 mg a.s./kg bw/day (females), respectively.

B.9.1.5 Risk assessment for birds

Routes of exposure

For the application in tomato (glass house), the routes of exposure to birds are considered to be:

- consumption of surface water containing residues of pyridaben (acute);
- consumption of fish contaminated with residues of pyridaben (long-term).

For the application in citrus, the routes of exposure to birds are considered to be:

- consumption of oversprayed insects with residues of pyridaben (short- and long-term);
- consumption of drinking water (surface water and puddles) containing residues of pyridaben (acute);
- consumption of fish contaminated with residues of pyridaben (long-term);
- consumption of earthworms contaminated with residues of pyridaben (long-term).

B.9.1.5.1 Risk of active substance for birds

Acute risk assessment (standard exposure scenario)

The acute risk assessment is based on an insectivorous bird of body weight (BW) 10 g feeding exclusively on insects, with a Food Intake Ratio (FIR) of 10.4 g fresh material/day, and a daily water intake (DWI) of 2.7 mL/day. The acute LD50 is >2250 mg/kg bw. The estimated theoretical exposure (ETE) for the acute time scale is calculated as $(FIR/bw) \cdot RUD \cdot \text{dose}$ or as $PEC_{dw} \cdot DWI/BW$. In the tables below, PEC_{feed} is equal to $RUD \cdot \text{dose}$. The residue values for acute intake represent 90th percentile values in agreement with the guidance in Sanco/4145/2000. The PEC_{sw} is the worst-case initial PEC_{sw} value (FOCUS Step 2, Northern and Southern Europe for tomato; FOCUS Step 3, Southern Europe for citrus, D6 ditch scenario) taken from Section B.8.6.1. The $PEC_{puddlewater}$ is calculated using a five-fold dilution of the spray liquid, where spray liquid concentration is 400 mg a.s./L. Residue values in food and water, ETE and TERa values are presented in the table below.

Table B.9.1.5.1-01 Acute Toxicity Exposure Ratios for exposure of birds to pyridaben due to consumption of contaminated insects and drinking water

appln.	dose (kg as/ha)	LD50 (mg/kg bw)	Route	PEC_{FEED} or PEC_{WATER} (mg/kg ww or mg/L)	ETE (mg/kg bw/d)	TERa
Tomato EU N & S	0.25	>2250	surface water	8E-05	2E-05	1E+08
Citrus EU S	0.30	>2250	Insects	16	16	139
			Surface water	0.010	0.0028	8E+05
			Puddles	80	21.6	>104

TERa values in Table B.9.1.5.1-01 are all far above the Annex VI 91/414 EEC trigger of 10. Hence, the acute risk to birds is considered to be acceptable.

Short-term risk assessment (standard exposure scenario)

Short-term risk assessment is based on the same bird, weighing 10 g feeding on insects. It is assumed however that in the course of some days, birds will not always be exposed to insects contaminated with pyridaben but also to uncontaminated food. Therefore, the arithmetic mean residue values are taken for residues in insects (in agreement with the guidance in Sanco/4145/2000). The toxicity endpoint used for risk assessment is the 5-day dietary NOEC for mortality (in analogy with the LC50) of 659 mg/kg bw/day. The ETE for the short-term time scale is calculated as $PEC_{\text{feed}} \cdot FIR/BW$, where $PEC_{\text{feed}} = RUD \cdot \text{dose}$. Residue values in food, ETE and TER_{st} values are presented in the table below.

Table B.9.1.5.1-02 Short-term Toxicity Exposure Ratios for exposure of birds to pyridaben due to consumption of contaminated insects

appln.	dose (kg as/ha)	NOEC (mg/kg bw/d)	route	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TER _{st}
Citrus EU S	0.30	659	Insects	8.7	9.0	73

The TER_{st} value in Table B.9.1.5.1-02 is above the Annex VI 91/414 EEC trigger of 10. Hence, the short-term risk to birds is considered to be acceptable.

Long-term risk assessment (standard exposure scenario)

Long-term risk assessment is based on the same bird weighing 10 g feeding on insects, and using arithmetic mean residue values for residues in insects (in agreement with the guidance in Sanco/4145/2000). Residues in insects are assumed to be stable in time, and no TWA correction factor is used. The NOEC is 10 mg a.s./kg bw/day. The ETE for the long-term time scale is calculated as $PEC_{\text{feed, initial}} \cdot DFI/BW$. Residue values in food, ETE and TER_{lt} values are presented in the table below.

Table B.9.1.5.1-03 Long-term Toxicity Exposure Ratios for exposure of birds to pyridaben due to consumption of contaminated insects

appln.	dose (kg as/ha)	NOEC (mg/kg bw/d)	Route	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TER _{lt}
Citrus EU S	0.30	10	Insects	8.7	9.0	1.1

The TER_{lt} value in Table B.9.1.5.1-03 is below the Annex VI 91/414 EEC trigger of 5. Hence, the risk assessment needs to be refined.

No information is available on (the time course of) residue levels in insects. The notifier is requested to provide further data for refined risk assessment of long-term exposure of birds to contaminated insects (e.g. measured residues on insects or PT or PD values).

Long-term risk assessment (bioaccumulation and food chain behaviour)

Earthworms

Long-term risk assessment is based on a bird weighing 100 g feeding exclusively on earthworms, with a daily food intake (DFI) of 113 g fresh material/day. The BCF for earthworms was calculated as $BCF = (0.84 + 0.01 \cdot Pow) / (f_{oc} \cdot K_{oc})$, where $K_{oc} = 66503 \text{ L/kg}$ (section B.8.2.1) and $f_{oc} = 0.02$ (standard value). Pow , calculated from $\log Pow > 6.37$ (section B.2.2; $\log Pow$ is not expected to be much higher than given value), was $2E+06$. The resulting BCF of pyridaben for earthworms was $>18 \text{ kg earthworm fresh weight per kg soil dry weight}$. Residue levels in earthworms were calculated from the 21-day TWA PEC_{soil} ($0.113 \text{ mg/kg dry weight}$ for the application in citrus, section B.8.3.1), as follows: $PEC_{FEED} = PEC_{worm} = PEC_{soil} \cdot BCF_{worm}$.

Fish

Long-term risk assessment is based on a fish-eating bird weighing 1000 g with a daily food intake (DFI) of 206 g fish/day. The BCF of pyridaben for fish is $<48 \text{ L/kg wet weight}$ (section B.9.2.3). Residue levels in fish were calculated from the 21-day TWA PEC_{sw} (2.179 and $0.0052 \text{ } \mu\text{g/L}$, respectively, for the application in citrus and tomato, see section B.8.6.1.) as follows: $PEC_{FEED} = PEC_{fish} = PEC_{sw} \cdot BCF_{fish}$.

The NOEC used for risk assessment is 10 mg/kg bw/day . The ETE for the long-term time scale is calculated as $PEC \cdot DFI / BW$. Residue levels in earthworms and fish and ETE and TERIt values are presented in the table below.

Table B.9.1.5.1-04 Long-term Toxicity Exposure Ratios for exposure of birds to pyridaben due to consumption of contaminated earthworms and fish

appln.	dose (kg as/ha)	NOEC (mg/kg bw/d)	Route	PEC_{FEED} (mg/kg ww)	ETE (mg/kg bw/d)	TERIt
Tomato EU N & S	0.25	10	Fish	$<2E-04$	$<5E-05$	$>2E+05$
Citrus EU S	0.30	10	Earthworms	>2.0	>2.3	<4.4
			Fish	<0.10	<0.02	>464

TERIt values for consumption of fish in Table B.9.1.5.1-04 are both far above the Annex VI 91/414 EEC trigger of 5. Hence, the long-term risk to birds as a result of bio-accumulation in fish is considered to be acceptable. The TERIt value for consumption of earthworms is <4.4 and therefore, a risk cannot be excluded. The notifier is requested to provide additional data for a refined risk assessment for this route (e.g. PT or PD values, measured residues in earthworms).

Bio-magnification in terrestrial food chains

The bioaccumulation potential reported in the List of Endpoints of the Toxicology section is stated to be low. Hence, according to the guidance provided in SANCO/4145/2000 – final (25 September 2002), the risk for biomagnification in terrestrial food chains is low.

B.9.1.5.2 Risk of metabolites for birds

According to the Guidance Document on terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10392/2002 rev 2 final of 17 October 2002), the risk of plant metabolites to birds should be addressed.

A risk assessment is performed for metabolites formed at a maximum level of at least 10% TRR in plants. In plant metabolism studies (section B.7.1), individual identified metabolites were at the most 5.1% TRR in apple fruit (25 days after treatment), 4.6% TRR in citrus fruit (7 days after the second treatment) and 1.1% TRR in tomato fruit (14 days after treatment). Hence no individual identified metabolite exceeded 10% TRR, and no assessment of plant metabolites is made.

B.9.1.6 Risk assessment for mammals

Procedures for risk assessment were identical to those for birds (see B.9.1.5), with the exception of the aspects indicated below.

B.9.1.6.1 Risk of active substance for mammals

Acute risk assessment (standard exposure scenario)

The acute risk assessment is based on a herbivorous mammal of body weight (BW) 25 g feeding exclusively on grasses (FIR 34.8 g/day). Assessment of exposure to pyridaben via intake of contaminated surface water however is based on a small mammal weighing 10 g, with a daily water intake (DWI) of 1.6 mL/day. The lowest acute oral LD50 is 161 mg a.s./kg bw for rats (see section B.6.5). The estimated theoretical exposure (ETE) for the acute time scale is calculated as $(FIR/bw) \cdot RUD \cdot \text{dose}$ or as $PEC_{sw} \cdot DWI/BW$. In the tables below, PEC_{feed} is equal to $RUD \cdot \text{dose}$. The residue values represent 90th percentile values, in agreement with the guidance in Sanco/4145/2000, where it is stated that for application of an insecticide on orchard tree stages without leaves an interception factor of 0.4 is assumed, while for later stages deposition is lower. Nexter Pro is applied when citrus trees are already infested with mites or whitefly, which is at a stage where leaves have already developed. No further guidance is given in Sanco/4145/2000, but in the Focus Groundwater Report (Sanco/321/2000) can be found that interception in that stage is 70%. Therefore $RUD = 142 \cdot 0.3 = 43$. The PEC_{sw} is the initial PEC_{sw} value (FOCUS Step 2, Northern and Southern Europe for tomato; FOCUS Step 3, Southern Europe for citrus) taken from Section B.8.6.1. The $PEC_{puddlewater}$ is calculated using a five-fold dilution of the spray liquid, where spray liquid concentration is 400 mg a.s./L. Residue levels in food and water, and ETE and TERa values are presented in the table below.

Table B.9.1.6.1-01 Acute Toxicity Exposure Ratios for exposure of mammals to pyridaben due to consumption of contaminated grasses and drinking water

appln.	dose (kg as/ha)	LD50 (mg/kg bw)	Route	PEC _{FEED} or PEC _{WATER} (mg/kg wwt or mg/L)	ETE (mg/kg bw/d)	TERa
Tomato EU N & S	0.25	161	Surface water	8E-05	1E-05	1E+07
Citrus EU S	0.30	161	Grasses	13	18	9.0
		161	Surface water	0.010	0.0016	1E+05
			Puddles	80	12.8	12.6

TERa values in Table B.9.1.6.1-01 are above the Annex VI 91/414 EEC trigger of 10 for consumption of contaminated water. Hence, the acute risk to mammals is considered to be acceptable for this route. A risk is expected due to consumption of contaminated grass, and therefore, the risk assessment needs to be refined for this route.

The lowest acute oral LD50 in rat was obtained in a study where animals were treated with pyridaben suspended in maize oil (LD50 values 161 and 181 mg/kg bw for males and females respectively). Two other studies were available, where animals were treated with pyridaben suspended in aqueous carboxymethyl cellulose. In this vehicle the toxicity of pyridaben was much lower: LD50 values were 570-820 and 1100-1350 mg/kg bw for males and females respectively. As there appears to be an effect of the vehicle on the toxicity of pyridaben, the most appropriate toxicity value for risk assessment is that determined in a vehicle resembling closely the environmental matrix through which mammals are exposed. The formulation is sprayed in an aqueous solution and grasses are a watery matrix, so the LD50 values determined with pyridaben suspended in aqueous carboxymethyl cellulose are considered to be most appropriate for evaluation. The TER based on the lowest LD50 for this vehicle (570 mg/kg bw) is shown in the table below.

Table B.9.1.6.1-02 Acute Toxicity Exposure Ratio for exposure of mammals to pyridaben due to consumption of contaminated grasses

appln.	dose (kg as/ha)	LD50 (mg/kg bw)	Route	PEC _{FEED} or PEC _{WATER} (mg/kg wwt or mg/L)	ETE (mg/kg bw/d)	TERa
Citrus EU S	0.30	570	Grasses	13	18	32

The TER value in Table B.9.1.6.1-02 is above the Annex VI 91/414 EEC trigger of 10 for consumption of contaminated grasses, and the risk is considered to be acceptable.

Long-term risk assessment (standard exposure scenario)

Long-term risk assessment is based on the same mammal weighing 25 g feeding on grasses. As explained in the acute risk assessment, for the application of pyridaben on citrus an interception value of 70% has to be taken into account on the arithmetic mean residue values for residues in leafy crops ($RUD = 76 \times 0.3 = 23$). Residue values are time-weighted over a period of 3 weeks assuming a DT50 of 10 days, which leads to a

TWA correction factor (f_{TWA}) of the initial residue values of 0.53 (in agreement with the guidance in Sanco/4145/2000).

From the toxicology section of the DAR (B.6), several toxicological endpoints are available that might be used for the ecotoxicological risk assessment. These values are presented in Table B.9.1.6.1-03.

Table B.9.1.6.1-03 Long-term toxicity values for mammals

Species		NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Critical effects	Reference
Semichronic oral toxicity					
13-week, rat (diet)		2.5 (F)	5.2 (F)	Decreased body weight gain and changes in clinical biochemistry (effects seen more or less from week 4).	Holmes, 1988a
13-week, mouse (diet)		3.9 (M)	12.6 (M)	Decreased body weight gain (effects seen more or less from week 4).	Holmes, 1988b
3-month, dog (capsule)		1.0	4.0	Decreased body weight gain, food-like emesis and ptyalism (no clear picture of when effects start).	Spicer, 1989b
1-year, dog (capsule)		1.0	4.0	Decreased body weight gain (no clear picture of when effects start).	Spicer, 1990, 1991 incl additional study
2-Generation reproduction toxicity					
rat	Parental	1.87	5.33	Decreased body weight gain and food consumption (effect clearly seen from week 4).	Willoughby, 1990
	developmental reproduction	5 ≥5.33	15	Decreased pup body weight. No effects.	
Teratogenicity					
rat	maternal	4.7	12.5	Decreased body weight gain and food consumption (effect clearly seen after 1 week).	Willoughby, 1988
	developmental	12.5	26.7	Decreased foetal weight, retarded ossification of various bones.	
	teratogenicity	≥ 26.7	-	No effects.	
rabbit (oral)	maternal	5	15	Decreased body weight gain and food consumption, reduced faeces (effect clearly seen after 1 week).	Ross, 1988
	developmental	15	-	No effects.	
	teratogenicity	15	-	No effects.	

The toxicity value used for risk assessment is taken to be 1.87 mg/kg bw/day, originating from the 2-generation reproduction study in rat with pyridaben (section B.6.6.1), and is based on adverse effects on a survival endpoint (decreased body weight gain) at the next higher dose. The effect on body weight gain is

clearly seen from four weeks of exposure. The choice for this endpoint is supported by the results of the other longer-term toxicity studies from the human toxicology section of the DAR (see table B.9.1.6.1-03) in which the NOAEL is set at values in the same range (from 1 to 5 mg/kg bw/d).

The ETE for the long-term time scale is calculated as $PEC_{feed, initial} * ftwa * DFI / BW$. Residue levels in food, and ETE and TERIt values are presented in the table below.

Table B.9.1.6.1-04 Long-term Toxicity Exposure Ratios (First Tier) for exposure of mammals to pyridaben due to consumption of contaminated grass

appln.	dose (kg as/ha)	NOEL (mg/kg bw/d)	Route	PEC _{FEED} (mg/kg ww)	ETE (mg/kg bw/d)	TERIt
Citrus EU S	0.30	1.87	Grasses	3.6	5.0	0.37

The TERIt value in Table B.9.1.6.1-04 is below the Annex VI 91/414 EEC trigger of 5. Hence, the long-term risk assessment needs to be refined. The notifier is requested to provide further data for refined risk assessment of long-term exposure of mammals to grass (e.g. PT or PD values, measured residues in grass).

Long-term risk assessment (bioaccumulation and food chain behaviour)

Earthworms

Long-term risk assessment is based on a mammal weighing 10 g feeding exclusively on earthworms, with a daily food intake (DFI) of 14 g fresh material/day. PEC calculations were identical to those for exposure of birds to earthworms (see B.9.1.5.1).

Fish

Long-term risk assessment is based on a fish-eating mammal weighing 3000 g with a daily food intake (DFI) of 390 g fish/day. PEC calculations were identical to those for exposure of birds to fish (see B.9.1.5.1).

The NOEL used for First Tier risk assessment is 1.87 mg/kg bw/day. The estimated theoretical exposure (ETE) for the long-term time scale is calculated as $PEC * DFI / BW$. Residue levels in earthworms and fish and ETE and TERIt values are presented in the table below.

Table B.9.1.6.1-05 Long-term Toxicity Exposure Ratios for exposure of mammals to pyridaben due to consumption of contaminated earthworms and fish

appln.	dose (kg as/ha)	NOEL (mg/kg bw/d)	Route	PEC _{FEED} (mg/kg ww)	ETE (mg/kg bw/d)	TERIt
Tomato EU N & S	0.25	1.87	Fish	<2E-04	<3E-05	>6E+04
Citrus EU S	0.30	1.87	Earthworms	>2.0	>2.8	<0.67
			Fish	<0.10	<0.014	>138

TERIt values for consumption of fish in Table B.9.1.6.1-05 are both far above the Annex VI 91/414 EEC trigger of 5. Hence, the long-term risk to mammals as a result of bio-accumulation in fish is considered to be

acceptable. The TERIt value for consumption of earthworms is <5 and therefore, the risk assessment needs to be refined. The notifier is requested to provide additional data for a refined risk assessment for this route (e.g. PT or PD values, measured residues in earthworms).

B.9.1.6.2 Risk of plants metabolites for mammals

According to the Guidance Document on terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10392/2002 rev 2 final of 17 October 2002), the risk of plant metabolites to mammals should be addressed.

A risk assessment is performed for metabolites formed at a maximum level of at least 10% TRR in plants. In plant metabolism studies (section B.7.1), individual identified metabolites were at the most 5.1% TRR in apple fruit (25 days after treatment), 4.6% TRR in citrus fruit (7 days after the second treatment) and 1.1% TRR in tomato fruit (14 days after treatment). Hence no individual identified metabolite exceeded 10% TRR, and no assessment of plant metabolites is made.

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

B.9.2.1 Acute toxicity to aquatic life (IIA 8.2)

B.9.2.1.1 Acute toxicity of the active substance

Study 1

Characteristics

Reference	: Willis C.A. (1987)	species	: Rainbow trout (<i>Salmo gairdneri</i>)
type of study	: Acute toxicity study	exposure duration	: 96 hours
year of execution	: 1986	nominal concentrations	: 0, 0.89, 1.4, 2.1, 3.3 and 5.0 µg/L
GLP statement	: Yes	dosing method	: Semi-static; stock solutions in acetone
Guideline	: EPA-540/9-85-006, OECD 203	acceptability	: Not acceptable
test substance	: NC-129 technical (pyridaben), lot no. 129T8605	96h-LC50	: 1.8 µg/L
Purity	: 98.0%		

Methods

A 96-hour acute toxicity test in rainbow trout (*Salmo gairdneri*) (2 replicates of five fish each per concentration) was conducted under semi-static conditions (renewal after 48 hours) with NC-129 technical (pyridaben; 98.0% purity) at nominal test concentrations of 0.89, 1.4, 2.1, 3.3 and 5.0 µg/L, with untreated and vehicle treated control (acetone).

Results

Measured concentrations in freshly prepared solutions were 98-138% of nominal, while measured concentrations in 48-hour old solutions had declined to 27-65% of nominal. Reported endpoints were based

on mean measured initial (freshly prepared) concentrations, and were recalculated by RMS based on mean measured concentrations using Finney (Finney, D.J., 1971: Probit analysis, Cambridge University Press, Cambridge, U.K., 3rd edition). Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. The results are summarised in Table B.9.2.1.1-01.

Table B.9.2.1.1-01 The acute toxicity of NC-129 technical (pyridaben) to fish

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC50 in µg/L	NOEC µg/L
<i>Salmo gairdneri</i>	Semi-static 96 hours (98.0%)	98-138% (fresh) 27-65% (48 h-old)	24 h: 4.0 ^(A) 48 h: 2.8 ^(A) 72 h: 2.1 ^(A) 96 h: 1.8 ^(A)	24 h: 1.9 ^(A) 48-96 h: 1.5 ^(A)

^(A) Based on mean measured concentrations

Conclusion

96-hour LC50: 1.8 µg/L, based on mean measured concentrations.

Guidelines and limitations

The report stated that control samples were fortified with pyridaben at 0.5, 2 and 5 µg/L and analysed concurrently with the study samples (SPE extraction followed by HPLC/UV analysis). The reporting on the results for these quality control samples was limited to the following information: RSD for duplicate samples at 0.5 and 5 µg/L <5%, recovery at low concentrations 20% with RSD for duplicate samples 30%. The information on RSD (repeatability) is contradictory. A recovery value " at low concentrations" of 20% is not acceptable. The study results are therefore not accepted.

Study 2

Characteristics

Reference	:	Ward S.G. (1994a)	species	:	Rainbow trout (<i>Oncorhynchus mykiss</i>)
type of study	:	Acute toxicity study	exposure duration	:	96 hours
year of execution	:	1993	nominal	:	0, 0.31, 0.52, 0.86, 1.4 and 2.4 µg
GLP statement	:	Yes	concentrations	:	a.s./L
guideline	:	EPA 72-1	dosing method	:	Flow-through; stock solutions in DMF
test substance	:	Pyridaben (BAS 300 I-Tech.), lot no. 900304	acceptability	:	Acceptable
purity	:	100.0%	96h-LC50	:	0.73 µg a.s./L

Methods

A 96-hour acute toxicity test in rainbow trout (*Oncorhynchus mykiss*) (twenty fish per concentration) was conducted under flow-through conditions with Pyridaben (100.0% purity) at nominal test concentrations of 0.31, 0.52, 0.86, 1.4 and 2.4 µg/L, with untreated and solvent-control (DMF).

Results

The measured concentrations were 0.23, 0.42, 0.70, 1.06 and 1.75 µg a.s./L at test initiation (representing 73-81% of nominal), and 0.34, 0.48, 0.74, 1.14 and 2.0 µg a.s./L at the end of exposure (representing 79-110% of nominal). Endpoints were based on mean measured concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. The results are summarised in Table B.9.2.1.1-02.

Table B.9.2.1.1-02 The acute toxicity of pyridaben to fish

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC50 in µg a.s./L	NOEC µg a.s./L
<i>Oncorhynchus mykiss</i>	Flow-through 96 hours (100.0%)	73-110	24 h: 1.5 ^(A) 48 h: 0.88 ^(A) 72 h: 0.78 ^(A) 96 h: 0.73 ^(A)	24-72 h: 0.45 ^(A) 96 h: 0.29 ^(A)

^(A) Based on mean measured concentrations

Conclusion

96-hour LC50: 0.73 µg a.s./L, based on mean measured concentrations.

Guidelines and limitations

The study is acceptable.

Study 3

Characteristics

reference	:	Willis C.A. (1988)	species	:	Bluegill sunfish (<i>Lepomis macrochirus</i>)
type of study	:	Acute toxicity study	exposure duration	:	96 hours
year of execution	:	1987	nominal concentrations	:	0, 1.8, 2.7, 4.2, 6.5 and 10 µg/L
GLP statement	:	Yes	dosing method	:	Semi-static; stock solutions in acetone
guideline	:	EPA-540/9-85-006, OECD 203	acceptability	:	Not acceptable
test substance	:	NC-129 technical (pyridaben), lot no. 129T8605	96h-LC50	:	2.6 µg/L
purity	:	98.0%			

Methods

A 96-hour acute toxicity test in bluegill sunfish (*Lepomis macrochirus*) (2 replicates of five fish each per concentration) was conducted under semi-static conditions (renewal after 48 hours) with NC-129 technical (pyridaben; 98.0% purity) at nominal test concentrations of 1.8, 2.7, 4.2, 6.5 and 10 µg/L, with untreated and treatment-control (acetone).

Results

Measured concentrations in freshly prepared solutions were 76-110% of nominal, while measured concentrations in 48-hour old solutions had declined to 36-61% of nominal. Reported endpoints were based on mean measured initial (freshly prepared) concentrations, and were recalculated by RMS based on mean measured concentrations using Finney (Finney, D.J., 1971: Probit analysis, Cambridge University Press,

Cambridge, U.K., 3rd edition). Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline with a minor deviation for the temperature that did not affect the results. The results are summarised in Table B.9.2.1.1-03.

Table B.9.2.1.1-03 The acute toxicity of NC-129 technical (pyridaben) to fish

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC50 in µg/L	NOEC µg/L
<i>Lepomis macrochirus</i>	Semi-static 96 hours (98.0%)	76-110% (fresh) 36-61% (48 h-old)	24 h: 3.3 ^(A) 48 h: 3.2 ^(A) 72 h: 2.7 ^(A) 96 h: 2.6 ^(A)	24-48 h: 1.5 ^(A) 72-96 h: 1.1 ^(A)

^(A) Based on mean measured concentrations

Conclusion

96-hour LC50: 2.6 µg/L, based on mean measured concentrations.

Guidelines and limitations

To verify that the reported analytical results are correct, information on recovery and repeatability for samples of test medium fortified with pyridaben at concentrations encompassing the tested range should be available. Data on validation of the analytical method to determine pyridaben concentrations in test medium was limited to the LOD (in terms of two times background signal) and linearity (coefficient of correlation 0.99, no plot provided however). This information is insufficient to accept the study results.

Study 4

Characteristics

reference	:	Ward G.S. (1994b)	species	:	Bluegill sunfish (<i>Lepomis macrochirus</i>)
type of study	:	Acute toxicity study	exposure duration	:	96 hours
year of execution	:	1993	nominal	:	0, 0.94, 1.6, 2.6, 4.3 and 7.2 µg
GLP statement	:	Yes	concentrations	:	a.s./L
guideline	:	EPA 72-1	dosing method	:	Flow-through; stock solutions in DMF
test substance	:	Pyridaben (BAS 300 I-Tech.), lot no. 900304	acceptability	:	Acceptable
purity	:	100.0%	96 h-LC50	:	3.5 µg a.s./L

Methods

A 96-hour acute toxicity test in bluegill sunfish (*Lepomis macrochirus*) (twenty fish per concentration) was conducted under flow-through conditions with Pyridaben (100.0% purity) at nominal test concentrations of 0.94, 1.6, 2.6, 4.3 and 7.2 µg/L, with untreated and solvent-control (DMF).

Results

The measured concentrations were 0.99, 1.46, 2.37, 3.16 and 5.16 µg a.s./L at test initiation (representing 72-105% of nominal), and 0.55-0.62, 0.85-1.19, 2.07-2.14, 2.76-3.24 and 5.31-5.42 µg a.s./L at the end of exposure (representing 53-82% of nominal). Endpoints were based on mean measured concentrations,

which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. The results are summarised in Table B.9.2.1.1-04.

Table B.9.2.1.1-04 The acute toxicity of pyridaben to fish

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC50 in µg a.s./L	NOEC µg a.s./L
<i>Lepomis macrochirus</i>	Flow-through 96 hours (100.0%)	53-105	24 h: 3.8 ^(A) 48, 72 & 96 h: 3.5 ^(A)	24, 48, 72 & 96 h: 2.2 ^(A)

^(A) Based on mean measured concentrations

Conclusion

96-hour LC50: 3.5 µg a.s./L, based on mean measured concentrations.

Guidelines and limitations

The study is acceptable.

Study 5

Characteristics

reference	:	Morrow J.E. & Ward S.G. (1993)	species	:	Sheepshead minnow (<i>Cyprinodon variegatus</i>)
type of study	:	Acute toxicity study	exposure duration	:	96 hours
year of execution	:	1993	nominal concentrations	:	0, 2.6, 4.3, 7.2, 12 and 20 µg a.s./L
GLP statement	:	Yes	dosing method	:	Flow-through; stock solutions in DMF
guideline	:	EPA 72-3	acceptability	:	Acceptable
test substance	:	Pyridaben (BAS 300 I-Tech.), lot no. 900304	96 h-LC50	:	17 µg a.s./L
purity	:	99.7%			

Methods

A 96-hour acute toxicity test in sheepshead minnow (*Cyprinodon variegatus*) (twenty fish per concentration) was conducted under flow-through conditions with Pyridaben (99.7% purity) at nominal test concentrations of 2.6, 4.3, 7.2, 12 and 20 µg/L, with untreated and solvent-control (DMF).

Results

The measured concentrations were 2.2, 3.4, 6.5, 11 and 17 µg a.s./L at test initiation (representing 80-93% of nominal), and 1.8-2.1, 2.6, 5.8-6.0, 7.0-7.8 and 17-19 µg a.s./L at the end of exposure (representing 58-93% of nominal). Endpoints were based on mean measured concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. The results are summarised in Table B.9.2.1.1-05.

Table B.9.2.1.1-05 The acute toxicity of pyridaben to fish

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC50 in µg a.s./L	NOEC µg a.s./L
<i>Cyprinodon variegatus</i>	Flow-through 96 hours (99.7%)	58-93	24-72 h: >18 ^(A) 96 h: 17 ^(A)	24 h: 9.2 ^(A) 48, 72 & 96 h: 6.2 ^(A)

^(A) Based on mean measured concentrations

Conclusion

96-hour LC50: 17 µg a.s./L, based on mean measured concentrations.

Guidelines and limitations

The study is acceptable.

Study 6

Characteristics

reference	: Willis C.A. & Wilson I. (1987)	species	: <i>Daphnia magna</i>
type of study	: Acute toxicity study	exposure duration	: 48 hours
year of execution	: 1986	nominal concentrations	: 0, 0.18, 0.27, 0.42, 0.65 and 1.0 µg/L
GLP statement	: Yes	dosing method	: Static; stock solutions in acetone
guideline	: EPA-540/9-85-005 (1), OECD 202	acceptability	: Not acceptable
test substance	: NC-129 technical (pyridaben), lot no. 129T8605	48 h-EC50	: 0.38 µg/L
purity	: 98.0%		

Methods

A 48-hour immobilisation test with *Daphnia magna* (4 replicates of 5 *Daphnia* each per concentration) was conducted under static conditions with NC-129 technical (pyridaben; 98.0% purity) at nominal test concentrations of 0.18, 0.27, 0.42, 0.65 and 1.0 µg/L, with untreated and vehicle treated -control (acetone).

Results

The measured concentrations were 0.12, 0.21, 0.30, 0.33 and 0.77 µg/L at test initiation (representing 51-78% of nominal), and 0.17, 0.30, 0.43, 0.85 and 1.1 µg/L at the end of exposure (representing 96-131% of nominal). Endpoints were based on initial measured concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance to the OECD 202 guideline. The results are summarised in Table B.9.2.1.1-06.

Table B.9.2.1.1-06 The acute toxicity of NC-129 technical to aquatic invertebrates

Species	Test type and duration. (purity)	Actual concn. (as % of nominal)	EC50 in µg/L	NOEC µg/L
<i>Daphnia magna</i>	Static 48 hours (98.0%)	51-78 (0 h) 96-131 (48 h)	24 h: >1.0 ^(A) 48 h: 0.38 ^(A)	24 & 48 h: 0.30 ^(A)

^(A) Based on initial measured concentrations

Conclusion

48-hour EC50: 0.38 µg/L, based on initial measured concentrations.

Guidelines and limitations

The report stated that control samples were fortified with pyridaben at 0.5 and 1 µg/L and analysed concurrently with the study samples (SPE extraction followed by HPLC/UV analysis). The following results were reported for method validation: RSD for duplicate samples at 0.5 and 1 µg/L approximately 5-10%; mean blank 0.094 µg/L; mean recovery at 0.5 µg/L 0.35±0.03 µg/L and at 1 µg/L 0.61±0.02 µg/L. It was also reported that concentrations for study samples were corrected by the value obtained for blank samples and for losses determined during the concentration step (as the overall mean recovery). The recovery results however were not corrected for residues in untreated blank samples. The apparent residues in untreated samples represented 78% of the measured initial pyridaben concentration in medium of the lowest test concentration. It is unclear whether these apparent residues represent true pyridaben residues or an interfering peak of another origin. Correction of concentrations in treated samples for a control value representing up to 78% of the measured initial pyridaben concentration is not acceptable. The recovery results are also not acceptable since these were not corrected for residues in untreated samples and therefore presumably overestimated. The study results are therefore not accepted.

Study 7

Characteristics

reference	:	Graves W.C. & Swigert J.P. (1993)	species	:	<i>Daphnia magna</i>
type of study	:	Acute toxicity study	exposure duration	:	48 hours
year of execution	:	1993	nominal concentrations	:	0, 0.23, 0.39, 0.65, 1.1 and 1.8 µg a.s./L
GLP statement	:	Yes	dosing method	:	Flow-through; stock solutions in acetone
guideline	:	EPA 72-2 (a)	acceptability	:	Acceptable
test substance	:	Pyridaben technical (NC-129), lot no. 900304	48 h-EC50	:	1.0 µg a.s./L
purity	:	99.7%			

Methods

A 48-hour immobilisation test with *Daphnia magna* (2 replicates of 10 *Daphnia* each per concentration) was conducted under flow-through conditions with pyridaben technical (99.7% purity) at nominal test concentrations of 0.23, 0.39, 0.65, 1.1 and 1.8 µg a.s./L, with untreated and vehicle control (acetone).

Results

The mean measured concentrations at 0, 24 and 48 hours were 83-105% of nominal. Endpoints were based on mean measured concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 202 guideline. The results are summarised in Table B.9.2.1.1-07.

Table B.9.2.1.1-07 The acute toxicity of Pyridaben technical to aquatic invertebrates

Species	Test type and duration. (purity)	Actual concn. (as % of nominal)	EC50 in µg a.s./L	NOEC µg a.s./L
<i>Daphnia magna</i>	Flow-through 48 hours (99.7%)	83-105	24 h: >1.6 ^(A) 48 h: 1.0 ^(A)	24 h: 0.6 ^(A) 48 h: 0.22 ^(A)

^(A) Based on mean measured concentrations

Conclusion

48-hour EC50: 1.0 µg a.s./L, based on mean measured concentrations.

Guidelines and limitations

The study is acceptable.

Study 8

Characteristics

reference	: Morrow J.E. (1993)	species	: <i>Mysidopsis bahia</i> (marine shrimp)
type of study	: Acute toxicity study	exposure duration	: 96 hours
year of execution	: 1993	nominal concentrations	: 0, 0.14, 0.24, 0.40, 0.66 and 1.1 µg/L
GLP statement	: Yes	dosing method	: Flow-through, stock solutions in DMF
guideline	: EPA 72-3	acceptability	: Acceptable
test substance	: Pyridaben (BAS 300 I-Tech.), Lot no. 900304	96-hour LC50	: 0.67 µg/L
purity	: 99.7%		

Methods

A 96-hour toxicity test in the salt-water shrimp *Mysidopsis bahia* (4 replicates of 5 shrimps each per concentration) was conducted under flow-through conditions with pyridaben (BAS 300 I-Tech.) at nominal test concentrations of 0.14, 0.24, 0.40, 0.66 and 1.1 µg/L, with untreated and vehicle control (DMF).

Results

The measured concentrations were 0.14, 0.21, 0.47, 0.65 and 0.87 µg/L at test initiation (representing 79-116% of nominal), and 0.16, 0.15, 0.36, 0.69 and 0.76 µg/L at the end of exposure (representing 63-113% of nominal). Endpoints were based on mean measured concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the EPA 72-3 guideline. The results are summarised in Table B.9.2.1.1-08.

Table B.9.2.1.1-08 The acute toxicity of pyridaben (BAS 300 I-Tech.) to aquatic invertebrates

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC50 in µg/L	NOEC µg/L
<i>Mysidopsis bahia</i> (marine shrimp)	Flow-through 96 hours (99.7%)	63-116	24-72 h: >0.82 ^(A) 96 h: 0.67 ^(A)	24-96 h: 0.15 ^(A)

^(A) Based on mean measured concentrations

Conclusion

96-hour LC50: 0.67 µg/L, based on mean measured concentrations.

Guidelines and limitations

The test is acceptable.

Study 9

Characteristics

reference	: Ward G.S. (1994c)	species	: <i>Crassostrea virginica</i> (mollusc)
type of study	: Acute toxicity study	exposure duration	: 96 hours
year of execution	: 1993	nominal concentrations	: 1 st test: 0, 2.6, 4.3, 7.2, 12 and 20 µg a.s./L, 2 nd test: 0.26, 0.43, 0.72, 1.2 and 2.0 µg a.s./L
GLP statement	: Yes	dosing method	: Flow-through, stock solution in DMF
guideline	: EPA 72-3 (b)	acceptability	: Acceptable
test substance	: Pyridaben (BAS 300 I-Tech.), Lot no. 900304	96-hour EC50	: 8.3 µg a.s./L
purity	: 100.0%		

Methods

Two 96-hour toxicity tests in *Crassostrea virginica* (20 oysters per concentration) were conducted under flow-through conditions with pyridaben (BAS 300 I-Tech.) at nominal test concentrations of 0.6, 4.3, 7.2, 12 and 20 µg a.s./L in the 1st test and 0.26, 0.43, 0.72, 1.2 and 2.0 µg a.s./L in the 2nd test. Both tests contained an untreated and vehicle control (DMF).

Results

Measured concentrations in the 1st test were 2.0, 4.2, 5.4, 10 and 15 µg a.s./L at test initiation (representing 75-99% of nominal), and 1.9, 3.8, 5.0, 10 and 14 µg a.s./L at the end of exposure (representing 70-88% of nominal). In the 2nd test measured concentrations were 0.22, 0.44, 0.61, 1.0 and 1.3 µg a.s./L at test initiation (representing 66-102% of nominal), and 0.18, 0.43, 0.64, 1.0 and 1.4 µg a.s./L at the end of exposure (representing 69-100% of nominal). Endpoints were based on mean measured concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration, temperature and salinity) were in accordance with the EPA 72-3 guideline. The results are summarised in Table B.9.2.1.1-09.

Table B.9.2.1.1-09 The acute toxicity of pyridaben (BAS 300 I-Tech.) to aquatic invertebrates

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	EC50 in µg a.s./L	NOEC µg a.s./L
<i>Crassostrea virginica</i> (marine oyster)	Flow-through 96 hours (100.0%)	66-102	96 h: 8.3 ^(A)	96 h: 0.20 ^(A)

^(A) Based on mean measured concentrations

Conclusion

96-hour EC50: 8.3 µg a.s./L, based on mean measured concentrations.

Guidelines and limitations

The test is acceptable.

Study 10

Characteristics

reference	:	Jenkins W.R. (1988)	exposure duration	:	96 hours
type of study	:	Algal growth inhibition test	nominal concentrations	:	0, 10, 56, 100, 560 and 1000 µg/L
year of execution	:	1987	dosing method	:	Static, stock solution in acetone
GLP statement	:	Yes	acceptability	:	Not acceptable
guideline	:	OECD 201, EPA 797.1060	96 h-EbC50	:	Not reported
test substance	:	NC-129 Technical, Lot no. 129T8605	96 h-ErC50	:	> 1000 µg/L
purity	:	98%	96 h-NOEbC	:	Not reported
species	:	<i>Selenastrum capricornutum</i>	96 h-NOErC	:	1000 µg/L

Methods

A 96-hour toxicity test on green algae (*Selenastrum capricornutum*) (3 replicates per test group, each containing 1.0×10^4 cells/mL at the start) was conducted with NC-129 Technical (98% pyridaben) at nominal test concentrations of 10, 56, 100, 560 and 1000 µg/L, with blank- and solvent-control (acetone). A positive control (Potassium dichromate: 0.3, 1.0 and 3.2 mg/L) was also included.

Results

The mean measured concentrations were 10, 37, 83, 454 and 866 µg/L at test initiation (representing 66-103% of nominal), and <LOD, <LOD, 21, 345 and 665 µg/L at the end of exposure (representing 21-67% of nominal). Endpoints were based on nominal concentrations. Water quality parameters were in accordance with the OECD guideline 201. The results are summarised in Table B.9.2.1.1-10.

Table B.9.2.1.1-10 The acute toxicity of NC-129 Technical to algae

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	EC50 in µg/L	NOEC µg/L
<i>Selenastrum capricornutum</i> (green alga)	Static 96 hours (98%)	66-103 (start) 21-67 (end)	96h-ErC50: >1000 ^(A)	96h-NOErC: 1000 ^(A)

^(A) Based on nominal concentrations

Conclusion

96-hour ErC50: >1000 µg/L, 96-hour NOErC: 1000 µg/L, 96-hour EbC50 and 96-hour NOEbC: not reported.

Guidelines and limitations

Algal cell densities in each of the three replicate vessels of the controls and the test solutions showed large variations, to an extent that a reliable estimation of EC50 values for both biomass and growth rate was considered to be impossible. The table below shows the cell densities after 96 hours. Cell densities in individual replicates of treatments differed by a factor of up to 12. The test result is therefore not accepted.

NC-129 treatment (µg/L)	Cell number (*10 ⁴) after 96 h			Factor between lowest and highest cell density
	Repl. 1	Repl. 2	Repl. 3	
Blank-control	229.3	121.8	78.3	2.9
Solvent-control	159.5	257.8	53.9	4.8
10	28.9	55.5	63.6	2.2
56	157.8	293.0	24.0	12
100	78.4	89.3	70.3	1.3
560	176.8	41.0	66.0	4.3
1000	132.5	69.9	29.6	4.5

Study 11

Characteristics

reference	:	Hughes J.S. & Jackson S.H. (1994)	exposure duration	:	5-14 days
type of study	:	Tier I Non-target plant toxicity study	nominal	:	0, 12 µg/L
year of execution	:	1993	concentrations	:	
GLP statement	:	Yes	dosing method	:	Static, stock solution in DMF
guideline	:	EPA 122-2	acceptability	:	Acceptable, except for <i>Lemna gibba</i>
test substance	:	BAS 300 I (Pyridaben), Lot no. 900304	EbC50 and ErC50	:	
purity	:	99.7%	<i>S. capricornutum</i>	:	>17 µg/L (72-hour)
species	:	<i>Selenastrum capricornutum</i> , <i>Anabaena flos-aquae</i> , <i>Navicula pelliculosa</i> , <i>Skeletonema costatum</i> , <i>Lemna gibba</i>	<i>A. flos-aquae</i>	:	>13 µg/L (120-hour)
			<i>N. pelliculosa</i>	:	>14 µg/L (120-hour)
			<i>S. costatum</i>	:	>16 µg/L (120-hour)

Methods

A Tier I non-target aquatic plant toxicity study on four algal species (*Selenastrum capricornutum*, *Anabaena flos-aquae*, *Navicula pelliculosa*, *Skeletonema costatum*) and duckweed (*Lemna gibba*) was conducted with BAS 300 I (pyridaben 99.7%) at a nominal concentration of 12 µg/L, with blank- and solvent-control (DMF). Algae (3 to 6 replicates per test group, containing 0.3-1.0x10⁴ cells/mL at the start) were exposed for 5 days and duckweed (3 replicates per test group, each with three plants and four fronds per plant) was exposed for 14 days to BAS 300 I. Additional replicates were exposed in light and dark conditions for analytical determination of the stability of BAS 300 I under test conditions.

Results

The initial measured concentrations ranged from 13.4 to 17.3 µg/L at the start of exposure (111-143% relative to nominal) and declined below the limit of detection within 5 days (algae tests) or within 7 days (duckweed test). The additional stability test indicated that BAS 300 I was unstable in light but relatively

stable while exposed in the dark with a recovery relative to initial of at least 83% at test termination. Endpoints were based on initial measured concentrations, which is acceptable. Water quality parameters were in accordance with the EPA 122-2 guideline. The growth of the test organisms during the tests was adequate.

Exposure to the nominal concentration of 12 µg/L resulted in 0.8% inhibition for *S. capricornutum* after 72 hours, 6% stimulation for *A. flos-aquae* after 120 hours, 6% stimulation for *N. pelliculosa* after 120 hours, 20% inhibition for *S. costatum* after 120 hours, and 3% inhibition for *L. gibba* after 14 days.

Conclusion

The 72-hour EbC50 and ErC50 for cell growth was >17 µg/L for *S. capricornutum*; the 120-hour EbC50 and ErC50 for cell growth was >13 µg/L for *A. flos-aquae*, >14 µg/L for *N. pelliculosa* and >16 µg/L for *S. costatum*; the 14-day EbC50 and ErC50 for frond growth of *Lemna gibba* based on frond numbers was >16 µg/L.

Guidelines and limitations

The reported EC50 for *L. Gibba* was based on frond number. Frond biomass measurements were not conducted as part of the above study. Measurement of frond biomass (dry weight, fresh weight or total frond area) however is also required according to the updated draft OECD 221 guideline (2002). The results of the *L. gibba* study will therefore not be taken to the list of endpoints. All other studies are acceptable.

B.9.2.1.2 Acute toxicity of metabolites

Study 1

Characteristics

reference	:	Jenkins C.A. (2003a)	exposure duration	:	96 hours
type of study	:	Acute toxicity study	nominal	:	0 & 10 mg/L
year of execution	:	2003	concentrations	:	
GLP statement	:	Yes	dosing method	:	Semi-static; dilutions in test medium
guideline	:	EEC C-1, OECD 203	acceptability	:	Acceptable
test substance	:	PB-7, batch PB-7-S001129	96 h-LC50	:	>8.0 mg/L
purity	:	99.0%			
species	:	Rainbow trout (<i>Oncorhynchus mykiss</i>)			

Methods

A 96-hour acute toxicity test in rainbow trout (*Oncorhynchus mykiss*) (ten fish per concentration) was conducted as a limit test under semi-static conditions with PB-7 (99.0% purity) at a nominal test concentration of 10 mg/L, with untreated control.

Results

Measured concentrations in solutions freshly prepared at 0 and 72 hours and in aged solutions from 24 and 96 hours were 61-93% of nominal. Endpoints were based on mean measured concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. The results are summarised in Table B.9.2.1.2-01.

Table B.9.2.1.2-01 The acute toxicity of PB-7 to fish

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	LC50 in mg/L	NOEC mg/L
<i>Oncorhynchus mykiss</i>	Semi-static 96 hours (99.0%)	61-93%	24-96 h: >8.0 ^(A)	24-96 h: 8.0 ^(A)

^(A) Based on mean measured concentrations

Conclusion

96-hour LC50: >8.0 mg/L, based on mean measured concentrations.

Guidelines and limitations

The study is acceptable.

Study 2

Characteristics

reference	:	Jenkins C.A. (2003b)	species	:	<i>Daphnia magna</i>
type of study	:	Acute toxicity study	exposure duration	:	48 hours
year of execution	:	2002	nominal concentrations	:	0, 10, 22, 48, 106, 234 and 515 µg/L
GLP statement	:	Yes	dosing method	:	Static; dilutions in test medium
guideline	:	EEC C2, OECD 202	acceptability	:	Acceptable
test substance	:	PB-7, batch PB-7-S001129	48 h-EC50	:	28 µg/L
purity	:	99.0%			

Methods

A 48-hour immobilisation test with *Daphnia magna* (4 replicates of 5 *Daphnia* each per concentration) was conducted under static conditions with PB-7 (purity 99.0%) at nominal test concentrations of 10, 22, 48, 106, 234 and 515 µg/L, with untreated control.

Results

The measured concentrations were 5, 13, 29, 86, 199 and 433 µg/L at test initiation (representing 50-85% of nominal) and 5, 12, 32, 73, 183 and 429 µg/L (representing 50-83% of nominal) at the end of exposure. Endpoints were based on mean measured concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 202 guideline. The results are summarised in Table B.9.2.1.2-02.

Table B.9.2.1.2-02 The acute toxicity of PB-7 to aquatic invertebrates

Species	Test type and duration. (purity)	Actual concn. (as % of nominal)	EC50 in µg/L	NOEC µg/L
<i>Daphnia magna</i>	Static 48 hours (99.0%)	50-85	24 h: 36 ^(A) 48 h: 28 ^(A)	24 & 48 h: 5 ^(A)

^(A) Based on mean measured concentrations

Conclusion

48-hour EC50: 28 µg/L, based on mean measured concentrations.

Guidelines and limitations

The study is acceptable.

Study 3

Characteristics

reference	:	Jenkins C.A. (2003c)	exposure duration	:	72 hours
type of study	:	Algal growth inhibition test	nominal	:	0, 0.64, 1.4, 3.1, 6.8 and 15 mg/L
year of execution	:	2003	concentrations	:	
GLP statement	:	Yes	dosing method	:	Static, dilutions in test medium
guideline	:	OECD 201, EEC C3	acceptability	:	Acceptable
test substance	:	PB-7, Batch PB-7-S001129	72h-EbC50	:	3.1 mg/L
purity	:	99.0%	72h-ErC50	:	>16 mg/L
species	:	<i>Selenastrum capricornutum</i>	72h-NOEbC	:	0.54 mg/L
			72h-NOErC	:	0.54 mg/L

Methods

A 72-hour toxicity test on green algae (*Selenastrum capricornutum*) (3 or 6 replicates per test group, each containing 1.0×10^4 cells/mL at the start) was conducted with PB-7 (purity 99.0%) in glassware pre-treated with siliconising agent (Surfasil, 2% in acetone) at nominal test concentrations of 0.64, 1.4, 3.1, 6.8 and 15 mg/L, with untreated control.

Results

The measured concentrations were 0.54, 1.15, 2.8, 5.9 and 16 mg/L at test initiation (representing 74-105% of nominal), and 0.09, 0.12, 0.44, 1.1 and 4.8 mg/L at the end of exposure (representing 9-32% of nominal). Endpoints were based on mean measured concentrations and re-calculated based on initial measured concentrations by the RMS using log-linear regression analysis with log-transformed concentrations according to the methods of OECD 201. Water quality parameters were in accordance with the OECD guideline 201. The results are summarised in Table B.9.2.1.2-03.

Table B.9.2.1.2-03 The acute toxicity of PB-7 to algae

Species	Test type and duration. (purity of test substance)	Actual concn. (as % of nominal)	EC50 in mg/L	NOEC mg/L
<i>Selenastrum capricornutum</i> (green alga)	Static 72 hours (99.0%)	74-105 (start) 9-32 (end)	72h-EbC50: 3.1 ^(A) 72h-ErC50: >16 ^(A)	72h-NOEbC & NOErC: 0.54 ^(A)

^(A) Based on initial measured concentrations

Conclusion

72-hour EbC50: 3.1 mg/L, 72-hour ErC50 >16 mg/L, 72-hour NOEbC and NOErC: 0.54 mg/L.

Guidelines and limitations

The study is acceptable.

Study 4

Characteristics

reference	: Albuquerque R. (2003a)	exposure duration	: 48 hours
type of study	: Acute toxicity study	nominal concentrations	: 0, 0.0016, 0.0080, 0.040, 0.20 and 1.0 mg/L
year of execution	: 2003	dosing method	: Static; serial dilution in test medium
GLP statement	: Yes		
guideline	: OECD 202, OECD 219		
test substance	: PB-7, Lot no. PB-7-S001129	acceptability	: Acceptable
purity	: 99.0%	48 h-LC50	: 0.14 mg/L
species	: <i>Chironomus riparius</i>		

Methods

A 48-hour immobilisation test with *Chironomus riparius* (4 replicates of 10 chironomid larvae each per concentration) was conducted under static conditions with PB-7 (99.0% purity) in beakers containing only test medium, no sediment. The nominal test concentrations were 0.0016, 0.0080, 0.040, 0.20 and 1.0 mg/L, with untreated control. All glassware was pre-treated with siliconising agent (Surfasil) and conditioned for ten days with the corresponding test substance concentration.

Results

The measured concentrations at 0.0016 and 0.0080 mg/L were below the LOQ (<0.01 mg/L) during the 48-hour test period. Measured concentrations at 0.040, 0.20 and 1.0 mg/L were 0.026, 0.14 and 0.86 mg/L at test initiation (representing 64-86% of nominal) and 0.025, 0.16 and 0.77 mg/L (representing 61-78% of nominal) at the end of exposure. Endpoints were based on mean measured concentrations, and were recalculated based on initial measured concentrations by the reviewer using Finney, D.J., 1971: Probit analysis, Cambridge University Press, Cambridge, U.K., 3rd edition. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 202 and 219 guidelines. The results are summarised in Table B.9.2.1.2-04.

Table B.9.2.1.2-04 The acute toxicity of PB-7 to sediment dwelling organisms

Species	Test type and duration (purity)	Actual concn. (as % of nominal)	LC50 in mg/L	NOEC mg/L
<i>Chironomus riparius</i>	Static 48 hours (99.0%)	61-86	24 h: 0.16 ^(A) 48 h: 0.14 ^(A)	24-48 h: <0.01 ^(A)

^(A) Based on measured initial concentrations

Conclusion

48-hour LC50: 0.14 mg/L, based on measured initial concentrations.

Guidelines and limitations

The study is acceptable.

B.9.2.1.3 Acute toxicity of the plant protection product (IIIA 10;2.1)

Study 1

Characteristics

reference	: Springer <i>et. al.</i> (1994)	exposure duration	: 96 hours
type of study	: Higher tier acute toxicity study	nominal concentrations	: 1 st test: 0, 1, 3, 6, 9 and 12 µg a.s./L; 2 nd test: 39, 78, 156, 312 and 467 µg a.s./L
year of execution	: 1991	dosing method	: Static; stocks in water applied by spray drift applicator
GLP statement	: Yes	acceptability	: Acceptable
guideline	: EPA 72-7(A)	96h-LC50	: 84 µg product/L ⇔ 64 µg a.s./L
test substance	: BAS 300 11 I (Pyridaben), lot no. AF137-50		
a.s. content	: 75.8%		
species	: Bluegill sunfish (<i>Lepomis macrochirus</i>)		

Methods

Two 96-hour acute toxicity tests in bluegill sunfish (*Lepomis macrochirus*) (2 replicates per concentration each containing 30 fish) were conducted outdoors in Alabama (USA) in September 1991 (1st test) or October 1991 (2nd test) under static conditions in 0.68 m³ microcosm tanks (coated stainless steel, partly buried) with BAS 300 11 I (75.8% pyridaben). Nominal test concentrations of 1, 3, 6, 9 and 12 µg a.s./L, and 39, 78, 156, 312 and 467 µg a.s./L, both including an untreated control were tested in the 1st and 2nd test, respectively. Approximately 5 cm clay (1.1% organic matter) and 5 cm topsoil (1.3% organic matter) were placed on the bottoms of the tanks and covered with 4 L of hydrosoil before the tanks were filled with 680 L water on July 29, 1991 (1st test) and October 2, 1991 (2nd test). The system was then fertilised with ammonium polyphosphate on August 1, 1991 (1st test) and October 23, 1991 (2nd test). Bluegill sunfish were introduced to the system 4-17 days prior to application of BAS 300 11 I. BAS 300 11 I was applied into each treatment tank from a 500 mL glass dispensing bottle with twelve entry points located three to eight cm below the water surface, equally spaced across the width of the tank on September 16, 1991 (1st test) and October 28, 1991 (2nd test). Water samples were collected using a column sampler inserted from the water surface to

near the soil level at the start and the end of both tests for analytical verification of the test substance concentration (extraction followed by HPLC; concurrently determined mean procedural recovery at 1, 10 and 50 µg a.s./L was 101%).

Results

In the 1st test no treatment related mortality or sublethal effects were observed during the 96-hour test period up to 12 µg a.s./L. No analysis was performed and a 2nd test was initiated. Results are presented for the 2nd test only in this summary.

Measured concentrations (means for 2 replicates; variation between replicates was low) declined from 31, 44, 68, 162 and 277 µg a.s./L at the start (representing 44-79% of nominal) to below the LOD (<1.0 µg a.s./L) for the lowest three concentrations and to 4.0 µg a.s./L (1% of nominal) for the two highest concentrations at the end of the test. Endpoints were based on measured initial concentrations, which is acceptable. Water quality parameters were: pH (8.4-9.0), dissolved oxygen (9.8-13 mg/L), temperature (21-24°C), TOC (6.2-10 mg/L), total alkalinity (59-78 mg/L), total phosphorous (105-460 µg/L) and turbidity (5-11 NTU's). The total amount of precipitation was 0 mm. The minimum and maximum air temperature were 7.6-14°C and 23-30°C. After 96 hours of exposure, 0, 3, 17, 65, 98 and 98% of the fish had died at 0, 31, 44, 68, 162 and 277 µg a.s./L, respectively. The results are summarised in Table B.9.2.1.3-01.

Table B.9.2.1.3-01 The acute toxicity of BAS 300-11 I (pyridaben) to fish

Species	Test type and duration. (a.s. content)	Actual concn. (as % of nominal)	LC50 in µg/L	NOEC µg/L
<i>Lepomis macrochirus</i>	Static 96 hours (75.8%)	44-79% (start) ≤1% (end)	24 h: 88 (formn) ^(A) ⇔ 67 (a.s.) ^(A) 48-72 h: 87 (formn) ^(A) ⇔ 66 (a.s.) ^(A) 96 h: 84 (formn) ^(A) ⇔ 64 (a.s.) ^(A)	24-96 h: <41 (formn) ^(A) ⇔ <31(a.s.) ^(A)

^(A) Based on measured initial concentrations

Conclusion

Under outdoor conditions in tanks with sediment (Alabama, USA, October-November 1991): 96-hour LC50: 84 µg product/L (64 µg a.s./L), 96-hour NOEC: <41 µg product/L (<31 µg a.s./L).

Guidelines and limitations

This study is acceptable.

Study 2

Characteristics

reference	: Jenkins C.A. (2002a)	exposure duration	: 96 hours
type of study	: Acute toxicity study	nominal	: 0, 0.97, 2.1, 4.7, 10, 23 and 50 µg product/L
year of execution	: 2000	concentrations	: product/L
GLP statement	: Yes	dosing method	: Semi-static; dilutions in test medium
guideline	: EEC C-1, OECD 203	acceptability	: Acceptable
test substance	: Pyridaben 75WP, batch OAJ 030	96h-LC50	: 2.9 µg product/L ⇔ 2.2 µg a.s./L
a.s. content	: 76.8%		
species	: Rainbow trout (<i>Oncorhynchus mykiss</i>)		

Methods

A 96-hour acute toxicity test in rainbow trout (*Oncorhynchus mykiss*) (seven fish per concentration) was conducted under semi-static conditions (daily renewal) with Pyridaben 75WP (76.8% pyridaben) at nominal test concentrations of 0.97, 2.1, 4.7, 10, 23 and 50 µg product/L, with untreated control.

Results

Measured concentrations in freshly prepared solutions represented 92-114% and 77-109% of nominal at the start of the test and after 72 hours, respectively. Measured concentrations in 24-hour old solutions had declined to 46-66% and 54-83% of nominal after 24 and 96 hours, respectively. Endpoints were based on mean measured concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 203 guideline. The results are summarised in Table B.9.2.1.3-02.

Table B.9.2.1.3-02 The acute toxicity of Pyridaben 75WP to fish

Species	Test type and duration. (a.s. content)	Actual concn. (as % of nominal)	LC50 in µg/L	NOEC µg/L
<i>Oncorhynchus mykiss</i>	Semi-static 96 hours (76.8%)	77-114% (fresh) 46-83% (24h-old)	24 h: 11 (formn) ^(A) ⇔ 8.8 (a.s.) ^(A) 48 h: 5.4 (formn) ^(A) ⇔ 4.2 (a.s.) ^(A) 72 h: 4.4 (formn) ^(A) ⇔ 3.4 (a.s.) ^(A) 96 h: 2.9 (formn) ^(A) ⇔ 2.2 (a.s.) ^(A)	24-48 h: 1.6 (formn) ^(A) ⇔ 1.2 (a.s.) ^(A) 72-96 h: 0.74 (formn) ^(A) ⇔ 0.57 (a.s.) ^(A)

^(A) Based on mean measured concentrations

Conclusion

96-hour LC50: 2.9 µg product/L (2.2 µg a.s./L), 96h-NOEC: 0.74 µg product/L (0.57 µg a.s./L).

Guidelines and limitations

This study is acceptable.

Study 3

Characteristics

reference	: Jenkins C.A. (2002b)	species	: <i>Daphnia magna</i>
type of study	: Acute toxicity study	exposure duration	: 48 hours
year of execution	: 2000-2001	nominal concentrations	: 0, 0.10, 0.22, 0.49, 1.1, 2.4 and 5.2 µg product/L
GLP statement	: Yes	dosing method	: Static; dilutions in test medium
guideline	: EEC C2, OECD 202	acceptability	: Acceptable
test substance	: Pyridaben 75WP, batch OAJ 030	48h-EC50	: 1.3 µg product/L ⇔ 0.99 µg a.s./L
a.s. content	: 76.5%		

Methods

A 48-hour immobilisation test with *Daphnia magna* (4 replicates of 5 *Daphnia* each per concentration) was conducted under static conditions with Pyridaben 75WP (76.5% pyridaben) at nominal test concentrations of 0.10, 0.22, 0.49, 1.1, 2.4 and 5.2 µg product/L, with untreated control.

Results

The measured concentrations of pyridaben were 0.086, 0.19, 0.35, 0.88, 1.9 and 4.4 µg a.s./L at test initiation (representing 95-114% of nominal) and 0.064, 0.083, 0.19, 0.28, 0.67 and 1.5 µg a.s./L (representing 35-51% of nominal) at the end of exposure. Endpoints were based on nominal concentrations, which is acceptable. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 202 guideline. The results are summarised in Table B.9.2.1.3-03.

Table B.9.2.1.3-03 The acute toxicity of Pyridaben 75WP to invertebrates

Species	Test type and duration. (a.s. content)	Actual concn. (as % of nominal)	EC50 in µg/L	NOEC µg/L
<i>Daphnia magna</i>	Static 48 hours (76.5%)	95-114 (start) 35-51 (end)	24 h: 3.2 (formn) ^(A) ⇔ 2.4 (a.s.) ^(A) 48 h: 1.3 (formn) ^(A) ⇔ 0.99 (a.s.) ^(A)	24 h: 0.64 (formn) ^(A) ⇔ 0.49 (a.s.) ^(A) 48 h: 0.22 (formn) ^(A) ⇔ 0.17 (a.s.) ^(A)

^(A) Based on nominal concentrations

Conclusion

48-hour EC50: 1.3 µg product/L (0.99 µg a.s./L), based on nominal concentrations.

Guidelines and limitations

The study is acceptable.

Study 4

Characteristics

reference	: Cunningham F.J. (1996)	species	: <i>Mysidopsis bahia</i> (marine shrimp)
type of study	: Acute toxicity study outdoors	exposure duration	: 96 hours
year of execution	: 1995	nominal concentrations	: 0, 6.5, 11, 18, 30 and 50 µg a.s./L
GLP statement	: Yes	dosing method	: Static; stocks in water
guideline	: EPA 72-2 with modifications	acceptability	: Acceptable
test substance	: BAS 300 11 I (Pyridaben), lot no. OAJ 030	96h-LC50	: 22 µg product/L ⇔ 16 µg a.s./L
a.s. content	: 75.4%		

Methods

A 96-hour acute toxicity test in the salt water shrimp *Mydisopsis bahia* (<24-hour old, 2 replicates of 15 shrimps each per concentration) was conducted outdoors in Florida (USA) in November-December, 1995, under static conditions in 56 L tanks with BAS 300 11 I (75.4% pyridaben) at nominal test concentrations of 6.5, 11, 18, 30 and 50 µg a.s./L, with untreated control. Test solutions were prepared by addition of stock solutions using a plastic syringe under the surface of the water followed by gentle stirring for 30 seconds. Test aquaria were covered with clear plexiglass at the end of each working day and during daytime rainfall events to prevent dilution. Water samples were collected for pyridaben analysis at the start and after 1, 2, 4, 8, 24, 48, 72 and 96 hours of exposure.

Results

Measured concentrations gradually declined from 5.9, 10, 16, 26 and 45 µg a.s./L at the start (representing 87-96% of nominal) to below the LOD (<0.50 µg a.s./L) within 48 to 96 hours of exposure. Endpoints were based on initial measured concentrations, which is acceptable. Water quality parameters were: salinity (20-23‰), pH (8.1-8.3), dissolved oxygen (5.4-7.5 mg/L) and temperature (24-27°C). After 96 hours of exposure, 0, 0, 7, 50, 93 and 100% of the mysids had died at 0, 5.9, 10, 16, 26 and 45 µg a.s./L, respectively. The results are summarised in Table B.9.2.1.3-04.

Table B.9.2.1.3-04 The acute toxicity of BAS 300 11 I (pyridaben) to invertebrates

Species	Test type and duration. (a.s. content)	Actual concn. (as % of nominal)	LC50 in µg/L	NOEC µg/L
<i>Mysidopsis bahia</i> (marine organism)	Static 96 hours (75.4%)	87-96% (start) <LOD (end)	24 h: >59 (formn) ^(A) ⇔ >45 (a.s.) ^(A) 48 h: 36 (formn) ^(A) ⇔ 27 (a.s.) ^(A) 72 h: 22 (formn) ^(A) ⇔ 17 (a.s.) ^(A) 96 h: 22 (formn) ^(A) ⇔ 16 (a.s.) ^(A)	24 h: 34 (formn) ^(A) ⇔ 26 (a.s.) ^(A) 48 h: 13 (formn) ^(A) ⇔ 10 (a.s.) ^(A) 72-96 h: 7.8 (formn) ^(A) ⇔ 5.9 (a.s.) ^(A)

^(A) Based on initial measured concentrations

Conclusion

Under outdoor conditions (Florida, November-December): 96-hour LC50: 22 µg product/L (16 µg a.s./L), based on initial measured concentrations.

Guidelines and limitations

This study is acceptable.

Study 5

Characteristics

reference	:	Jenkins C.A. (2002c)	exposure duration	:	72 hours
type of study	:	Algal growth inhibition test	nominal	:	0, 1.9, 4.3, 9.4, 21, 46 and 100 mg
year of execution	:	2000	concentrations	:	product/L
GLP statement	:	Yes	dosing method	:	Static, dilutions in test medium
guideline	:	OECD 201, EEC C3	acceptability	:	Acceptable
test substance	:	Pyridaben 75WP, batch OAJ 030	72h-EbC50	:	46 mg product/L ⇔ 35 mg a.s./L
a.s. content	:	76.8%	72h-ErC50	:	89 mg product/L ⇔ 68 mg a.s./L
species	:	<i>Selenastrum capricornutum</i>	72h-NOEbC	:	21 mg product/L ⇔ 16 mg a.s./L
			72h-NOErC	:	21 mg product/L ⇔ 16 mg a.s./L

Methods

A 72-hour toxicity test on green algae (*Selenastrum capricornutum*) (3 or 6 replicates per test group, each containing 1.0×10^4 cells/mL at the start) was conducted with Pyridaben 75WP (76.8% pyridaben) at nominal test concentrations of 1.9, 4.3, 9.4, 21, 46 and 100 mg product/L, with untreated control.

Results

The measured concentrations of pyridaben were 1.4, 3.4, 7.3, 15, 37 and 69 mg a.s./L at test initiation (representing 90-106% of nominal), and 1.9, 4.2, 8.5, 21, 31 and 69 mg a.s./L at the end of exposure (representing 89-131% of nominal). Endpoints were based on nominal concentrations, which is acceptable. Water quality parameters were in accordance with the OECD guideline 201. The results are summarised in Table B.9.2.1.3-05.

Table B.9.2.1.3-05 The acute toxicity of Pyridaben 75WP to algae

Species	Test type and duration. (a.s. content)	Actual concn. (as % of nominal)	EC50 in mg/L	NOEC mg/L
<i>Selenastrum capricornutum</i> (green alga)	Static 96 hours (76.8%)	89-131	72h-EbC50: 46 (formn) ^(A) ⇔ 35 (a.s.) ^(A) 72h-ErC50: 89 (formn) ^(A) ⇔ 68 (a.s.) ^(A)	72h-NOEbC & 72h-NOErC: 21 (formn) ^(A) ⇔ 16 (a.s.) ^(A)

^(A) Based on nominal concentrations

Conclusion

72-hour EbC50: 46 mg product/L (35 mg a.s./L), 72-hour ErC50: 89 mg product/L (68 mg a.s./L), 72-hour NOEbC & NOErC: 21 mg product/L (16 mg a.s./L), based on nominal concentrations.

Guidelines and limitations

The study is acceptable.

B.9.2.2 Chronic toxicity**B.9.2.2.1 Chronic toxicity of the active substance****B.9.2.2.1.1 Fish (IIA 8.2.2)****Study 1****Characteristics**

reference	:	Jenkins W.R. (1989)	species	:	Rainbow trout (<i>Salmo gairdneri</i>)
type of study	:	Chronic toxicity study	exposure duration	:	21 days
year of execution	:	1988	nominal concentrations	:	0, 0.38, 0.80, 1.6, 3.1 and 6.3 µg/L
GLP statement	:	Yes	dosing method	:	Flow-through, stock solution in acetone
guideline	:	OECD 204	acceptability	:	Not acceptable
test substance	:	technical grade NC-129, batch not reported; ¹⁴ C-NC-129, batch CP-928	NOEC	:	0.84 µg eq./L
purity	:	NC-129: purity not reported; ¹⁴ C-NC-129: radiochemical purity ≥ 99.2%	LC50	:	1.6 µg eq./L

Methods

A 21-day flow-through study was undertaken with rainbow trout (*Salmo gairdneri*). Ten fish per replicate (one replicate per concentration) were exposed to NC-129 (pyridaben; mixture of 2.8% ¹⁴C-pyridaben and 97.2% non-labeled pyridaben) at nominal concentrations of 0.38, 0.80, 1.6, 3.1 and 6.3 µg/L plus control and solvent control (acetone). Samples for analyses of actual exposure concentrations were taken at the start and after 3, 7, 14 and 21 days. Samples were extracted with hexane and dissolved in methanol before liquid scintillation counting (LSC). Survival and fish behaviour were monitored daily.

Results

Mean measured concentrations were 0.18, 0.39, 0.84, 2.0 and 5.1 µg eq./L, representing 47 to 82% of nominal. The variability between measurements at individual sampling days was limited for concentrations at the reported NOEC and above. Endpoints were based on mean measured concentrations. Survival was not affected at and below 0.84 µg eq./L when compared to the control groups, but was reduced by 70% and 100% at 2.0 and 5.1 µg eq./L, respectively. Symptoms including effects on swimming behaviour, respiratory movement and colour, were observed at and above 2.0 µg eq./L. Water quality parameters (pH, oxygen concentration and temperature) were in accordance with the OECD 204 guideline. Fish length and weight measurements were not conducted.

Conclusion

The NOEC and LOEC for survival and symptoms were 0.84 and 2.0 µg eq./L, respectively.

Guidelines and limitations

1. Fish length and weight measurements were not conducted, which disagrees with the recommendations by OECD 204.
2. Analysis of test concentrations was based on a non-specific method (liquid scintillation counting). These measurements of total radioactivity are not specific and may include degradation products of pyridaben.
3. The report did not contain any data on the purity and the batch number of the unlabelled test substance.
4. The report stated that the radiochemical purity of the radiolabel pyridaben in the stock solution was confirmed prior to the test, but experimental data to verify this measurement (chromatograms, results of radiochemical purity measurements) were not reported.
5. Overall evaluation. There were important deviations from the OECD 204 guideline (no measurements of fish growth, insufficient characterization of test material, non-specific analytical measurements). The study result is therefore not accepted.

Study 2

Characteristics

reference	:	Rhodes J.E. <i>et al.</i> (1995)	species	:	Fathead minnow (<i>Pimephales promelas</i>)
type of study	:	Chronic toxicity study (FLC)	exposure duration	:	301 days
year of execution	:	1993-1994	nominal concentrations	:	0, 0.088, 0.18, 0.35, 0.70 and 1.4 µg/L
GLP statement	:	Yes	dosing method	:	Flow-through, stock solution in acetone
guideline	:	EPA 72-5	acceptability	:	Acceptable
test substance	:	¹⁴ C BAS 300 I (Pyridaben), Lot no. CP-1068; unlabelled BAS 300 I (Pyridaben), Lot no. 129S8604 and 900304	NOEC	:	0.28 µg/L
purity	:	¹⁴ C-labelled: 93.2%; unlabelled: 99.5-99.8%			

Methods

A 301-day fish full life cycle flow-through study was undertaken with fathead minnow (*Pimephales promelas*). Newly fertilised eggs (<24 hours post-fertilisation, four replicates/concentration, 50 eggs/replicate) were exposed to a mixture of ¹⁴C BAS 300 I (Pyridaben) and non-radiolabeled pyridaben at nominal concentrations of 0.088, 0.18, 0.35, 0.70 and 1.4 µg/L plus control and solvent control (acetone). Water quality parameters were according to the EPA 72-5 guideline. The total duration of the exposure period was 301 days. Survival, abnormal appearance and behaviour were monitored daily for both the parent and F1 generation. Fry were thinned to 25 per replicate on day 7. Fish length of the parent generation was measured at 30, 58, 80 and 296 days post-hatch and fish wet weight was determined at 80 and 296 days post-hatch. Sex of the parent generation was verified at 296 days post-hatch. On day 87, individual males and females were paired (1 ♂ and 2 ♀ per replicate, 6 replicates per treatment) and kept in spawning aquaria until day 100. Lots of 35 newly fertilised eggs obtained from the parent generation obtained between days 87 and 100 were used to determine egg hatchability, survival and growth effects in the F1 generation. Fish length of the F1 generation was measured at 56 days post-hatch.

An additional acute study was performed by placing embryos from the same lot as used to initiate the 301-day full life cycle test into a hatching tray and 30 days later sub-samples of actively growing fry were used to determine the 96h-LC50 for pyridaben. Target concentrations of 0.38, 0.75, 1.5, 3.0 and 6.0 µg/L were tested plus a control and solvent-control. Concentrations were verified by LSC measurements at the start and at the end of the test period. Further information on the design and performance of this test (e.g. number of fish per concentration) was not available.

Results

Mean measured concentrations, analysed by LSC, were 0.087, 0.15, 0.28, 0.56 and 1.2 µg eq/L, representing 79 to 99% of nominal. Monthly performed HPLC/UV analysis of test water extracts confirmed the analytical results obtained for the lowest and middle concentration by LSC with mean measured concentrations of 0.082 and 0.255 µg/L, representing 73% and 93% of nominal.

Hatchability and survival of the parental generation were not reduced at any concentration. A statistically significant reduction in parental fish length was observed at 1.2 µg/L after 30, 58 and 80 days post-hatch and at 0.56 µg/L at 30 days post-hatch, when compared to the pooled control group. Parental wet weight was statistically significantly reduced at 1.2 µg/L after 80 days post-hatch. Reproduction of the parental generation was not significantly affected at any concentration based on days to first spawn, number of spawning days, number of spawn and number of eggs per spawn. Egg hatchability and survival of the F1 generation after 56 days post-hatch were statistically significantly reduced at 0.56 and 1.12 µg/L. Length and wet weight of the F1 generation after 56 days post-hatch were not significantly reduced at any concentration.

The 96-hour acute study, conducted with the same lot of fish used to initiate the full life cycle test, resulted in a 96h-LC50 of 2.3 µg/L. Mean measured radioactivity concentrations were 0.45, 0.83, 1.6, 3.3 and 5.7 µg/L, representing 95-118% of nominal. The LC50 is not taken to the List of Endpoints because of lack of information on test procedures and the use of a non-specific analytical method.

Conclusion

Based on reduced fry growth of the parent generation, and reduced F1, egg hatchability and survival of the F1 generation, the NOEC was 0.28 µg/L and the LOEC was 0.56 µg/L.

Guidelines and limitations

The study was conducted in accordance to the EPA 72-5 guideline and is acceptable.

B.9.2.2.1.2 Invertebrates (IIA 8.2.5)**Study 1****Characteristics**

reference	:	Jenkins W.R. <i>et al.</i> (1989)	species	:	<i>Daphnia magna</i>
type of study	:	Chronic toxicity study	exposure duration	:	21 days
year of execution	:	1988	nominal concentrations	:	0, 0.005, 0.01, 0.05, 0.1 and 0.5 µg/L
GLP statement	:	Yes	dosing method	:	Semi-static, stock solution in acetone
guideline	:	OECD 202	acceptability	:	Not acceptable
test substance	:	¹⁴ C-NC-129, batch no. CP-928	NOEC	:	0.043 µg/L
Radiochemical purity	:	≥99.2%			

Methods

The chronic toxicity of ¹⁴C-NC-129 (radiochemical purity ≥99.2%) to *Daphnia magna* was assessed in a 21-day semi-static study. First instar daphnids (≤24 hours old, 40 per treatment, 10 daphnia per replicate vessel) were used to initiate the study. The nominal concentrations were 0.005, 0.01, 0.05, 0.1 and 0.5 µg/L plus a blank- and solvent-control (acetone). Mean measured concentrations were calculated by RMS as the average of the mean concentrations measured at the start and the end of three renewal intervals. Mean measured concentrations, determined by LSC of hexane extracts, were 0.015, 0.014, 0.043, 0.073 and 0.432 µg eq./L, representing 303%, 135%, 86%, 73% and 86%, respectively, of the nominal concentrations. Water quality parameters were in accordance with the OECD 211 guideline.

Results

Mobility of adult daphnia was statistically significantly reduced at 0.432 µg/L, when compared to the solvent-control. Male daphnia were found in test vessels of 0.073 and 0.432 µg/L. A slight delay in time to first brood (one day) was observed at 0.432 µg/L. Reproductive success, as measured by the mean number of young per surviving adult, was significantly reduced at and above 0.073 µg/L when compared to the blank-control group.

Conclusion

The overall NOEC, based on parental immobility and effects on reproduction, was 0.043 µg/L.

Guidelines and limitations

1. Analysis of test concentrations (LSC of hexane extracts) involves measurement of total radioactivity. This method is not specific and the results may include degradation products of pyridaben.
2. The length of the parental daphnids was not measured at the end of the test as is required according to the OECD 211 guideline.

3. Overall evaluation. Because of the above deviations from the test guideline, the study result is not accepted.

Study 2

Characteristics

reference	:	Drottar K.R. & Swigert J.P. (1994)	species	:	<i>Daphnia magna</i>
type of study	:	Chronic toxicity study	exposure duration	:	21 days
year of execution	:	1993	nominal concentrations	:	0, 0.025, 0.050, 0.10, 0.20 and 0.40 µg a.s./L
GLP statement	:	Yes	dosing method	:	Flow-through, stock solution in acetone
guideline	:	EPA 72-4			
test substance	:	Unlabelled: BAS 300 I, Lot no. 900304, Labelled: ¹⁴ C Pyridaben, Lot no. CP 1007			
(Radiochemical) purity	:	Unlabelled: 100% Labelled: >99%	acceptability	:	Acceptable
			NOEC	:	0.086 µg a.s./L

Methods

The chronic toxicity of Pyridaben (as a mixture of ¹⁴C-labelled and unlabelled pyridaben) to *Daphnia magna* was assessed in a 21-day flow-through study. First instar daphnids (≤24 hours old, 22 per treatment, 11 daphnia per replicate vessel) were used to initiate the study. The nominal concentrations were 0.025, 0.050, 0.10, 0.20 and 0.40 µg a.s./L plus a blank- and solvent-control (acetone). The mean measured radioactivity concentrations in the water during exposure (determined by LSC) were 0.022, 0.044, 0.086, 0.18 and 0.32 µg eq./L, representing 80 to 90% of nominal. The mean percentage of parent compound determined by HPLC in diluter stock solutions was 101-105%. Water quality parameters were in accordance with the OECD 211 guideline.

Results

Survival of adult daphnia was statistically significantly reduced at 0.32 µg a.s./L when compared to the controls. No delay in time to first brood was observed at any of the concentrations. Reproductive success, as measured by the mean number of young per adult, was significantly reduced at and above 0.18 µg a.s./L when compared to the pooled control group. No concentration dependent statistically significant reductions in body length or body weight were observed at any concentration.

Conclusion

The NOEC for parental mortality was 0.18 µg a.s./L, the NOEC for growth was 0.32 µg a.s./L and the NOEC for reproduction (overall NOEC) was 0.086 µg a.s./L.

Guidelines and limitations

The radioactivity in the test solutions was not identified, but the radioactivity in stock solutions consisted entirely of pyridaben. Analytical results from acute flow-through and static tests with *Daphnia magna* (studies 6 and 7, section B.9.2.1.1) indicated that under the test conditions of the above study pyridaben may be assumed to be stable. The test is therefore acceptable.

Study 3

Characteristics

reference	:	Holmes C.M. & Machado M.W. (1995)	species	:	<i>Mysidopsis bahia</i>
type of study	:	Chronic toxicity study	exposure duration	:	35 days
year of execution	:	1994	nominal concentrations	:	0, 0.0094, 0.019, 0.038, 0.075 and 0.15 µg/L
GLP statement	:	Yes	dosing method	:	Flow-through, stock solution in acetone
guideline	:	EPA 72-4 (c)	acceptability	:	Acceptable
test substance	:	[¹⁴ C]Pyridaben (BAS 300 I) Technical, Lot no. CP1007	NOEC	:	0.047 µg a.s./L
purity	:	Radiochemical purity 99.6%, chemical purity >99%			

Methods

The chronic toxicity of [¹⁴C]Pyridaben (BAS 300 I) Technical (radiochemical purity >99%) to *Mysidopsis bahia* was assessed in a 35-day flow-through study. Mysids (≤24 hours old, 60 per treatment, 30 mysids per replicate vessel) were used to initiate the study. The nominal concentrations were 0.0094, 0.019, 0.038, 0.075 and 0.15 µg/L plus a blank- and solvent-control (acetone). Mean measured radioactivity concentrations, determined by LSC, were 0.0086, 0.017, 0.033, 0.070 and 0.13 µg eq./L, representing 86 to 93% of nominal. HPLC analysis confirmed that the stock solution contained the nominal pyridaben concentration at the start and the end of the test, but the mean measured concentration of the test solutions of the highest test concentration during the test period was 0.10 µg a.s./L, representing only 67% of nominal. Radioactivity in test solutions of lower concentrations was not analysed by HPLC. Water quality parameters were in accordance with the EPA 72-4 guideline. On day 15, males and females were paired and redistributed into glass pairing jars (1 pair from each exposure aquarium per jar). The remaining mysids were pooled and placed in one of the initial retention chambers until study end. Survival and sub-lethal effects were assessed during the first 15 days of the study, reproduction and mortality of males and females were assessed after pairing (day 15) and body length and dry weight were assessed at the end of the test.

Results

Survival and growth of mysids were not affected at any concentration when compared to the pooled controls.

Results on reproductive success are summarised in Table B.9.2.2.1.2-01. At termination of the standard 28 day exposure, reproduction among solvent control organisms did reach the minimum requirement of the OPPTS 850.1350 guideline (≥75% of females should be producing young), but that of the dilution water control organisms did not (55% of females were producing young). For this reason, the study was extended

from 28 to 35 days, but there was no improvement in the dilution water control. The other validity criterion of the OPPTS 850.1350 guideline however (at least 3 young per female) was satisfied by both controls. Therefore, the test is accepted.

Reproduction was reduced by 47% and 46% at 0.13 µg eq./L, when compared to the pooled control group, after 28 and 35 days, respectively. This difference was not statistically significant due to large variation in the control and treated groups (the number of offspring per female per reproductive day in the two replicates of the blank-control, solvent-control and 0.13 µg eq./L, respectively, was 0.15-0.17, 0.12-0.26 and 0.05-0.13 at day 28, and 0.10-0.11, 0.08-0.19 and 0.03-0.10 at day 35). The reported NOEC value was 0.13 µg eq./L, based on the lack of statistically significant effects. However, the effect at 0.13 µg eq./L on reproduction was almost 50%, and the results at the lower test concentration do not provide a justification to discount this large reduction as a random finding. Therefore, the RMS set the NOEC at 0.070 µg eq./L, which is equivalent to 0.047 µg a.s./L when taking into consideration the percentage of pyridaben in the test solution of the highest test concentration (67%, as measured by HPLC; no HPLC measurements were performed at lower concentrations).

Table B.9.2.2.1.2-01 Reproductive success of *Mysidopsis bahia* exposed to pyridaben

Mean measured radioactivity concentration (µg eq./L)	Offspring/female/ reproductive day		% deviation from control ^(A)					
			Day 28			Day 35		
	Day 28	Day 35	Blank-control	Solvent-control	Pooled control	Blank-control	Solvent-control	Pooled control
Untreated control	0.16	0.11	-	-	-	-	-	-
Solvent control	0.19	0.14	-	-	-	-	-	-
Pooled control	0.17	0.12	-	-	-	-	-	-
0.0086	0.19	0.14	+19	0	+12	+27	+27	+17
0.017	0.13	0.10	-19	-32	-24	-9	-9	-17
0.033	0.15	0.10	-6	-21	-12	-9	-9	-17
0.070	0.18	0.12	+13	-5	+6	+9	+9	0
0.13	0.09	0.07	-44	-53	-47	-41	-41	-46

^(A) The RMS calculated the % deviation from the control groups

Conclusion

The overall NOEC for mysid mortality, reproduction and growth was 0.070 µg eq./L, equivalent to 0.047 µg a.s./L.

Guidelines and limitations

Deviations from the guideline and their impact on the study results were discussed above. The test is acceptable.

B.9.2.2.1.3 Effects on sediment dwelling organisms (IIA 8.2.7)

Characteristics

reference	:	Albuquerque R. (2003b)	species	:	<i>Chironomus riparius</i>
type of study	:	Chronic toxicity study on <i>Chironomus riparius</i>	exposure duration	:	28 days
year of execution	:	2002	nominal concentrations	:	0, 0.51, 1.1, 2.5, 5.5 and 12.0 µg/L
GLP statement	:	Yes	dosing method	:	Static, stock solution in acetone, spike to water
guideline	:	Draft OECD 219	acceptability	:	Acceptable
test substance	:	¹⁴ C-Pyridaben, Lot no. CP-1504	NOEC	:	5.1 µg/L
radiochemical purity	:	>97%			

Methods

The chronic toxicity of ¹⁴C-Pyridaben (radiochemical purity >97%) to *Chironomus riparius* (<36 hours old, 1st instar larvae) was assessed in a 28-day water/sediment system under static conditions. The nominal concentrations were 0.51, 1.1, 2.5, 5.5 and 12.0 µg/L, with untreated and solvent-control. The test was performed in glass beakers, with four replicates per treatment and eight for the controls each containing 10 midge larvae and approximately 2 cm of sediment (artificial OECD soil) and approximately 8 cm of overlying water. Additional replicates at 0.49 and 12.7 µg/L were set up for analytical measurements on sediment and pore water on days 1 and 7. Midge larvae were added to the test system one day prior to the addition of test substance. Treatment was performed by spiking the water with an aliquot (0.08 mL) of a stock solution of the test substance in acetone/water (1:1). Water quality parameters during the study were in accordance with the OECD 219 guideline.

The measured concentrations in the report were based on a non-specific analytical method (LSC). The study conclusion is therefore valid for radioactivity concentrations. Identification of the radioactivity in the treatment solutions or in the test beakers was not performed during this study. However, radiochemical purity of the test substance (same batch no. as used in study) was confirmed using three TLC systems (values 97.9, 99.6 and 99.7%, respectively) in april 2002. Performance of the study was between February and September 2002. There is sufficient evidence that the test systems were treated with pyridaben of sufficiently high radiochemical purity.

Results

Based on LSC analysis of the stock solutions used for spiking the water, the measured applied concentrations were 0.49, 1.1, 2.7, 5.8 and 12.7 µg eq./L. On the day of application, immediately after application, the analytically determined concentrations (based on LSC) of the test substance in the overlying water were 0.28, 0.48, 1.0, 2.5 and 5.1 µg eq./L, representing 39-57% of the measured applied

concentrations. After 28 days, the measured concentrations in the overlying water had fallen to 15-18% of the measured applied concentrations.

At 0.49 and 12.7 µg/L on day 0 and 7, and on day 28 in all test concentrations, measured concentrations in pore water were <1% of measured applied concentrations. At 0.49 and 12.7 µg/L, concentrations in sediment increased from 7.5-<12.5% AR on day 0 to 38-61% AR on day 7. After 28 days, concentrations in sediment (determined by combustion/LSC) at all tested concentrations were 37-48% AR. The overall recovery at study end (sum of RA in overlying water, pore water and sediment) was in the range 55-65% AR. In an attempt to explain the poor recovery, selected day 28 samples were re-analysed, whereby sediment was extracted and the radioactivity in extracts and PES was determined separately. Using this procedure, the recoveries increased to 70-99% AR. In an additional experiment it was shown that ¹⁴C pyridaben was also present in significant amounts on the walls of the test vessels (mean of 9.2% AR).

No significant differences in emergence of male and female midges were observed in any of the treatments. Emergence rate and development rate were not statistically significantly reduced at any of the tested concentrations, when compared to the pooled control.

Conclusion

The NOEC for emergence and development rate was 5.1 µg eq./L, based on initial measured concentrations.

Guidelines and limitations

The study is acceptable.

B.9.2.2.1.4 Microcosm studies

Study 1

Reference	: Rand G.M. & Holmes C.M. (1995); Healey G.F. (2004)	exposure duration	: 12 weeks
type of study	: outdoor microcosm study	test concentrations	: 0.34, 3.4 and 34 µg a.s./L
year of execution	: 1992-1993 (experimental part) & 2003-2004 (additional statistical analysis)	treatment regime	: 2X (29 days interval) with suspension of test substance in water
GLP statement	: yes	conclusion	: NOEC <0.34 µg a.s./L, NOEAEC 0.34 µg a.s./L, EAC 0.11 µg a.s./L (2 X, 29 days' interval)
Guideline	: EPA 72-7		
test substance	: BAS 300 11 I (pyridaben based 75% WP. Lot no. OAJ 030)		
a.s. content	: 74.9% pyridaben	acceptability	: acceptable

The objective was to assess the effects of the insecticide and acaricide pyridaben on the population and community dynamics of phytoplankton, periphyton, zooplankton, macroinvertebrates and fish in outdoor

microcosms, on the functioning of phytoplankton (primary productivity), and to study the rate of dissipation of pyridaben in pond water and sediment.

1. Extended summary

Methods

Design and treatment

The study was conducted in 1992 and 1993 at Toxikon Environmental Sciences Field Station in Jupiter, Florida, USA, in 24 23-m³ outdoor fibreglass tanks (L x W x H = 6.7 x 1.9 x 1.9 m), buried 1 m into the ground. The tanks were covered with a translucent roof (no walls), which allowed sunlight in on a natural photoperiod. Using a randomised block design, each treatment was assigned to 6 tanks. On May 18-20 1992, each tank was lined with 10-15 cm of sand and about 10-15 cm of sediment from a nearby pond (characteristics for composite sample from 24 tanks determined on 20 April 1993: 89% sand, 8% clay, 3% silt, pH 7.9, 0.79% organic matter, textural class loamy sand). On May 20-22 1992, the tanks were filled with water from an on-site pond. Each tank was stocked with 20 bluegill sunfish (*Lepomis macrochirus*; mean length 4.9 cm, mean weight 1.50 g) on June 12, 1992. Any fish that were found dead prior to the first treatment were replaced. During the study, water levels were maintained as required by adding rain- and/or groundwater. Meteorological data were collected daily on-site. Measurements on sampling days showed that the water temperature from T1 until study end ranged between 17 and 31°C, and the overall mean solar irradiance was 692 μ Einstein.

On May 4 (T1) and June 2 (T2), 1993, each microcosm was treated by applying a mixture of Pyridaben 75 WP in water directly below the water surface of each microcosm. The control enclosures were treated with water only. The nominal treatment rates were 0.34, 3.4 and 34 μ g a.s./L. Biotic and abiotic parameters were monitored from 46 weeks before the first treatment (16 June, 1992) until 8 weeks after the second treatment (July 28, 1993).

Residue analysis

Samples from each application solution were taken for residue analysis. Water samples for residue analysis (composite from depth integrated subsamples from each quadrat per tank) were collected from all tanks 1 day before T1, 0-30 min and 12 hours after T1 and T2, 1, 2, 4, 7, 14, 21 and 28 days after T1 and T2, and 5, 6, 7 and 8 weeks after T2. Sediment samples (composite of 5 cores/tank) were collected from all tanks 1 day before T1, 1, 4, 7, 14 and 28 days after T1 and T2, and 56 day after T2. The top 6 cm of each core was removed for analysis. Field spikes were prepared by fortifying sediment samples (n=3 on each sediment sampling day, 200 μ g /kg) and water samples (n=3 at 0, 7, 14, 21 and 28 days after T1 and T2, 34 μ g/L) from control tanks with pyridaben analytical standard and carrying these fortified samples through transport and shipping along with the other samples. All samples were stored frozen prior to analysis but storage stability data were submitted demonstrating that pyridaben was stable in frozen spiked water and sediment for 18 and 12 months respectively (encompassing the duration of storage of study samples).

Pyridaben was extracted from water samples with dichloromethane and from sediment with methanol. Sediment extracts were partitioned with dichloromethane. The dichloromethane phases were cleaned up on a florisil column and analysed by GC/ECD. Concurrent validation using laboratory fortified microcosm water and sediment was reported and supported LOQs of 0.1 µg/L (water) and 0.01 mg/kg (sediment). Application solutions were diluted with acetonitrile and analysed by HPLC/UV.

Water chemistry

Samples were collected from all tanks starting in week -46 at 1-2 weekly intervals prior to T1, 1 day before T1, 1, 2, 7, 14, 21 and 28 days after T1 and T2, and 5, 6, 7 and 8 weeks after T2. Measurements involved: alkalinity, hardness, total suspended solids, total dissolved solids, turbidity, DOC, TOC, total N, NO₂, NO₃, nitrogen kjeldahl, ammonia, total P, orthophosphate. Temperature, pH, conductivity and O₂ were measured daily in each tank until study end.

Phytoplankton and zooplankton

The species composition and abundance of zooplankton, phytoplankton and phytoplankton pigments (chlorophyll a and phaeophytin; photometrically) were determined in water samples collected from all tanks starting in week -46 at 1-2 weekly intervals prior to T1, 1 day before T1, 1, 2, 7, 14, 21 and 28 days after T1 and T2, and 6 and 8 weeks after T2.

An additional measure of primary productivity was obtained by suspending three sets of light and dark bottles filled with microcosm water under the water surface for 3 hours. Dissolved oxygen was measured at the start and end of the three hour period in each bottle. Gross primary productivity was calculated as the sum of net productivity (i.e. the increase in oxygen concentration in the light bottle) and respiration (i.e. the loss of oxygen in the dark bottle).

Periphyton

The species composition and abundance of periphyton and periphyton pigments (chlorophyll a and phaeophytin; photometrically) were determined in floating artificial periphyton samplers collected from all tanks starting at 9 time points prior to week -1, 1 day before T1, 7 and 28 days after T1 and T2, and 5 and 8 weeks after T2.

Macroinvertebrates

Stationary artificial substrate samplers suspended near the sediment water interface, and emergent insect traps were used to monitor changes in abundance and composition of macroinvertebrates. Samples were collected from all tanks at 9 time points prior to week -1, 1 day before T1, 7 and 28 days after T1 and T2, and 8 weeks after T2. Emergent insects were also monitored on day 14 and 21 after T1 and T2, and 5, 6 and 7 weeks after T2.

Fish

At test termination, the number of surviving fish and fish length and weight were recorded. Two *in situ* 96-hour acute toxicity tests were conducted starting on the treatment days. Ten juvenile bluegills were placed in a cage in each of two replicate tanks from each treatment and the controls. Mortality was assessed daily.

Data analysis

Results are based on nominal concentrations of the test substance (for justification see results of residue analysis). In the initial report from 1995, log-transformed abundance data were analysed by ANOVA followed by Dunnett's multiple t-test to test differences at taxon or parameter level ($p \leq 0.05$; one sided) and to calculate the NOEC. Effects on the diversity of the communities of phytoplankton, zooplankton and macroinvertebrates were analysed using the Simpson's diversity index.

A report supplement from 2004 presented statistical analysis on the zooplankton and benthic invertebrate community level involving multivariate analysis using Principal Response Curves (PRC). For analysis at the community level, taxon abundance data were $\ln(ax+1)$ transformed and analysed using Redundancy Analysis (RDA). The Monte Carlo permutation test was performed using the first principal component to test for an overall effect of treatment at the community level on each sampling occasion. To test for a dose response trend and investigate differences between the control and the treatment groups, $\ln(ax+1)$ transformed taxon abundance data were analysed using Principal Components Analysis (PCA). For the calculation of $\text{NOEC}_{\text{community}}$ per sampling date the Williams test was applied to the sample scores of the first principal component of each sampling date (as calculated by the computer program CANOCO version 4). For analysis at the taxon level, $\ln(ax+1)$ transformed abundance data of those taxa having a high correlation with the PRCs were separately analysed by the Williams test to determine the $\text{NOEC}_{\text{taxon}}$.

Proposed procedure for evaluation^[1]

In the Evaluation the concentration-response relationships observed are classified according to the effect-classes presented in Table B.9.2.2.1.4-01. This Table is an adapted version of the classification of the EU-guidance document (see Annex 2 of de Jong et al. ^[5]). The occurrence of an effect on consecutive time points is likely to be more related to substantial damage to the ecosystem than an effect that is observed once or with longer intervals. For aquatic mesocosm studies, a duration of 8 weeks may be chosen as an assessment endpoint for recovery (EU, 2002^[2]). In the review papers that introduced the Effect classes (Brock *et al.*, 2000a^[3]), (Brock *et al.*, 2000b^[4]) a recovery period of 8 weeks was chosen, since in most of the micro/mesocosm evaluated invertebrates were sampled at intervals of 2 to 4 weeks. Consequently, a period of 8 weeks allows a few sampling dates to show consistent recovery. By taking both the duration of the effects after the first and last application into account in Effect classes 3 and 5 (A or B), the ecological impact of repeated applications could be evaluated more realistically. The potential for recovery is recognised as an important issue for the determination of the NOEAEC.

Table B.9.2.2.1.4-01 Proposed effect classes to evaluate the treatment-related responses observed in aquatic micro/mesocosm tests

Effect class	Description	Criteria
1	Effects could not be demonstrated (NOEC _{micro/mesocosm})	• No (statistically significant) effects observed as a result of the treatment • Observed differences between treatment and controls show no clear causal relationship
2	Slight and transient effects	• Effects reported as 'slight' or 'transient', or other similar descriptions • Short-term and/or quantitatively restricted response of one or a few sensitive endpoints, and only observed at individual samplings
3A	Pronounced effects; recovery within 8 weeks after first application	• Clear response of sensitive endpoints, but full recovery within 8 weeks after the first application, • Effects reported as 'temporary effects on several sensitive species', 'temporary effects on less sensitive species/endpoints' or other similar descriptions • Effects observed at some subsequent sampling instances
3B	Pronounced effects; recovery within 8 weeks after last application	• Clear effects of sensitive endpoints, but full recovery within 8 weeks post last application. In the case of repeated treatments a total duration of the effects of > 8 weeks is possible, • Effects reported as 'temporary effects on several sensitive species', 'temporary effects on less sensitive species/endpoints' or other similar descriptions • Effects observed at some subsequent sampling instances
4	Pronounced effects ; study too short to demonstrate recovery within 8 weeks after the last application	• Clear effects (such as large reductions in densities of sensitive species) observed, but the study is too short to demonstrate complete recovery within 8 weeks after the (last) application
5A	Pronounced effects ; no recovery within 8 weeks after the last application; full recovery within the test period	• Clear response of sensitive endpoints and recovery time is longer than 8 weeks after the last application, • Full recovery is reported before the end of the study • Effects reported as 'long-term effects followed by recovery on several sensitive and less sensitive species/endpoints' or other similar descriptions • On consecutive time-points
5B	Pronounced effects ; no recovery within 8 weeks after the last application; no full recovery demonstrated within the test period	• Clear response of sensitive endpoints and recovery time is longer than 8 weeks after the last application, • Full recovery is not reported before the end of the study • Effects reported as 'long-term effects followed by recovery on several sensitive and less sensitive species/endpoints' or other similar descriptions • On consecutive time-points

In the tables showing effects consistent statistically significant NOECs (Dunnett's test or William's test, $p < 0.05$) per sampling date are shown. An effect was considered to be consistent when statistical significant deviations pointed in the same direction for at least two consecutive sampling dates. Concentrations >NOEC showed significant increases (↑) or reductions (↓). The blank cells indicate no statistical significance at the highest tested concentration of 34 µg/L. Statistically significant changes which had a doubtful relationship to the treatment (e.g., observed at a single sampling date only and/or involving very low numbers or irregular abundances) are not included. An exception were the results for periphyton and macroinvertebrates. The spacing between samplings was high, and for this reason single occurrences of statistically significant effects could have indicated a biologically relevant effect. Single occurrences of statistically significant effects are therefore included for these groups, unless it was obvious that they had a doubtful relationship to the treatment (e.g., involving very low numbers or irregular abundances and/or without a dose-relationship).

Results

Residue analysis

Application solutions

The levels of pyridaben ranged between 87-97% of the theoretical value (mean 93%, SD 4%).

Field fortifications

The mean recovery from untreated microcosm water samples fortified at 34 µg/L was 81% (SD 13%). The mean recovery from untreated sediment samples fortified at 200 µg/kg was 85% (SD 12%).

Microcosm water

Residues in water from treated tanks on day -1, and from untreated tanks, were <0.1 µg/L, except for one sample at 33 days after T1 (0.2 µg/L). A summary of the main results for treated tanks is shown in Table B.9.2.2.1.4-02. Residue values are not corrected for recoveries. The residues 0-30 minutes after T1 and T2 were far higher than the nominal values. Since the measured concentrations in the application solutions were close to the expected values, this is likely to be attributable to the fact that the residues were not yet homogeneously distributed in the water of each tank at this early sampling time. At 12 hours after application, the measured values in the water were in fair agreement with the nominal treatment values (81-112%, except low dose after T2: 168%). It is acceptable therefore, to base the study conclusions on nominal concentrations.

DT50 values for dissipation of pyridaben from the water were calculated following the recommendations and procedures of the "Guidance document on estimating persistence and degradation kinetics from Environmental Fate studies on pesticides in EU registration" (SANCO/10058/2005). The procedures described in this document for estimation of persistence DT50 values in water from water-sediment studies were followed (SFO = single first-order, FOMC = first-order multi-compartment, DFOP = double first-order parallel model, HS = hockey stick). All calculations were performed with ModelMaker v 4.0 software. SFO, FOMC, and where required DFOP and HS models were evaluated by the RMS. The results for the best fit model are presented in Table. The mean DT50 value (persistence) was 0.88 days at the two lowest doses and 1.3 days at the highest dose.

Table B.9.2.2.1.4-02 Concentrations of pyridaben in microcosm water treated twice (T1 and T2) with pyridaben 75 WP

nominal concentration (µg/L)	0.34	3.4	34
measured concn 0-30 min after T1 (µg/L)			
range	0.49-2.96 ^(A)	4.2-5.9	34-68
mean	1.1	4.9	53
meas concn 12 hours after T1 (µg/L)			
range	0.20-0.38	2.3-3.0	20-41
mean	0.30	2.8	29
meas concn 0-30 min after T2 (µg/L)			
range	0.48-1.2	3.4-7.4	39-78
mean	0.8	5.4	58
meas concn 12 hours after T2 (µg/L)			
range	0.39-0.77	2.8-6.3	28-34
mean	0.57	3.8	29
DT50 (persistence) after T1			
best fit model	SFO	SFO	SFO
DT50 (d)	0.93	0.85	1.17
r ²	0.32	0.96	0.91
chi ² error	51.03	16.6	28.90
DT50 (persistence) after T2			
best fit model	SFO	SFO	SFO
DT50 (d)	0.87	0.91	1.45
r ²	0.72	0.77	0.97
chi ² error	40.24	46.91	16.08
mean DT50 (d)	0.88	0.88	1.3

^(A) 0.49-1.1 µg/L for 5 of 6 tanks, 2.96 µg/L (mean of triplicate analysis) for remaining tank

Microcosm sediment

Residues (on dry weight basis) in sediment from untreated tanks were always <0.01 mg/kg. Residues in sediment from treated tanks of the mid and high dose at 1 day prior to T1 were also <0.01 mg/kg in 5 of 6 tanks, with residues of 0.027 and 0.016 mg/kg in the remaining two tanks. A summary of the main results for treated tanks after T1 is shown in Table B.9.2.2.1.4-03. At the mid dose, mean residues were ≤0.01 mg/kg at all samplings. At the high dose, mean residues reached maximum levels of 0.12 and 0.053 mg/kg after T1 and T2, respectively, and at study end (111 days after T2) residues were still detected in 5 out of 6 tanks (mean residue 0.017 mg/kg).

Table B.9.2.2.1.4-03 Concentrations (on dry weight basis; means of 6 tanks(A); mg/kg) of pyridaben in microcosm sediment treated twice (T1 and T2) with pyridaben 75 WP

nominal concentration 3.4 µg/L		nominal concentration 34 µg/L	
Time after treatment	Residue (mg/kg)	Time after treatment	residue (mg/kg)
1DAT1	0.006	1DAT1	0.074
4DAT1	0.009	4DAT1	0.103
7DAT1	0.010	7DAT1	0.065
14DAT1	0.010	14DAT1	0.033
28DAT1	0.006	28DAT1	0.044
1DAT2	0.007	1DAT2	0.053
4DAT2	0.006	4DAT2	0.047
7DAT2	0.006	7DAT2	0.023
14DAT2	0.006	14DAT2	0.030
28DAT2	0.005	28DAT2	0.014
54DAT2	0.005	56DAT2	0.022
		84DAT2	0.012
		111DAT2	0.017

xDATy: day x after treatment y
 (A) Residues <LOQ (<0.01 mg/kg) were set at 0.005 mg/kg for calculation of the mean.

Functional parameters

A clear reduction of the variability of the pre-test values of the physical and chemical parameters indicated that the microcosms had stabilised at the time of the first treatment. The average water temperature rose from about 25°C at T1 to about 28°C at T2, and remained at about 28°C for the remainder of the study. Dissolved oxygen values at T1 were about 10-12 mg/L. They declined to about 9 mg/L just before T2, and fluctuated between 8 and 11 mg/L for the remainder of the study. The pH declined from about 9.1-9.5 at T1 to 8.8-9.1 at 14 days after T2, and then rose to about 9.1-9.3 at 56 days after T2. Conductivity was about 240 µmhos/cm at T1, it reached a maximum of about 280 µmhos at 28 days after T2, and was about 270 µmhos/cm at 56 days after T2. It was not clear whether the above physical parameters were statistically analysed, but inspection of the data did not suggest any biologically significant treatment related trends. There were no statistically significant differences between the treated and the control tanks for the water chemistry parameters (alkalinity, hardness, total suspended solids, total dissolved solids, turbidity, DOC, TOC, total N, NO₂, NO₃, nitrogen kjeldahl, ammonia, total P, orthophosphate). NOEC_{water chemistry} is 34 µg/L; effect class is 1.

Chlorophyll a and phaeophytin; primary productivity

It was stated in the report that concentrations of chlorophyll a and phaeophytin in samples of phytoplankton and periphyton were insignificant or undetectable for many of the measurements and that there was no consistent pattern or trend. An overview of mean concentrations per sampling day per treatment however was not presented but inspection of the study raw data indicated that indeed the levels were generally too low to allow any meaningful conclusion regarding effects of treatments.

There were no consistent effects or patterns in primary productivity (according to the light and dark bottle method). NOEC_{primary productivity} is 34 µg/L; effect class is 1.

Structural parameters

Species richness in the microcosms and abundance were sufficiently high to provide valid data.

Phytoplankton

Phytoplankton species richness in the microcosms: 72 phytoplankton genera. Abundance data of the following major taxa were subjected to statistical analysis: Bacillariophyceae, Chlorophyta, Chrysophyta, Cryptophyta, Cyanophyta, Euglenophyta, Pyrrophyta and Xanophyceae. Abundance data of individual genera were not analysed statistically. However, during laboratory studies algae were the least sensitive of the aquatic organisms tested (EbC50 35000 µg/L versus chronic NOEC values of 0.047-0.28 µg/L for other aquatic species). Therefore direct effects on primary producers are not anticipated at the concentrations tested during the microcosm study. Moreover, the only adverse effect on phytoplankton major taxa recorded during this study was a reduction of cryptophyta at 14DAT2 and 21DAT2 (see below), which may be fortuitous. From 21DAT1 through 28DAT2, the pyrrophyta increased statistically significant. This correlates with the reduction in zooplankton abundance. The data analysis for phytoplankton is therefore accepted. Table B.9.9.2.2.1.4-04 presents a summary of consistent NOECs (Dunnett's test, $p < 0.05$) per sampling date.

At 34 µg/L, the abundance of cryptophyta was reduced at 14 and 21 days after T2, whilst that of pyrrophyta was increased between 21 days after T1 through 28 days after T2. The latter effect may have been the result of reduced predation by zooplankton. The NOEC_{population} is 3.4 µg/L, the effect class at one concentration higher is 3A. There were no consistent statistically significant effects on species richness and diversity (Simpson's index). Based on this data, the NOEC_{community} is 34 µg/L, the effect class is 1.

Table B.9.2.2.1.4-04 NOECs (Dunnett's test, $p < 0.05$) per sampling date for phytoplankton populations (nominal treatment levels, µg/L). Concentrations >NOEC showed significant increases (↑) or reductions (↓). NOECs based on poor data are not shown.

parameter	day after treatment T1:						day after treatment T2:							
	1	2	7	14	21	28	1	2	7	14	21	28	41	56
cryptophyta										3.4 (↓)	3.4 (↓)			
pyrrophyta					3.4 (↑)	3.4 (↑)	3.4 (↑)	3.4 (↑)	3.4 (↑)	3.4 (↑)	3.4 (↑)	3.4 (↑)		

Periphyton

Periphyton species richness in the microcosms: 64 genera. Abundance data of 7 major taxa were analysed: Bacillariophyceae, Chlorophyta, Chrysophyta, Cryptophyta, Cyanophyta, Euglenophyta and Pyrrophyta. There were no indications of a treatment related effect. The NOEC_{population} is 34 µg/L, the effect class is 1.

Zooplankton

Zooplankton species richness in the microcosms: 46 genera, mainly representing Cladocera, Copepoda, and Rotifers. In Table B.9.9.2.2.1.4-05 a summary is presented of consistent statistically significant NOECs (Dunnett's test, $p < 0.05$) per sampling date.

PRC/PCA analysis showed significant treatment related negative effects on the community level starting on the first day after the first treatment at the two highest doses, up to and including 21 days after the second treatment at the highest dose. A significant effect was again observed at the last sampling date at all doses, but this represented a positive effect on the community level, and hence was considered to be not adverse. Consistent negative effects of the treatment on the population levels were noted at all three test doses for Polyarthra, at the mid and high dose for Nauplii, Latonopsis, Trichocerca, Diaptomus and Ceriodaphnia, and at the high dose only for Keratella and Acanthocylops. At the high dose, occasionally increased abundances were recorded for Monostyla, Synchaetidae and Lecanidae between 28 days after T1 and 21 days after T2. The abundance of Ilyocryptus was increased at the mid and high dose between 28DAT1 through 28DAT2, and at the high dose also at 41DAT2. The author of the report suggested that these increases might be due to reduced predation by fish. The $\text{NOEC}_{\text{population}}$ is $< 0.34 \mu\text{g/L}$, effect class at one concentration higher is 3A since the recovery period at $0.34 \mu\text{g/L}$ is < 8 weeks after T1. The $\text{NOEC}_{\text{community}}$ is $0.34 \mu\text{g/L}$, effect class at one concentration higher 3A since the recovery period at $3.4 \mu\text{g/L}$ is < 8 weeks after T1.

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Table B.9.2.2.1.4-05 NOECs (Dunnett's test, $p < 0.05$) per sampling date for zooplankton populations (nominal treatment levels of pyridaben; $\mu\text{g/L}$). Concentrations $>$ NOEC showed statistically significant increases ($\uparrow$) or reductions ($\downarrow$). NOECs based on poor data are not shown.

parameter	day after treatment T1:							day after treatment T2:						
	1	2	7	14	21	28		1	2	7	14	21	28	56
species richness	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)		3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	
diversity ^(B)														
PRCs/PCAs ^(A)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)		0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	$< 0.34 (\uparrow)$
Nauplii	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)		0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	
Polyartha	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)		0.34 ($\downarrow$)	$< 0.34 (\downarrow)$	$< 0.34 (\downarrow)$	$< 0.34 (\downarrow)$	0.34 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)
Latonopsis	3.4 ($\downarrow$)	3.4 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)		3.4 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	3.4 ($\downarrow$)	
Keratella	3.4 ($\downarrow$)	3.4 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)		3.4 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	3.4 ($\downarrow$)	
Trichocerca	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)		0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	3.4 ($\downarrow$)	
Acanthocyclops	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)		3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	3.4 ($\downarrow$)	
Diaptomus	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)		0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	0.34 ($\downarrow$)	3.4 ($\downarrow$)	
Lepadella														
Ceriodaphnia					0.34 ($\downarrow$)	0.34 ($\downarrow$)								
Ilyocryptus					0.34 ($\uparrow$)	0.34 ($\uparrow$)		0.34 ($\uparrow$)	0.34 ($\uparrow$)	0.34 ($\uparrow$)	0.34 ($\uparrow$)	0.34 ($\uparrow$)	0.34 ($\uparrow$)	3.4 ($\uparrow$)
Monostyla					3.4 ($\uparrow$)	3.4 ($\uparrow$)		3.4 ($\uparrow$)	3.4 ($\uparrow$)	3.4 ($\uparrow$)	3.4 ($\uparrow$)	3.4 ($\uparrow$)	3.4 ($\uparrow$)	
Lecanidae					3.4 ($\uparrow$)	3.4 ($\uparrow$)		3.4 ($\uparrow$)	3.4 ($\uparrow$)	3.4 ($\uparrow$)	3.4 ($\uparrow$)	3.4 ($\uparrow$)	3.4 ($\uparrow$)	

^(A) Determined by William's test applied to the sample scores of the first principal component of each sampling date ($p < 0.05$). The PRC captured 44% of the variance explained by the treatment.

^(B) Simpson's diversity index, statistically analysed by Dunnett's t-test; tabulated statistically significant differences from the control were not statistically significant when data were analysed using Kendall's non-parametric procedure. When using Pearson correlations, the Simpson's diversity index was significant on 7DAT1 and 14DAT, and on 28DAT2 to 56DAT2.

Macroinvertebrates

The abundances of emergent insects were low preventing firm conclusions on effects on this group. Benthic invertebrate species richness in the microcosms: 57 genera, with representatives of insecta, annelida, mollusca, hydracarina, bryozoa, cnidaria, nematoda and turbellaria. In Table B.9.2.2.1.4-06 a summary is presented of statistically significant NOECs (Dunnett's test, $p < 0.05$) per sampling date for benthic invertebrates.

In the original report from 1995, it was stated that results for diversity and richness measurements were generally not significant, but no detail of the corresponding statistical analyses was presented. According to the statistical report from 2004, PRC captured 31% of the variance explained by the treatment, and PRC/PCA analysis showed that the benthic invertebrate community was not significantly affected by the pyridaben treatment at any sampling date ($\text{NOEC}_{\text{community}} 34 \mu\text{g/L}$, effect class 1).

Statistically significant reductions of individual taxonomic groups were observed for Hydra (high dose: at 7 and 28 days after T1; two highest doses at 28 days after T2) and Oxyethira (at all doses on day 7 after T1, and at the high dose on day 7 after T2). Since the sampling frequency of the macroinvertebrates was low, evaluation is based on significant effects observed on single sampling dates. The $\text{NOEC}_{\text{population}}$ is $< 0.34 \mu\text{g/L}$, effect class at one concentration higher 3A. At 56 days after treatment T2, the Hydra populations in the control and the low dose had virtually collapsed, which was the result of natural population development. The last sampling on day 56 after treatment T2 is therefore not suitable to conclude that at the mid and high dose level recovery of Hydra occurred within 8 weeks after T2 (effect class for these 2 dose levels is therefore 5B). The NOEC for this species is $0.34 \mu\text{g/L}$.

Table B.9.2.2.1.4-06 NOECs (Dunnett's test, $p < 0.05$) per sampling date for benthic macroinvertebrate populations (nominal treatment levels of pyridaben, $\mu\text{g/L}$). Concentrations $> \text{NOEC}$ showed significant increases ($\uparrow$) or reductions ($\downarrow$). NOECs based on poor data are not shown.

Parameter	day after treatment T1:		day after treatment T2:		
	7	28	7	28	56
Hydra	3.4 ($\downarrow$)	3.4 ($\downarrow$)		0.34 ($\downarrow$)	
Oxyethira	< 0.34 ($\downarrow$)		3.4 ($\downarrow$)		
Dero	3.4 ($\downarrow$)				
Oribatei	3.4 ($\uparrow$)				

No statistically significant effects were seen on the macroinvertebrate community: $\text{NOEC } 34 \mu\text{g a.s./L}$; effect class 1.

Fish

Fish mortality was observed at the high dose within 8 days (mostly within 48 hours) after T1 and T2 (25 and 6 fish, respectively). Fish mortality was observed in the control and the low dose 7 or more weeks after T2 (23 fish), but these mortalities are unrelated to treatment as low mortality (2 fish) was observed in the mid dose. Mean mortality rate at study end was 4%, 14%, 2% and 26% in the control, low, mid and high dose, respectively.

Prior to T1 a representative sample of fish from each treatment was caught and length and weight was determined: >7 cm and >4 g respectively, without differences between treatments. At study end, mean fish length and weight were significantly higher at the high dose compared to the other treatments, probably due to reduced competition for food.

The first *in situ* test was not successful due to high escape rate (7-10 per tank, only 10-23% of the added fish were recovered). Mean % mortality in the second *in situ* test was 0%, 0%, 5% and 70% in the control, low, mid and high dose, respectively. Comparison of this data to results of laboratory testing with bluegill is hampered by the fact that length and weight of the fish used for the *in situ* tests was not clearly specified ("juvenile").

The NOEC_{population} is 3.4 µg/L, effect class at one concentration higher 3A.

2. Evaluation

Evaluation of the treatment-related effects observed in the indoor microcosm study

a. Evaluation of the scientific reliability of the field study

Criteria for a suitable (semi)field study

The following 5 questions are important in the decision whether the study can be used for an appropriate risk assessment.

1. Does the test system represent a realistic freshwater community?

Answer: yes, however without macrophytes.

The study was performed in outdoor aquatic microcosms of sufficient volume. The systems housed an ecosystem that was representative for a freshwater community with algae (phytoplankton, periphyton), zooplankton, macroinvertebrates, and fish.

2. Is the experimental set-up adequate and unambiguous?

Answer: not completely.

The experiment is well designed, state-of-the-art at the time it was carried out (1992 to 1993). The frequency of sampling/monitoring of residues in water and sediment, water chemistry, phytoplankton, zooplankton and fish was adequate following two applications spaced 29 days apart. The sampling frequency of macroinvertebrates was rather low. This has hampered evaluation since now it was not possible to follow the proposed procedure for evaluation (statistically significant effect on consecutive time points). Variability of the pre-test values of the physical and chemical parameters indicated that the microcosms had stabilised at the time of the first treatment. Species richness and abundance were sufficiently high to provide valid data. Exposure levels were acceptable; however effects were seen at all exposure levels. Zooplankton and fish (bluegill sunfish) were most susceptible, while no statistically significant effect was observed on phytoplankton, periphyton, primary productivity, and on the macroinvertebrate community.

In laboratory tests, bluegill were not the most sensitive fish species tested (96-hour LC50 a factor of about 5 higher than that of the most sensitive species rainbow trout). In addition, the fish used for stocking the microcosms were larger (measured length prior to T1 7.2-7.6 cm) than those used for laboratory testing (recommended length 2.0 cm). The size of the fish used for the *in situ* tests was not reported. These aspects should be taken into consideration when comparing the results on fish testing from this study with those from laboratory testing.

The study was performed according to general principles of GLP.

3. Is the exposure regime adequately described?

Answer: yes.

The residues 0-30 minutes after T1 and T2 were far higher than the nominal values, probably due to an inhomogeneous distribution in the water column. From 12 hours after both applications the measured values were in fair agreement with the nominal treatment values. Half-life (mean values) ranged from 0.88 to 1.3 days. Therefore using nominal values is acceptable.

4. Are the investigated endpoints sensitive and in accordance with the working mechanism of the compound?

Answer: yes.

In laboratory toxicity studies *Daphnia magna* and fish were most susceptible to pyridaben (both acute and chronic). *Chironomus riparius* was less susceptible; algae were least susceptible.

5. Is it possible to evaluate the observed effects statistically?

Answer: yes.

The recommendations of the Classic workshop^[6] (1999) propose that 5 levels of the test compound are studied (besides controls), with the minimum of two replicates per treatment. The design chosen was 3 levels of pyridaben, with 6 replications. The experimental design adopted allows univariate and

multivariate analysis of the responses observed. The techniques applied were univariate (ANOVA, Dunnett's t-test) and multivariate analysis (Principal Response Curves and Redundancy Analysis) of the data sets. The latter analysis was performed in 2004 and state-of-the-art in 1993.

It is concluded that the outdoor microcosm study can be used for the ecological risk assessment of the test compound to aquatic invertebrates, fish, and primary producers.

b. Evaluation of the results of the study;

Evaluation of the treatment-related responses observed in the aquatic microcosm test according to the proposed effect classes:

Treatment level of 0.34 µg a.s./L nominal

At this treatment level the statistically significant decreases were seen for the rotifer (zooplankton) *Polyarthra* (NOEC < 0.34 µg a.s./L; effect class at one concentration higher 3A) and for the macroinvertebrate *Oxyethira* (NOEC < 0.34 µg/L, effect class at one concentration higher 3A).

Treatment level of 3.4 µg a.s./L nominal

Besides the above mentioned effects on *Polyarthra* (effect class 3B at this treatment level) and *Oxyethira* (effect class 3A), a decrease was seen in zooplankton numbers of the *Latonopsis* (effect class 3A and *Ceriodaphnia* (effect class 2; both cladocerans), *Trichocerca* (rotifer; effect class 3A), nauplii (copepod; effect class 3A) and *Diaptomus* (copepod; effect class 3A). For the zooplankton community the NOEC_{community} is 0.34 µg a.s./L; effect class at one concentration higher 3A. The taxon *Ilyocryptus* (*Cladocera*) showed an increase in numbers from day 28 after T1 through day 28 after T2. A reduction of the numbers of the macroinvertebrate *Hydra* was recorded on day 28 after T2 (effect class 5B since the data did not allow to conclude that at the mid and high dose level recovery of *Hydra* occurred within 8 weeks after T2).

Treatment level of 34 µg a.s./L nominal

In the microcosms treated with 34 µg a.s./L the same effects were found as in the 3.4 µg a.s./L treatment, however for several taxa the time to recover was longer. Most importantly, *Polyarthra* did not fully recover at the highest treatment level (effect class 5B at this exposure level).

In addition to those effects, consistent negative effects were also observed for the zooplankton taxa *Keratella* and *Lepadella* (both rotifers; NOEC 3.4 µg a.s./L; effect class at one concentration higher 3A), and the copepod *Acanthocyclops*: NOEC 3.4 µg a.s./L; effect class at one concentration higher is 3B. The zooplankters *Ilyocryptus* (*Cladocera*), *Monostyla*, and *Lecanidae* (both rotifers) showed an increase in numbers on several sampling dates from day 28 after T1. This is presumably an indirect effect. The NOEC for zooplankton species richness and diversity is 3.4 µg a.s./L (both parameters effect class at one concentration higher 3B). The number of cryptophyta (phytoplankton) was reduced (NOEC 3.4 µg a.s./L; effect class at one concentration higher 3A); the number of pyrrophyta increased. No statistically significant effects were seen on individual periphyton taxa: NOEC_{population} 34 µg a.s./L; effect class 1. No statistically

significant effects were seen on the macroinvertebrate and phytoplankton community and periphyton species richness: NOEC 34 µg a.s./L; effect class 1. For fish the NOEC is 3.4 µg a.s./L; effect class at one concentration higher 3A. No treatment-related effects were observed for functional endpoints (water chemistry and primary productivity). These parameters are classified as class 1 effects.

c. Summary of effects

A summary of the effects according to the proposed classification is given in the table below.

Table B.9.2.2.1.4-07 Summary of the effect classes observed for several endpoints in the outdoor microcosm study with pyridaben

Species/group	Nominal concentration [µg a.s./L]		
	0.34	3.4	34
Chemico-Physical	1	1	1
Primary productivity	1	1	1
Phytoplankton abundance	1	1	3A
Phytoplankton community	1	1	1
Periphyton abundance	1	1	1
Periphyton richness	1	1	1
Zooplankton abundance	3A(↓)	3B(↓)	5B(↓)
Zooplankton community	1	3A(↓)	3A(↓)
Zooplankton diversity	1	1	3B(↓)
Zooplankton richness	1	1	3B(↓)
Macroinvertebrates abundance	3A(↓)	5B(↓)	5B(↓)
Macroinvertebrate community	1	1	1
Fish	1	1	3A(↓)

On the basis of the univariate analysis of the data sets, **an overall NOECmicrocosm of <0.34 µg a.s./L** can be derived from the evaluated microcosm experiment. At the exposure level of 0.34 µg a.s./L and above, the populations of the macroinvertebrate *Oxyethira* were reduced at 7 days after treatment, but recovery occurred at all dose levels before study end. At the exposure level of 0.34 µg a.s./L and above, a statistically significant effect on the abundance of the rotifer *Polyarthra* was seen from day 2 after T2 through day 14 after T2, but at the next dose of 3.4 µg a.s./L full recovery of *Polyarthra* within the study was observed (but not at 34 µg a.s./L). At 3.4 µg a.s./L, adverse effects without demonstrated recovery prior to study end were recorded only for the macroinvertebrate *Hydra*.

The NOEAEC of the evaluated microcosm study with pyridaben is therefore: 0.34 µg a.s./L. Since only a single high quality micro/mesocosm study is available, a precautionary extrapolation factor of 3 is considered to be necessary for the spatio-temporal extrapolation of the Effect class 3 NOEAEC, to ensure that at this short-term concentration level no Class 4 – 5 effects will occur (in agreement with guidance provided in De Jong et al.⁽⁵⁾). The EAC is therefore 0.11 µg a.s./L.

The relevance of this study for the proposed treatment regime of Nexter Pro will be discussed in the risk assessment.

A summary of endpoints as derived from this study is presented in the table below.

Table B.9.2.2.1.4-08 Summary of endpoints in the outdoor microcosm study with pyridaben; values based on nominal concentrations

Group	NOEC [$\mu\text{g a.s./L}$]	NOEAEC [$\mu\text{g a.s./L}$]
Phytoplankton	3.4	34
Periphyton	34	
Zooplankton	<0.34	3.4
Macroinvertebrates	<0.34	0.34
Fish (bluegill)	3.4	3.4
Community	0.34	34

The data on the effects at community level indicate that zooplankton was most susceptible: NOEC is 0.34 $\mu\text{g a.s./L}$. Since at this exposure level recovery was observed at the highest exposure level within 8 weeks after the first application of pyridaben the NOEAEC at community level is 34 $\mu\text{g a.s./L}$.

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6. 1999. Community-Level Aquatic System Studies - Interpretation Criteria (CLASSIC), Fraunhofer Institute, Schmallenberg, Germany, (Giddings, J., Heger, W., Brock, T., Heimbach, F., Maund, S., Norman, S., Ratte, H., Schäfers, C. and Streloke, M., Eds.), SETAC, Pensacola, FL, USA.

Study 2

Characteristics

reference	: Singh M., 1994 (IIIA 10.2.2/02)	exposure duration	: 56 days
type of study	: Fate and effects on fish in outdoor microcosms	treatment rates	: 1.7 & 2.0 µg/L
year of execution	: 1991-1994	dosing method	: 2X (20 hour interval) with suspension of test substance in water
GLP statement	: Yes	acceptability	: Acceptable
guideline	: EPA 164-2	conclusion	: See study conclusion in this summary
test substance & purity	[benzene-U-14C]-pyridaben, lot no. CP-1007, chemical purity not reported, radiochemical purity 97.29%; [pyridazinone-3,6-14C]-pyridaben, lot no. CP-1008, chemical purity not reported, radiochemical purity 96.17%.		

The fate and effects on fish of [benzene-U-14C]-pyridaben and [pyridazinone-3,6-14C]-pyridaben formulated as WP was investigated in outdoor microcosms in Alabama (USA).

Methods

Each of three microcosms consisted of a partially buried galvanized steel tank coated with epoxy paint (L x W x H 236 x 87 x 53 cm). In each tank 5 cm of clay was placed, and 5 cm of topsoil was placed over the clay (soil properties see table B.9.2.2.1.4-09). The tanks were inoculated with 4 L of hydrosol from an on-site pond, and then filled with water from the same pond, to a depth of 36 cm (nominal tank volume 680 L). To insure primary production, the microcosms were fertilised with 2.5 mL of liquid ammoniated polyphosphate fertiliser on 1 August 1991. Thirty bluegill sunfish (*Lepomis macrochirus*, mean length 5.6 cm) were stocked into each microcosm on 26 August 1991. Any fish found dead prior to application was replaced. No fish mortality was observed during the 17 days prior to treatment.

Treatment with [benzene-U-14C]-pyridaben and [pyridazinone-3,6-14C]-pyridaben was assigned to one tank each, whilst the remaining tank was the control tank. The tanks were treated with a simulated spray drift application (treatment I) on 16 September 1991, and with a simulated runoff (treatment II) 20 hours later, on 17 September 1991. The first treatment was applied by injecting 400 mL of an aqueous suspension containing 1157 µg of the radiolabeled compound and 0.4 mg of inert WP powder (clay, lignosulfonate and silica) just below the water surface (nominal treatment rate 1.7 µg/L based on water volume of 380 L). The control tank was left untreated. The second treatment was applied by pouring 400 mL of an aqueous suspension containing 1389 µg of the radiolabeled compound, 0.5 mg of inert WP powder and 101 g of sieved soil over the water surface (nominal treatment rate 2.0 µg/L based on water volume of 680 L). At this time, the control tank was treated with a similar slurry mixture without test substance. The treatment solutions contained 3.75% v/v methanol. The radiochemical purity of the test compound was confirmed to be >96% prior to each application.

Water physico-chemical parameters were monitored in all microcosms *in situ* at residue sampling days and were as follows: temperature (20.2-32.8°C); pH (8.2-8.9); dissolved oxygen (7.2-11.4 mg/L).

Length and weight of twenty fish from the lot used for stocking, the 15 fish collected for residue analysis on day 4 after treatment I, and all remaining fish at study end (56 days after treatment I, 12 November 1991) were measured. Tanks were checked daily for fish mortality.

Water samples were collected at 1, 4, 8, 20, 28 and 48 hours, and at 4, 7, 14, 28, 42 and 56 days after treatment I. Hydrosol samples were collected at 8, 20, 28 and 48 hours, and at 4, 7, 14, 28, 42 and 56 days after treatment I. At each sampling, 3 water samples and 2 hydrosol cores were collected from each tank. The distribution of the radioactive residues and metabolite identification in water and hydrosol was determined for selected samples (as shown in the tables with results).

The water layer was extracted twice with ethyl acetate (second extraction after acidification to pH 2). Radioactivity in the initial water sample, ethyl acetate extract and remaining aqueous phase was determined by LSC. The organic phase and the aqueous phase (after lyophilisation) was analysed by silica gel TLC using two solvent systems. Another aliquot of the water sample was extracted using C18-Empore discs and (for later samples) SAX-Empore discs, and the purified sample was analysed by reversed phase HPLC.

Hydrosol samples were extracted three times with acetone. Radioactivity in extracts was determined by LSC and, in extracted hydrosol, by combustion/LSC. The extracts were lyophilised and analysed by silica gel TLC using two solvent systems.

Fish samples (day 4 and 56) were extracted with acetone/hexane (1:1) followed by two extractions with acetone. Radioactivity in extracts was determined by LSC and, in extracted fish residues, by combustion/LSC. The acetone/hexane fish extracts from day 4 were analysed by silica gel TLC using three solvent systems.

In TLC co-chromatography reference standards PB-1 (pyridaben), PB-4, PB-7, PB-9, PB-14, B-1, B-3, P-1, P-13, P-14 and P-15 were used, but in HPLC co-chromatography only PB-1 (pyridaben) was used. TLC analyses were reported to be qualitative, whereas HPLC analyses were reported to be quantitative.

Samples of hydrosol and water were stored frozen prior to extraction and analysis, but storage stability data were submitted. These data showed that pyridaben is stable for 18 months in pond water stored at -10°C to -15°C, and for 12 months in sediment stored below -5°C. These periods are sufficiently long to demonstrate the stability of pyridaben in samples of hydrosol and water stored under study conditions.

Table B.9.2.2.14-09 Properties of clay and top soil added to microcosms

Parameter	Topsoil	Clay
% sand/silt/clay ^(A)	53/34/14	31/27/42
Texture ^(A)	sandy loam	clay
pH	8.1	5.2
% organic carbon	1.1	1.3
CEC (meq/100 g)	3.36	14.15
FC at 0.33 bar (%)	17.86	35.94

^(A) Classification system not reported, presumably USDA

Results

The distribution of radioactivity is shown in Tables B.9.2.2.1.4-10 to B.9.2.2.1.4-11. The radioactivity level in water decreased from a maximum of 92-96% AR after 8 hours to 42-45% AR on day 56. The amount of radioactivity partitioning into the hydrosol increased from 9.5-21% AR after 8 hours to a maximum of 29% AR on day 56 (BEN-label) or 55% AR on day 28 (PYR label; 31% AR on day 56). The total recovered radioactivity was 92-113% AR between 8 hours and 7 days and 71-110% AR after 28-56 days (recoveries were <80% AR for BEN-label after 28 days and for both labels after 56 days). The non-extractable hydrosol radioactivity increased from 3.0-8.0% AR after 8 hours to a maximum of 24% AR on day 56 (BEN-label) or 36% AR on day 28 (PYR label; 24% AR on day 56).

The radioactivity concentrations in water, hydrosol and fish, as well as the BCF values for radioactivity in fish are shown in Tables B.9.2.2.1.4-12 to B.9.2.2.1.4-13. Radioactivity concentrations in water reached a maximum of 2.90-3.21 µg eq./L at 21-28 hours after the first treatment, and declined to 1.57-1.67 µg eq./L after 56 days. Radioactivity concentrations in hydrosol ranged between 1.91 and 5.30 µg eq./kg after the first treatment. Radioactivity concentrations in fish were 123-127 µg eq./kg 4 days after the first treatment and 151-272 µg eq./kg 56 days after the first treatment. Pyridaben was not detectable in fish extracts from day 4. BCF values for radioactivity were 45 L/kg (both labels) after 4 days, and 173 and 90 L/kg (BEN- and PYR-label respectively) after 56 days.

Qualitative TLC analysis and quantitative HPLC showed complete disappearance of pyridaben from the water phase within an hour of exposure. TLC and HPLC chromatograms of water extracts showed the presence of numerous degradation products, with tentative identification of PB-4, PB-9, PB-14, PB-22, B-1, B-3, B-7, B-11, P-1, P-14 and P-16. TLC analysis of soil extracts showed the presence of low levels of pyridaben after the first treatment up to day 28 (see Table B.9.2.2.1.4-14). Levels were 2.09-3.64% AR at 28 hours after the first treatment, declined to 0.44-2.12% AR after 28 days, but were not detectable anymore at 56 days after treatment. Extensive degradation of pyridaben in hydrosol was also apparent from TLC chromatograms.

During the study three dead fish were noted, two in the control tank and one in the tank treated with Pyr-labeled pyridaben. At the end of the study, based on the total number of fish accounted for, there was apparently one unobserved mortality in the control, and two in the tank treated with Ben-labeled pyridaben. Hence the overall mortality was 3/30 (10%) in the control and 2/30 (7%) in both pyridaben treatments. Fish lengths and weights in the pyridaben treatments were not different from those in the control.

Table B.9.2.2.1.4-10 Distribution of radioactivity (% AR) in water and hydrosol of outdoor microcosms treated with [U-benzene-14C] pyridaben (twice with 20 hour interval at 1.7 and 2.0 µg/L respectively)

time	treat- ment	water			hydrosol			total recovered ^(A)
		Total	organo- extract.	aqueous	total	extractable	non-extract.	
1 h	# 1	78	63	8.2				
4 h		77						
8 h		92	74	13	21	13	8.0	113
20 h		88	72	12	23	16	7.1	111
28 h	# 2	71	58	8.8	21	14	7.0	92
4 d		75	58	12	20	7.3	13	95
7 d		73	51	15	25	8.4	16	97
14 d		57						
28 d		49	33	12	28	5.5	23	77
56 d		42	21	29	29	5.3	24	71

Empty cell: not analysed

^(A) Values given only for sampling times with analysis of water and soil**Table B.9.2.2.1.4-11 Distribution of radioactivity (% AR) in water and hydrosol of outdoor microcosms treated with [pyridazinone-3,6-14C] pyridaben (twice with 20 hour interval at 1.7 and 2.0 µg/L respectively)**

time	treat- ment	water			hydrosol			total recovered ^(A)
		Total	organo- extract.	aqueous	total	extractable	non-extract.	
1 h	# 1	74	52	19				
4 h		73						
8 h		96	64	28	9.5	6.5	3.0	106
20 h		86	56	26	14	9.9	4.3	100
28 h	# 2	77	50	23	25	15	10	103
4 d		73	36	32	28	13	14	101
7 d		75	39	32	25	9.0	16	100
14 d		57						
28 d		55	26	25	55	19	36	110
56 d		45	18	27	31	6.3	24	75

Empty cell: not analysed

^(A) Values given only for sampling times with analysis of water and soil

Table B.9.2.2.1.4-12 Concentrations of radioactivity in water (µg/L) and hydrosol and fish (µg/kg) and BCF values (L/kg) in fish of outdoor microcosms treated with [U-benzene-14C] pyridaben (twice with 20 hour interval at 1.7 and 2.0 µg/L respectively)

time	Treatment	water	hydrosol	fish (µg eq/kg)			fish BCF (L/kg)
		(µg eq/L)	(µg eq/kg)	extracable	non-extr.	total	
1 h	# 1	1.32					
4 h		1.30					
8 h		1.57	0.92				
20 h		1.49	1.00				
21 h	# 2	3.21					
28 h		2.66	2.04				
4 d		2.81	1.91	89.9	37.4	127	45.3
7 d		2.72	2.39				
14 d		2.13					
28 d		1.83	2.73				
56 d		1.57	2.85	66.2	206	272	173

Empty cell: not analysed

Table B.9.2.2.1.4-13 Concentrations of radioactivity in water (µg/L) and hydrosol and fish (µg/kg) and BCF values (L/kg) in fish of outdoor microcosms treated with [pyridazinone-3,6-14C] pyridaben (twice with 20 hour interval at 1.7 and 2.0 µg/L respectively)

time	Treatment	water	hydrosol	fish (µg eq/kg)			fish BCF (L/kg)
		(µg eq/L)	(µg eq/kg)	extracable	non-extr.	total	
1 h	# 1	1.27					
4 h		1.24					
8 h		1.63	0.41				
20 h		1.45	0.63				
21 h	# 2	2.20					
28 h		2.90	2.44				
4 d		2.74	2.67	86.5	36.3	123	44.8
7 d		2.81	2.40				
14 d		2.14					
28 d		2.05	5.30				
56 d		1.67	2.98	47.2	104	151	90.3

Empty cell: not analysed

Table B.9.2.2.1.4-14 Levels of Pyridaben (% AR) in hydrosol of outdoor microcosms treated with [U-benzene-14C] or [pyridazinone-3,6-14C] pyridaben (twice with 20 hour interval at 1.7 and 2.0 µg/L respectively)

Time	treatment	BEN-label	PYR-label
8 h	# 1	nd	nd
28 h	# 2	2.09	3.64
4 d		0.41	2.08
28 d		0.44	2.12
56 d		nd	nd

nd: not detectable

Conclusions

The fate and effects on bluegill sunfish of [benzene-U-14C]-pyridaben and [pyridazinone-3,6-14C]-pyridaben formulated as Pyridaben WP was investigated in outdoor microcosms in Alabama (USA). The tanks were treated with a simulated spray drift application (nominal treatment rate 1.7 µg/L), followed by a simulated runoff treatment 20 hours later (nominal treatment rate 2.0 µg/L). Radioactivity concentrations in water reached a maximum of 2.90-3.21 µg eq./L at 21-28 hours after the first treatment, and declined to 1.57-1.67 µg eq./L after 56 days. Radioactivity concentrations in hydrosol ranged between 1.91 and 5.30 µg eq./kg after the first treatment. Complete disappearance of pyridaben from the water phase had occurred within an hour of exposure. Low levels of pyridaben were found in hydrosol up to 28 days after the first treatment (max. 2.09-3.64% AR at 28 hours after the first treatment, declining to 0.44-2.12% AR after 28 days, not detectable at 56 days after treatment). Extensive degradation of pyridaben into numerous degradation products had occurred in water and hydrosol. Radioactivity concentrations in fish were 123-127 µg/kg 4 days after the first treatment and 151-272 µg/kg 56 days after the first treatment. Pyridaben was not detectable in fish extracts from day 4. BCF values for radioactivity were 45 L/kg (both labels) after 4 days, and 173 and 90 L/kg (Ben- and Pyr-label respectively) after 56 days. Fish mortality was 10% in the control and 7% in both pyridaben treatments. Fish weights in the pyridaben treatments were not different from those in the control. The biological results should be interpreted with caution (see Guidelines and Limitations below, point 1).

Guidelines & Limitations

1. In laboratory tests, bluegill was not the most sensitive fish species tested (96-hour LC50 a factor of about 5 higher than that of the most sensitive species rainbow trout). In addition, the fish used for stocking the microcosms were larger (measured length 4 days after treatment 4.2-6.8 cm) than those used for laboratory testing (recommended length according to OECD 203 2.0 cm). These aspects should be taken into consideration when comparing the results on fish testing from this study with those from laboratory testing.
2. An explanation for the differences in behaviour of pyridaben in the present and the next study is provided in the first comment under "Guidelines and Limitations" of the next study.
3. Pyridaben was formulated as WP, but without the wetting agent present in Pyridaben 75 WP in WSB. The treatment solutions contained 3.75% v/v methanol, but the concentration of methanol in the microcosms was very low (0.002% v/v) and any effects of methanol (e.g. enhancement of solubility or increased susceptibility to photolysis) are likely to be negligible at this concentration.
4. Pyridaben was the only reference compound used for HPLC co-chromatography, and TLC analysis was reported to be qualitative. The assignment of identity of metabolites of pyridaben in this study should therefore be considered tentative.
5. The following information was not available: recovery of concentration procedures; HPLC column recovery.

Study 3

Characteristics

reference	: Singh M., 1995 (IIIA 10.2.2/03)	exposure	: 28 days
type of study	: Fate in outdoor microcosms	duration	
year of execution	: 1993-1994	treatment rates	: 24.9 & 15.1 µg/L
GLP statement	: Yes	dosing method	: 1 x with suspension of test substance in water
guideline	: EPA 164-2	acceptability	: Acceptable
test substance & purity	: [benzene-U-14C]-pyridaben, lot no. CP-1007, chemical purity not reported, radiochemical purity >99%; [pyridazinone-3,6-14C]-pyridaben, lot no. CP-928, chemical purity not reported, radiochemical purity >99%.	conclusion	: See study conclusion in this summary

The fate of [benzene-U-14C]-pyridaben and [pyridazinone-3,6-14C]-pyridaben formulated as WP was investigated in outdoor microcosms in Florida (USA), to support the outdoor microcosm study conducted at the same site (cf. study 1 of this section).

Methods

Each of two microcosms consisted of a glass cylinder (152 cm x 15 cm i.d.) with glass base, placed vertically in the same control tank number 24 from the field site at Toxikon Environmental Sciences (measurements field tank 670 x 190 x 190 cm). The test system was covered by a translucent plastic roof. To each cylinder 2 L of sediment (from control tanks 18, 19 and 24) and 21 L of water (from control tank 24) were added. The cylindrical tanks were allowed to equilibrate for 30 days prior to treatment. Shortly before treatment the algal growth on the inside of the cylinders was removed.

The water in one cylinder each was treated with [benzene-U-14C]-pyridaben or [pyridazinone-3,6-14C]-pyridaben on 16 June 1993, by injecting 35-36 mL of an aqueous suspension of the radiolabeled compound formulated as 75% inert WP powder (clay, lignosulfonate and silica) just below the water surface. The actual treatment rate based on LSC analysis of day 0 samples (taken 15-30 minutes after treatment) was 24.9 and 15.1 µg/L for Ben- and Pyr-label, respectively, equivalent to 91% and 96% of the expected rate (27.4 and 15.7 µg/L respectively) based on LSC analysis of the treatment solutions.

Water samples were collected after 15-30 minutes (day 0) and after 1, 3, 7, 14, 21 and 28 days. The entire sediment was sampled after 28 days and all glassware was rinsed with acetone. Radioactivity in an aliquot of each water sample was quantified by LSC. Another aliquot of each water sample was extracted using C18-Empore discs and, where this extraction was not quantitative, by SAX-Empore discs. Radioactivity in non-retained water and in retained and then eluted material was quantified by LSC. The eluates were concentrated and analysed by reversed phase HPLC. HPLC fractions were collected and analysed by LSC using pyridaben as the only co-chromatographed reference compound. Day 3 and Day 28 water samples were also qualitatively analysed by HPLC co-chromatography using a range of reference standards.

Day 28 sediment samples were shaken three times with water for 20 minutes. The water layers were separated by centrifugation and discarded without analysis. The remaining sediment was extracted three

times with acetone/water (9/1) and once with acetone/1N phosphoric acid (4/1). Radioactivity in extracts was determined by LSC and in extracted sediment by combustion/LSC. The acetone/water extracts were concentrated and analysed by reversed phase HPLC. HPLC fractions were collected and analysed by LSC using pyridaben as the only co-chromatographed reference compound.

Results

At the end of the study (day 28), a large water plant was observed growing in the cylinder treated with Bz-label.

The distribution of radioactivity and the levels of pyridaben in water and sediment extracts are shown in Tables B.9.2.2.1.4-16 and B.9.2.2.1.4-17. The total recovered radioactivity was 104-120% AR on day 28. The radioactivity in water decreased to 59-68% AR on day 28. At the most 4.8% AR could not be extracted from water by C-18 or SAX empore discs. The amount of radioactivity in sediment on day 28 was 36% AR (Ben-label) and 59% AR (PYR label). The non-extractable sediment radioactivity on day 28 was low (1.8-3.1% AR). The levels of pyridaben in the water decreased from 86-119% AR immediately after treatment to 5.3-6.1% AR on day 3 and 2.0-3.3% AR after 28 days, when pyridaben levels in sediment were still 20.9-47.2% AR.

Level P-I DT₅₀ values of pyridaben were calculated following the recommendations and procedures of the "Guidance document on estimating persistence and degradation kinetics from Environmental Fate studies on pesticides in EU registration" (SANCO/10058/2005). The calculations were performed with ModelMaker v 4.0 software. SFO (single first-order) and FOMC (first-order multi-compartment) models were evaluated by the RMS, and they gave nearly identical results. The results for SFO model are presented in Table B.9.2.2.1.4-15. DT₅₀ and DT₉₀ values were 0.67-0.68 and 2.23-2.27 days respectively.

Table B.9.2.2.1.4-15 Half-lives (dissipation) for pyridaben in water of microcosms treated with [U-benzene-14C] or [pyridazinone-3,6-14C] pyridaben at 24.9 and 15.1 µg/L, respectively

label	compartment	process	best fit kinetics	Chi ² (error)	r ²	DT50 (d)	DT90 (d)
BEN	water	degradation & mass transfer	SFO	4.15	1.000	0.67	2.23
PYR	water	degradation & mass transfer	SFO	11.00	0.991	0.68	2.27

Besides pyridaben, HPLC chromatograms of water extracts from Ben-and Pyr-label, respectively, showed the presence of up to 7 and 6 degradation products, each accounting for at the most 5.0 and 7.0% AR, except a fraction of 13.0% AR in day 21 water from Pyr-label. The latter fraction however was the first eluting peak from the HPLC column, with little retention, and is likely to have represented a mixture of several polar compounds. Besides pyridaben, HPLC chromatograms of day 28 sediment samples showed the presence of 2 fractions, each representing at the most 1% AR. In the water samples of day 3 and day 28, PB-4, PB-7, PB-9 and P-14 were characterised by HPLC cochromatography with reference standards.

Table B.9.2.2.1.4-16 Distribution of radioactivity (% AR) in water and sediment of outdoor microcosms treated with [U-benzene-14C] pyridaben at 24.9 µg/L

day	water				sediment				total recovered
	total	extrac-table	pyrid-aben	non-extr.	total	extrac-table	pyrid-aben	non-extr.	
0	100.0	122.4	119.0	2.5	-	-	-	-	100
1	57.9	63.9	42.2	2.5	-	-	-	-	
3	80.2	65.1	5.3	1.1	-	-	-	-	
7	ns	ns	ns	ns	-	-	-	-	
14	73.0	65.4	1.1	2.4	-	-	-	-	
21	69.4	62.7	2.9	4.8	-	-	-	-	
28	67.5	56.3	2.0	2.6	36.2	33.1	20.9	3.1	103.6

- : not sampled at this time point

ns = no sample, sample lost

Table B.9.2.2.1.4-17 Distribution of radioactivity (% AR) in water and sediment of outdoor microcosms treated with [pyridazinone-3,6-14C] pyridaben at 15.1 µg/L

day	water				sediment				total recovered
	total	extrac-table	pyrid-aben	non-extr.	total	extrac-table	pyrid-aben	non-extr.	
0	100.0	124.1	86.0	3.1	-	-	-	-	100
1	46.0	41.4	30.2	4.2	-	-	-	-	
3	62.2	54.2	6.1	0.8	-	-	-	-	
7	60.1	49.9	3.7	1.6	-	-	-	-	
14	68.2	62.0	4.0	2.1	-	-	-	-	
21	56.8	47.9	2.3	3.1	-	-	-	-	
28	58.8	52.6	3.3	2.5	58.6	56.9	47.2	1.8	120.0

- : not sampled at this time point

Conclusions

The fate of [benzene-U-14C]-pyridaben and [pyridazinone-3,6-14C]-pyridaben formulated as WP was investigated in Florida (USA), in outdoor microcosms consisting of a glass cylinder placed vertically in a large tank, to support the outdoor microcosm study conducted at the same site (cf. study 1 of this section). The water was treated with [benzene-U-14C]-pyridaben or [pyridazinone-3,6-14C]-pyridaben formulated as 75% inert WP at a rate of 24.9 and 15.1 µg/L, respectively. The levels of pyridaben in water decreased from 86-119% AR immediately after treatment to 5.3-6.1% AR on day 3 and 2.0-3.3% AR after 28 days, when pyridaben levels in sediment were still 20.9-47.2% AR. DT50 and DT90 values for dissipation of pyridaben from water were 0.67-0.68 and 2.23-2.27 days respectively.

The study is acceptable to support the outdoor microcosm study conducted at the same site (cf. study 1 of this section).

Guidelines & Limitations

1. The behaviour of pyridaben in the present study was different from that in study 2 of this section (complete disappearance of pyridaben within an hour from treatment, at the most 3.6% pyridaben in sediment). In order to explain these differences, the author of the report supposed that the large amount of pyridaben in the sediment resulted from precipitation of pyridaben shortly after application. The treatment rates (24.9 and 15.1 µg/L) exceeded the water solubility of the compound (12 µg/L at 20°C),

whereas the treatment rate in the previous study was 3.7 µg/L. The author of the report also stated that the test system in the present study was covered by a translucent plastic roof, whereas there was no roof over the test system of the previous study. Finally, the author of the report stated that the tank in this study was much deeper (1.8 versus 0.6 m). Consequently, light intensity was much less in this study. These explanations are acceptable.

2. Pyridaben was formulated as WP, but without the wetting agent present in Pyridaben 75 WP in WSB. The lack of wetting agent may have enhanced precipitation of applied test material. The rate of disappearance of pyridaben from water may therefore have been overestimated.
3. No information was given on: characteristics of water and sediment; pH and temperature of test water during the study; the presence of algal growth on the cylinder walls during the study; the amount of radioactivity in sediment prior to extraction as determined by combustion analysis; radioactivity levels in rinses of sampling equipment and test cylinders at study end.
4. In order to wash the day 28 sediment, it was shaken 3 times in succession for 20 minutes with water, and the water layers were discarded without quantification of radioactivity by LSC. Although this extraction is not likely to have removed much of the pyridaben, it may have extracted polar degradation products. The total radioactivity in sediment (estimated as the sum of extractable and non-extractable radioactivity) may therefore have been underestimated.

B.9.2.2.2 Chronic toxicity of metabolites

No data were submitted.

B.9.2.3 Bioaccumulation (IIA 8.2.3)

Study 1

Characteristics

reference	:	Jenkins W.R. (1994)	species	:	Rainbow trout (<i>Oncorhynchus mykiss</i>)
type of study	:	Bioconcentration and metabolism in fish	exposure duration	:	28 days
year of execution	:	1990-1991	nominal concentrations	:	0 & 0.16 µg/L
GLP statement	:	Yes	dosing method	:	Flow-through, stock in acetone
guideline	:	EPA 165-4, OECD 305E	acceptability	:	Acceptable
test substance & purity	:	Main & 1st supplementary study: [benzene-U-14C]-pyridaben, lot no. CP-1007, chemical purity not reported, radiochemical purity ≥99.2%; [pyridazinone-3,6-14C]-pyridaben, lot no. CP-1008, chemical purity not reported, radiochemical purity ≥98.9%.	conclusion	:	See study conclusion

Methods

Studies were conducted to investigate the bioconcentration and metabolism of [benzene-U-14C]-pyridaben and [pyridazinone-3,6-14C]-pyridaben in rainbow trout.

Main study

Two groups of 50 rainbow trout (*Oncorhynchus mykiss*) were exposed to [benzene-U-14C]-pyridaben and [pyridazinone-3,6-14C]-pyridaben (nominal concentration 0.16 µg/L) for 28 days in a flow-through system, followed by 38 days of depuration in untreated water. Solvent (acetone) and dilution water controls were included. Water quality parameters were: temperature (11.3-13.1°C), dissolved oxygen (7.80-10.8 mg/L), pH (7.4-8.2). Water and 4-6 fish were sampled on day 3, 7, 14, 21 and 28 of uptake and 1, 3, 7, 10, 14, 27 and 38 of depuration. On day 0, water and 16 fish were sampled. Total radioactive residues in hexane extracts from water (by LSC), and in visceral portions, muscle and residual carcass from fish (by solubilisation/LSC), were determined at all sampling times.

Extraction and metabolite identification was performed on visceral samples of 3 fish of each label from day 28 of uptake. The samples were extracted twice with acetone/hexane (1/4 v/v). The extract was evaporated and partitioned between 0.1M H₃PO₄ and ethyl acetate. Radioactivity in aqueous and organic layers was quantified by LSC and in post-extraction solids by combustion/LSC. The ethyl acetate extract was analysed by silica gel TLC using one solvent system.

First supplementary study

In the main study, the recovery of pyridaben from water samples fortified at 0.15 and 6 µg/L and extracted with hexane was acceptable (mean 94-96%). However, the hexane extracts were not chromatographed and there is no evidence therefore, that the radioactivity in the hexane extracts consisted of pyridaben. In addition, chromatography of visceral samples of fish from the main study showed that pyridaben was extensively metabolised. Prior to analysis however these visceral samples were stored for 11 months. The aim of the first supplementary study was to identify the radioactivity in water under conditions identical to those of the main study, and to identify radioactivity in fish samples that were processed immediately.

To that end, two groups of 20 rainbow trout were exposed to [benzene-U-14C]-pyridaben and [pyridazinone-3,6-14C]-pyridaben for 28 days in a flow-through system to a nominal concentration of 0.16 µg/L, plus solvent (acetone) and dilution water control. Water quality parameters were: temperature (11.1-12.3°C), dissolved oxygen (7.0-10.4 mg/L), pH (7.3-7.8). Water was sampled on day 0, 7, 14, 21 and 28. Four fish were sampled on day 0, 7 and 28. Total radioactive residues in dichloromethane extracts from water (by LSC), and in visceral portions, muscle and residual carcass from fish (by solubilisation/LSC) were determined at all sampling times.

Extraction and metabolite identification was performed on visceral portions, muscle and residual carcass of a pooled sample from all fish (both labels) from day 7 and 28. The samples were extracted and analysed as described for the main study. Radioactivity in the dichloromethane extracts of water samples from day 7 and 28 (both labels) was identified by silica gel TLC using two solvent systems.

Second supplementary study

In the above studies, the measured concentrations in the water were lower than the nominal concentrations. The aim of the second study was to identify possible causes for this difference. This study part is considered to be of little relevance for the present evaluation. Methods and results will therefore be described briefly.

The same flow-through dosing systems as described above was employed with [pyridazinone-3,6- ^{14}C]-pyridaben only, but without fish. One dosing system was silanised to minimise loss of test material due to adsorption, the other one was untreated. Each system was operated as in the above studies (14 days of conditioning, 28 days of exposure). Radioactivity levels in acetone stock solutions, secondary aqueous stock solutions and in dichloromethane extracts from test water were determined regularly, and the adsorption to fish food and test vessel walls vessels was investigated.

Results

No abnormal behaviour and fish mortality was observed for control and test fish throughout the studies. Fish weight in test and control fish was comparable.

Main study

The mean measured radioactivity concentrations in hexane extracts from the test water during exposure were 0.109 and 0.113 $\mu\text{g eq./L}$ (standard deviation 0.012 and 0.023 $\mu\text{g eq./L}$, respectively).

A steady-state situation for ^{14}C concentration in whole fish appeared to be reached after 14 days (ben-label) or 3 days (pyr-label) of exposure. The reported values for the bioconcentration factor (BCF) were calculated by dividing the ^{14}C -concentration in fish at a particular sampling time by the overall mean ^{14}C -concentration in hexane extracts from water during exposure (day 0-28). The reported steady state BCF values for whole fish were 439 and 342 L/kg wwt for fish from ben- and pyr-label, respectively. The RMS recalculated BCF values however by dividing the ^{14}C -concentration in fish at a particular sampling time by the overall mean ^{14}C -concentration in water during exposure up to the sampling time concerned. The steady-state BCF value then was obtained as the arithmetic mean for the BCF values during steady state. The steady state BCF for radioactivity in whole fish (day 0-28) was 398 and 293 L/kg wwt for ben-label and pyr-label, respectively. After 3 days in uncontaminated water, 94.0% and 90.7 of the radioactivity present at the end of the uptake phase in fish from ben- and pyr-label was cleared, and after 10 days 99.3% and 99.8%. The CT50 and CT90 values were calculated by the RMS using first order regression analysis on depuration day 0-3 data. For fish from ben- and pyr-label, respectively, the CT50 was 0.7 and 0.9 days, and the CT90 2.4 and 3.0 days.

The report presented also bioconcentration factors from kinetic modelling using two-compartment non-linear regression analysis, according to the methods outlined in the OECD 305E guideline. For both labels, a plateau value for residues in fish was estimated to have been reached by day 7. For ben- and pyr-label, respectively, the estimated uptake rate constant k_1 was 254 and 297 day^{-1} , the estimated depuration rate constant was 0.634 and 0.960 day^{-1} , and the estimated BCF was 401 and 309 L/kg wwt.

Chromatography of visceral samples of fish from day 28 of uptake showed that pyridaben was extensively metabolised, but these visceral samples were stored for 11 months prior to analysis. The first supplementary study provides additional data on metabolite identification in fish.

First supplementary study

The mean measured radioactivity concentrations in dichloromethane extracts of the test water during exposure were 0.0846 and 0.0866 $\mu\text{g eq./L}$ (standard deviation 0.008 and 0.011 $\mu\text{g eq./L}$, respectively). The recovery of pyridaben from water samples fortified at 0.13 $\mu\text{g/L}$ and extracted with dichloromethane was acceptable (mean 89%). The radioactivity in the dichloromethane extracts of test water consisted nearly entirely (97% on average) of pyridaben. These results confirm the validity of the main study (i.e. radioactivity in hexane extracts from test water may be assumed to have represented pyridaben, and BCF values need not be corrected for the percentage of parent compound in the hexane extracts).

The radioactivity concentrations in fish (36-47 $\mu\text{g/kg}$ on day 7-28) were comparable to those measured in the main study (28-48 $\mu\text{g/kg}$ on day 7-28). The time for residues in fish to reach plateau levels as determined in the main study were confirmed (plateau reached by day 7 for pyr-label, not reached by day 7 for ben-label). The steady state BCF for radioactivity in whole fish (day 28 value for ben-label, day 7 and 28 average for pyr-label) was 544 and 451 L/kg ww for ben-label and pyr-label, respectively (calculation by RMS using methods described above).

Extraction removed 77-91% of the TRR from fish samples, leaving 8-32% TRR non-extractable residues. Recoveries of the work-up of extracts of fish samples were variable (20-113%, mean 74%). The majority of the extracted radioactivity (16-100% TRR, mean 51% TRR) partitioned into the organic phase, which was subjected to TLC. The report presented results for both solvent systems, expressed in percent of radioactivity applied to the plate, but not in % TRR. Not all reference substances were well separated, and most spots were assigned to more than one reference compound. Therefore only a general statement can be given concerning the identification of the radioactivity. Parent pyridaben was not identified in any sample. The main metabolite in fish was PB-10. Other metabolites identified were PB-5, PB-7, PB-9, PB-11, PB-12, PB-13 and PB-14.

The absence of parent pyridaben in fish tissues indicates that this compound has a very low potential for bioconcentration.

Second supplementary study

Exposure levels in test vessels from silanised and untreated systems were comparable, hence adsorption to glass walls was insignificant. Acetone and acid washes of test systems at the end of exposure contained insignificant amounts of test material (about 2% of total amount dosed). Radioactivity levels in test water were essentially unaffected by the addition of food. The aqueous stocks contained only 84% of the theoretical value of 6.4 $\mu\text{g/L}$. The reason for this was not identified, but the author of the report suggested that it is presumably related to the low water solubility of pyridaben (12 $\mu\text{g/L}$ at 20°C).

Conclusion

For ben-labeled and pyr-labeled ^{14}C -pyridaben respectively: BCF for radioactivity in whole fish 398 and 293 L/kg ww; CT₅₀ 0.7 and 0.9 days, CT₉₀ 2.4 and 3.0 days; 99.3% and 99.8% clearance after 10 days in uncontaminated water. The absence of parent pyridaben in fish tissues indicates that this compound has a very low potential for bioconcentration.

Guidelines and limitations

1. The lipid content of the fish was not determined.
2. No good chromatographic separation of metabolites was obtained for most fish extracts, and the presentation of the results of metabolite identification did not have the required format. The results however allow the conclusion that pyridaben was not present in extracts from fish samples, which is sufficient for the present evaluation.

Study 2

Characteristics

reference	: Ohuchiyama N. (1987)	species	: Carp (<i>Cyprinus carpio</i>)
type of study	: Bioconcentration in fish	exposure duration	: 29 days
year of execution	: 1987	nominal concentrations	: 1 µg/L
GLP statement	: No	dosing method	: Flow-through, stock in DMSO
guideline	: Japanese Industrial Standard K 0102	acceptability	: Acceptable
test substance & purity	: Pyridaben, lot no. 129T8604, purity not reported.	conclusion	: BCF in carp <48 L/kg (normalised for 6% lipid content)

Methods

A study was conducted to investigate the bioconcentration of pyridaben in carp.

A group of 25 carp (*Cyprinus carpio*), mean weight 17.2 g, mean length 8.7 cm, lipid content 3.1%) were exposed to pyridaben (unspecified purity, nominal concentration 1 µg/L) for 29 days in a flow-through system in a temperature controlled room (25±2°C), followed by 14 days of depuration in untreated water. The concentration of the solvent DMSO in the test solution was 0.004% v/v. No controls were included. Dissolved oxygen in the test tank was 6.5–7.5 mg/L during the test. Water and 2 fish were sampled on day 1, 4, 8, 15, 22 and 29 of uptake. Two fish were sampled on day 1, 3, 7, 10 and 14 of depuration.

Water samples were extracted with hexane, the organic layer was evaporated, reconstituted in acetonitrile and analysed by reversed phase HPLC. Whole fish were chopped and extracted with ethyl acetate/acetonitrile (1/9 v/v). After filtration of the extract and addition of water, pyridaben residues were partitioned into hexane. The hexane layer was cleaned up by alumina column chromatography, evaporated, reconstituted in acetonitrile and analysed by reversed phase HPLC. The recovery of pyridaben from water samples fortified at 1 µg/L and fish samples fortified at 12.5 and 125 µg/kg and extracted by the above methods was acceptable (water: n=2, 96–96%, mean 96%; fish: n=2 at each level, 86–90% at 125 µg/kg,

mean 88%, 100-111% at 12.5 µg/kg, mean 106%). The LOQ of the analytical method was reported to be 20 µg/kg for the test fish.

Results

No abnormal behaviour or appearance of the test fish was observed. Concentrations of pyridaben in water and fish during uptake are shown in Table B.9.2.3-01. The BCF during steady state may be set at <25 L/kg at 3.1% lipid content (normalised to 6% lipid content: <48 L/kg). Concentrations in fish were <20 µg/kg at all samplings during depuration.

Table B.9.2.3-01 Concentrations of pyridaben in water and fish during the uptake phase of a bioaccumulation study with carp

Day	1	4	8	15	22	29
Concentration in water (µg/L)	0.897	0.611	0.754	0.776	0.892	0.848
Concentration in fish (µg/kg)	<20	28.45	<20	<20	<20	<20
BCF ^(A) (L/kg)	<22	<38	<27	<26	<25	<25

^(A) Calculated by RMS by dividing the concentration in fish at a particular sampling time by the overall mean concentration in water during exposure up to the sampling time concerned.

Conclusion

BCF for pyridaben in carp <48 L/kg (normalised value for 6% lipid content).

Guidelines and limitations

1. The report was pre-dated to the GLP and the study was not conducted under GLP. The study procedures however were scientifically acceptable, and the results are considered to be valid.
2. No tank with solvent and/or untreated control fish was included in the test. The origin of the untreated control fish used for the recovery tests was not identified in the report (presumably carp from the same batch used to initiate the flow-through study).
3. The purity of the test material was not reported. Since the analysis of water and fish was specific for pyridaben, this is accepted.

B.9.2.4 Risk assessment

B.9.2.4.1 Risk assessment of the active substance

B.9.2.4.1.1 Acute risk

Measurement of treatment concentrations during the course of the flow-through tests with technical pyridaben in fish (3 species), *Daphnia magna*, *Mysidopsis bahia* and *Crassostrea virginica* showed that concentrations of pyridaben were maintained within acceptable limits, and results were based on mean measured concentrations. In static tests in algae (4 species), pyridaben was not stable, and results were based on measured initial concentrations.

Tests with the proposed Pyridaben 75WP formulation were also submitted. In the semi-static test with fish (1 species), analytical measurements showed that initial concentrations of pyridaben were generally >80% of nominals in fresh solutions, but decreased during the renewal periods, and results were based on mean measured concentrations. In the static tests with *Daphnia magna* (1 study) and *Mysidopsis bahia* (1 study), analytical measurements showed that initial concentrations were >80% of nominals but declined to below 80% during the test period, and results were based on nominal concentrations (*Daphnia*) or measured initial concentrations (*Mysidopsis*). In the test with algae (1 species), analytical measurements showed that initial and mean measured concentrations of pyridaben were >80% of nominals, and results were based on nominal concentrations. The results of these tests (see Tables B.9.2.1.3-01– B.9.2.1.3-05) showed that the formulation is of comparable toxicity to *Daphnia*, but less toxic to fish, shrimp and algae.

Acute risk assessment will be based on the lowest LC/EC50 values for each group of organisms, except for algae. Due to the low water solubility, toxic concentrations could not be reached in tests with the active substance. Therefore, the risk assessment for algae will be based on the results of the study with the formulated product. Acute risk assessment for the other organisms (fish, *Daphnia* and shrimp) will be based on the results of the studies with the active substance. Since *C. virginica* was less sensitive than the other invertebrates *M. bahia* and *D. magna*, *C. virginica* is not included separately in the risk assessment.

For the initial risk assessment the following endpoints will be used:

96 hr LC50 for rainbow trout (*Oncorhynchus mykiss*) of 0.73 µg a.s./L;

48 hr EC50 for *Daphnia magna* of 1.0 µg a.s./L;

96 hr LC50 for *Mysidopsis bahia* of 0.67 µg a.s./L;

72 hr EC50 for *Selenastrum capricornutum* of 35000 µg a.s./L.

The acute TERs for spray drift at 1 metre for fish, *Daphnia magna*, *Mysidopsis bahia*, and algae are shown in Table B.9.2.4.1.1-01. For the application in citrus, the PECsw value for spray drift at 1 metre was taken from section B.8.6.1. This value was calculated for the single application at 0.30 kg a.s./ha in citrus using FOCUS STEP 3, since STEP 1 and STEP 2 calculations resulted in PECsw concentrations of pyridaben (15.7-16.8 µg/L), which exceeded the water solubility of 12 µg/L. No drift percentages are available at EU level for the greenhouse scenario. In the Netherlands an emission rate to surface water of 0.1% is used (emission via condensation water and evaporation/volatilisation). PECsw values for the greenhouse application in tomato were calculated for this emission rate of 0.1% using FOCUS STEP 2 (as opposed to FOCUS STEP 1 no run off/drainage, which is considered appropriate for greenhouse treatments).

Table B.9.2.4.1.1-01 Acute TERs for pyridaben for fish, *Daphnia*, shrimp and algae

Crop	Dose (kg a.s./ha)	dist. (m)	% drift	LC/EC50 (µg as/L)				PECsw (µg a.s./L)	TER			
				Fish	<i>Daphnia</i>	<i>M. bahia</i>	Algae		Fish	<i>Daphnia</i>	<i>M. bahia</i>	Algae
Tomato	0.25	-	0.1 ^(A)	0.73	1.0	0.67	35000	0.0833	8.8	12	8.0	4E+05
Citrus ^(B)	0.30	1	15.73	0.73	1.0	0.67	35000	10.402	0.070	0.096	0.064	3365
Citrus ^(C)	0.30	1	15.73	0.73	1.0	0.67	35000	7.820	0.093	0.128	0.086	4476

^(A) Emission rate to surface water

^(B) D6 ditch scenario (FOCUS STEP 3)

^(C) R4 stream scenario (FOCUS STEP 3)

The acute TERs for algae are above the Annex VI trigger of 10 for both applications and the risk is considered acceptable. The acute TERs for fish, *Daphnia magna* and *M. bahia* are all below the relevant Annex VI trigger of 100 for both applications and the risk assessment needs to be refined.

Fish and invertebrates were the most sensitive species. A higher tier study on the acute toxicity of BAS 300 11 I (75.8% pyridaben) to Bluegill sunfish (*Lepomis macrochirus*) under outdoor conditions in tanks with sediment was submitted. The resulting 96-hour LC50 was 64 µg a.s./L, i.e. under outdoor conditions (DT50 for disappearance from water phase presumably much less than one day), bluegill sunfish was almost a factor of 20 less sensitive to pyridaben than under standard flow-through test conditions in the laboratory. In an *in situ* test in a microcosm study (study 1, section B.9.2.2.1.4), 5 and 70% of the bluegill died after exposure to 3.4 and 34 µg a.s./L (DT50 for disappearance from water phase 0.9-1.5 days), respectively, resulting in a 96-hour LC50 of 19 µg/L. No higher tier study with rainbow trout, the most sensitive fish species (4.8 times more sensitive than bluegill sunfish in acute testing), was submitted. Trout is also likely to be less sensitive to a pulse-dose exposure to pyridaben under outdoor conditions, as compared to the flow-through or semi-static exposure in acute laboratory tests, but the risk for rainbow trout cannot be refined in the absence of higher tier studies (e.g., exposure to a realistic dosing regime under outdoor conditions).

A higher tier study on the acute toxicity of BAS 300 11 I (75.8% pyridaben) to *Mysidopsis bahia* under outdoor conditions (DT50 for disappearance from water phase much less than one day) gave a 96-h LC50 of 16 µg a.s./L. Shrimps were a factor of 24 less sensitive to pyridaben than under standard flow-through test conditions in the laboratory. Based on a LC50 value of 16 µg a.s./L, the application in tomato (TER = 192) would satisfy the Annex VI trigger of 100 for shrimp, but treatment of citrus would not (TERs 1.5-2.0).

The results of a microcosm study are evaluated at the end of section B.9.2.3.1.2.

B.9.2.4.1.2 Long-term risk

The proposed use in tomato and citrus involves a single seasonal application (at 0.25 and 0.30 kg a.s./ha) only. In water/sediment studies, pyridaben dissipated from the water column with an overall DT50 of 2.5 days, hence >2 days. According to the Guidance Document on Aquatic Ecotoxicology (Working Document, Sanco/3268/2001), chronic exposure to pyridaben is therefore likely to occur. Chronic toxicity data for the technical substance were submitted and are summarised in Table B.9.2.4.1.2-01. A first-tier chronic risk assessment has been performed for fresh water and marine organisms.

Table B.9.2.4.1.2-01 The chronic toxicity of pyridaben to aquatic life

Species	Type of test	NOEC (µg a.s./L)
<i>Pimephales promelas</i>	301-day full life cycle test	0.28
<i>Daphnia magna</i>	21-day reproduction test	0.086
<i>Chironomus riparius</i>	28-day chronic toxicity test	5.1
<i>Mysidopsis bahia</i>	35-day reproduction test	0.047

The long-term TERs based on the initial PEC_{sw} (covering the worst-case situation that chronic effects are caused by the peak concentration directly after application, and taken from section B.8.6.1), and based on the NOEC values of the active substance, are shown in Table B.9.2.4.1.2-02.

Table B.9.2.4.1.2-02 Long-term TERs for pyridaben based on initial PECs

Crop	Dose (kg a.s./ha)	dist. (m)	% drift	NOEC (µg as/L)				PEC _{sw} (µg a.s./L)	TER			
				Fish	<i>Daphnia</i>	<i>Chironomus</i>	<i>M. bahia</i>		Fish	<i>Daphnia</i>	<i>Chironomus</i>	<i>M. bahia</i>
Tomato	0.25	-	0.1 ^(A)	0.28	0.086	5.1	0.047	0.0833	3.4	1.0	61	0.56
Citrus ^(B)	0.30	1	15.73	0.28	0.086	5.1	0.047	10.402	0.027	0.008	0.49	0.0045
Citrus ^(C)	0.30	1	15.73	0.28	0.086	5.1	0.047	7.820	0.036	0.011	0.65	0.0060

(A) Emission rate to surface water

(B) D6 ditch scenario (FOCUS STEP 3)

(C) R4 stream scenario (FOCUS STEP 3)

The long-term TER for fish, *Daphnia*, *Chironomus* and *M. bahia* are all below the Annex VI trigger of 10, except for the long-term risk for *Chironomus* in tomato. For the other organisms and uses, the long-term risk assessment needs to be refined.

For tomato, refinement of the risk for fish and invertebrates using refinement of the PEC (additional FOCUS calculations) is not possible since only one scenario could be evaluated (glasshouses). Refinement using 21-day TWA instead of the initial PEC_{sw} values is considered not appropriate in this case, since in the microcosm study (two applications with 29 days interval, DT50 (water) 0.88-1.3 days, study 1 in section B.9.2.2.1.4) reductions of the populations of certain species (e.g. *Ceriodaphnia*, *Lepadella*) were observed 1-3 weeks after the initial pulse exposure to pyridaben (DT50 0.7-1.5 days), hence it cannot be excluded that effects on *Daphnia* could result from exposure occurring early on in the exposure period.

For refinement of the risk assessment for invertebrates, data from the microcosm study (study 1 in section B.9.2.2.1.4) can be used. Following two treatments T1 and T2 (29 days' interval) of microcosms populated by phytoplankton, periphyton, zooplankton, macroinvertebrates and fish (bluegill sunfish) with BAS 300 11 I at dose levels of 0.34, 3.4 and 34 µg a.s./L, no adverse effects were observed on periphyton at nominal concentrations up to and including 34 µg a.s./L. Phytoplankton populations (reduction of cryptophyta and increase of pyrrophyta, the latter possibly an indirect effect) were significantly affected at 34 µg a.s./L. Significant treatment related negative effects on zooplankton at the community level were observed at 3.4 and 34 µg a.s./L, and significant negative effects at the population level at and above 0.34 µg a.s./L. No statistically significant effects were seen on macroinvertebrates at the community level up to a concentration

of 34 µg a.s./L, but statistically significant reductions of individual taxonomic groups were observed for *Hydra* (at and above 3.4 µg a.s./L) and *Oxyethira* (at and above 0.34 µg a.s./L). Increased fish mortality was observed at 34 µg a.s./L, and mean fish length and weight were significantly higher at 34 µg a.s./L as compared to the control and lower treatments, probably due to reduced competition for food. An overall NOEC_{microcosm} of <0.34 µg a.s./L can be derived from the evaluated microcosm experiment. At the exposure level of 0.34 µg a.s./L and above, the populations of the macroinvertebrate *Oxyethira* were reduced at 7 days after treatment, but recovery occurred at all dose levels before study end. At the exposure level of 0.34 µg a.s./L and above, a statistically significant effect on the abundance of the rotifer *Polyarthra* was seen from day 2 after T2 through day 14 after T2, but at the next dose of 3.4 µg a.s./L full recovery of *Polyarthra* within the study was observed (but not at 34 µg a.s./L). At 3.4 µg a.s./L, adverse effects without demonstrated recovery prior to study end were recorded only for the macroinvertebrate *Hydra*. The NOEAEC of the evaluated microcosm study with pyridaben is therefore: 0.34 µg a.s./L. In the study summary of the monograph, the effects classes proposed by De Jong *et al.* were applied to the treatment regime of the study (two applications). The setting of the NOEAEC however was based on recovery after the second application. Therefore the NOEAEC is also applicable to the treatment regime of Nexter Pro (single application).

Taking a precautionary extrapolation factor of 3 into account, the EAC was determined to be 0.11 µg a.s./L. The EAC is higher than the PEC_{sw} for the application in tomato (0.0833 µg a.s./L), but lower than the PEC_{sw} for the application in citrus (7.820 µg a.s./L for the R4 stream scenario and 10.58 µg a.s./L for the D6 ditch scenario). Therefore, the risk for the application in tomato is considered to be acceptable for invertebrates, but the risk assessment for the application in citrus needs to be refined. Refinement could be performed using buffer zones. The smallest buffer zone at which the risk is acceptable is 225 m with a spray drift of 0.016%, resulting in a PEC_{sw} of 0.01087 µg a.s./L (D6 ditch scenario) or 0.0080 µg a.s./L (R4 stream scenario). TERs based on the EAC of 0.11 µg a.s./L are then above 10 (10.27 and 13.75, respectively).

The notifier is requested to submit additional data for a refined risk assessment for invertebrates and sediment-dwelling organisms for the application in citrus. This may be a FOCUS step 4 calculation for the application in citrus or any other data considered appropriate.

Two microcosm studies with fish are available. In microcosms containing sediment, no treatment related mortality of fish was observed after treatment of 1.7 + 2.0 µg a.s./L. The highest tier endpoint available for fish is the NOEC for fish of 3.4 µg a.s./L from the microcosm study containing also phytoplankton, zooplankton, periphyton and macroinvertebrates.

Only one fish species, bluegill sunfish, was included in these microcosms. This species was not the most sensitive fish species (bluegill sunfish 4.8 times less sensitive in acute testing than rainbow trout, the most sensitive species). Therefore a factor of 10 for interspecies variability should be applied to the endpoint. Furthermore, not the most sensitive fish stages were tested and only effects on mortality, length and weight and behaviour were studied, not chronic toxicity effects like reproduction. Therefore, also a factor of 3 should be applied for extrapolation from acute to chronic toxicity.

The resulting endpoint for fish is 0.11 µg a.s./L, as for invertebrates. It has already been shown that with this endpoint, triggers are not met.

The notifier argues that the relevant endpoint for fish acute risk assessment is 14.6 µg a.s./L: the lowest acute LC50 for rainbow trout (0.73 µg a.s./L) multiplied with 20 to reflect the difference in toxicity seen between lab and higher tier studies. A comparison between acute indoor and outdoor studies is made to support this. For mysid shrimp, LC50 values are 0.67 and 16 µg a.s./L for indoor and outdoor, respectively. For bluegill sunfish, these values are 3.5 and 64 µg a.s./L. For both species, the ratio between indoor and outdoor toxicity is about 20. According to the notifier, this reduction of toxicity observed under field conditions is independent of species, but reflects the dissipation process and subsequent bioavailability. Furthermore, the notifier states that the focus of the risk assessment should be at acute risk assessment and not chronic, because the ratio between acute and chronic LC50-values is <3 and because the fish life cycle study conducted with fathead minnow demonstrated limited effects over prolonged periods of exposure.

The argumentations of the notifier are not accepted. To determine whether chronic risk assessment is relevant, acute endpoints should be compared with chronic endpoints, so LC50s with NOECs. The NOEC from the FLC-study with fathead minnow is 0.28 µg a.s./L based on reduced fry growth of the parent generation, and reduced F1, egg hatchability and survival of the F1 generation. A 96-h LC50 of 2.3 µg a.s./L was determined in the FLC-study. This endpoint was not considered reliable, but is used since no acceptable study with this species is available. It is clear that for fathead minnow the ratio between acute and chronic toxicity is quite high. Therefore, chronic risk assessment should be performed. As shown above, the risk for fish is not acceptable.

Therefore, the notifier is requested to submit additional data for a refined risk assessment for fish for both applications (e.g. exposure of sensitive stages and species to a realistic dosing regime (possibly under outdoor conditions), or a FOCUS step 4 calculation for the application in citrus).

B.9.2.4.1.3 Bioaccumulation

Pyridaben has a DT90 in the whole system of a water/sediment system of ≥ 210 days. The risk for bioaccumulation should therefore be considered. Two bioconcentration studies with pyridaben under flow-through conditions were submitted. In the first study, the mean BCF for radioactivity was 346 L/kg wet weight for whole fish, but pyridaben was not detected in fish tissues. In the second study, the BCF based on parent pyridaben was <48 L/kg wet weight for whole fish (carp: 6% lipid). The Annex VI trigger factor is 100 L/kg for non-readily biodegradable substances. In birds and mammals, the risk of bioaccumulation due to the intake of contaminated fish, based on the worst-case BCF value of <48 L/kg, was considered to be low (section B.9.1.5.2 and B.9.1.6.2). Therefore, bioaccumulation is considered to be of no concern.

B.9.2.4.2 Risk assessment of metabolites

A risk assessment should be performed for metabolites formed at a maximum level of at least 10% AR (major metabolites), for metabolites formed at at least two successive samplings at a level of at least 5% AR, and for metabolites for which an increasing trend of formation is observed in water-sediment studies.

PB-7 was shown to be the only major (>10%) metabolite in the water phase of a water/sediment system (Section B.8.4.3.2), with a maximum level of 17.2% AR in water and 14.1% AR in sediment. In an aquatic photolysis study (Section B.8.4.2), the DT50 for aquatic photolysis of pyridaben at 25°C (Xenon light, 425 W/m²) was 6.8 minutes, and major photodegradates were W-1 (max. 27.9% AR, DT50 <1.5 hours) and B-3 (max. 29.2% AR, photolytically stable). The risk of these metabolites must be considered. The metabolites PB-9 and P-16 were found at 8.5% and 5.2% AR, respectively, in the water phase of water-sediment systems, but since levels >5% AR were not observed at two successive samplings and levels did not increase in time, the risk of these metabolites is not considered.

B.9.2.4.2.1 Acute risk

PB-7

The LC/EC50s for PB-7 are summarised in Table B.9.2.4.2.1-01.

Table B.9.2.4.2.1-01 The acute toxicity of PB-7 to aquatic life

Species	Test duration	LC/EC50 (mg/L)
<i>Oncorhynchus mykiss</i>	96 hours	>8.0
<i>Daphnia magna</i>	48 hours	0.028
<i>Selenastrum capricornutum</i>	72 hours	3.1
<i>Chironomus riparius</i>	48 hours	0.15

Acute risk assessment will be based on these LC/EC50 values. The worst-case TERs for this metabolite are summarised in Table B.9.2.4.2.1-02. The PEC_{sw} values presented in the table are taken from section B.8.6.1.

Table B.9.2.4.2.1-02 Acute TERs for PB-7

Crop	LC/EC50 (µg/L)				PECsw (µg/L)	TER			
	Fish	<i>Daphnia</i>	Algae	<i>Chironomus</i>		Fish	<i>Daphnia</i>	Algae	<i>Chironomus</i>
Tomato	>8000	28	3100	150	0.0153	>5E+05	1830	2E+05	9804
Citrus	>8000	28	3100	150	3.0982	>2582	9.0	1001	48

For the application in tomato, the acute TERs of PB-7 for fish, *Daphnia*, algae and *Chironomus* are all far above the relevant Annex VI triggers (100, 100, 10 and 100, respectively). Hence the acute risk should be low for this application. For the application in citrus, a risk is considered to be present for *Daphnia* and *Chironomus* and the risk assessment needs to be refined.

Exposure of aquatic organisms to this metabolite during the microcosm study is likely to have occurred. The EAC was however lower than the PECsw for pyridaben for the application in citrus, and the risk for this application was concluded to need further refinement. Therefore, the risk for PB-7 for the application in citrus awaits fulfilment of the requirements for pyridaben.

W-1 and B-3

No studies on acute toxicity with W-1 and B-3 are available. The photolytic DT50 of pyridaben was 6.8 minutes at a light intensity of 425 W/m². This light intensity is comparable to the light intensity measured on earth in Europe (450 W/m², midsummer, 40°N). Therefore, the toxicity of these photolytic metabolites should be covered by the microcosm study (study 1 in section B.9.2.2.1.4), and the risk of both metabolites is considered to be low for algae and invertebrates for the application in tomato, for which the PEC is covered by the EAC of 0.11 µg a.s./L. As the EAC does not cover the PEC for the application in citrus, a separate assessment is given below.

The relative toxicities of these metabolites cannot be predicted by structural considerations alone. Therefore, the toxicities of these metabolites were estimated by RMS using EPA EPI Suite software (Ecosar). The results are summarised in Table B.9.2.4.2.1-03.

Table B.9.2.4.2.1-03 Structures of pyridaben and it's metabolites, and experimentally determined and estimated toxicity endpoints

Structure	Name (max. % AR in water)	Endpoint	Test (µg a.s./L) ^(B)	Estimate (µg a.s./L) ^(C)
	Pyridaben	Fish 96 hr LC50 <i>Daphnia</i> 48 hr EC50 Algae 72 hr EC50	0.73 1.0 35000	40-43 17-738 152-697
	PB-7 (17.2%)	Fish 96 hr LC50 <i>Daphnia</i> 48 hr EC50 Algae 72 hr EC50	>8000 28 3100	2376-4937 16712-20618 10924-29175
	B-3 (29.2%)	Fish 96 hr LC50 <i>Daphnia</i> 48 hr EC50 Algae 72 hr EC50		5152-13935 15989 10582
	W-1 (27.9%)	Fish 96 hr LC50 <i>Daphnia</i> 48 hr EC50 Algae 72 hr EC50		1177-1698 2131 1520

(A) Maximum level in whole system (water/sediment)

(B) Endpoint from submitted studies

(C) Estimated by RMS using EPA EPI Suite software (Ecosar)

As can be read from Table B.9.2.4.2.1-03, estimated endpoints for pyridaben are lower than those for all metabolites. Furthermore, the differences in sensitivity for pyridaben between algae, fish and daphnia were the same for estimated and experimentally determined endpoints. The estimated endpoints for PB-7 for fish and algae (but not for *Daphnia*) are in reasonable agreement with the experimentally determined endpoints. Since the estimated lower toxicity of PB-7 is confirmed by experimental data, the estimated toxicity values for the other two metabolites B-3 and W-1 may be used in a qualitative risk assessment. The maximum levels of the aqueous photodegradates W-1 and B-3 were respectively 27.9% and 29.2% AR.

Both metabolites are estimated to be far less toxic to algae than parent pyridaben, and of comparable or slightly higher toxicity to algae than PB-7. Considering the large safety margins calculated for the risk of parent pyridaben and PB-7 to algae (TER values 3E+03 and 1001, respectively), and the PEC_{sw} values for W-1 and B-3 (4.2286 and 2.2128 µg/L), which are lower than that of pyridaben (7.820-10.420 µg/L) and slightly higher or lower than that of PB-7 (3.0982 µg/L), the risk of W-1 and B-3 to algae should be low for the treatment of citrus.

B-3 is estimated to be far less toxic to fish than parent pyridaben, and of comparable toxicity to fish as PB-7. Considering the large safety margin calculated for the risk of PB-7 to fish (TER >2582) and the PEC_{sw} value for B-3 (2.2128 µg/L), which is lower than that of PB-7 (3.0982 µg/L), the acute risk of B-3 to fish should be low for the treatment of citrus (and hence for that of tomato). B-3 is estimated to be far less toxic to daphnia than parent pyridaben, but of slightly higher toxicity to daphnia than PB-7. Since the TER of PB-7 to *Daphnia* for treatment of citrus was 9.0, the estimates indicate a possible risk of B-3 to *Daphnia* for treatment of citrus, even though the PEC_{sw} value for B-3 (2.2128 µg/L) is slightly lower than that of PB-7 (3.0982 µg/L).

W-1 is estimated to be far less toxic to fish than parent pyridaben, but of higher toxicity (by a factor of about 5) to fish than PB-7. Although the safety margin calculated for the risk of PB-7 to fish was large for treatment of citrus (TER >2582, i.e. a factor of 26 above the trigger of 100), there is insufficient ground to conclude with certainty that the risk of W-1 for treatment of citrus to fish will also be low, in spite of its short-lived existence (DT₅₀ <1.5 hours). The reasons are that there may be a large uncertainty in the toxicity estimate, and that there is very close structural similarity between W-1 and parent pyridaben, suggesting that they may be of comparable toxicity. The risk of W-1 to fish for treatment of tomato should be low however, considering the large safety margin for PB-7 (TER >5E+05) and the only slightly higher PEC_{sw} value for W-1 (4.2286 µg/L) as compared to that of PB-7 (3.0982 µg/L). W-1 is estimated to be less toxic (factor of about 3) to daphnia than parent pyridaben, but of higher toxicity to daphnia (factor of about 10) than PB-7. Since the TER of pyridaben and PB-7 to *Daphnia* for treatment of citrus was 0.095 and 9.0, respectively, the estimates indicate a possible risk of W-1 to *Daphnia* for treatment of citrus.

The risk of B-3 and W-1 to *Daphnia*, and that of W-1 to fish should be addressed. This may be done by submitting acute toxicity studies with these metabolites, but fulfillment of the data requirements for parent pyridaben (e.g., restriction on label, or studies in which exposure to metabolites is also addressed) may also address the issues for metabolites.

B.9.2.3.2.2 Long-term risk

According to the Guidance Document on Aquatic Ecotoxicology (Working Document, Sanco/3268/2001), chronic toxicity testing is only required where the metabolite is acutely more toxic than the parent compound. For metabolites in sediment, it is stated that testing is required where a metabolite is present in the sediment at a level of more than 10% of the parent applied radioactivity at day 14 or later. An assessment is required for PB-7 (water and sediment) and W-1 and B-3 (aqueous photodegradates).

PB-7

PB-7 is acutely not more toxic than parent pyridaben. Therefore, chronic testing with water organisms is not required for this metabolite. PB-7 was a major metabolite in sediment, with a maximum level of 14% AR after 120 days in a water/sediment study, and testing with sediment-dwelling organisms is required. Toxicity of this metabolite should be covered by the microcosm study (study 1 in section B.9.2.2.1.4). Therefore, the risk for this metabolite is considered to be low for the application in tomato, but the risk assessment for the application in citrus awaits fulfillment of the data requirement for parent pyridaben. Alternatively, a test with *Chironomus riparius* may be submitted.

W-1 and B-3

Since the acute toxicity of W-1 and B-3 is expected to be lower than that of pyridaben (Table B.9.2.4.2.1-03), testing with these metabolites is not necessary.

Conclusion long-term risk assessment of metabolites

Chronic exposure of aquatic organisms to metabolites is considered to be of no concern for both applications. The chronic risk of PB-7 to sediment-dwelling organisms needs to be considered after fulfillment of the data requirements of parent pyridaben (e.g., restrictions on label, studies in which exposure to metabolites is also addressed, or a chronic study with *Chironomus riparius*).

B.9.2.4.2.3 Bioaccumulation

No experimentally determined BCF values in fish are available for PB-7, B-3 and W-1. LogPow values were estimated by the RMS using EPA EPI Suite software and Pallas 3.0 software (see Table B.9-01). The BCF for fish can be estimated according to the formula $\log BCF = 0.85 \cdot \log Pow - 0.7$ (USES 2.0). The estimated logPow values and estimated BCF values are presented in Table B.9.2.4.2.3-01.

Table B.9.2.4.2.3-01 LogPow and BCF-values for metabolites of pyridaben

Metabolite	EPA EPI Suite		Pallas	
	LogPow ^(A)	BCF (L/kg)	logPow ^(B)	BCF (L/kg)
PB-7	3.86	381	3.67	263
B-3	2.99	69	2.94	63
W-1	4.29	884	4.96	3281

^(A) Estimated by RMS using EPA EPI Suite software

^(B) Estimated by RMS using Pallas 3.0 software

The estimated BCF value of B-3 is below the Annex VI trigger of 100, while those of PB-7 and W-1 are above 100. W-1 was a photolytic degradate of pyridaben that was formed at it's maximum level of 16% AR within 0.5 hours. W-1 has a DT50 of <1.5 hours, and therefore, it's risk for bioaccumulation should be low.

In order to further assess the risk for bioaccumulation, the concentration in fish for PB-7 is estimated by multiplying the mean BCF with the concentration of the metabolite in water. Since presence of PB-7 in rats and hens was not unequivocally confirmed (see sections B.6.1 and B.7.2), and PB-7 was only found at 0.7% AR in faeces and 3.8% TRR in livers of goats dosed with parent pyridaben, the toxicity of this metabolite cannot be assumed to be covered by parent pyridaben, and the concentration of PB-7 in fish cannot be

compared to the NOEC for the parent. Therefore, the maximum NOEC value that would indicate a risk due to bioaccumulation for birds and mammals is calculated from the concentration in fish, according to $NOEC < 5 \cdot ETE$, where $ETE = PEC_{feed} \cdot DFI/BW$ (see sections B.9.1.5 and B.9.1.6). The results for the application in citrus are shown in Table B.9.2.3.2.3-02.

Table B.9.2.4.2.3-02 Mean BCF-values and maximum NOEC that leads to risk for metabolites of pyridaben

Application in citrus				Birds			Mammals		
Metabolite	Mean BCF	PEC _{sw} (mg/L)	PEC _{fish} (mg/kg)	NOEC pyridaben (mg/kg)	NOEC metabolite for TER<5	factor more toxic	NOEC pyridaben (mg/kg)	NOEC metabolite for TER<5	factor more toxic
PB-7	322	3.1E-03	1.0	10	1.03	9.7	1.87	0.65	2.9

The results in Table B.9.2.4.2.3-02 show that a risk due to bioaccumulation of PB-7 exists if the long-term toxicity of PB-7 is at least a factor of 2.9 higher than that of parent pyridaben. PB-7 was less toxic than parent pyridaben to fish, daphnia and algae by a factor of at least 28 (section B.9.2.1). In rat, PB-7 was also less toxic by a factor of 2.8-4.8 than parent pyridaben (LD50 for pyridaben in carboxymethylcellulose 570-1100 mg/kg bw; LD50 for PB-7 tested in carboxymethylcellulose 2728-3086 mg/kg bw). The available toxicity data for PB-7 do not indicate that it has a higher toxicity than pyridaben. Furthermore, the BCF calculated for PB-7 is based on the assumption that PB-7 is not further degraded in fish, which is not likely. Therefore, the risk due to bioaccumulation for PB-7 should be low.

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

No data were submitted. The risk assessment for mammals is presented under B.9.1.6.

B.9.4 Effects on bees (Annex IIA 8.3.1, IIA 10.4)**B.9.4.1 Toxicity****B.9.4.1.1 Acute toxicity of the active substance****Study 1****Characteristics**

reference	:	Hoxter K.A. <i>et al.</i> (1993)	species	:	Honey bee (<i>Apis mellifera</i>)
type of study	:	Acute contact toxicity study	exposure duration	:	44 hours
year of execution	:	1992	nominal	:	0, 0.008, 0.016, 0.031, 0.062, 0.12
GLP statement	:	Yes	concentrations	:	and 0.25 µg a.s./bee
guideline	:	EPA 141-1	dosing method	:	Stock solution in acetone
test substance	:	BASF Pyridaben Tech, Lot #900304	acceptability	:	Acceptable
purity	:	99.7%	LD50 contact	:	0.024 µg a.s./bee

Methods

A 44-hour acute contact toxicity test on honeybees (*Apis mellifera*) (2 replicates of 25 bees each per concentration) was conducted with pyridaben technical at doses of 0.008, 0.016, 0.031, 0.062, 0.12 and 0.25 µg a.s./bee, with solvent-control and untreated control.

Results

No bees died in the control groups. At 0.008 and 0.016 µg a.s./bee, bees were immobile or lethargic. Further results are shown in the table below.

Table B.9.4.1.1-01 The acute contact toxicity of pyridaben technical to honeybees (*Apis mellifera*).

Test type (purity of test substance)	LD50 (µg a.s./bee)
Acute contact, 44 hr (99.7%) in acetone	0.024

Conclusion

44-Hour LD50: 0.024 µg a.s./bee

Guidelines and limitations

Current guidelines (EPPO (2000), OECD 214 (1998) and EC C.16 (2001)) all state that a toxic standard should be included and that the LD50 of the toxic standard should meet a specified range in order for a test to be valid. The sensitivity of the bees and the test procedures were not verified using a toxic standard in the above test. However, as clear effects were seen, exposure obviously took place. Therefore, results can be used for risk assessment.

Study 2**Characteristics**

reference	: Gough H.J. <i>et al.</i> (1990a)	nominal concentrations	: 0, 0.05, 0.1, 0.2, 0.5, 1 and 2 µg/bee
type of study	: Acute contact and oral toxicity study	dosing method	: contact: stock solution in acetone oral: stock solution in 50% sucrose solution
year of execution	: 1989	acceptability	: Acceptable
GLP statement	: Yes	contact LD50	: 0.317 µg/bee
guideline	: Not reported	oral LD50	: 0.535 µg/bee
test substance	: Technical NC-129, lot 129T8902		
purity	: 98.0%		
species	: Honey bee (<i>Apis mellifera</i>)		
exposure duration	: 48 hours		

Methods

A 48-hour acute contact and oral toxicity test on honeybees (*Apis mellifera*) (3 replicates of 10 bees each per concentration) was conducted with technical NC-129 (pyridaben). Nominal doses in both tests were 0.05, 0.1, 0.2, 0.5, 1 and 2 µg/bee, with solvent control and positive control (dimethoate, 0.075, 0.15 and 0.3 µg/bee).

Results

Slight mortality (2 bees) was observed in the control groups. Dose-related paralysis was observed in the contact test at and above 0.2 µg/bee and in the oral test at and above 0.5 µg/bee. Further results are shown in the table below. The positive control gave the expected response.

Table B.9.4.1.1-02 The acute contact toxicity of technical NC-129 to honeybees (*Apis mellifera*)

Test type (purity of test substance)	LD50 (µg a.s./bee)
Acute contact, 48 hr (98.0%) in acetone	0.317
Acute oral, 48 hr (98.0%) in 50% sucrose solution	0.535

Conclusion

The 48-hour contact LD50 was 0.317 µg/bee, the 48-hour oral LD50 was 0.535 µg/bee.

Guidelines and limitations

The test is acceptable.

Study 3**Characteristics**

reference	:	Hinken C. (1987)	exposure duration	:	48 hours
type of study	:	Acute contact toxicity study	nominal	:	0, 0.0049, 0.0098, 0.0195, 0.0391,
year of execution	:	1986	concentrations	:	0.0781, 0.1563 and 0.3125 µg
GLP statement	:	Yes		:	a.s./bee
guideline	:	EPA 141-1	dosing method	:	Stock solution in acetone
test substance	:	NC-129 Technical, Lot No. 129T8605	acceptability	:	Acceptable
purity	:	98.0%	LD50	:	
species	:	Honey bee (<i>Apis mellifera</i>)	contact	:	0.1163 µg a.s./bee

Methods

A 48-hour acute contact toxicity test on honeybees (*Apis mellifera*) (2 replicates of 50 bees each per concentration) was conducted with NC-129 Technical at doses of 0.0049, 0.0098, 0.0195, 0.0391, 0.0781, 0.1563 and 0.3125 µg a.s./bee, with solvent-control and untreated control.

Results

No bees died in the control groups. At 0.1563 and 0.3125 µg a.s./bee, bees were lethargic. Further results are shown in the table below.

Table B.9.4.1.1-03 The acute contact toxicity of NC-129 Technical to honeybees (*Apis mellifera*)

Test type (purity of test substance)	LD50 (µg a.s./bee)
Acute contact, 48 hr (98.0%) in acetone	0.1163

Conclusion

48-Hour LD50: 0.1163 µg a.s./bee

Guidelines and limitations

Current guidelines (EPPO (2000), OECD 214 (1998) and EC C.16 (2001)) all state that a toxic standard should be included and that the LD50 of the toxic standard should meet a specified range in order for a test to be valid. The sensitivity of the bees and the test procedures were not verified using a toxic standard in the above test. However, as clear effects were seen, exposure obviously took place. Therefore, results can be used for risk assessment.

B.9.4.1.2 Acute toxicity of the plant protection product**Study 1****Characteristics**

reference	: Gough H.J. <i>et al.</i> (1990b)	nominal concentrations	: contact: 0, 0.05, 0.1, 0.2, 0.5, 1 and 2 µg a.s./bee
type of study	: Acute contact and oral toxicity study		oral: 0, 0.05, 0.1, 0.2, 0.5, 1 and 2 µg a.s./bee and 0, 0.2, 0.5, 1, 2, 5 and 10 µg a.s./bee
year of execution	: 1989		
GLP statement	: Yes		
guideline	: Not reported		
test substance	: Formulated NC-129 (JF 10708C), lot 129T8902, SC formulation	dosing method	: contact: stock solution in deionised water
content a.s.	: 23.1% w/w		oral: stock solution in 50% sucrose solution
species	: Honey bee (<i>Apis mellifera</i>)	acceptability	: Acceptable
exposure duration	: 48 hours	Contact LD50	: 3.0 µg/bee ⇔ 0.691 µg a.s./bee
		Oral LD50	: 19 µg/bee ⇔ 4.45 µg a.s./bee

Methods

A 48-hour acute contact and oral toxicity test on honeybees (*Apis mellifera*) (3 replicates of 10 bees each per concentration) was conducted with formulated NC-129 (JF 10708C). Nominal doses in the contact test were 0.05, 0.1, 0.2, 0.5, 1 and 2 µg a.s./bee, with solvent control and positive control (dimethoate, 0.075, 0.15 and 0.3 µg/bee). Two oral tests were performed. Nominal doses were 0.05, 0.1, 0.2, 0.5, 1, 2 and µg a.s./bee in the first test and 0.2, 0.5, 1, 2, 5 and 10 µg a.s./bee in the second test, with solvent control and positive control (dimethoate, 0.075, 0.15 and 0.3 µg/bee).

Results

Slight to no mortality (0-2 bees) was observed in the control groups. Dose-related paralysis was observed in the contact test at and above 0.2 µg a.s./bee but not in the oral test. Further results are shown in the table below. The positive control gave the expected response.

Table B.9.4.1.2-01 The acute contact toxicity of formulated NC-129 (SC with 23.1% w/w pyridaben) to honeybees (*Apis mellifera*)

Test type (a.s. content of test substance)	LD50 (µg/bee)
Acute contact, 48 hr (23.1% w/w) in deionised water	3.0 µg formn/bee ⇔ 0.691 µg a.s./bee
Acute oral, 48 hr (23.1% w/w) in 50% sucrose solution	19 µg formn/bee ⇔ 4.45 µg a.s./bee

Conclusion

The 48-hour contact LD50 was 0.691 µg a.s./bee, the 48-hour oral LD50 was 4.45 µg a.s./bee.

Guidelines and limitations

The test is acceptable for the tested product (pyridaben based 23.1% w/w suspension concentrate). Due to the difference in formulation type (SC versus WP) the results may not be extrapolated to Pyridaben 75 WP.

Study 2

Characteristics

reference	: Halsall N. (1999a)	nominal concentrations	: 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 µg a.s./bee
type of study	: Acute contact and oral toxicity study	dosing method	: contact: stock solution in water oral: stock solution in 50% sucrose solution
year of execution	: 1999		
GLP statement	: Yes		
guideline	: EPPO 170, EPA 141-1, OECD 213 and OECD 214		
test substance	: Pyridaben 20 WP formulation, lot 01B009	acceptability	: Acceptable
content a.s.	: 20.86%	Oral LD50	: 10.2 µg/bee ⇔ 2.12 µg a.s./bee
species	: Honey bee (<i>Apis mellifera</i>)	Contact LD50	: 17.6 µg/bee ⇔ 3.68 µg a.s./bee
exposure duration	: 48 hours		

Methods

A 48-hour acute contact and oral toxicity test on honeybees (*Apis mellifera*) (3 replicates of 10 bees each per concentration) was conducted with Pyridaben 20 WP formulation. Nominal doses were 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 µg a.s./bee, with solvent control, untreated control and positive control (dimethoate, 0.10, 0.12, 0.14, 0.16, 0.18 and 0.20 µg/bee).

Results

Mortality in the control groups was ≤3.3%. Slow movements and lethargy were observed at 4.0 and 8.0 µg a.s./bee in the contact test. Further results are shown in the table below. The positive control gave the expected response.

Table B.9.4.1.2-02 The acute contact toxicity of Pyridaben 20 WP formulation to honeybees (*Apis mellifera*)

Test type (a.s. content of test substance)	LD50 (µg/bee)
Acute contact, 48 hr (20.86% w/w) in deionised water	17.6 µg formn/bee ⇔ 3.68 µg a.s./bee
Acute oral, 48 hr (20.86% w/w) in 50% sucrose solution	10.2 µg formn/bee ⇔ 2.12 µg a.s./bee

Conclusion

The 48-hour contact LD50 was 3.68 µg a.s./bee, the 48-hour oral LD50 was 2.12 µg a.s./bee.

Guidelines and limitations

The study is acceptable.

Study 3

Characteristics

reference	:	De Wael L. <i>et al.</i> (1993a)	nominal concentrations	:	0, 0.05 and 0.12% in sucrose solution, acetone or water
type of study	:	Acute oral, contact, direct exposure and inhalation toxicity study	dosing method	:	Oral: solution in 50% sucrose Contact and direct exposure: solution in acetone Inhalation: solution in water
year of execution	:	July 1993			
GLP statement	:	No			
guideline	:	Not reported			
test substance	:	Sanmite (Protex), batch not reported	acceptability	:	Supplemental information
content a.s.	:	150 g/L pyridaben, EC formulation	oral LD50	:	<0.005 µg/bee
species	:	Honey bee (<i>Apis mellifera</i>)	contact LD50	:	>0.12%
exposure duration	:	72 hours	direct exposure LD50	:	<0.05%
			inhalation LD50	:	>0.12%

Methods

A 72-hour acute oral, contact, direct exposure and inhalation test on honeybees (*Apis mellifera*) (2 replicates of 10 bees each per concentration) was conducted with Sanmite (Protex).

Oral toxicity was assessed in individually housed bees and in groups of 10 bees, at nominal doses of 0.05 and 0.12% Sanmite in 50% sucrose, with sucrose control and positive control (dimethoate, 0.32 g a.s./L in 50% sucrose). The individually housed bees were starved for 1-2 hours and then fed 10 µl of test solution. After consumption of the test solution they were given untreated sucrose solution for the remainder of the test. The groups of 10 bees were fed treated test solution for 24 hours *ad libitum*, and untreated sucrose solution for the remainder of the test.

Contact toxicity was assessed in bees exposed to dry residues on absorbing paper treated with 0.05 and 0.12% Sanmite in acetone, with solvent control and positive control (ethylparathion, 0.18 g a.s./L in acetone).

Toxicity after direct exposure was assessed in groups of 10 bees sprayed with 1 mL of 0.05 and 0.12% Sanmite in acetone, with solvent control and positive control (ethylparathion, 0.18 g a.s./L in acetone).

Inhalation toxicity was assessed in bees kept in test units with netted floors placed over 0.05 and 0.12% Sanmite in water, with water control and positive control (ethylparathion, 0.18 g a.s./L in water).

Results

No mortality was observed in the control groups in the individual oral test and direct exposure test, 1-2 bees died in the control group in the contact and inhalation test, and 4 bees (20%) died in the control group for the *ad libitum* 24-hour oral exposure test. The high control mortality invalidates the result of the latter test. Paralysis was observed at both concentrations in the oral tests. Further results are shown in the table below. The positive control gave the expected response.

Table B.9.4.1.2-03 The acute toxicity of Sanmite (Protex) to honeybees (*Apis mellifera*)

Test type	LD50
Acute oral, 72 hr, individual test in 50% sucrose solution	<0.005 µg/bee ^(A)
Acute oral, 72 hr, group test in 50% sucrose solution	Not valid (20% control mortality)
Acute contact, 72 hr on paper treated with solution in acetone	45% mortality at highest test dose of 0.12%
Acute direct exposure (overspray), 72 hr in acetone	<0.05%
Acute inhalation, 72 hr vapour of solution in water	>0.12%

^(A) Calculated by RMS from test concentration (0.05%) and dosed volume (10 µl/bee)

Conclusion

The 72-hour oral LD50 was <0.005 µg/bee, the 72-hour contact LD50 was approximately 0.12%, the 72-hour direct exposure LD50 was <0.05% and the 72-hour inhalation LD50 was >0.12%. The study is not acceptable (see below).

Guidelines and limitations

The report lacked documentation on the time of conduct of the study and data on the composition of Sanmite (Protex). The report was from 1993, hence after GLP had come into force, but the test was not conducted under GLP. No batch number was reported, however it was stated that the same batch number was used as in study nr. 4 (De Wael L. *et al.* 1993b). Therefore the study result is only accepted as supplemental information for comparison of the toxicity between honeybees and bumblebees.

Study 4

Characteristics

reference	:	De Wael L. <i>et al.</i> (1993b)	nominal concentrations	:	0, 0.05 and 0.12% in sucrose solution, acetone or water
type of study	:	Acute oral, contact, direct exposure and inhalation toxicity study	dosing method	:	Oral: solution in 50% sucrose
year of execution	:	July 1993		:	Contact and direct exposure: solution in acetone
GLP statement	:	No		:	Inhalation: solution in water
guideline	:	Not reported		:	Supplemental information
test substance	:	Sanmite (Protex), batch not reported	acceptability	:	
content a.s.	:	150 g/L pyridaben, EC formulation	lowest oral LD50	:	<0.05%
species	:	Bumble bee (<i>Bombus terrestris</i>)	contact LD50	:	>0.12%
exposure duration	:	72 hours	direct exposure LD50	:	>0.12%
			inhalation LD50	:	>0.12%

Methods

A 72-hour acute oral, contact, direct exposure and inhalation test on bumble bees (*Bombus terrestris*) (2 replicates of 10 bees each per concentration) was conducted with Sanmite (Protex).

Oral toxicity was assessed in individually housed bees and in groups of 10 bees, at nominal doses of 0.05 and 0.12% Sanmite in 50% sucrose, with sucrose control and positive control (dimethoate, 0.32 g a.s./L in 50% sucrose). The individually housed bees were starved for 1-2 hours and then fed 30 µl of test solution. After consumption of the test solution they were given untreated sucrose solution for the remainder of the test. The groups of 10 bees were fed treated test solution for 24 hours *ad libitum*, and untreated sucrose solution for the remainder of the test.

Contact toxicity was assessed in bees exposed to dry residues on absorbing paper treated with 0.05 and 0.12% Sanmite in acetone, with solvent control and positive control (ethylparathion, 0.18 g a.s./L in acetone).

Toxicity after direct exposure was assessed in bees sprayed with 1 mL of 0.05 and 0.12% Sanmite in acetone, with solvent control and positive control (ethylparathion, 0.18 g a.s./L in acetone).

Inhalation toxicity was assessed in bees kept in test units with netted floors placed over 0.05 and 0.12% Sanmite in water, with water control and positive control (ethylparathion, 0.18 g a.s./L in water).

Results

No mortality was observed in the control groups in the oral tests, contact test and direct exposure test, but 1 bee died in the control group in the inhalation test. No sublethal effects were observed in any test. Further results are shown in the table below. The LD₅₀ was calculated by the RMS using Finney, D.J., 1971: Probit analysis, Cambridge University Press, Cambridge, U.K., 3rd edition. The positive controls gave the expected responses.

Table B.9.4.1.2-04 The acute contact toxicity of Sanmite (Protex) to bumble bees (*Bombus terrestris*)

Test type	LD ₅₀
Acute oral, 72 hr, individual test in 50% sucrose solution	0.0067 µg/bee ^(A)
Acute oral, 72 hr, group test in 50% sucrose solution	<0.05%
Acute contact, 72 hr, on paper treated with solution in acetone	>0.12%
Acute direct exposure, 72 hr in acetone	>0.12%
Acute inhalation, 72 hr vapour of solution in water	>0.12%

^(A) Calculated by RMS from reported concentration (0.067%) and dosed volume (10 µl/bee)

Conclusion

The 72-hour individual oral LD50 was 0.0067 µg/bee, the group 72-hour oral LD50 was <0.05%, the 72-hour contact LD50 was >0.12%, the 72-hour direct exposure LD50 was >0.12% and the 72-hour inhalation LD50 was >0.12%.

Guidelines and limitations

The report was from 1993, hence after GLP had come into force, but the test was not conducted under GLP. No batch number was reported, however it was stated that the same batch number was used as in study nr. 3 (De Wael L. *et al.* 1993a). Therefore the study result is only accepted as supplemental information for comparison of the toxicity between honeybees and bumblebees.

B.9.4.2 Residue studies

Characteristics

reference	:	Mayer D.F. (1993)	species	:	Honey bee (<i>Apis mellifera</i>)
type of study	:	Contact toxicity study with aged residues on field treated foliage	exposure duration	:	24 hours
year of execution	:	1993	nominal concentrations	:	0.56 kg a.s./ha
GLP statement	:	Yes	dosing method	:	Aged, dry residues on field-sprayed alfalfa
guideline	:	EPA 141-2	acceptability	:	Acceptable
test substance	:	BAS 300 11 I (pyridaben WP), lot OAJ 030	LD50	:	<0.56 kg a.s./ha
content a.s.	:	76.8% w/w			

Methods

A study was conducted to evaluate the effect of field aged residues of BAS 300 11 I on alfalfa foliage on honey bees.

Two studies were conducted with an interval of 6 days. Different groups of honey bees (*Apis mellifera*) were exposed under laboratory conditions for 24 hours to alfalfa leaves that were taken from plots (2 per treatment) previously treated with BAS 300 11I at a rate of 0.56 kg a.s./ha. The leaves used in the laboratory studies were aged in the field under ambient conditions for 2, 8 or 24 hours. Control plots were left untreated. No rainfall occurred during the study. The laboratory test was conducted in wire screen cages containing about 500 cm³ of chopped detached foliage, with three replicates of 50 bees per plot (hence six replicates per treatment). Survival was assessed after 24 hours.

Results

Mortality in control groups was low (max. 2.3%). In the treatment groups, bees were observed to be unable to walk or fly normally, regurgitate the honey stomach and clump together. Further results are shown in the table below.

Table B.9.4.2-01 The acute contact toxicity of BAS 300 11I to honeybee (*Apis mellifera*) exposed to field treated alfalfa leaves aged for 2, 8 and 24 hours

Treatment	Dose (kg a.s./ha)	Mortality (%)	
		First trial	Second trial
2 hours aged residues			
Water control	0	1.3	0.6
BAS 300 11I	0.56	62*	81*
8 hours aged residues			
Water control	0	1.0	2.3
BAS 300 11I	0.56	36*	50*
24 hours aged residues			
Water control	0	1.0	1.3
BAS 300 11I	0.56	16*	24*

* Significantly different from control at 5% level

Conclusion

Survival was significantly reduced at 0.56 kg a.s./ha. Survival increased with aging of residues, but was still significantly reduced after 24 hours of aging.

Guidelines and limitations

1. The report did not contain any information to verify the dose tested (e.g. calibration of the sprayer or measurement of the amount of liquid sprayed). However, the study does provide useful information about the duration of contact toxicity of residues under field weathering conditions.
2. No toxic standard was used. However, since clear effects were seen after two hours, this is not deemed necessary.

B.9.4.3 Tunnel studies

Study 1

Characteristics

reference	:	Stute K. (1988a)	exposure duration	:	48 hours
type of study	:	Tunnel test	nominal	:	6.0 kg/ha
year of execution	:	1988	concentrations	:	
GLP statement	:	No	dosing method	:	<i>Phacelia</i> plants treated in a tent
guideline	:	Not reported	acceptability	:	Acceptable in combination with the results of studies 2 and 3.
test substance	:	BAS 9078 0 I (A), batch not reported	conclusion	:	NOEC <6.0 kg/ha ⇔ <1.2 kg a.s./ha
content a.s.	:	20% pyridathioben (pyridaben)			
species	:	honeybee			

Methods

The effect of BAS 9078 0 I (A) on foraging behaviour and survival of honeybees was determined in a tent (tunnel) test.

Treatments with BAS 9078 0 I (A) at 1.2 kg a.s./ha and a positive control (not identified) were tested in 667 L water /ha against controls (method of treatment of control not identified). Each treatment was performed in one 12 m² tent with blooming *Phacelia*. A bee colony with 3 comb sections was placed in each tent three days before treatment, on 7 August 1988. Flying behaviour and mortality were assessed at regular intervals during 48 hours after treatment which took place at 3:50 pm on 10 August 1988.

Results

It was reported that about 150 bees in each tent were directly hit by the spray. The results of the post-treatment observations are shown in the table below. During the first 48 hours after treatment, a total of 36 bees died in the control group, while 67 bees died in the treatment group and 452 bees died in the positive control group. Increased mortality in the treatment group was mainly seen directly after spraying. Between 24 and 48h, an equal amount of bees died in treatment and control group (17 vs. 19). After treatment, visits to blossoms decreased in the pyridaben treatment and the positive control. Number of visits to blossoms in pyridaben treatment group was still lower than in the control group at the end of the test. The report stated that additional tests should be conducted for conclusive evaluation.

Table B.9.4.3-01 The toxicity of BAS 9078 0 I (A) to honey bees foraging in a tent on *phacelia* treated with pyridaben at 1.2 kg a.s./ha

Treatment	Dose (kg a.s./ha)	Number of bees per m ²			Cumulative mortality ^(A)		
		2 hr	24 hr	48 hr	2 hr	24 hr	48 hr
Water control	0	10-12	12-15	12	9	17	36
BAS 9078 0 I (A)	1.2	1-2	3-4	6-8	34	50	67
Positive control	Not reported	0	6-8	10-12	285	412	452

^(A) Number of bees

Conclusion

Increased bee mortality (initial effect) and reduced foraging behaviour at 1.2 kg a.s./ha.

Guidelines and limitations

1. The performing laboratory and the study location were not identified in this non-GLP test report. The information on test design and test procedures was essentially limited to what is included in this summary (e.g. no data on identity and dose of positive control, no information on spraying procedures).
2. Compliance with a guideline was not claimed. The study design however basically agreed with that of BBA VI, 23-1 and EPPO (2000) guidelines for tunnel tests, except that the test was unreplicated (the next study however was reported to be a replicate for the present study) and that the duration of observations was not long enough (after 48 hours foraging behaviour was still reduced and continuation up to 72 hours at least would have been preferred).
3. Overall evaluation by RMS. The study indicates that a pyridaben dose of 1.2 kg a.s./ha has adverse effects (increased mortality and reduced foraging behaviour) on honey bees under semi-field conditions. The test is unreplicated, but this can be accepted since two other unreplicated tests (studies 2 and 3) were performed, giving a total of three tunnel tests. The lack of information on the identity of the positive control is accepted since the results of all three tunnel tests showed that a pyridaben dose of 1.2 kg a.s./ha gave adverse effects on bees (the lack of a positive control is more essential when no adverse effects are found). Prior to implementation of GLP it was not uncommon that spraying procedures were not described in detail and this does not invalidate the results. The conclusion of the three combined studies 1, 2 and 3 (adverse effects on foraging behaviour and survival at 1.2 kg a.s./ha) is therefore taken to the List of Endpoints.

Study 2

Characteristics

reference	:	Stute K. (1988b)	exposure duration	:	52 hours
type of study	:	Tunnel test	nominal	:	6.0 L/ha
year of execution	:	1988	concentrations	:	
GLP statement	:	No	dosing method	:	<i>Phacelia</i> plants treated in a tent
guideline	:	Not reported	acceptability	:	Acceptable in combination with the results of study 1 and 3
test substance	:	BAS 9078 4 I (A), batch not reported	conclusion	:	NOEC <6.0 kg/ha ⇔ <1.2 kg a.s./ha
content a.s.	:	200 g/L pyridathioben (pyridaben)			
species	:	honeybee			

Methods

The effect of BAS 9078 0 I (A) on foraging behaviour and survival of honey bees was determined in a tent (tunnel) test.

Treatments with BAS 9078 0 I (A) at 1.2 kg a.s./ha and a positive control (not identified) were tested in 667 L water /ha against controls (method of treatment of control not identified). Each treatment was performed in one 12 m² tent with blooming *Phacelia*. A bee colony with 3 comb sections was placed in each tent three days before treatment, on 18 July 1988. Flying behaviour and mortality were assessed at regular intervals during 52 hours after treatment which took place at 12:10 pm on 21 July 1988.

Results

It was reported that about 150 bees in each tent were directly hit by the spray. The results of the post-treatment observations are shown in the table below. During the first 52 hours after treatment, a total of 53 bees died in the control group, while 66 bees died in the treatment group and 513 bees in the positive control group. Mortality in the treatment group was comparable to the control group. After treatment, visits to blossoms decreased in the pyridaben treatment and the positive control. At the end of the test, the number of visits to blossoms in pyridaben treatment group was equal to the control group. The report stated that additional tests should be conducted to fully clarify toxicity to bees.

Table B.9.4.3-02 The toxicity of BAS 9078 0 I (A) to honey bees foraging in a tent on *phacelia* treated with pyridaben at 1.2 kg a.s./ha

Treatment	Dose (kg a.s./ha)	Number of bees per m ²			Cumulative mortality ^(A)		
		6 hr	28 hr	52 hr	6 hr	28 hr	52 hr
Water control	0	6-8	10-12	12-15	11	15	53
BAS 9078 4 I (A)	1.2	3-4	0	10-12	20	31	66
Positive control	Not reported	0	6-8	12-15	317	474	513

^(A) Number of bees**Conclusion**

Reduced foraging behaviour at 1.2 kg a.s./ha (initial effect); no effect on bee mortality.

Guidelines and limitations

1. The performing laboratory and the study location were not identified in this non-GLP test report. The information on test design and test procedures was essentially limited to what is included in this summary (e.g. no data on identity and dose of positive control, no information on spraying procedures).
2. Compliance with a guideline was not claimed. The study design however basically agreed with that of BBA VI, 23-1 and EPPO (2000) guidelines for tunnel tests, except that the test was unreplicated (the previous study however was reported to be a replicate for the present study) and that the duration of observations was not long enough (preferably 72 hours, but on the last study day foraging behaviour in the pyridaben treatment and the control were comparable).
3. Overall evaluation by RMS. The study indicates that a pyridaben dose of 1.2 kg a.s./ha has adverse effects (reduced foraging behaviour) on honey bees under semi-field conditions. The test is unreplicated, but this can be accepted since two other unreplicated tests (studies 1 and 3) were performed, giving a total of three tunnel tests. The lack of information on the identity of the positive control is accepted since the results of all three tunnel tests showed that a pyridaben dose of 1.2 kg a.s./ha gave adverse effects on bees (the lack of a positive control is more essential when no adverse effects are found). Prior to implementation of GLP it was not uncommon that spraying procedures were not described in detail and this does not invalidate the results. The conclusion of the three combined studies 1, 2 and 3 (adverse effects on foraging behaviour and survival at 1.2 kg a.s./ha) is therefore taken to the List of Endpoints.

Study 3**Characteristics**

reference	: Stute K. (1988c)	exposure duration	: 52 hours
type of study	: Tunnel test	nominal concentrations	: 6.0 kg/ha
year of execution	: 1988	dosing method	: <i>Phacelia</i> plants treated in a tent
GLP statement	: No	acceptability	: Acceptable in combination with the results of study 1 and 2
guideline	: Not reported	conclusion	: NOEC <6.0 kg/ha ⇔ <1.2 kg a.s./ha
test substance	: BAS 9078 0 I (A), batch not reported		
content a.s.	: 20% pyridathioben (pyridaben)		
species	: honeybee		

Methods

The effect of BAS 9078 0 I (A) on foraging behaviour and survival of honey bees was determined in a tent (tunnel) test.

Treatments with BAS 9078 0 I (A) at 1.2 kg a.s./ha and a positive control (not identified) were tested in 667 L water /ha against controls (method of treatment of control not identified). Each treatment was performed in one 12 m² tent with blooming *Phacelia*. A bee colony with 3 comb sections was placed in each tent three days before treatment, on 18 July 1988. Flying behaviour and mortality were assessed at regular intervals during 52 hours after treatment which took place at 12:05 pm on 21 July 1988.

Results

It was reported that about 150 bees in each tent were directly hit by the spray. The results of the post-treatment observations are shown in the table below. During the first 52 hours after treatment, a total of 53 bees died in the control group, while 78 bees died in the treatment group and 523 bees died in the positive control group. Increased mortality in the treatment group was mainly seen directly after spraying. Between 24 and 52h, an equal amount of bees died in treatment and control group (43 vs. 38). After treatment, visits to blossoms decreased in the pyridaben treatment and the positive control. At the end of the test, the number of visits to blossoms in pyridaben treatment group was equal to the control group. The report stated that additional tests should be conducted to fully clarify toxicity to bees.

Table B.9.4.3-03 The toxicity of BAS 9078 0 I (A) to honey bees foraging in a tent on *phacelia* treated with pyridaben at 1.2 kg a.s./ha

Treatment	Dose (kg a.s./ha)	Number of bees per m ²			Cumulative mortality ^(A)		
		6 hr	28 hr	52 hr	6 hr	28 hr	52 hr
Water control	0	6-8	10-12	12-15	11	15	53
BAS 9078 4 I (A)	1.2	2-3	10-12	12-15	27	35	78
Positive control	Not reported	0	6-8	12-15	317	474	523

^(A) Number of bees

Conclusion

Increased bee mortality and reduced foraging behaviour at 1.2 kg a.s./ha. Both back to control levels at the end of the test.

Guidelines and limitations

1. The performing laboratory and the study location were not identified in this non-GLP test report from 1988. The information on test design and test procedures was essentially limited to what is included in this summary (e.g. no data on identity and dose of positive control, no information on spraying procedures).
2. Compliance with a guideline was not claimed. The study design however basically agreed with that of BBA VI, 23-1 and EPPO (2000) guidelines for tunnel tests, except that the test was unreplicated (the previous two studies however may be considered to represent replicates for the present study) and that the duration of observations was not long enough (preferably 72 hours, but on the last study day foraging behaviour and mortality in the pyridaben treatment and the control were comparable).
3. Overall evaluation by RMS. The study indicates that a pyridaben dose of 1.2 kg a.s./ha has adverse effects (increased mortality and reduced foraging behaviour) on honey bees under semi-field conditions. The test is unreplicated, but this can be accepted since two other unreplicated tests (studies 1 and 2) were performed, giving a total of three tunnel tests. The lack of information on the identity of the positive control is accepted since the results of all three tunnel tests showed that a pyridaben dose of 1.2 kg a.s./ha gave adverse effects on bees (the lack of a positive control is more essential when no adverse effects are found). Prior to implementation of GLP it was not uncommon that spraying procedures were not described in detail and this does not invalidate the results. The conclusion of the three combined studies 1, 2 and 3 (adverse effects on foraging behaviour and survival at 1.2 kg a.s./ha) is therefore taken to the List of Endpoints.

Study 4

Characteristics

reference	:	Tisseur M. <i>et al.</i> (1992)	species	:	Honey bee (<i>Apis mellifica</i>)
type of study	:	Tunnel test	exposure duration	:	7 days
year of execution	:	1991	nominal	:	0.15 kg a.s./ha
GLP statement	:	No	concentrations	:	
guideline	:	Not reported	dosing method	:	<i>Phacelia</i> plants treated in a tent
test substance	:	Nexter, batch not reported	acceptability	:	Acceptable
content a.s.	:	200 g a.s./L	NOEC	:	<0.15 kg a.s./ha

Methods

The effect of Nexter (200 g/L pyridaben) on bee activity, behaviour, mortality and general status of the bee colony was determined in a tunnel test at a dose of 0.15 kg a.s./ha.

Bee-hives equipped with Todd dead bee traps (one colony with one queen and 10,000-14,000 worker bees per treatment) were placed in tunnels with *Phacelia tanacetifolia* eight days before treatment. Each tunnel of 160 m² contained two treated plots with *Phacelia* of each 16 m² and two untreated plots with *Phacelia* of

each 16 m², designated as shelter plots; the remaining 96 m² of each tunnel consisted of paths covered with plastic sheeting. Treatments with Nexter at 0.15 kg a.s./ha and a positive control (Zolone flo (a.s. phosalone, 1.2 L/ha) were tested against water-treated controls. The treatments were made in 375 L of water per ha, when the *Phacelia* was in full bloom, in the morning. Bee activity and behaviour were assessed once daily on -3DAT and -2DAT, twice on -1DAT (morning and afternoon), five times on 0DAT (once before and four times after treatment), and once daily on +1DAT, +2DAT and +5DAT. Mortality was assessed once daily on -3DAT, -2DAT, -1DAT, +1DAT, +2DAT, +3DAT, +4DAT and +5DAT and twice on 0DAT. Assessments on brood-comb development were made at -3DAT and +7DAT (comparison of total brood area, estimation of the percentage of the brood area occupied by eggs, young and old larvae and capped cells).

Results

Activity and behaviour

The results are shown in Table B.9.4.3-04. Visiting activity in the positive control was reduced on the day of treatment, but not on any following day. Treatment with Nexter caused decreased bee activity on the day of treatment only. The difference in activity between treated and shelter plots in the control and positive control was attributed by the author of the report to a higher flower density on these plots.

Table B.9.4.3-04 Flight activity of honey bees foraging in a tent on *Phacelia* treated with pyridaben at 0.15 kg a.s./ha

Treatment Dose		Number of field bees per m ²					
		Water control 0 kg a.s./ha		Nexter 0.15 kg a.s./ha		Positive control 1.2 L/ha	
DAT	Time	Treated	Shelter	Treated	Shelter	Treated	Shelter
-3	Afternoon	15	17	14	14	11	16
-2	Afternoon	19	23	16	17	15	21
-1	Morning	14	19	11	8	12	13
-1	Afternoon	16	18	18	18	14	18
0	-30'	14	14	14	12	12	13
0	+30'	19	21	9	12	15	17
0	+90'	19	21	12	12	11	17
0	+4 hr	18	22	16	14	12	16
0	+6 hr	18	22	17	15	16	21
+1	Morning	26	26	20	25	18	27
+2	Morning	15	19	16	14	15	20
+3	Morning	12	14	10	8	8	11

Mortality

The results are shown in Table B.9.4.3-05. Mortality was high before treatment in all groups, which was attributed by the authors to the intense activity of the worker bees, the high temperature and the season. This is acceptable. The positive control did not cause an increase in bee mortality, but the authors stated that this was not surprising in the case of tests on flowers. Nexter caused increased bee mortality on the day of treatment.

Table B.9.4.3-05 Mortality of honey bees foraging in a tent on *Phacelia* treated with pyridaben at 0.15 kg a.s./ha

Treatment	Number of dead bees		
	Water control	Nexter	Positive control
Dose	0 kg a.s./ha	0.15 kg a.s./ha	1.2 L/ha
-3DAT	173	144	209
-2DAT	186	214	224
-1DAT	310	264	214
0DAT	345	494	332
+1DAT	264	174	217
+2DAT	343	249	229
+3DAT	356	327	306
+4DAT	327	332	401
+5DAT	299	236	325

Brood development

The results are shown in Table B.9.4.3-06. In all groups, a decrease in total brood area was observed during the post-treatment assessment at +7DAT. The authors considered the decrease for the Nexter treatment to be unrelated to treatment, since, according to them, this decrease (39%) has generally been observed in these tests. The decrease in the Nexter treatment (39%) was far higher than that of the concurrent control (9%). However, only one colony per treatment was tested, while in general high variability occurs in brood area, especially for egg and larvae stages. Furthermore, the authors also stated that the strong decrease for the positive control could be explained by a full stop of oviposition, and that the presence of royal cells showed that this colony wanted to renew the queen, which was stated to be rather frequent in the region. This may be acceptable but implies that the results on brood development from the positive control hive are not suitable for evaluation. In summary, results on brood development from this study are not considered suitable for risk assessment.

Table B.9.4.3-06 Effects on brood of honey bees foraging in a tent on *Phacelia* treated with pyridaben at 0.15 kg a.s./ha

Treatment	Brood area (dm ²)		% of brood area covered by							
			Eggs		Young larvae		Old larvae		Capped cells	
	-3DAT	+7DAT	-3DAT	+7DAT	-3DAT	+7DAT	-3DAT	+7DAT	-3DAT	+7DAT
Water control	33.9	30.7	20	37	8.6	7.5	8.3	5.9	63	50
Nexter	29.2	17.7	26	13	20	0	6.2	6.2	49	81
Positive control	19.9	6.0	15	0	13	0	8.0	0	56	100

Conclusion

Nexter (0.15 kg pyridaben/ha) caused increased bee mortality on the day of treatment. Results for brood development were considered not suitable for risk assessment.

Guidelines and limitations

1. The report was not signed and the test was not GLP compliant (but performed prior to GLP implementation). The description of study methods and results however was sufficiently detailed, except

that information on spraying methods was limited. Prior to implementation of GLP however it was not uncommon that spraying procedures were not described in detail and this does not invalidate the results.

2. The results for the positive control are equivocal (no increase in bee mortality, interpretation of bee brood development hampered by renewal of queen). The lack of an adequate positive control is considered to be not essential since clear adverse effects on bee survival were found in the pyridaben treatment.
3. Each tunnel contained two treated and two untreated (shelter) plots. This does not represent a worst-case exposure regime since bees are not forced to visit treated plots only. It was further noted that at some point in the report it was stated that the flower density in the control and the positive control was higher in the shelter plots than in the treated plots, but no statement was made on the flower density on the shelter plots and treated plots of the tunnel in which pyridaben was applied.
4. Overall evaluation. The study did not include replicates, which is in disagreement with the recommendations by the EPPO (2000) guideline. However, the finding of increased bee mortality in the present study agrees with the results from previous tunnel tests (studies 1 and 3). This study result is therefore taken to the List of Endpoints. Due to lack of replicates, results for brood development are not considered relevant for risk assessment.

B.9.4.4 Field studies

Characteristics

reference	:	Stute K. (1989)	species	:	bee
type of study	:	Field test	exposure duration	:	48 hours
year of execution	:	1989	nominal concentrations	:	3.0 L/ha
GLP statement	:	No	dosing method	:	Field treated <i>Phacelia</i> plants
guideline	:	Not reported	acceptability	:	Not acceptable
test substance	:	BAS 9078 6 A, batch not reported	NOEC	:	No reliable conclusion may be drawn
content a.s.	:	200 g/L pyridathioben (pyridaben)			

Methods

Two field studies were performed in Hetendorf near Hermannsburg (Germany) in June 1989 to assess the effects of BAS 9078 6 A (200 g/L pyridaben) on the behaviour and mortality of bees and the development of bee colonies. The treatment was assigned to one *Phacelia* field measuring 2500 m² (first trial) or 3200 m² (second trial). The treatments in both trials involved a single dose of 0.6 kg a.s./ha in 667 L of water made to blooming *Phacelia* at 13:00 hr. Each trial used four test colonies and an unspecified number of control colonies. It was reported that the test field was not situated within the flight range of strange bee colonies. Flying behaviour was assessed the day before treatment, 7 times on the day of treatment, 3 times at +1DAT, and once daily up to +6DAT (end of trial). Bee mortality "in front of the test field" was assessed the day before treatment, 4 times on the day of treatment after treatment, 3 times at +1DAT, and once daily up to +6DAT. Bee mortality "in front of the reference hives" was assessed the day before treatment, 4 times on the day of treatment after treatment, 3 times at +1DAT, and once daily up to +6DAT. Colony strength was assessed the day before treatment and at +2DAT and +6DAT. Microscopical pollen analysis was conducted in both trials.

Results

Results on flight activity, bee mortality and colony strength are shown in the table below. Flower visit diminished only for a short time immediately after spraying. There were no adverse effects on colony strength and bee survival. Brood condition was reported to be either well developed (first trial) or not changed by injuries (second trial) but details on the method of observation or measurement to reach this conclusion were not available in the report. Flying behaviour was evaluated as high activity and no signs of poisoning. *Phacelia* pollen represented 76-85% of the pollen samples that were identified but since it is unclear which crops were grown in the neighbouring fields, this information is of limited value.

Table B.9.4.4-01 Effects of BAS 9078 6 A (0.6 kg pyridaben/ha) on bee colonies near treated *Phacelia*

DAT	First trial				Second trial			
	No. of bees per m ²	Cumulative mortality (no. of bees)		Colony strength	No. of bees per m ²	Cumulative mortality (no. of bees)		Colony strength
		Test field	Reference hive ^(A)			Test field	Reference hive ^(A)	
-1	22-23	14	24	Normal & good	18-20	25	21	Good
0 ^(B)	18-22	37	52	-	17-20	51	43	-
+1	24-27	67	81	-	15-18	72	70	-
+2	20-25	89	102	Normal & good	10-13	101	103	Good & unaffected
+3	12-14	120	120	-	12-15	126	132	-
+4	15-17	147	147	-	18-21	148	151	-
+5	9-12	185	180	-	15-17	171	171	-
+6	7-10	204	209	Normal & good	10-11	192	188	Good & unaffected

^(A) Negative control

^(B) At the end of the day

Conclusion

No conclusions may be drawn (see below)

Guidelines and limitations

1. The performing laboratory was not identified in this non-GLP test report from 1989. Compliance with a guideline was not claimed. On many points, the study design lacked information. The notifier stated that at the time of performance it was not usual in Germany to give details in the study report, because they used standard reporting forms. The study was performed by the same person who wrote the BBA-bee testing guidelines. The notifier concludes that the study therefore complied with BBA-guidelines (1991). Nowadays, EPPO-guidelines should be followed. Below, all points on which information is lacking in the report will be noted, followed by requirements on this point from BBA and EPPO guidelines. a) the configuration of the test plots (in particular the distance to neighbouring fields and the nature of the crops on neighbouring fields). BBA: study area should be at least 3 km from competitive sources of forage; EPPO: plots should be separated and not be close to other flowering crops, and distance between plots should be recorded. b) The place where the test colonies were located. BBA: colonies should be set up in a single line at least 3 or 4 days before treatment; EPPO: colonies should be placed in or on the edge of the flowering crop only a few days before the trial. c) The number of control hives and the place where

they were located. BBA: four control colonies (for area of 0.25 ha) set up in the home apiary; EPPO: at least three colonies per treatment (for area of at least 1500 m²), should be placed in or on the edge of a field with flowering crop. d) The size and the number of frames of the test and control colonies. BBA: healthy normal-strength and normally developed colonies on at least two frames; EPPO: healthy, well-fed queen-right colonies in normal condition, containing at least 10000-15000 bees, at least 10-12 frames per colony with at least 5-6 brood frames. e) Method of measurement of bee mortality ("in front of test field"). BBA: linen shading 1.5 m in width are laid in front of the hives and a trap to catch dead bees is suspended in front of the entrance; EPPO: mortality should be assessed using dead-bee traps. NB the notifier indicated that in the study, mortality was assessed using sheets but did not mention dead-bee traps. f) Methods to determine colony strength. BBA: count the number of comb sides occupied, recording accurately the state of the brood at that time and the pollen stores on each brood frame; EPPO/current brood test guideline: Brood status checked using detailed counting sheets. In summary, for some of the points it can be assumed that the study complies with current guidelines, but for other points there are major deviations. Direct comparison of mortality in test fields and reference hives is not possible since mortality data in the pyridaben treatments were obtained as "number of dead bees in front of test field", whereas control mortality was reported as the number of dead bees "in front of reference hives". It is not clear whether dead bee traps as recommended by the EPPO (2000) guideline were used. Measurements of the brood status in the test colonies were not reported, which however would be required considering that pyridaben has ovicidal and larvicidal action against insects.

2. Overall evaluation. There were important guideline deviations (a.o. possibly no dead bee traps, no detailed assessment of effects on bee brood). Therefore the study is not accepted.

B.9.4.5 Bree brood studies

Characteristics

reference	:	Halsall N. (1999b)	observation duration	:	21 days
type of study	:	Bee brood study	nominal	:	25, 50 and 100 mg a.s./L
year of execution	:	1999	concentrations	:	
GLP statement	:	Yes	dosing method	:	Solution in sucrose 50%
guideline	:	EPPO 22 (Oomen <i>et al.</i> , 1992)	acceptability	:	Acceptable
test substance	:	Pyridaben 20 WP, Lot 01B009	NOEC	:	
content a.s.	:	20.86%	brood development	:	100 mg a.s./L
species	:	Honey bee (<i>Apis mellifera</i>)			

Methods

In a field honey bee (*Apis mellifera*) brood study, bee colonies (four per treatment and three for the reference product) were exposed for 21 days to Pyridaben 20 WP in a 50% sucrose solution in water (1 L of solution per hive) at nominal concentrations of 25, 50 and 100 mg a.s./L. The test included a negative control (fed with 50% sucrose solution in water) and a reference product (diflubenzuron at 576 mg a.s./L). Each colony contained one queen and 10,000-15,000 honey bees. Seven days before application, the hives were located on a grass field in Cambridgeshire, England. Environmental conditions during the test were: temperature (8-31°C), relative humidity (27-103%), soil temperature (15-24°C) and precipitation (0-17 mm). Before application, the colonies were opened and at least 110 cells containing eggs, young larvae or old larvae on one frame per colony were marked using a transparent acetate sheet. Consumption of the sucrose solution was monitored on a daily basis. Dead adults and larvae were counted and removed on a daily basis, and brood development was assessed in marked cells twice weekly for a period of 21 days. Flight intensity, worker bee behaviour and queen behaviour was assessed on the days of brood development assessment.

Results

The sucrose control dose was fully consumed after 6 days, and the positive control dose within 24 hours. However, the test substance had a repellent effect on worker bees, and not all Pyridaben 20 WP doses were consumed within 21 days. The mean quantity of pyridaben active substance consumed per colony after 21 days was 23.6, 43.0 and 54.5 mg a.s. at concentrations of 25, 50 and 100 mg a.s./L, respectively. In an additional experiment that investigated repellency at lower pyridaben 20 WP doses, full consumption of the dosing solution was observed within 3 days at 1 mg a.s./L. The mean quantity of pyridaben consumed per hive during the above study after 3 days was 6.5, 8.1 and 13.5 mg a.s. at concentrations of 25, 50 and 100 mg a.s./L, respectively. The author of the report stated that, based on the data of this additional experiment, evaluation was based on the dose levels offered, although the repellent effect was observed. This is considered to be acceptable for the product tested (pyridaben 20 WP), but the repellency prevents extrapolation of the result to other pyridaben based formulations since it is unclear whether the repellency is due to pyridaben or to co-formulants.

Flight intensity and worker bee and queen behaviour were not affected at any concentration. Based on marked cells, the development of the various stages was reported to be normal for all treatments except for diflubenzuron. Further results are summarised in Table B.9.4.5-01.

Table B.9.4.5-01 Effects of Pyridaben 20 WP on brood of honey bees (*Apis mellifera*)

Parameter	Control	Pyridaben 25 mg a.s./L	Pyridaben 50 mg a.s./L	Pyridaben 100 mg a.s./L	Diflubenzuron 576 mg a.s./L
Sucrose consumption (mg a.s./colony)	0	23.6	43.0	54.5	576
Successful eggs (%)	57.0	78.2	82.1	78.3	0.9*
Successful young larvae (%)	82.7	80.5	89.6	90.4	0.0*
Successful old larvae (%)	96.8	90.2	90.3	89.2	56.9*
Number of dead adults ^(A)	4.4	2.4	6.3	4.7	2.3
Number of dead drones ^(A)	0.3	0.3	0.1	0.1	0.5

* Significantly different from control at 5% level

^(A) Per assessment day

Conclusion

Pyridaben 20 WP did not have an adverse effect on the development of the brood of honey bees at concentrations up to 100 mg a.s./L. Actually ingested dose at this concentration was 54.5 mg a.s.

Guidelines and limitations

The test was performed according to the current guideline (EPPO, 1992). The study conclusion (NOEC 100 mg a.s./L) is acceptable for the product tested (pyridaben 20 WP), but the repellency prevents extrapolation of the result to other pyridaben based formulations since it is unclear whether the repellency is due to pyridaben or to co-formulants.

B.9.4.6 Risk assessment

B.9.4.6.1 Acute risk of the active substance

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002). Data on the acute toxicity of the active substance pyridaben and formulated products to honeybees are presented in Tables B.9.4.1.1-02, B.9.4.1.2-01 and B.9.4.1.2-02. The acute contact and oral LD50 values determined in the study with the active substance were lower than those determined in the studies with formulated NC-129 and Pyridaben 20WP. The calculation of hazard quotients (HQs) is therefore based on the acute oral and contact LD50 values, determined for the active substance (0.535 and 0.024 µg a.s./bee, respectively). The hazard quotients for the treatment of tomato and citrus are presented in Table B.9.4.6.1-01.

Table B.9.4.6.1-01 Hazard quotients for honey bees using laboratory toxicity studies on pyridaben

Crop	dose (g a.s./ha)	oral toxicity		contact toxicity		Annex VI trigger
		LD50 (µg a.s./bee)	HQ	LD50 (µg a.s./bee)	HQ	
Tomato EU N & S	250	0.535	467	0.024	10417	50
Citrus EU S	300	0.535	561	0.024	12500	50

The HQs are all above the trigger of 50 specified in Annex VI of 91/414 EC, and higher tier testing is required to evaluate the risk to bees.

Higher tier risk assessment

An aged residue study, four tunnel tests, one field test and one bee brood study with formulated products were submitted. The residue study showed that survival, which was significantly reduced at 0.56 kg a.s./ha, increased with aging of residues, but was still significantly reduced after 24 hours of aging. The field test was not acceptable due to insufficient information on test procedures and important guideline deviations. A bee brood study with Pyridaben 20 WP showed no adverse effects on brood of honey bees fed sucrose solution with 100 mg/L pyridaben. Repellency was observed however during the study and it was uncertain that the study result could be extrapolated to other pyridaben based formulations, since it is unclear whether the repellency is due to pyridaben or to co-formulants. Furthermore, the NOEC (100) mg a.s./L is lower than the concentration in the spray liquid of Nexter Pro (400 mg a.s./L) and thus does not cover the uses applied for anyway. Therefore, the risk assessment will be based on the tunnel tests with BAS 9078 0 I, BAS 9078 4 I and Nexter, all containing 20% pyridaben. In three tunnel tests, where bee survival and behaviour were assessed, increased bee mortality during the first day and reduced foraging behaviour were observed at dose rates of 1.2 kg a.s./ha (four times the maximum recommended rate for pyridaben). In the fourth tunnel test, which included assessments of brood-comb development, increased bee mortality on the day of treatment and adverse effects on bee brood development were observed at a dose rate of 0.15 kg a.s./ha. This dose rate is lower than the proposed doses in citrus in Southern Europe (1 x 0.30 kg a.s./ha) and tomato in Northern and Southern Europe (1 x 0.25 kg a.s./ha). Therefore, a risk to bees is considered to be present.

The notifier is therefore requested to submit additional data for an adequate risk assessment (e.g. an acceptable field study). Alternatively, the notifier may include a phrase on the label stating that application is only allowed in greenhouses equipped with insect mesh and in which bees are not used as pollinators, whilst treatment in the field should not be made on flowering crops, and spraying of flowering weeds must be avoided.

B.9.4.2.4 Risk of plant metabolites

According to the Guidance Document on terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10392/2002 rev 2 final of 17 October 2002), the risk of plant metabolites to bees should be addressed.

A risk assessment is performed for metabolites formed at a maximum level of at least 10% TRR in plants. In plant metabolism studies (section B.7.1), individual identified metabolites were at the most 5.1% TRR in apple fruit (25 days after treatment), 4.6% TRR in citrus fruit (7 days after the second treatment) and 1.1%

TRR in tomato fruit (14 days after treatment). Hence no individual identified metabolite exceeded 10% TRR, and no assessment of plant metabolites is made.

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

B.9.5.1 Laboratory toxicity studies

Study 1

Characteristics

reference	:	Sharples A. (2001a)	species	:	<i>Typhlodromus pyri</i>
type of study	:	Laboratory toxicity study	exposure duration	:	14 days
year of execution	:	2000	nominal	:	0, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 g
GLP statement	:	yes	concentrations	:	a.s./ha
guideline	:	Louis and Ufer (1995), Blümel <i>et al.</i> (2000)	exposure method	:	Dry residues on glass plates
test substance	:	Pyridaben 75WP, Lot no. OAJ 030	acceptability	:	Acceptable
a.s. content	:	76.5% pyridaben	LR50	:	0.59 g a.s./ha
			ER50	:	>0.4 g a.s./ha

Methods

The effect of fresh residues of Pyridaben 75WP (76.5% pyridaben) on survival and reproduction of the predatory mite *Typhlodromus pyri*, confined under laboratory conditions over laboratory-treated glass plates, was determined.

Treatments with Pyridaben 75WP at 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 g a.s./ha and a positive control (dimethoate, 4 g a.s./ha) were tested in 200 L spray solution/ha against water-treated controls. Laboratory treated glass plates were allowed to dry and incorporated as a floor into test units (4/treatment). A thin layer of surrounding insect trap glue provided an exposure area into which twenty protonymphs were added per test unit. The test units were kept in a controlled environment room until study end. Survival was assessed over a 7-day period. Fecundity assessments were carried out in the control and Pyridaben 75WP treatments up to 0.4 g a.s./ha during a second week in the same treated test units.

Results

Test results are shown in the table below. The reference substance gave the expected response. Mortality rates were increased in comparison to the control at and above 0.4 g a.s./ha. The LR50 was 0.59 g a.s./ha. No statistically significant effects on mite fecundity were observed at any concentration tested. The ER50 exceeded 0.4 g a.s./ha.

Table B.9.5.1-01 Effects of Pyridaben 75WP on *Typhlodromus pyri* under laboratory conditions

Treatment	Dose (g a.s./ha)	Mortality (%)	Corrected mortality (%) ^(A)	Mean number of eggs per female	Reduction of reproduction (%) ^(B)
Water control	-	13.8	-	7.97	-
Pyridaben 75WP	0.05	17.5	4.3	5.06	36.5
Pyridaben 75WP	0.1	17.5	4.3	5.89	26.1
Pyridaben 75WP	0.2	10.0	-4.4 ^(C)	5.36	32.8
Pyridaben 75WP	0.4	25.0	13.0	5.11	35.9
Pyridaben 75WP	0.8	86.3	84.1	Not tested	-
Pyridaben 75WP	1.6	98.8	98.6	Not tested	-
Dimethoate	4	97.5	97.1	Not tested	-

(A) According to the Abbott formula

(B) Reduction = 100%*(1-R), where R = Rt/Rc, Rt and Rc = average number of eggs per female in treated (t) and control (c)

(C) Note: negative %, hence no adverse effect

Conclusion

LR50 0.59 g a.s./ha, ER50 >0.4 g a.s./ha.

Guidelines and limitations

The study is acceptable.

Study 2

Characteristics

reference	: Sharples A. (2001b)	species	: <i>Aphidius rhopalosiphi</i>
type of study	: Laboratory toxicity study	exposure duration	: 2 days
year of execution	: 2000	nominal	: 0, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 g
GLP statement	: yes	concentrations	: a.s./ha
guideline	: Mead-Briggs (1992), Mead-Briggs <i>et al.</i> (2000)	exposure method	: Dry residues on glass plates
test substance	: Pyridaben 75WP, Lot no. OAJ 030	acceptability	: Acceptable
a.s. content	: 76.5% pyridaben	LR50	: 0.24 g a.s./ha
		ER50	: 0.2 g a.s./ha

Methods

The effect of fresh residues of Pyridaben 75WP (76.5% pyridaben) on survival and reproduction of the parasitic wasp *Aphidius rhopalosiphi*, confined under laboratory conditions over laboratory-treated glass plates, was determined.

Treatments with Pyridaben 75WP at 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 g a.s./ha and a positive control (dimethoate, 0.12 g a.s./ha) were tested in 200 L spray solution/ha against water-treated controls. Laboratory treated glass plates were allowed to dry and incorporated into test units (4/treatment), forming floor and ceiling respectively. Ten adult wasps (five of each sex) were added to each test unit. The test units were kept in a controlled environment room until study end. Mortality was assessed during 48 hours. Fifteen surviving females of the control, the 0.05 and 0.1 g a.s./ha treatments and eight and four surviving females for the 0.2 and 0.4 g a.s./ha treatment, respectively, were removed and individually confined over untreated

aphid-infested barley plants for 24 hours. The wasps were then removed and the plants were left for a further 11 days before the number of aphid mummies was recorded.

Results

Mortality and reproduction data are summarised in table B.9.5.1-02. Mortality was increased at and above 0.2 g a.s./ha (LR50 0.24 g a.s./ha). Reproduction success was reduced at 0.2 and 0.4 g a.s./ha. Based on a reduction rate of 48% at 0.2 and 0.4 g a.s./ha, the ER50 is set by the RMS at 0.2 g a.s./ha. The reference substance gave the expected response.

Table B.9.5.1-02 Effects of Pyridaben 75WP on *Aphidius rhopalosiphii* under laboratory conditions

Treatment	Dose (g a.s./ha)	Mortality (%)	Corrected mortality (%) ^(A)	Mean number of mummies per female	Reduction of reproduction (%) ^(B)
Water control	-	0	-	6.3	-
Pyridaben 75WP	0.05	0	0	4.1	35
Pyridaben 75WP	0.1	5	5	7.4	-17 ^(C)
Pyridaben 75WP	0.2	60	60	3.25	48
Pyridaben 75WP	0.4	72.5	72.5	3.25	48
Pyridaben 75WP	0.8	87.5	87.5	Not tested	-
Pyridaben 75WP	1.6	100	100	Not tested	-
Dimethoate	0.12	100	100	Not tested	-

(A) According to Abbott formula

(B) Reduction = 100%*(1-R), where R=Rt/Rc, Rt and Rc being the average number of mummies per female

(C) Note: negative %, hence no adverse effect

Conclusion

LR50 0.24 g a.s./ha, ER50 0.2 g a.s./ha.

Guidelines & Limitations

Reduction of reproduction was calculated by RMS. The study is acceptable.

Study 3

Characteristics

Reference	: Bourgoign B. <i>et al.</i> (1996)	exposure duration	: 3 days
type of study	: Toxicity study	nominal	: 0, 0.2 g product/ha
year of execution	: 1996	concentrations	
GLP statement	: No	exposure method	: Sprayed residues on rearing boxes
Guideline	: Not reported	acceptability	: Not acceptable
test substance	: BAS 300 I, batch: not reported	LR50	: See conclusion
a.s. content	: Not reported	ER50	: See conclusion
Species	: <i>Neoseiulus californicus</i>		

Methods

The short-term effect of BAS 300 I on the survival of *Neoseiulus californicus*, exposed to sprayed residues in rearing boxes, was determined.

Rearing boxes (8 per treatment) were sprayed with BAS 300 I at 0.2 g product/L and a positive control (MAITAC 20 HF; 4 mL Amitraz/L) and tested against water-treated controls. Each box contained 10 adult *N. californicus* at test initiation. Mortality assessments were carried out 1.5, 24, 48 hours and 3 days following treatment.

Results

Corrected mortality percentages of 0, 1.25, 3.75 and 7.5% were obtained after 1.5, 24, 48 hours and 3 days, respectively. The positive control gave the expected response.

Conclusion

BAS 300 I caused 7.5% corrected mortality after 3 days.

Guidelines and limitations

This non-GLP report lacked the following essential information: batch and a.s. content of the test substance, test conditions (design of rearing boxes, material they were made of, ventilation, temperature, rH), mortality in the controls, sprayer calibration, spray volume. Further, the report did not state whether *N. californicus* were introduced into the rearing boxes before or after treatment. Therefore the study result is not accepted.

Study 4

Characteristics

Reference	:	Van de Veire M. & Degheele D.	species	:	<i>Encarsia formosa</i> , <i>Orius niger</i>
type of study	:	Greenhouse and laboratory toxicity studies	exposure duration	:	1-50 days
year of execution	:	Not reported	nominal concentrations	:	<i>E. formosa</i> : 0, 1 g prod./L; <i>O. niger</i> : 0, 75 g prod./L
GLP statement	:	No	exposure method	:	Various
Guideline	:	Not reported	acceptability	:	Usefull as supplementary information
test substance	:	Pyridaben (Sanmite 15 EC), batch: not reported	LR50	:	See conclusion
a.s. content	:	Not reported	ER50	:	See conclusion

Methods

The effects of Pyridaben (Sanmite 15 EC) were tested in two laboratory tests and two greenhouse experiments on *Encarsia formosa*, and in two laboratory tests on *Orius niger*.

Laboratory tests with *E. formosa*:

The first laboratory test with *E. formosa* was carried out in small cages with sprayed residues at a dose of 1 g product/L on the side glass walls. Adults were exposed to dried fresh residues for 24 hours after which the survivors were brought in contact with host larvae (*T. vaporariorum*) for 4 hours. Mortality of wasps and percentage parasitisation of host scales were determined.

In the second test, five rows of 20 just blackened scales, collected by hand from tobacco leaves, were stuck on a cardboard using saccharose solution, and sprayed with a dose of 1 g product/L. The cardboards were

kept in petridishes, without ventilation, in a climatic chamber and the numbers of hatched and dead scales were counted 10-14 days after treatment.

Greenhouse experiments with *E. formosa*:

In the first experiment, the residual toxicity of *E. formosa* was tested on tomato leaves sprayed in a greenhouse at a dose of 1 g product/L against water-treated controls. Leaves were collected 3, 9 and 12 days after treatment and 15 adult (0-1 day old) *E. formosa* were placed on the leaves in 3 replicates per treatment (possibly in a laboratory experiment, unclear from report). Mortality was assessed 24 hours after exposure.

A second experiment was performed in an isolated glasshouse compartment on tomato plants with 12 leaves "transplanted in grow bags". It was reported that in this compartment, not only pyridaben was tested, but also three experimental pesticides (not further specified). Adult whiteflies (250) were introduced into the plants on 21 February. On 4 April, pyridaben was sprayed to run-off on 8 plants at a dose of 1 g product/L, and 8 plants were left untreated. On 19 April (+15DAT), 200 *E. Formosa* were introduced into the plants. A second treatment with pyridaben was performed on 31 May (i.e. +57 DAT1). Mortality of whiteflies and *E. formosa* parasitisation of scales was checked on leaves containing the oldest whitefly stages at regular intervals during the experiment.

Laboratory tests with *Orius niger*:

The first laboratory test was performed with 5 male and 5 female 1-day old adult *O. niger* per replicate test container (two replicates per treatment). Glass plates were treated with the test product at 75 g product/ha in 100 L/ha, and two treated plates with freshly dried spray residue were inserted into a plexiglass frame to form the test containers. An untreated control was included. The treated glass plates were replaced with untreated glass plates after two days and a small rooted sweet pepper plant was put in the container as oviposition medium. Dead insects were counted and the survivors were kept on the plants for a further three days for egg deposition. Five days later the number of eggs and hatched larvae were counted and compared with untreated controls.

The second test was performed in a set-up similar to the first test, but in this case with nymph stages instead of adults. In the second test, 30 *O. niger* nymphs were exposed to dried residues at 75 g product/ha using 3 replicate test containers per treatment. An untreated control was included. After 4 days, treated glass plates were replaced with untreated ones, oviposition medium (small rooted sweet pepper plants) was introduced and insects were kept for a further 5-6 days. The number of eggs/female and the emergence rate were determined 6-7 days later.

Results

Laboratory tests with *E. formosa*:

First experiment: mortality of *E. formosa* was 100% in the first laboratory test after 24 hours. No mortality was observed in the untreated control.

Second experiment: the mean reduction of emergence rate (after Abbott's correction) of the parasitic wasps in the second test was 82%.

Greenhouse experiments with *E. formosa*:

First experiment: exposure during 7 days of adult *E. formosa* to 3, 9 and 12 day-aged residues on tomato leaves, resulted in mortality percentages of 75, 70 and 65%, respectively.

Second experiment: results are shown in the table below. Excellent larvicidal activity against L2 and L3 larval stages of the whitefly was observed with 100% mortality observed 15 days after the first treatment. The experiment further showed long residual toxicity of Pyridaben against whitefly. Due to killing of the host whitefly population, the parasitisation rate of *E. formosa* was reduced, but the population of the parasitic wasps was not eradicated.

Table B.9.5.1-03 Effects of Pyridaben on whiteflies and *E. Formosa* parasitisation in a greenhouse experiment

	Days after 1 st treatment						Days after 2 nd treatment	
	+7DAT	+15DAT	+21DAT	+28DAT	+37DAT	+50DAT	+6DAT	+20DAT
Pyridaben (Sanmite 15 EC) 1 g product/L								
Whitefly mortality (%)	69	100	94	94	n.d.	79	100	100
<i>E. formosa</i> parasitisation (%)	0	13	0	0	n.d.	0	36	44
Untreated control								
Whitefly mortality (%)	8	n.d.	66	0	22	5	56	n.d.
<i>E. formosa</i> parasitisation (%)	0	n.d.	8	39	11	100	83	n.d.

n.d. not determined

Laboratory tests with *Orius niger*:

Pyridaben did not adversely affect the survival or fecundity of *O. niger* adults, but it reduced survival and fecundity of *O. niger* nymphs. Results are shown in Table B.9.5.1-04.

Table B.9.5.1-04 Effects of Pyridaben on *O. niger* survival and fecundity in a laboratory experiment

Adult exposure				Nymph exposure		
Mortality (%) at +2DAT	Fecundity ^(A)	Hatching rate (%)	Reduction of reproduction ^(B) (%)	Mortality (%) at +5DAT	Fecundity ^(C)	Reduction of fecundity ^(D) (%)
Pyridaben (Sanmite 15 EC) 75 g product/ha						
0	43	94	-4 ^(E)	6.7	11	21
Untreated control						
0	39	90	-	0	14	-

^(A) Mean number of eggs deposited in 5 days (mean of two replicates)

^(B) Reduction = 100%*(1-R), where R=Rt/Rc, Rt and Rc being the average number of hatched eggs

^(C) Mean number of eggs deposited per female in 5 days

^(D) Reduction = 100%*(1-R), where R=Rt/Rc, Rt and Rc being the average number of eggs per female

^(E) Note: negative effect %, hence no adverse effect

Conclusion

Sanmite 15 EC, 1 g product/L:

Laboratory test, inert substrate: 100% mortality of adult *E. Formosa*.

Laboratory test, natural substrate (leaves): 82% reduction of emergence rate of *E. Formosa*.

Greenhouse test: 75, 70 and 65% mortality, respectively, of adult *E. formosa* exposed to 3, 9 and 12 day-aged residues on tomato leaves.

Greenhouse test on tomato plants, two treatments 57 days apart to control whitefly: Due to killing of the host whitefly population, the parasitisation rate of *E. formosa* was reduced, but the population of the parasitic wasps was not eradicated.

Sanmite 15 EC, 75 g product/ha:

Laboratory test with adults: no mortality after 2 days, 4% increase in reproduction after 5 days.

Laboratory test with nymphs: 6.7% mortality after 5 days, 21% reduction of fecundity after 5 days.

Guidelines and limitations

This non-GLP report, which was neither signed nor dated, lacked information on amongst other things: batch number and a.s. content of the test substance; test conditions (e.g. temperature, rH, detailed information on test container); method of treatment of the control and results for the control treatment in the second laboratory test and the first greenhouse experiment with *E. Formosa*; the spray volume applied, and hence the areal dose (g a.s./ha). Therefore the study results are not taken to the list of endpoints, but may be used as supplementary information.

Study 5

Characteristics

reference	:	Bargen H. (2002a)	exposure duration	:	14 days
type of study	:	Extended laboratory toxicity study	nominal	:	0, 46.5, 200 and 300 g a.s./ha
year of execution	:	2001	concentrations	:	
GLP statement	:	Yes	exposure method	:	Fresh and aged residues on apple leaf discs
guideline	:	Overmeer (1988) and Oomen (1988), Blümel <i>et al.</i> (2000), Candolfi <i>et al.</i> (2001)	acceptability	:	Acceptable
test substance	:	Pyridaben 75WP, batch OAJ030	LR50	:	0-, 7-, 14-, 21- & 28-day aged residue: <46.5, <46.5, 155, 300 & >300 g a.s./ha.
a.s. content	:	76.5% pyridaben		:	
species	:	<i>Typhlodromus pyri</i>	ER50	:	0-, 7-, 14-, 21- & 28-day aged residue: <46.5, <46.5, >46.5, >300 & >300 g a.s./ha,

Methods

The effect of fresh and aged residues of Pyridaben 75WP (76.5% pyridaben) on survival and reproduction of the predatory mite *Typhlodromus pyri*, exposed under laboratory conditions on laboratory-treated leaves from apple seedlings, was determined.

Treatments with Pyridaben 75WP at 46.5, 200 and 300 g a.s./ha and a positive control (dimethoate, 32 g a.s./ha) were tested in 400 L water/ha against water-treated controls. Bioassays were performed with fresh (0DAT) and aged (+7DAT, +14DAT, +21DAT and +28DAT) residues. Treatments were applied to potted

Results

Table B.9.5.1-05 Effects of Pyridaben 75WP on *Typhlodromus pyri* under extended laboratory conditions (exposure to aged residues on excised apple leaf discs)

* Statistically significant at 5% level
(A) According to Abbott formula
(B) Reduction = $100 \times (1 - R)$, where $R = R_t/R_c$. R_t and R_c = average number of eggs per treated or control female

Table B.9.5.1-06 LR50 and ER50 values of Pyridaben 75WP for *Typhlodromus pyri* under extended laboratory conditions (exposure to aged residues on excised apple leaf discs)

Ageing period	LR50 (g a.s./ha)	ER50 (g a.s./ha)
0DAT	<46.5	<46.5
+7DAT	<46.5	<46.5
+14DAT	155	>46.5
+21DAT	300 ^(A)	>300
+28DAT	>300	>300

^(A) LR50 set by RMS at 300 g a.s./ha based on 46.2% (i.e. about 50%) corrected mortality at this dose, which was the highest tested dose

Conclusion

Pyridaben 75WP caused significant effects on survival and reproduction of *Typhlodromus pyri* directly after exposure (LR50 and ER50 <46.5 g a.s./ha). Effects decreased with aging of residues, reaching LR50 and ER50 >300 g a.s./ha after 28 days.

Guidelines & Limitations

Reduction of reproduction was calculated by RMS from the reported data. The LR50 value at +14DAT was calculated by RMS using log-linear regression analysis with log-transformation of the dose levels. The study is acceptable.

Study 6

Characteristics

reference	:	Bargen H. (2002b)	exposure duration	:	48 hours
type of study	:	Extended laboratory toxicity study	nominal	:	0, 46.5, 200 and 300 g a.s./ha
year of execution	:	2001	concentrations	:	
GLP statement	:	Yes	exposure method	:	Fresh and aged residues on apple leaves
guideline	:	Polgar (1988), Mead-Briggs (1996), Candolfi <i>et al.</i> (2001), Mead-Briggs <i>et al.</i> (2000)	acceptability	:	Acceptable
test substance	:	Pyridaben 75WP, batch OAJ030	LR50	:	0-, 7-, 14-, 21-, 28-, 35-, 42- & 49-day aged residue: <46.5, <46.5, 56, 123, 172, 208, 272 & >300 g a.s./ha.
a.s. content	:	76.5% pyridaben	ER50	:	0-, 7-, 14-, 21-, 28-, 35-, 42- & 49-day aged residue: <46.5, <46.5, <46.5, 104, 42, 261, >300 & >300 g a.s./ha.
species	:	<i>Aphidius rhopalosiphi</i>		:	

Methods

The effect of fresh and aged residues of Pyridaben 75WP (76.5% pyridaben) on survival and reproduction of the parasitic wasp *Aphidius rhopalosiphi*, exposed under laboratory conditions on laboratory-treated leaves from potted apple seedlings, was determined.

Treatments with Pyridaben 75WP at 46.5, 200 and 300 g a.s./ha and a positive control (dimethoate, 8 g a.s./ha) were tested in 400 L water/ha against water-treated controls. Bioassays were performed with fresh (0DAT) and aged (+7DAT, +14DAT, +21DAT, +28DAT, +35DAT, +42DAT and +49DAT) residues.

Treatments were applied to potted apple seedlings (Golden Delicious) using a calibrated laboratory sprayer, and seedlings were placed outdoors under a UV-permeable rain-cover for aging. For each bioassay, leaves (4 replicates per treatment) were used as floor in glass exposure cages. Five male and five female *Aphidius rhopalosiphi* (<48 h old) were exposed to each of 4 replicate units per treatment. Survival was assessed in each bioassay during the 48-hour exposure period. Fecundity assessments were carried out in the control and in the treatment groups with $\leq 75\%$ corrected mortality. For this purpose, a maximum of 15 surviving females per treatment group were removed from the units after 48 hours and individually confined over untreated aphid-infested barley plants for 24 hours. The wasps were then removed and the plants were left for a further 10-12 days before the number of aphid mummies was recorded. The test units were kept in a controlled environment room until study end. The positive control was included in the first bioassay only.

Results

The results are summarised in the table below. Statistically significant increases in mortality were observed at 46.5 g a.s./ha up to +14DAT, at 200 g a.s./ha in all but +42DAT and at 300 g a.s./ha in all but +49DAT. Reproduction success was statistically significantly affected at 300 g a.s./ha on +14DAT and +28DAT, at 200 g a.s./ha on +21DAT, +28DAT and +42DAT and at 300 g a.s./ha on +35DAT. The reference substance gave the expected response.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must be completed before this document.

Table B.9.5.1-07 Effects of Pyridaben 75WP on *Aphidius rhopalosiphi* under extended laboratory conditions (exposure to aged residues on excised apple leaf discs)

Treatment	Dose (g a.s./ha)	Mortality (%)	Corrected mortality (%) ^(A)	Mean number of mummies per female	Reduction of reproduction ^(B)
Bioassay 1 (0DAT)					
Water control	-	10.0	-	Not tested	-
Pyridaben 75WP	46.5	92.5*	91.7	Not tested	-
Pyridaben 75WP	200	100*	100	Not tested	-
Pyridaben 75WP	300	100*	100	Not tested	-
Dimethoate	8	100*	100	Not tested	-
Bioassay 2 (+7DAT)					
Water control	-	0	-	Not tested	-
Pyridaben 75WP	46.5	70.0*	70.0	Not tested	-
Pyridaben 75WP	200	100*	100	Not tested	-
Pyridaben 75WP	300	100*	100	Not tested	-
Bioassay 3 (+14DAT)					
Water control	-	0	-	12.6	-
Pyridaben 75WP	46.5	42.5*	42.5	3.7	71
Pyridaben 75WP	200	97.5*	97.5	Not tested	-
Pyridaben 75WP	300	100*	100	Not tested	-
Bioassay 4 (+21DAT)					
Water control	-	5.0	-	5.7	-
Pyridaben 75WP	46.5	12.5	7.9	5.7	0
Pyridaben 75WP	200	70.0*	68.4	0.5*	91
Pyridaben 75WP	300	92.5*	92.1	Not tested	-
Bioassay 5 (+28DAT)					
Water control	-	2.5	-	9.5	-
Pyridaben 75WP	46.5	15.3	13.1	4.5*	52
Pyridaben 75WP	200	37.5*	35.9	2.1*	78
Pyridaben 75WP	300	82.5*	82.1	Not tested	-
Bioassay 6 (+35DAT)					
Water control	-	5.0	-	5.6	-
Pyridaben 75WP	46.5	5.0	0	5.1	8
Pyridaben 75WP	200	38.1*	34.8	4.9	12
Pyridaben 75WP	300	75*	73.7	1.7*	70
Bioassay 7 (+42DAT)					
Water control	-	12.5	-	9.1	-
Pyridaben 75WP	46.5	Not tested	-	Not tested	-
Pyridaben 75WP	200	28.3	18.1	2.1*	77
Pyridaben 75WP	300	65*	60	6.3	32
Bioassay 8 (+49DAT)					
Water control	-	10.0	-	5.1	-
Pyridaben 75WP	46.5	Not tested	-	Not tested	-
Pyridaben 75WP	200	47.5*	41.7	12.5	-144 ^(C)
Pyridaben 75WP	300	12.5	2.8	10.4	-103 ^(C)

* Statistically significant at 5% level

^(A) According to Abbott formula^(B) Reduction = 100%*(1-R), where R = Rt/Rc, Rt and Rc = average number of mummies per treated or control female^(C) Note: negative %, hence no adverse effect

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Table B.9.5.1-08 LR50 and ER50 values of Pyridaben 75WP for *Aphidius rhopalosiphi* under extended laboratory conditions (exposure to aged residues on excised apple leaf discs)

Ageing period	LR50 (g a.s./ha)	ER50 (g a.s./ha)
0DAT	<46.5	<46.5
+7DAT	<46.5	<46.5
+14DAT	56	<46.5
+21DAT	123	104
+28DAT	172	42
+35DAT	208	261
+42DAT	272	>300
+49DAT	>300	>300

Conclusion

Pyridaben 75WP caused significant effects on survival and reproduction of *Aphidius rhopalosiphi* directly after exposure (LR50 and ER50 <46.5 g a.s./ha). Effects decreased with aging of residues, reaching LR50 and ER50 >300 g a.s./ha after 49 and 42 days respectively.

Guidelines & Limitations

Reduction of reproduction was calculated by RMS from the reported data. The LR50 values for +14DAT, +21DAT, +28DAT, +35DAT and +42DAT and the ER50 values for +21DAT, +28DAT and +35DAT were calculated by RMS using log-linear regression analysis with log-transformation of the dose levels. The study is acceptable.

Study 7

Characteristics

reference	:	Hirth N. (2004)	species	:	<i>Chrysoperla carnea</i>
type of study	:	Extended laboratory toxicity study (exposure to fresh and 15-day aged residue on apple leaves)	exposure duration	:	Until adult emergence
			nominal concentrations	:	0, 53, 247 and 308 g a.s./ha
year of execution	:	2003	exposure method	:	Dry residues on field treated apple leaves
GLP statement	:	Yes			
guideline	:	Vogt <i>et al.</i> (2000), Kemmeter <i>et al.</i> (2000), Barret <i>et al.</i> (1994), Candolfi <i>et al.</i> (2001)	acceptability	:	Acceptable
			LR50 & ER50	:	fresh and 15-day aged residue: >308 g a.s./ha
test substance	:	Nexter (BAS 300 11 I), batch OAJ030			
a.s. content	:	76.5% pyridaben			

Methods

The effect of fresh and 15-day aged residues of Nexter (76.5% pyridaben) on survival and reproduction of the green lacewing *Chrysoperla carnea*, confined under laboratory conditions over field-treated apple leaves, was determined.

Treatments with Nexter at 53, 247 and 308 g a.s./ha and a positive control (dimethoate, 80 g a.s./ha) were tested in 800 L water/ha against water-treated controls. Bioassays were performed with fresh (0DAT) and 15-day aged (+15DAT) residues. The positive control was only tested on freshly treated leaves (0DAT).

Potted apple trees (Retina, 3-5 years old) were treated with the test substance using a calibrated knapsack sprayer and allowed to dry. Aging was conducted outdoors under a UV-permeable rain-cover. Leaves were collected from the mid area of each tree and into each test unit one leaf was incorporated forming its floor. One <2-3 day old early second instar larva was added to each test unit (50 replicate units per treatment). The test units were kept in a controlled environment room until study end. Assessments of mortality were made up to the emergence of adults. Surviving adults (≤ 49 per treatment) were removed and confined over two untreated oviposition boxes. The number of eggs was counted over two 24-hour periods during one week. Hatching success of the larvae was assessed at least 4 days later.

Since the hatching rate in the control was insufficient (28%) during the first bioassay (exposure to non-aged residues), this assay was prolonged and reproduction was re-assessed during two further weeks. During the first prolongation week, the hatching rate in the control was still too low (45%), but during the assessment of the last week, the hatching rate in the control was adequate (87%), and the study is therefore acceptable. Conclusions concerning effects on reproduction in this first bioassay should be based on the last assessment. The author of the report stated that "it can be assumed that the low hatching rate in the controls was due to the climatic conditions which were not in the optimum range at this time" (i.e. during the first two assessments).

Results

The results are summarised in the table below. For completeness sake, results for the two first assessments on reproduction are shown in small italic print between brackets. These data however are not used for evaluation, for reasons given above. Pre-imaginal mortality was not increased and reproduction was not reduced up to 308 g a.s./ha in both bioassays. The LR50 for mortality and the ER50 for reproduction for exposure to non-aged and 15-day aged residues were >308 g a.s./ha. The reference substance gave the expected response.

Table B.9.5.1-09 Effects of Nexter on *Chrysoperla carnea* under extended laboratory conditions (exposure to field treated apple tree leaves)

Treatment	Dose (g a.s./ha)	Pre-imaginal mortality (%)	Corrected mortality (%) ^(A)	No. of eggs/female/day ^(D)	Hatching rate (%)	Reduction of reproduction ^(B)
Bioassay 1 (0DAT)						
Water control	0	14	-	(22.8) (22.8) 18.7	(27.9) (44.6) 86.5	(-) (-) -
Nexter	53	22	9.3	(23.9) (22.0) 18.2	(30.3) (40.8) 83.2	(-14 ^(C)) (12) 6
Nexter	247	14	0	(28.4) (26.0) 26.4	(36.2) (32.4) 81.0	(-62 ^(C)) (17) -32^(C)
Nexter	308	28.6	17	(28.5) (34.6) 26.6	(25.0) (37.2) 81.8	(-12 ^(C)) (-27 ^(C)) -35(C)
Dimethoate	80	71.4*	66.9	Not tested	-	-
Bioassay 2 (+15DAT)						
Water control	0	4	-	19.8	90.2	-
Nexter	53	2	-2.1 ^(C)	11.5	89.8	42
Nexter	247	8	4.2	22.0	84.4	-4 ^(C)
Nexter	308	2	-2.1 ^(C)	14.0	81.8	36

* Significantly different from control at 5% level

(A) According to Abbott formula

(B) Reduction = 100%*(1-R), where R = Rt/Rc, Rt and Rc = average number of hatched eggs per treated or control female

(C) Note: negative value, hence no adverse effect

(D) For completeness sake, results for the two first assessments on reproduction in bioassay 1 are shown in small italic print between brackets. These data however are not used for evaluation, for reasons given above

Conclusion

For exposure to non-aged and 15-day aged residues: LR50 >308 g a.s./ha, ER50 (reproduction) >308 g a.s./ha.

Guidelines and limitations

Reduction of reproduction was calculated by RMS from the reported data. The study is acceptable.

Study 8

Characteristics

reference	:	Anonymous	exposure duration	:	1-3 days
type of study	:	Extended laboratory toxicity study	nominal	:	Not reported
year of execution	:	1990	concentrations	:	
GLP statement	:	No	exposure method	:	NC-129 residues on tomato leaves
guideline	:	Not reported	acceptability	:	Not acceptable
test substance	:	NC-129, batch: not reported	LR50	:	Not reported
a.s. content	:	Not reported	ER50	:	Not reported
species	:	<i>Encarsia formosa</i>			

Methods

The effect of 3-, 8- and 14-day aged residues of NC-129 on survival of *Encarsia formosa*, confined under laboratory conditions over treated tomato leaves, was determined.

Tomato plants were sprayed with NC-129 and leaves were collected at +3DAT, +8DAT or +14DAT. The leaves were placed in a Petri dish and 20 *E. Formosa* were placed on the leaves in each dish. Three replicates with a total of 60 *E. Formosa* were tested per aging period. Mortality of *E. formosa* was determined 0 to 3 days later by counting the number of dead organisms.

Results

Exposure of *E. formosa*:

to 3- day aged residue: 90% after 3 days;

to 8-day aged residue 78% after 1 day;

to 14-day aged residues 0% after 0 days.

Conclusion

No reliable conclusions may be drawn (see below)..

Guidelines and limitations

The information on test methods and the description of the results in the non-GLP report of this study was limited to six lines of text. On the basis of such limited information the scientific acceptability of the test procedures cannot be verified. The report lacked the following essential information: dose, batch and purity of the test substance, age, condition and origin of plants and organisms, detailed description of the application method including apparatus, exposure to a negative and positive control, author, personnel and test facility involved. The study is not acceptable.

Study 9

Characteristics

reference	:	Anonymous	species	:	<i>Encarsia Formosa</i> and others
type of study	:	Greenhouse toxicity study	exposure duration	:	1-3 days
year of execution	:	1990	nominal	:	750 µl NC-129/L
GLP statement	:	No	concentrations	:	
guideline	:	Not reported	exposure method	:	NC-129 residues on tomato plants
test substance	:	NC-129 (Sanmite), batch: not reported	acceptability	:	Not acceptable
a.s. content	:	Not reported	Conclusion	:	See study conclusion

Methods

The effect of sprayed residues of NC-129 (Sanmite) on presence, survival and/or parasitisation of beneficial insects (*Encarsia Formosa*, *Phytoseiulus persimilis* and *Dacnusa sibirica*) and pest organisms (whitefly, *T. vaporariorum*; spider mite, *T. urticae*; leafminer *L. huidobrensis*), confined in a greenhouse over treated tomato plants, was determined.

Forty tomato plants were transplanted in growth bags in a greenhouse. The plants were infested with high numbers of white flies (*T. vaporariorum*: ≥1300) and spider mites (*T. urticae*) during a period of five weeks following transplantation. In a following 3-week period, two introductions of 100 *E. formosa* and two

introductions of 800 *P. persimilis* were performed. After introduction of both lots of *E. formosa* and the first lot of *P. persimilis*, plants were sprayed with 5 litres of the acaricide NC-129 at a dose of 750 µl product per litre.

The following observations were made:

1. The number of dead and alive whitefly larvae was counted on samples of 6 top leaves (heavily infested) and of 10 older leaves collected at +9DAT, and from 4 top leaves collected at +15DAT,
2. The number of the pest organism *T. urticae* were counted on samples of 10-30 tomato leaves collected at +9DAT, +15DAT, +28DAT and +34DAT,
3. Since leafminers (another pest organism) appeared in the test plot (which was reported to have happened through immigration from the adjacent commercial greenhouse), at +6DAT leafminer larvae were collected and grown to pupation in order to examine the rate of parasitisation of leafminers with the beneficial *D. sibirica*.
4. In order to determine parasitisation of whitefly with *E. formosa*, black and white whitefly scales were counted in samples of leaves collected at 0DAT and +25DAT. Eclosion rates of *E. formosa* from black scales were determined at +9DAT and +15DAT.
5. On the day of treatment (0DAT) a yellow sticky plate was hung near the top of the plants. This plate was replaced at +8DAT, +15DAT, +28DAT, +34DAT and +40DAT, and beneficial insects and pest organisms stuck onto the plate were counted.

Results

1. Whitefly. At +9DAT and +15DAT, only dead whitefly larvae were found on heavily infested young leaves. At +9DAT, on older leaves, alive larvae were found (5 out of a total of 20 larvae, present on 10 leaves), and also whitefly scales were found, of which 21% were parasitized. This indicates that the treatment had a strong effect against whitefly larvae.
2. *T. urticae*. Mortality was 100% on +9DAT and +15DAT. Information on *T. urticae* counts at further samplings (+28DAT and +34DAT) was not provided but it was stated that on these days *P. persimilis* were found on 23-34 leaves. This indicates that the treatment had a strong effect against *T. urticae*. Interpretation of the observations of *P. persimilis* is hampered by the lack of information on the occurrence of *P. persimilis* at earlier samplings (prior to +28DAT), and the possibility of re-colonisation by this species from the neighbouring greenhouse (see comment under "Guidelines and Limitations"),
3. Leafminers. From 11 out of 20 leafminers (55%), collected at +6DAT for further growth until pupation, the endoparasite *Dacnusa sibirica* emerged. Interpretation of this finding, in particular of the effect of the treatment on the beneficial *D. sibirica*, is hampered by the fact that it is unclear whether leafminers were already present at the time of treatment.
4. *E. formosa*. *E. Formosa* parasitisation rate of whitefly scales was 17.8 and 31.2% at 0DAT and +25DAT, respectively. Eclosion rates of black scales were 67% and 45% at +9DAT and +15DAT, respectively. This may indicate that the treatment did not have an important impact on *E. formosa* populations.
5. Monitoring of insect pests and beneficials with yellow sticky traps. The results are shown in the table below. The interpretation is hampered by the fact that it is unclear to what extent re-colonisation from the neighbouring greenhouse occurred (see comment under "Guidelines and Limitations").

Table B.9.5.1-10 Effects of NC-129 on a variety of insects in a greenhouse experiment

Date	Number of insects counted on the yellow sticky traps			
	Adult whiteflies (pest organism)	<i>L. huidobrensis</i> (leafminer, pest organism)	<i>D. sibirica</i> (beneficial, parasitizes leafminers)	<i>C. parksii</i> (beneficial)
May 11 (+8DAT)	532	57	2	1
May 18 (+15DAT)	76	96	3	1
May 31 (+28DAT)	129	5	0	0
June 6 (+34DAT)	172	27	8	0
June 12 (+40DAT)	192	369	79	1

Conclusion

NC-129 was effective against the greenhouse whitefly (*T. vaporariorum*) and the spider mite (*T. urticae*). No further conclusions may be drawn (see below).

Guidelines and limitations

This non-GLP report lacked the following information: batch and purity of the test substance; application method; negative and positive control; author, personnel and test facility involved. From the report, it could not be identified to what extent the compartment of the greenhouse in which the test was performed, was isolated, so that migration and re-colonisation of affected species could not occur. As a matter of fact, it was stated in the report that leafminers appeared in the test plot through immigration from the adjacent commercial greenhouse. Without this information it is not possible to evaluate the results on counts of beneficial insects. The study is not acceptable.

B.9.5.2 Semi-field and field studies**Study 1****Characteristics**

reference	:	Bourgouin B. & Brun V. (1996)	exposure duration	:	44 days
type of study	:	Field toxicity study	nominal	:	200 g product/ha
year of execution	:	1996	concentrations	:	
GLP statement	:	No	exposure method	:	Single spray treatment of apple orchard with naturally occurring beneficial insect and spider mite populations
guideline	:	Not reported		:	
test substance	:	BAS 300 I, batch: not reported		:	
a.s. content	:	Not reported		:	
species	:	<i>Neoseiulus californicus</i> , <i>Panonychus ulmi</i>	acceptability	:	Not acceptable
			Conclusion	:	No reliable conclusions may be drawn

Methods

The effects of a single spray treatment with BAS 300 I on 23 July 1996 on the populations of the beneficial insect *Neoseiulus californicus* (phytoseiidae) and its prey the red spider mite *Panonychus ulmi* was determined during a period of 44 days after treatment in an apple orchard in Balma, Midi-Pyrénées, Southern France.

Each treatment consisted of 4 replicate rows with six apple trees per row. Apple trees treated with BAS 300 I at 200 g product/ha and two positive controls (MAÏTAC 20 HF, a.s. amitraz, 4 L product/ha, toxic reference on *N. californicus*; OMITE 30 WP, a.s. propargite, 4 kg product/ha, toxic reference on *P. ulmi*, neutral on *N. californicus*) were tested in 750 L water/ha against water-treated controls. Starting one day before treatment (-1DAT) and at +2DAT, +15DAT, +21DAT, +30DAT and +44DAT, 30 leaves were collected from the two central trees of each replicate (120 leaves per treatment). The number of mobile *P. ulmi* and *N. Californicus* were counted in each leaf sample. It was reported that identification of Phytoseiidae was performed on -1DAT and +44DAT, but results of this identification were not presented in the report.

Results

The results are shown in the table below. Phytoseiidae counts in the control were always >30 per sample of 120 leaves. The two positive controls gave the expected response. Effect percentages for BAS 300 I according to Abbot and the Henderson-Tilton coefficient were calculated by the RMS from the reported mean numbers of mobile phytoseiidae per leaf.

BAS 300 I was effective against *P. ulmi*, with a strong reduction (93%) within 2 days after treatment, and a nearly full control within 15 days.

Since pretest counts of phytoseiidae were comparable between treatments, the E-values calculated according to Abbot may be used for evaluation. Effects of >50% (52%) were already noted at the first evaluation (2 days after treatment), and increased to a maximum of 81% at 15 days after treatment. Thereafter effect percentages decreased but were still close to 50% (i.e. 46%) at study end (44 days after treatment). Results for individual replicate counts and statistical analysis of the test results were not reported which precludes a full evaluation.

Table B.9.5.2-01 Effects of BAS 300 I (1X 200 g product/ha) on phytoseiidae and red spider mite in an apple orchard in Southern France

Treatment	Organism	Mean number of mobile stages per leaf at:						
		-1DAT	+2DAT	+7DAT	+15DAT	+21DAT	+30DAT	+44DAT
water control	Spider mite	6.85	4.95	10.86	2.6	0.1	0.07	0.04
	phytoseiidae	1.48	1.88	3.9	3.94	0.64	0.56	0.28
MAITAC 20 HF ^(A)	Spider mite	6	0.68	1	1.1	0.12	0.58	0.17
	phytoseiidae	1.46	0.1	0.58	0.88	0.37	0.8	0.17
OMITE 30 WP ^(B)	Spider mite	3.09	0.38	0.12	0.02	0	0.02	0
	phytoseiidae	1.4	1.6	1.78	0.77	0.24	0.39	0.17
BAS 300 I	Spider mite	6.39	0.47	0.62	0.08	0.03	0.05	0
	phytoseiidae	1.39	0.9	1.12	0.73	0.29	0.35	0.15
	effect phytoseiidae (Abbot)	na	52	71	81	55	38	46
	effect phytoseiidae (Hend.-Tilton)	na	49	69	80	52	33	43

na = not applicable

(A) Toxic reference on *N. californicus*

(B) Toxic reference on *P. ulmi*, neutral on *N. californicus*

Conclusion

Maximum 81% reduction of phytoseiidae after 15 days; no full recovery apparent (46% reduction after 44 days (study end)).

Guidelines and limitations

1. The report was not signed. The study was conducted in 1996, after GLP regulations had come into force, but it was not performed under GLP. The results can therefore not be taken to the List of Endpoints. The study information may be used as supplementary information.
2. The test product was identified as the "commercial product BAS 300 I". Its batch number and a.s. content were not specified. The name BAS 300 I refers to the active substance pyridaben, not to a formulation containing pyridaben. The tested dose was reported to be 200 g BAS 300 I/ha. Considering the low water solubility of pyridaben (12 µg/L), it is not practically possible to apply the unformulated technical active substance. The nature of the test material, and therefore also the tested dose in terms of g a.s./ha, are not unequivocally established on the basis of the information in the report.
3. Results for individual replicate counts and statistical analysis of the test results were not reported which precludes a full evaluation. Evaluation of the study results is also hampered by the lack of information on the lay out of the study. In particular, the size of the orchard was not reported, and the location of the individual test plots within the orchard. For this reason it was unclear how cross contamination and edge effects were avoided. Information on the methods of leaf sampling and of counting of spider mites and beneficial insects was not available. Information on preparation of spray solutions and on spraying procedures was not available. Results for species identification of phytoseiidae, claimed to have been performed on samples from -1DAT and +44DAT, were not reported. Meteorological conditions during the trial were not reported. This non-GLP study result however, although lacking in documentation, may indicate that BAS 300 I applied at 200 g/ha has an effect on phytoseiidae, possibly without full recovery after 44 days.

Study 2

Characteristics

reference	:	Anonymous	exposure duration	:	44 days
type of study	:	Field toxicity study	nominal	:	0, 0.26 L/ha
year of execution	:	1996	concentrations	:	
GLP statement	:	No	exposure method	:	Single spray treatment of apple orchard with naturally occurring beneficial insect populations
guideline	:	Not reported		:	
test substance	:	BAS 300 I, batch: not reported	acceptability	:	Not acceptable
a.s. content	:	Not reported	Conclusion	:	No reliable conclusions may be drawn
species	:	<i>Typhlodromus pyri</i>		:	

Methods

The effects of a single spray treatment with BAS 300 I on 26 June 1996 on the populations of *Typhlodromus pyri* was determined during a period of 44 days after treatment in an apple orchard in Messimy (69), Rhône-Alpes, Southern France.

Each treatment was assigned to 4 replicate rows with six apple trees per row. A single treatment with BAS 300 I at 0.26 L/ha and a positive control (Deltamethrin; 9.75 g a.s./ha) were tested in 1300 L water/ha against water-treated controls. Starting on the day of treatment (0DAT) and at +2DAT, +7DAT, +15DAT, +21DAT, +30DAT and +44DAT, 30 leaves were collected from two central trees of each replicate and *Typhlodromus pyri* were counted under a stereomicroscope.

Concerning the verification of the absence of prey, it was stated in the report that "the presence of *P. ulmi* (red spider mite, prey animal of *T. pyri*) was detected only on the test units of the toxic reference at +44DAT (2-6 individuals) and for an experimental unit 46 individuals. The absence of Eriophyidae was checked at 0DAT and +21DAT". This information is too concise for interpretation.

Results

The results are shown in the table below. Phytoseiidae counts in the control were sufficiently high to produce valid data. All phytoseiidae collected on day 0 and 44 were identified to be *T. pyri*. The positive control gave the expected response. Effect percentages for BAS 300 I according to Abbot and the Henderson-Tilton coefficient were calculated by the RMS from the reported mean numbers of mobile phytoseiidae per 30 leaves.

Since day 0 counts of phytoseiidae were comparable between treatments, the E-values calculated according to Abbot may be used for evaluation. Effects (36% reduction) were already noted at the first evaluation (2 days after treatment), and remained at comparable levels until study end (44 days after treatment). The difference from the control was statistically significant from day 7 onwards.

Table B.9.5.2-02 Effects of BAS 300 I on *Typhlodromus pyri* under field conditions

Treatment	Water control	Deltamethrin	BAS 300 I		
	Mean <i>T.pyri</i> per 30 leaves	Mean <i>T.pyri</i> per 30 leaves	Mean <i>T.pyri</i> per 30 leaves	% Effect (Abbot)	% Effect (Henderson-Stilton)
0DAT	22.75	21.50	22.50	n.a.	n.a.
+2DAT	20.27	0.50*	13.00	36	35
+7DAT	35.25	0.75*	18.75*	47	46
+15DAT	26.75	0.00*	17.50*	35	34
+21DAT	32.53	1.50*	18.25*	44	43
+30DAT	47.50	2.25*	24.25*	49	48
+44DAT	68.75	6.75*	38.00*	45	44

* Significantly different from control at 5% level

Conclusion

From 2 days after treatment until 44 days after treatment (study end): reduction of *T. pyri* ranging between 35% and 49% (no recovery). The study result is not taken to the List of Endpoints (see below).

Guidelines and limitations

1. The report was not signed. The study was conducted in 1996, after GLP regulations had come into force, but it was not performed under GLP. The results can therefore not be taken to the List of Endpoints. The study information may be used as supplementary information. The usefulness is limited since the dose in terms of g a.s./ha is unclear (see below), and meteorological conditions during the trial were not reported.
2. The test product was identified as "BAS 300 I". Its batch number and a.s. content were not specified. The name BAS 300 I refers to the active substance pyridaben, not to a formulation containing pyridaben. The tested dose was reported to be 0.02 L of BAS 300 I/hL in 1300 L water/ha. This suggests that the tested product was a liquid. The technical active substance BAS 300 I however is a solid at room temperature. Considering the low water solubility of pyridaben (12 µg/L), it is not practically possible to apply the unformulated technical active substance. The nature of the test material, and therefore also the tested dose in terms of g a.s./ha, are not unequivocally established on the basis of the information in the report.

Study 3

Characteristics

reference	:	Müther J. (2003a)	species	:	Field populations of arthropods in citrus orchard
type of study	:	Field study	exposure duration	:	139 days
year of execution	:	2001	dose	:	4.5 & 46.5 g a.s./ha
GLP statement	:	Yes	exposure method	:	Spraying (1X) of citrus trees
guideline	:	Blümel <i>et al.</i> (2000), Candolfi <i>et al.</i> (2000)	acceptability	:	Acceptable
test substance	:	BAS 300 11 I, batch OAJ030	conclusion	:	No significant effect on non-target arthropod population up to 46.5 g a.s./ha
a.s. content	:	765 g pyridaben/kg			

Methods

The effects of BAS 300 11 I (4.5 and 46.5 g pyridaben/ha, equivalent to 1.5% and 15.5% drift rate of 300 g a.s./ha) on non-target arthropods were studied under field conditions in a citrus (mandarin) orchard near Valencia in Spain. The study included a toxic reference (0.15% Dafeine, a.s. dimethoate, and 0.15% Kelthane, a.s. dicofol), and a water treated control. In a randomised block design, based on the pre-test number of predatory mites, the control and pyridaben treatments were allocated to three plots, and the toxic reference to two plots. Each plot consisted of ≥ 49 trees in at least 5 rows, with sampling limited to the inner rows. All treatments were applied once on 17 May 2001 in 2500 L water/ha using a mist blower at growth stage BBCH 73.

The sampling schedule took into consideration the normal decline in arthropod populations occurring in this region in the hot and dry period of July and August. The following samplings were performed:

1. Inventory sampling of the whole fauna of one tree per plot by knockdown treatment with dichlorvos, at -1DAT, +6DAT and +139DAT (3 October). The fauna was analysed on different taxonomic levels. Statistical evaluation (ANOVA followed by Dunnett's test, or, for inhomogeneous samples, U-test, $p \leq 0.05$) was undertaken for units with at least one organism per tree on average and which occurred at each sampling date.
2. Yellow water trap sampling for assessment of aerial arthropods: during a period of 3-5 days, traps filled with water and detergent were placed within the rows, at 2/3 of the crown height. Sampling was performed once before and 12 times after treatment, up to +141DAT. Statistical analysis was performed as for the inventory samples.
3. The number of motile stages and eggs of predatory mites and the number of spider mites were counted on 200-300 leaves per plot using a washing method, at -3DAT, +4DAT, +20DAT, +33DAT, +48DAT, +83DAT, +103DAT, +124DAT, +131DAT and +137DAT. Statistical analysis was performed as for the inventory samples. Efficacy values were calculated according to Abbott. Species determination was performed on in total 150 predatory mites from different samples.

In addition to the above observations, visual control for determination of foliar arthropods was also performed: 25 one year old branches per plot were searched for predatory/parasitic arthropods and for aphids at -3DAT, and at 12 occasions until +140DAT. As the numbers were low, no statistical analysis was

performed, and the observations were mainly used to check the development of arthropod populations in the field and to choose an optimum time for the final set of assessments, including the last inventory sampling.

Results

Inventory sampling

In total 1443 spiders were caught, comprising 14 families and 57 taxa; statistical analysis could be performed on 2 families and 2 taxa. In total 17808 insects were caught, comprising 13 orders and 124 families; statistical analysis could be performed on 4 orders/suborders, 10 families and 3 species.

Results for inventory samplings are summarised in Table B.9.5.2-03. This table shows statistically significant changes. Statistically significant changes, however, which were considered to be unrelated to treatment, have not been included (e.g., a change at pyridaben low dose without a corresponding change at the high dose). For hymenoptera, the two main groups considered separately were hymenoptera without ants and ants. The reason is that ants (formicidae) may have a negative impact on the abundance of non-target arthropods in trees due to their tendency to drive off potential natural enemies of aphids, which they harvest for honeydew as well as consume directly for food.

Table B.9.5.2-03 Statistically significant ($p \leq 0.05$) increases (↑) or reductions (↓), compared to the control, of arthropod populations from inventory sampling, after pyridaben low dose (T1), high dose (T2) or the toxic reference (TOX) treatment

Parameter	-1DAT			+6DAT			+139DAT		
	T1	T2	TOX	T1	T2	TOX	T1	T2	TOX
Arachnida, aranea						↓			
Arachnida, aranea, Salticidae						↓			↓
Arachnida, aranea, Salticidae, <i>Baltus chalybeius</i>									↓
Arachnida, aranea, Philodromidae						↓		↓	
Insecta, Coleoptera			↑			↓			
Insecta, Coleoptera, Coccinellidae			↑			↓			↑
Insecta, Coleoptera, Coccinellidae, <i>Scymnus</i> spp.					↓	↓			
Insecta, Diptera, Brachycera, Syrphidae			↑			↓			
Insecta, Diptera, Brachycera, Tachinidae								↓	
Insecta, Homoptera, <i>Aphis spiraeicola</i>			↑		↓	↓			↑
Insecta, Hymenoptera (excluding ants)		↑				↓		↓	
Insecta, Hymenoptera, Formicidae					↓	↓			↓
Insecta, Hymenoptera, Braconidae			↑					↓	↓
Insecta, Hymenoptera, Chalcididae									↓
Insecta, Hymenoptera, Dryinidae		↑		↓	↓	↓	↓	↓	↓
Insecta, Hymenoptera, Evaniidae			↑			↓			
Insecta, Hymenoptera, Ichneumonidae		↑	↑					↓	↓
Insecta, Hymenoptera, Pteromalidae		↑			↓	↓	↓	↓	
Insecta, Hymenoptera, Trichogrammatidae			↑		↓	↓			
Insecta, Psocoptera		↑			↓	↓			

The pyridaben treatments had no significant effects on spider populations, except for a reduction of Philodromidae at +139DAT at the highest dose. Since no effects were seen immediately after treatment, the relationship of this reduction to the treatment is uncertain.

The reduction of the Coccinellida *Scymnus* spp at +6DAT may have been correlated with the reduction of a possible prey for this species, the aphid *Aphis spiraeicola*, at the same time point. This is also suggested by the fact that at the 2nd and 3rd inventory sampling, the pattern of abundance for *Scymnus* and *A. spiraeicola* found in the various treatments coincided. The same may apply to the reduction in ant (Formicidae) numbers at the high pyridaben dose at +6DAT.

Reductions of several hymenopteran parasitic wasp families were observed. Some reductions were only observed at +139DAT, mostly at the highest pyridaben dose, and may be fortuitous or not a direct effect. Other reductions of hymenopteran families (and that of Psocoptera) were observed at +6DAT at the high dose, but not anymore at +139DAT. At the low pyridaben dose the numbers of Pteromalidae at +6DAT exceeded those of the control, but at +139DAT they were significantly lower than those of the control. It is uncertain therefore, that the statistically significant reduction of Pteromalidae at the low pyridaben dose at +139DAT is (directly) related to the treatment. Since at +139DAT the numbers of Pteromalidae at the low pyridaben dose were lower than those of the high pyridaben dose, the statistically significant reduction at the latter dose may also not be (directly) related to the treatment. A consistent effect at both samplings was only observed for Driinidae (Hymenoptera), at both pyridaben doses.

The toxic reference treatment had significant effects on spider and insect populations at +6DAT and +139DAT.

Yellow water traps for aerial insects

Results for yellow water trap samplings are summarised in Table B.9.5.2-04, which shows statistically significant changes. Statistically significant changes, however, which were considered to be unrelated to treatment, have not been included (e.g., due to a particularly high control value). At the high pyridaben dose, the total number of arthropods was reduced at +15DAT - +19DAT, and Tachinidae populations were reduced at +6DAT.

Table B.9.5.2-04 Statistically significant ($p \leq 0.05$) increases ($\uparrow$) or reductions ($\downarrow$), compared to the control, of aerial arthropod populations from yellow water traps, after pyridaben low dose (T1), high dose (T2) or the toxic reference (TOX) treatment

Parameter	DAT	T1	T2	TOX
Arthropods, total number ^(A)	+15		$\downarrow$	$\downarrow$
	+19		$\downarrow$	$\downarrow$
Insecta, Diptera, Brachycera, Tachinidae	+6		$\downarrow$	

^(A) A statistically significant reduction at +141DAT at the high pyridaben dose (16 insects/trap versus 27 in the control) was considered unrelated to the treatment. This was confirmed by the lack of effect at previous assessments up to +127DAT.

Predatory mite counts

The predatory mite density in the test plots was 8-11 mites per 100 leaves before treatment. This density is low, but likely to be normal for the ecological conditions of the trial site. Due to climatic conditions, the predatory mite populations decreased during summer and almost disappeared in the very hot month of August. The author of the report stated that this can be considered as normal in the region of the test site. Effects on predatory mites are summarised in Table B.9.5.2-05. Efficacy values calculated for sampling dates with very low predatory mite numbers in the control ($< 2/100$ leaves) are not reliable.

Table B.9.5.2-05 Effect (efficacy according to Abbott) on predatory mites of pyridaben low dose (T1), high dose (T2) or the toxic reference (TOX) treatment

DAT	Date	T1	T2	TOX
+4	21-May	12	13	34
+20	6-Jun	4	18	80*
+33	19-Jun	-49	-29	67
+48	4-Jul	19	0	64
+83	8-Aug	(33) ^(A)	(67) ^(A)	(100) ^(A)
+103	28-Aug	(0) ^(A)	(75) ^(A)	(12) ^(A)
+124	18-Sep	(-256) ^(A)	(-56) ^(A)	(50) ^(A)
+131	25-Sep	(-75) ^(A)	(-101) ^(A)	(25) ^(A)
+137	1-Oct	24	10	74

* Statistically significant difference from the control at the 5% level

^(A) Values calculated with very low predatory mite numbers in the control ($< 2/100$ leaves) and hence not reliable

There were no statistically significant effects of pyridaben treatments on predatory mite populations. The highest percent reduction was 24% and 18% at the low and high dose, respectively. The toxic reference treatment had the expected effect (80% reduction at +20DAT, $> 50\%$ at later times).

There were no statistically significant effects of pyridaben treatments on predatory mite eggs. The toxic reference treatment only had a statistically significant effect at +83DAT, but this is unreliable as it was based on very low egg numbers in the control.

The number of spider mites was very low during the study (control: 1 per sample of 100 leaves before treatment, increasing to 14 per sample of 100 leaves at +48DAT, then falling to 0.33 per sample of 100 leaves at +137DAT). There were no statistically significant effects of pyridaben treatments on spider mite populations. The toxic reference treatment only had a statistically significant reduction at +83DAT.

The predatory mites were determined to be 94.7% *Euseius stipulatus* and 5.3% *Typhlodromus phialatus*.

Overall evaluation by RMS

In laboratory studies, pyridaben was highly toxic to predatory mites and parasitic wasps (14-day and 48-h LR50 0.59 and 0.24 g a.s./ha). Significant effects in the field, if any, are therefore expected to occur early after treatment. The early inventory sampling at +6DAT is therefore appropriately scheduled. This sampling, together with the last inventory sampling (3 October), provided data to assess effects on and recovery of a sufficient number of arthropod families/species. Data from yellow water traps supported this assessment. The initial population level of predatory mites was sufficiently high to provide valid data for this category, which was monitored sufficiently frequently and up to the end of the season.

Treatment with the toxic reference product gave significant reductions of the predatory mite populations from 20 days after treatment, confirming the validity of the study. Treatment under field conditions with pyridaben up to 46.5 g a.s./ha had no significant effect on predatory mite populations. It had also no significant effect on spider populations. Reductions of several families or species of insects (e.g., the Coccinellida *Scymnus* spp. and several hymenopteran parasitic wasps) were observed at 6-19 days after treatment, mostly at the high pyridaben dose. They could however be the results of treatment related reduced prey (aphid) availability, whilst the lack of effects at 139 days showed that the populations had the potential to recover. A direct relationship to the treatment is uncertain for effects on insects noted only at 139 days after treatment. A consistent effect at both samplings was only observed for Driinidae (Hymenoptera), at both pyridaben doses. In all, the following were subjected to statistical analysis: 2 families and 2 species of spiders; predatory mites (2 species); 4 orders/suborders, 10 families and 3 species of insects. Many more insect species and families however were present in the orchard, but not subjected to statistical evaluation due to low numbers and/or because they were not found in the control at all sampling times. No consistent effects could be demonstrated for any of these insect families/species, except for the parasitic wasp family of Driinidae, which represented 0.9% of all insects in the control. Based on these results, it is concluded that pyridaben applied in citrus orchard in Europe-S up to 46.5 g a.s./ha will not have any significant effects at the overall non-target arthropod population level.

Conclusion

Pyridaben applied in citrus orchard in Spain up to 46.5 g a.s./ha did not have any significant effects on non-target arthropod populations.

Guidelines and limitations

The level of replication (3 plots for each pyridaben treatment) is lower than e.g. recommended by Candolfi *et al.* (IOBC, 2000) for field trials on effects on predatory mites in orchards (at least 5 per treatment). The results for predatory mites are reliable, as effect percentages were low throughout and the reduced statistical power is compensated by a high number of observation times. Visual inspection of the population levels in control and pyridaben treatments did not suggest that any biologically relevant reductions were left out of the evaluation.

Study 4

Characteristics

reference	:	Müther J. (2003b)	species	:	Field populations of arthropods in apple orchard
type of study	:	Field study	exposure duration	:	63 days
year of execution	:	2001	dose	:	3.75 & 39 g a.s./ha
GLP statement	:	Yes	exposure method	:	Spraying (1X) of apple trees
guideline	:	Blümel <i>et al.</i> (2000), Candolfi <i>et al.</i> (2000)	acceptability	:	Acceptable
test substance	:	BAS 300 11 I, batch OAJ030	conclusion	:	No significant effect on non-target arthropod population up to 39 g a.s./ha
a.s. content	:	765 g pyridaben/kg			

Methods

The effects of BAS 300 11 I (3.75 and 39 g pyridaben/ha, equivalent to 1.5% and 15.5% drift rate of 250 g a.s./ha) on non-target arthropods were studied under field conditions in an apple orchard in Traenheim in the Alsace region of Northern France. The study included a toxic reference (Decis, a.s. deltamethrin, 11.25 g a.s./ha) and a water treated control. In a randomised block design, based on the pre-test number of predatory mites, the control and pyridaben treatments were allocated to three plots, and the toxic reference to two plots. Each plot consisted of 5 rows with 14 trees in each of the inner rows, with sampling limited to the inner rows. All treatments were applied once on 14 June 2001 in 1000 L water/ha using a radial sprayer at growth stage BBCH 73-74.

The following samplings were performed:

1. Inventory sampling of the whole fauna of one tree per plot by knockdown treatment with dichlorvos, at -1DAT, +7DAT and +63DAT (16 August). The fauna was analysed on different taxonomic levels.
2. Visual control for determination of foliar arthropods: 25 one year old branches per plot were searched for predatory/parasitic arthropods and for aphids at -2DAT, +5DAT, +12DAT, +22DAT, +27DAT, +36DAT, +41DAT, +50DAT, +55DAT and +61DAT.
3. Yellow water trap sampling for assessment of aerial arthropods: during a period of 3-5 days, traps filled with water and detergent were placed within the rows, at 2/3 of the crown height. Sampling was performed at the same times as visual control observations.
4. The number of motile stages and eggs of predatory mites and the number of spider mites were counted on ≥50 leaves per plot using a washing method, at -3DAT, +5DAT, +22DAT, +36DAT, +50DAT and +61DAT. Efficacy values were calculated according to Abbott. Species determination was performed on in total 166 predatory mites from different samples.

Statistical evaluation (ANOVA followed by Dunnett's test, or, for inhomogeneous samples, U-test, $p \leq 0.05$) was undertaken for units with at least one organism per tree on average and which occurred at each sampling date.

Results

Inventory sampling

In total 529 spiders were caught, comprising 12 families. Statistical analysis could only be performed on the mean number of spiders per tree, as the number of spiders in single families or on species level was too low. This analysis showed no statistically significant effect of any treatment, except for a reduction in the toxic reference substance at +63DAT.

In total 53639 insects were caught, comprising 13 orders and 104 families; statistical analysis could be performed on 5 orders/suborders, 11 families and 2 species. Results for inventory samplings of insects are summarised in Table B.9.5.2-06, which shows all statistically significant changes. For hymenoptera, the two main groups considered separately were hymenoptera without ants and ants. The reason is that ants (formicidae) may have a negative impact on the abundance of non-target arthropods in trees due to their tendency to drive off potential natural enemies of aphids, which they harvest for honeydew as well as consume directly for food.

Table B.9.5.2-06 Statistically significant ($p \leq 0.05$) increases (↑) or reductions (↓) of insects, compared to the control, of arthropod populations from inventory sampling, after pyridaben low dose (T1), high dose (T2) or the toxic reference (TOX) treatment

Parameter	-1DAT			+7DAT			+63DAT		
	T1	T2	TOX	T1	T2	TOX	T1	T2	TOX
Coleoptera, Curculionidae, <i>Polydrusus impressifrons</i>				↑	↑	↓			
Diptera, Brachycera, Syrphidae		↓					↑	↑	↑
Diptera, Brachycera, Muscidae		↑			↓	↓	↑	↑	↑
Diptera, Brachycera, Empididae								↑	↑
Diptera, Nematocera, Mycetophilidae			↑	↓	↓	↓			
Diptera, Nematocera, Sciaridae						↓			
Homoptera, Sternorrhyncha	↓	↓							
Hymenoptera						↓	↓		
Hymenoptera (without ants)		↑	↑			↓	↓		
Hymenoptera, Formicidae						↓	↓		
Hymenoptera, Pteromalidae		↑					↓		
Hymenoptera, Ichneumonidae	↓					↓			
Hymenoptera, Eupelmidae		↑	↑			↓		↑	↑
Hymenoptera, Aphelinidae	↓	↓	↓		↓	↓	↓		

At both pyridaben doses, increases of Coleoptera, *P. impressifrons* and reductions of Diptera, Nematocera, Mycetophilidae were observed at +7DAT, but not anymore at +63DAT. At the high pyridaben dose, reductions of Diptera, Brachycera, muscidae and Hymenoptera, Aphelinidae were observed at +7DAT, but the levels of the latter populations were already reduced at the pre-test count, and the levels of both populations were not reduced anymore at +63DAT. The reductions of several populations at the low dose pyridaben at +63DAT are considered to be unrelated to treatment, without a corresponding change at the

high dose. Increases of several populations at the high pyridaben dose or at both pyridaben doses at +63DAT are not considered to be adverse effects. It is concluded that the inventory counts revealed no significant effects on non-target arthropod populations. The toxic reference treatment had significant effects on insect populations at +7DAT.

Visual control assessments

At no time point during the study, there were remarkable differences in aphid infestation between the different treatments. The numbers observed of most orders/suborders of beneficial arthropods were too low for statistical analysis, which, therefore, was only undertaken for the total number of arthropods, and for predatory larvae of the species *Aphidoletes aphidimyza* (Diptera, Cecidomyiidae), which reached a peak level in the control at +22DAT, then declined to low numbers at +27DAT and afterwards. Statistically significant changes for visual control assessments are summarised in Table B.9.5.2-07.

Table B.9.5.2-07 Statistically significant ($p \leq 0.05$) increases (↑) or reductions (↓) of insects, compared to the control, of arthropod populations from visual control assessments, after pyridaben low dose (T1), high dose (T2) or the toxic reference (TOX) treatment

Parameter		T1	T2	TOX
Beneficial arthropods	+36DAT	↑		
Beneficial arthropods	+41DAT		↑	
Beneficial arthropods	+61DAT	↓		↓
Diptera, Cecidomyiidae, <i>Aphidoletes aphidimyza</i>	+22DAT		↓ ^(A)	↓ ^(B)
Diptera, Cecidomyiidae, <i>Aphidoletes aphidimyza</i>	+27DAT		↓	↓

^(A) Reduction of 60% compared to the control, but not statistically significant

^(B) Reduction of 86% compared to the control, but not statistically significant

Increases of the total number of beneficial arthropods at the low and high pyridaben dose at +36DAT and +41DAT are not considered to be adverse effects. A reduction at the low dose pyridaben at +61DAT is considered to be unrelated to treatment, without a corresponding change at the high dose. Reductions of *A. aphidimyza* at +22DAT - +27DAT high pyridaben dose had a temporary character. It is concluded that the visual assessments revealed no significant effects on non-target arthropod populations.

Yellow water traps for aerial insects

In total 7460 insects were caught and 3 predatory mites (excluded from analysis), comprising 9 orders and 113 families; statistical analysis could be performed on the total number of insects and on 2 orders and 1 family. Results for yellow water trap samplings are summarised in Table B.9.5.2-08, which shows statistically significant changes.

Table B.9.5.2-08 Statistically significant ($p \leq 0.05$) increases (↑) or reductions (↓), compared to the control, of aerial arthropod populations from yellow water traps, after pyridaben low dose (T1), high dose (T2) or the toxic reference (TOX) treatment

Parameter	DAT	T1	T2	TOX
Insecta	+12	↓		
	+41			↓
Insecta, Hymenoptera	+5		↑	
	+22	↓		
	+36		↑	
Insecta, Coleoptera	+12	↓	↓	
	+22			↓

Increases of the total number of certain populations at the high pyridaben dose are not considered to be adverse effects. A reduction at the low dose pyridaben of certain populations, without a corresponding change at the high dose, is considered to be unrelated to treatment. Reductions of Coleoptera at +12DAT at both pyridaben doses had a temporary character. It is concluded that there were no significant effects on aerial non-target arthropod populations.

Predatory mite counts

The predatory mite density in the test plots was 2.2-2.8 mites per leaf before treatment. Effects on predatory mites are summarised in Table B.9.5.2-09.

Table B.9.5.2-09 Effect (efficacy according to Abbott) on predatory mites of pyridaben low dose (T1), high dose (T2) or the toxic reference (TOX) treatment

DAT	Date	T1	T2	TOX
+5	19-Jun	-14	-21	51
+22	6-Jul	-20	13	50*
+36	20-Jul	-1	25	82*
+50	3-Aug	6	12	36
+61	14-Aug	-16	-8	36

* Statistically significant difference from the control at the 5% level

There were no statistically significant effects of pyridaben treatments on predatory mite populations. The highest percent reduction was 6% and 25% at the low and high dose, respectively. The toxic reference treatment had the expected effect.

The only statistically significant reduction of the number of predatory mite eggs in pyridaben treatments was found at the low dose at +22DAT, but numbers at the high pyridaben dose at this time point were comparable, and not statistically different from the control. Egg numbers in the toxic reference treatment were lower than the control at all times. The number of spider mites was very low during the study and there were no statistically significant effects of pyridaben treatments or the toxic reference treatment on spider mite populations.

The predatory mites were determined to be 100% *Typhlodromus pyri*.

Overall evaluation by RMS

In laboratory studies, pyridaben was highly toxic to predatory mites and parasitic wasps (14-day and 48-h LR50 0.59 and 0.24 g a.s./ha). Significant effects in the field, if any, are therefore expected to occur early after treatment. The early inventory sampling at +7DAT is therefore appropriately scheduled. This sampling, together with the last inventory sampling (+63DAT, 16 August) provided data to assess effects on and recovery of a sufficient number of arthropod families/species. Data from yellow water traps supported this assessment. The initial population level of predatory mites was sufficiently high to provide valid data for this category, which was monitored sufficiently frequently and long enough.

Treatment with the toxic reference product gave significant reductions of the predatory mite populations from 5 days after treatment, confirming the validity of the study. Treatment under field conditions with pyridaben up to 39 g a.s./ha had no significant effect on populations of predatory mites and other beneficial arthropods.

Conclusion

Pyridaben applied in an apple orchard in France-N up to 39 g a.s./ha did not have any significant effects on non-target arthropod populations.

Guidelines and limitations

The level of replication (3 plots for each pyridaben treatment) is lower than e.g. recommended by Candolfi *et al.* (IOBC, 2000) for field trials on effects on predatory mites in orchards (at least 5 per treatment). The results for predatory mites are reliable, as effect percentages were low throughout, the possibly reduced statistical power is compensated by a high number of observation times, and standard deviations for predatory mite numbers in control and pyridaben treatments were low. The numbers of other beneficial arthropods during the study were variable, but visual inspection of the population levels in control and pyridaben treatments did not suggest that any biologically relevant reductions were left out of the evaluation.

B.9.5.3 Risk assessment

B.9.5.3.1 Risk of the active substance

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002) and ESCORT 2. For the proposed use in tomato (single application rate of 0.25 kg a.s./ha) and citrus (single application rate of 0.30 kg a.s./ha), it is assumed for in-field environments that 100% of the dose will be deposited. For the use in citrus, it is assumed for off-field environments that 15.73% (90th percentile, 1 application, 3 m buffer zone, late stage) of the dose will be deposited in the non-target area. For the application in tomato (greenhouse), off-field exposure is not applicable. Table B.9.5.3.1-01 below summarises the results of standard laboratory testing with non-target arthropod species.

Table B.9.5.3.1-01 Summary of effects of Pyridaben 75WP on non-target arthropod species during laboratory testing on inert substrate

Species	Life stage	Test type, substrate and duration	Actual dose tested (g a.s./ha)	Endpoint	Effect (%) at respective dose in test and LR50 and ER50 values
<i>Typhlodromus pyri</i>	Protonymphs	Laboratory, glass, 14 days	0.05; 0.10; 0.20; 0.40; 0.80; 1.6 (fresh residues)	Mortality Reduction of reproduction	4.3; 4.3; -4.4 ^(A) ; 13; 84; 99 LR50 (g a.s./ha): 0.59 37; 26; 33; 36; n.a. ^(B) ; n.a. ^(B) ER50 (g a.s./ha): >0.40
<i>Aphidius rhopalosiphi</i>	Adults	Laboratory, glass, 2 days	0.05; 0.10; 0.20; 0.40; 0.80; 1.6 (fresh residues)	Mortality Reduction of reproduction	0; 5; 60; 73; 88; 100 LR50 (g a.s./ha): 0.24 35; -17 ^(A) ; 48; 48; n.a. ^(B) ; n.a. ^(B) ER50 (g a.s./ha): 0.20

^(A) Note: negative effect %, hence no adverse effect

^(B) n.a. = not applicable (insufficient survivors from initial phase to assess reproduction)

According to Annex II of 91/414, data must be submitted on two sensitive species and two species that are relevant to the intended use. Annex II refers to the SETAC Guidance Document on Regulatory Testing Procedures for Pesticides with Non-target Arthropods (Barrett *et al.*, 1994) as a source of guidance for testing. Since several limitations have been identified in this Guidance document it is proposed that the risk to non-target arthropods both in and off-field should be addressed according to the recommendations of ESCORT 2.

A Tier I assessment is performed using the data from the initial laboratory studies on inert substrate with *T. pyri* and *A. rhopalosiphi*. For this standard risk assessment, the following scenarios are used to describe the exposure in-field and off-field. For both the key input is the nominal field application rate supplemented by various factors:

in-field exposure = Application rate * MAF

off-field exposure = Application rate * MAF * (drift factor / vegetation distribution factor)

As the proposed worst-case use of the Pyridaben 75 WP formulation involves 1 application at the highest dose (citrus in Southern Europe, 300 g a.s./ha), the multiple application factor (MAF) for the maximum dose is 1. The drift factor for off-field exposure was based on the 90th percentile drift data and corrected by a “vegetation distribution factor” of 10%.

The LR50 for *Typhlodromus pyri* and *Aphidius rhopalosiphi* on fresh dried residue on glass was determined as 0.59 and 0.24 g a.s./ha, respectively. These values were used for hazard quotient (HQ) calculation for in-field and off-field exposure: in-field HQ= in-field exposure/LR50 and off-field exposure= (off-field exposure/LR50)*uncertainty factor. An uncertainty (safety) factor of 10 was included in the off-field Hazard Quotient calculations to account for uncertainty with the extrapolation from *Typhlodromus pyri* and *Aphidius rhopalosiphi* as indicator species, to all off-field non-target arthropods. The risk is considered acceptable if HQ <2. The table below summarises the results.

Table B.9.5.3.1-02 Risk to *Typhlodromus pyri* and *Aphidius rhopalosiphi* as a result of in-field (0 m) and off-field (3 m) exposure of the proposed Pyridaben 75 WP formulation in tomato in Northern and Southern Europe and citrus in Southern Europe (Hazard Quotient Approach; vegetation distribution factor off-field of 10), based on LR50 and ER50 values from inert substrate

Crop / species	dose g as/ha	distance (m)	% drift	Exposure (g a.s./ha)	LR50 (g a.s./ha)	HQ	trigger value
<i>Typhlodromus pyri</i>							
Tomato EU N & S	250	0	-	250	0.59	424	2
Citrus EU S	300	0	-	300	0.59	508	2
		3	15.73	4.7	0.59	80	2
<i>Aphidius rhopalosiphi</i>							
Tomato EU N & S	250	0	-	250	0.24	1042	2
Citrus EU S	300	0	-	300	0.24	1250	2
		3	15.73	4.7	0.24	197	2

The HQs for in-field exposure are above the trigger of 2 for both applications. The HQs for off-field exposure are above 2 for the application in citrus. The off-field HQ values for *T. pyri* and *A. rhopalosiphi* are only below the trigger value of 2 if buffer zones of at least 40 and 75 m are used, respectively (*T. pyri*, 40 m and *A. rhopalosiphi*, 75 m: HQ=1.6 and 1.4, respectively). Such large buffer zones are considered unrealistic, and therefore, a risk is considered to be present.

Since the in-field HQ values for both indicator species are above the trigger of 2 for both applications, and the off-field HQ values are above 2 for the application in citrus, higher tier data are required for these two species, as well as for two additional species. Data were submitted from extended laboratory studies with *T. pyri*, *A. rhopalosiphi*, *C. carnea* and *E. formosa*, and from greenhouse studies with *E. formosa* and *O. niger*. In addition, data were submitted from field studies with *N. californicus* & *P. ulmi* and *T. pyri*, as well as from field studies with field populations of arthropods in a citrus orchard in Spain and an apple orchard in France. The extended and greenhouse study with *E. formosa* and the field studies with *N. californicus* & *P. ulmi* and *T. pyri* were not acceptable due to the lack of information on study procedures and test substance identification, and the greenhouse studies with *E. formosa* and *O. niger* were concluded to be useful only as supplementary information.

Therefore, the data from the extended laboratory studies with *T. pyri*, *A. rhopalosiphi* and *C. carnea*, and the field studies with field populations of arthropods in a citrus orchard in Spain and an apple orchard in France are used for a higher tier risk assessment. Results from these studies are summarised in the table below.

Table B.9.5.3.1-03 Summary of effects of Pyridaben 75WP (*T. pyri* and *A. rhopalosiph*) and Nexter (BAS 300 11 I; *C. carnea*) on non-target arthropod species during extended laboratory testing

Species	Life stage	Test type, substrate and duration	Actual dose tested (g a.s./ha)	Endpoint	Effect (%) at respective dose in test and LR50 and ER50 values
<i>Typhlodromus pyri</i>	Protonymphs	Extended laboratory, fresh residues on apple leaf discs, 14 days	47; 200; 300	Mortality Reduction of reproduction	80; 96; 93 LR50 (g a.s./ha): <47 n.a. ^(A) ; n.a. ^(A) ; n.a. ^(A) ER50 (g a.s./ha): <47
		Extended laboratory, 7-day aged residues on apple leaf discs, 14 days	47; 200; 300	Mortality Reduction of reproduction	86; 96; 99 LR50 (g a.s./ha): <47 n.a. ^(A) ; n.a. ^(A) ; n.a. ^(A) ER50 (g a.s./ha): <47
		Extended laboratory, 14-day aged residues on apple leaf discs, 14 days	47; 200; 300	Mortality Reduction of reproduction	3.2; 56; 80 LR50 (g a.s./ha): 155 2; n.a. ^(A) ; n.a. ^(A) ER50 (g a.s./ha): >47
		Extended laboratory, 21-day aged residues on apple leaf discs, 14 days	47; 200; 300	Mortality Reduction of reproduction	1.1; 7.7; 46 LR50 (g a.s./ha): 300 1; 11; 17 ER50 (g a.s./ha): >300
		Extended laboratory, 28-day aged residues on apple leaf discs, 14 days	300	Mortality Reduction of reproduction	32 LR50 (g a.s./ha): >300 22 ER50 (g a.s./ha): >300
<i>Aphidius rhopalosiph</i>	Adults	Extended laboratory, fresh residues on apple leaves, 2 days	47; 200; 300	Mortality Reduction of reproduction	92; 100; 100 LR50 (g a.s./ha): <47 n.a. ^(A) ; n.a. ^(A) ; n.a. ^(A) ER50 (g a.s./ha): <47
		Extended laboratory, 7-day aged residues on apple leaves, 2 days	47; 200; 300	Mortality Reduction of reproduction	70; 100; 100 LR50 (g a.s./ha): <47 n.a. ^(A) ; n.a. ^(A) ; n.a. ^(A) ER50 (g a.s./ha): <47
		Extended laboratory, 14-day aged residues on apple leaves, 2 days	47; 200; 300	Mortality Reduction of reproduction	43; 98; 100 LR50 (g a.s./ha): 56 71; n.a. ^(A) ; n.a. ^(A) ER50 (g a.s./ha): <47
		Extended laboratory, 21-day aged residues on apple leaves, 2 days	47; 200; 300	Mortality Reduction of reproduction	7.9; 68; 92 LR50 (g a.s./ha): 123 0; 91; n.a. ^(A) ER50 (g a.s./ha): 104
		Extended laboratory, 28-	47; 200; 300	Mortality	13; 36; 82 LR50 (g a.s./ha): 172

Species	Life stage	Test type, substrate and duration	Actual dose tested (g a.s./ha)	Endpoint	Effect (%) at respective dose in test and LR50 and ER50 values
		day aged residues on apple leaves, 2 days		Reduction of reproduction	52; 78; n.a. ^(A) ER50 (g a.s./ha): 42
		Extended laboratory, 35-day aged residues on apple leaves, 2 days	47; 200; 300	Mortality Reduction of reproduction	0; 35; 74 LR50 (g a.s./ha): 208 8; 12; 70 ER50 (g a.s./ha): 261
		Extended laboratory, 42-day aged residues on apple leaves, 2 days	200; 300	Mortality Reduction of reproduction	18; 60 LR50 (g a.s./ha): 272 77; 32 ER50 (g a.s./ha): >300
		Extended laboratory, 49-day aged residues on apple leaves, 2 days	200; 300	Mortality Reduction of reproduction	42; 2.8 LR50 (g a.s./ha): >300 -144 ^(B) ; -103 ^(B) ER50 (g a.s./ha): >300
<i>Chrysoperla carnea</i>	2 nd instar larvae	Extended laboratory, fresh residues on field treated apple leaves, until adult emergence	53; 247; 308	Reduction of reproduction	6; -32 ^(B) ; -35 ^(B) ER50 (g a.s./ha): >308
		Extended laboratory, 15-day aged residues on field-treated apple leaves, until adult emergence	53; 247; 308	Reduction of reproduction	42; -4 ^(B) ; 36 ER50 (g a.s./ha): >308

^(A) n.a. = not applicable (insufficient survivors from initial phase to assess reproduction)

^(B) Note: negative effect %, hence no adverse effect

For the higher tier risk assessment, scenarios are similar to those used in the Tier I assessment, with the following exceptions: the application rate is multiplied by a factor of 0.5 for the application in citrus (3-dimensional crop situation); for off-field, the vegetation distribution factor is not included since in the studies, whole plants were treated; for off-field, the uncertainty factor is 5. The risk is considered acceptable if the effects at the calculated in- and off-field rates are <50% (ESCORT 2). The in-field rate calculated for the application in tomato is 250 g a.s./ha. The in- and off (3 m) -field exposure rates for citrus, calculated according to the methods in ESCORT 2 (i.e. for off-field with omission of the vegetation distribution factor of 10), are 150 and 118 g a.s./ha, respectively.

Table B.9.5.3.1-04 gives an overview of the LR50 and ER50 values obtained in the extended laboratory studies with *T. pyri*, *A. rhopalosiphi* and *C. carnea*.

Table B.9.5.3.1-04 LR50 and ER50 values for *Typhlodromus pyri*, *Aphidius rhopalosiphi* and *C. carnea* as a result of exposure to the proposed Pyridaben 75 WP formulation in extended laboratory studies with fresh and aged residues

Ageing of residues (days)	<i>T. pyri</i>		<i>A. rhopalosiphi</i>		<i>C. carnea</i>	
	LR50 (g a.s./ha)	ER50 (g a.s./ha)	LR50 (g a.s./ha)	ER50 (g a.s./ha)	LR50 (g a.s./ha)	ER50 (g a.s./ha)
0	<47	<47	<47	<47	>308	>308
7	<47	<47	<47	<47		
14	155	>47	56	<47	>308 ^(A)	>308 ^(A)
21	300	>300	123	104		
28	>300	>300	172	42		
35			208	261		
42			272	>300		
49			>300	>300		

^(A) 15-day aged residues instead of 14-day aged residues

Effects >50% on survival and reproduction were observed in the extended laboratory studies with *T. pyri* and *A. rhopalosiphi* when exposed to fresh residues at a dose rate of 47 g a.s./ha, which is below the calculated worst-case in- and off-field rates of 250 and 118 g a.s./ha, respectively. With ageing of the residues, the effects decreased. After 21 and 42 days, respectively, the LR50 and ER50 values for *T. pyri* and *A. rhopalosiphi* were above the in-field dose rates in tomato and citrus of 250 and 150 g a.s./ha. After 21 and 35 days, respectively, the LR50 and ER50 values for *T. pyri* and *A. rhopalosiphi* are above the off-field rate of 118 g a.s./ha as well. Effects <50% were observed in the extended laboratory study with *C. carnea* at 308 g a.s./ha, which is above the highest in- and off-field rates of 250 and 118 g a.s./ha, respectively. This data for the two indicator species and for one additional species is sufficient to demonstrate that there is potential of recovery of affected in-field populations of non-target arthropods after treatment of tomato. However, for off-field populations, aged-residue studies are not sufficient to address recovery, because for off-field it is less sure that recolonisation is possible. Off-field areas will often be narrow, and especially for the less mobile surface dwellers recolonisation will be difficult. Therefore, for off-field actual recovery must be shown, not just potential for recovery as done with an aged-residue test. Also, since the Tier I assessment for citrus showed that there was an in-field and an off-field risk, testing of two additional species under extended laboratory conditions is required.

During field studies with field populations of arthropods in a citrus orchard in Spain and an apple orchard in France, no significant effects were observed at the highest tested dose rates of 47 and 39 g a.s./ha, respectively. These dose rates are lower than the in- and off-field rates calculated for citrus. However, bigger buffer zones can be taken into account. The off-field rate for citrus using a buffer zone of 10 m (drift value 3.60%) is 27 g a.s./ha. Therefore, these field studies address the potential for recovery of affected arthropod populations for the application in citrus, provided that a buffer zone of 10 m is used.

It should be noted that in this approach, the calculation of the off-field rate includes the correction factor of 5. This factor, meant to cover inter-species variability in sensitivity of off-field non-target arthropod species, could be lowered considering the fact that in the field tests many different species were tested. However, ESCORT 2 does not specify how much it should be lowered, and no consensus has been reached so far.

about this. RMS is of the opinion that some kind of safety factor should still be applied, for the following reasons: 1) In the field studies, surface dwellers were not included in the sampling, while surface dwellers make up an important part of the off-field fauna. 2) The species sensitivity distributions of in-field and off-field fauna might differ considerably. Since citrus needs regular use of PPPs, it is to be expected that the in-field species present in a citrus crop have been selected on their high capacity for recovery after disturbance (from e.g. PPPs), which means that they have a higher recovery capacity than the off-field species. Also, the off-field community is more diverse than the in-field community.

In the case of pyridaben, using a correction factor of 2 would lead to an off-field drift rate of 47 g a.s./ha and thus to an acceptable risk without the use of buffer zones. However, due to the uncertainties and lack of knowledge surrounding the issue of correction factors, RMS does not propose any lowered correction factor.

Overall conclusion

The in-field risk to non-target arthropods for the application in tomato is acceptable. For this greenhouse application, off-field exposure is not applicable. Tier I assessment indicates an in- and an off-field risk to non-target arthropods as a result of treatment of citrus, but extended laboratory studies with two standard species and one additional species (*C. carnea*) suggest that there is potential of recovery of affected in- and off-field populations of non-target arthropods. Two field studies confirm this. However, the test dose rates in these field studies are lower than the in- and off-field rates for citrus. Therefore, the in- and off-field risk in citrus is also acceptable, provided that a buffer zone of 10 m is used.

B.9.5.3.2 Risk of metabolites

According to the Guidance Document on terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10392/2002 rev 2 final of 17 October 2002), the risk of plant metabolites to arthropods should be addressed.

A risk assessment is performed for metabolites formed at a maximum level of at least 10% TRR in plants. In plant metabolism studies (section B.7.1), individual identified metabolites were at the most 5.1% TRR in apple fruit (25 days after treatment), 4.6% TRR in citrus fruit (7 days after the second treatment) and 1.1% TRR in tomato fruit (14 days after treatment). Hence no individual identified metabolite exceeded 10% TRR, and no assessment of plant metabolites is made.

B.9.6 Effects on earthworms (IIA 8.4, IIIA 10.6.1)**B.9.6.1 Toxicity****B.9.6.1.1 Toxicity of the active substance****Characteristics**

Reference	: Coulson J.M. <i>et al.</i> (1989)	exposure duration	: 14 days
type of study	: Acute toxicity	nominal	: 0, 1.0, 10, 18, 32, 56 and 100 mg
year of execution	: 1989	concentrations	: a.s./kg soil
GLP statement	: Yes	dosing method	: Test substance was dissolved in acetone and mixed with soil.
Guideline	: OECD 207	acceptability	: Acceptable
test substance	: NC-129, Lot no. 129T8702	LC50	: 38 mg a.s./kg soil
Purity	: 97.9%		
Species	: <i>Eisenia foetida</i>		

Methods

In an acute toxicity study, earthworms (*Eisenia fetida*) were exposed to NC-129 (pyridaben) for 14 days in artificial soil (OECD 207 substrate, with 10% organic matter, 5.8% oc). The test was conducted in 1 L glass beakers closed with perforated cling film, containing 750 g artificial moist soil. Nominal soil concentrations of 1.0, 10, 18, 32, 56 and 100 mg a.s./kg soil, the control and the positive control (2-chloroacetamide, 32, 56 and 100 mg a.s./kg soil) were tested in 4 replicates of 10 worms each. Jars were maintained at 20±2°C under continuous light. Soil moisture was maintained at 50±5% of the dry weight. The study was conducted according to OECD 207 and GLP.

Results

No behavioural changes were observed at any concentration. The positive control gave the expected response. Other results are summarised in Table B.9.6.1.1-01. The LC50 was 38 mg a.s./kg soil.

Table B.9.6.1.1-01 Acute toxicity of NC-129 to *Eisenia fetida*

Nominal concentration (mg a.s./kg soil)	Mortality at 14 days (%)	Mean weight change survivors over 14 days (%)
Control	0	-2.2
1.0	0	-0.6
10	0	-7.3
18	0	-12.6*
32	28	-22.6*
56	93	-
100	100	-

* Significantly different from control at 5% level

Conclusion

LC50 38 mg a.s./kg soil (5.8% oc).

Guidelines and limitations

The test is acceptable.

B.9.6.1.2 Toxicity of metabolites (IIA 8.4)

No data were submitted.

B.9.6.1.3 Toxicity of the plant protection product (IIIA 10.6.1)

Study 1

Characteristics

Reference	: Rodgers M.H. (2002)	exposure duration	: 14 days
type of study	: Acute toxicity	nominal	: 0, 15, 24, 38, 61 and 98 mg
year of execution	: 2001	concentrations	: product/kg soil
GLP statement	: Yes	dosing method	: Test substance was suspended in water and mixed with soil.
Guideline	: OECD 207	acceptability	: Acceptable
test substance	: Pyridaben 75 WP, Lot no. OAJ030	LC50	: 110 mg product/kg soil ⇔ 84 mg a.s./kg soil
a.s. content	: 76.5%		
Species	: <i>Eisenia foetida</i>		

Methods

In an acute toxicity study, earthworms (*Eisenia foetida*) were exposed to Pyridaben 75 WP for 14 days in artificial soil (OECD 207 substrate, with 10% organic matter). The test was conducted in 1 L glass beakers closed with perforated plastic, containing 737 g artificial moist soil. Nominal soil concentrations of 15, 24, 38, 61 and 98 mg product/kg soil and the control were tested in 4 replicates of 10 worms each. Jars were maintained at 19.5-21.9°C under continuous light. Soil moisture was 34-35% and 31-32% of the dry weight at the start and the end of the test, respectively. A positive control (chloroacetamide) was tested in a separate study.

Results

At and above 24 mg product/kg soil, dead worms were observed on the sides of the jars. At and above 38 mg product/kg, live worms were visible on the sides of the jars and on one occasion one worm at 38 mg product/kg was observed on the surface of the soil. The positive control gave the expected response. Other results are summarised in Table B.9.6.1.3-01. The LC50 was 110 mg product/kg soil, corresponding to 84 mg a.s./kg soil.

Table B.9.6.1.3-01 Acute toxicity of Pyridaben 75 WP to *Eisenia fetida*

Nominal concentration (mg product/kg soil)	Mortality at 14 days (%)	Mean weight change survivors over 14 days (%)
Control	0	+6
15	0	-3
24	18	-2
38	15	-6
61	15	-8
98	53	-9

Conclusion

The LC50 is 110 mg product/kg soil corresponding to 84 mg a.s./kg soil.

Guidelines and limitations

The test is acceptable.

Study 2**Characteristics**

reference	:	Fisher E.R. (2003)	exposure duration	:	8 weeks
type of study	:	Chronic toxicity in earthworms	nominal	:	0, 0.13, 0.20, 0.29, 0.44, 0.66 and
year of execution	:	2002-2003	concentrations	:	0.99 mg a.s./kg soil
GLP statement	:	Yes	dosing method	:	Test substance was suspended in
guideline	:	BBA VI, 2-2; OECD 222 (draft)		:	water and sprayed on soil.
test substance	:	Pyridaben 75 WP, Lot no. OAJ030	acceptability	:	Acceptable
a.s. content	:	76.5%	NOEC	:	0.29 mg a.s./kg soil (2.9% oc)
species	:	<i>Eisenia foetida</i>		:	

Methods

In a chronic toxicity study, earthworms (*Eisenia fetida*) were exposed to Pyridaben 75 WP (76.5% pyridaben) for 4 weeks in artificial soil (OECD 207 substrate, with 5% organic matter equivalent to 2.9% oc). After this period, the adults were removed and cocoons remained in the test vessels for 4 more weeks. The test was conducted in plastic containers covered with tight fitting lids containing 500 g of soil (based on d.w.). The product was dissolved in water and sprayed on the soil using a calibrated laboratory sprayer (600 L/ha). A water treated control and nominal soil concentrations of 0.13, 0.20, 0.29, 0.44, 0.66 and 0.99 mg a.s./kg soil were tested in 4 replicates of 10 worms each. Vessels were maintained at 19.4-20.6°C under a 12 hours light (539 lux) and 12 hours dark photoperiod. Soil moisture was 27-28% and 25-26% of dry weight at the start and the end of the test, respectively (equivalent to 40-43% of MWHC). Worms were fed with cattle manure on the day after treatment and once weekly thereafter until day 28. After four weeks, survival and growth rate of adults were determined, and after eight weeks the number of offspring. In a separate study, a positive control (carbendazim) was tested.

Results

Adult worms were visible at the sides of the containers throughout the study and once on the surface (dose not specified in the report). At and above 0.44 mg a.s./kg soil, food consumption was reduced. The positive control gave the expected response. Other results are summarised in Table B.9.6.1.3-02. No treatment related mortality occurred. Mean weight change and reproduction rate were reduced at 0.44 mg a.s./kg soil and above. The NOEC is 0.29 mg a.s./kg soil (in substrate with 2.9% organic carbon).

Table B.9.6.1.3-02 Chronic toxicity of Pyridaben 75 WP to *Eisenia fetida*

Treatment	Nominal dose (mg a.s./kg soil)	Mortality at 28 days (%)	Mean weight change over 28 days (%)	No. of juveniles per surviving adult
Control	0	0	+8	16
Pyridaben 75 WP	0.13	0	+12	19
Pyridaben 75 WP	0.20	0	+13	17
Pyridaben 75 WP	0.29	2.5	+10	14
Pyridaben 75 WP	0.44	0	+2	6*
Pyridaben 75 WP	0.66	0	+1	4*
Pyridaben 75 WP	0.99	0	-4	3*

* Significantly different from control at 5% level

Conclusion

The NOEC is 0.29 mg a.s./kg soil (in substrate with 2.9% organic carbon).

Guidelines and limitations

The study is acceptable.

B.9.6.2 Field studies (IIIA 10.6.1)

Characteristics

reference	:	Carter J.N. (2005)	test organism	:	Earthworms
type of study	:	Earthworm field study	exposure duration	:	16 months
year of execution	:	2004	nominal	:	104 and 349 g a.s./ha equivalent to
GLP statement	:	Yes	concentrations	:	0.139 and 0.4634 mg a.s./kg soil
guideline	:	ISO 11268-3	dosing method	:	Sprayed on soil
test substance	:	Pyridaben 75 WP, batch OAJ 030	acceptability	:	Not acceptable
a.s. content	:	76.5%			

Methods

Data were submitted from a study on the effect of Pyridaben 75 WP (formulated product containing 76.5% pyridaben) on earthworm populations under field conditions. The study was conducted on a field with a loam soil (pH 7.9, 1.5% organic carbon), located in Cambridgeshire, England, which was divided into 5 x 5 m plots. The current study was an additional sampling event for study 3 summarised in section B.9.8.1.3.

The number of earthworms was determined 16 months after application in two separate 0.5 m² subplots of each of three plots per treatment, which were not sampled during previous sampling events in study 3 (section B.9.8.1.3; sampling events after 1, 6 and 12 months).

Results

The report did not contain any data concerning the climatic conditions during the period between the end of study 3 in section B.9.8.1.3 (12 months after application) and the sampling point after 16 months in the present study.

The results for earthworm density are summarised in the table below. Earthworm density after 16 months in treated plots was comparable to that in untreated plots. Three worm species were present: *Dendrodrillus rubidus* and *Allophora chlorotica* were found to be the most abundant species, while lower numbers of *Lumbricus terrestris* were present.

Evaluation by RMS. Most of the comments made during the evaluation of the report describing the earlier parts of the study (cf. study 3 of section B.9.8.1.3) apply also to this study. In the present study, worm species were identified although no numerical data were given to confirm the statement that *D. rubidus* and *A. chlorotica* were the most abundant species. The study result is considered to be insufficiently reliable to be taken to the List of Endpoints, in particular because of the following shortcomings: no pre-test worm counts, applied dose not verified by chemical analysis of the soil, no positive control.

Table B.9.6.2-01 Earthworm density (mean of 3 plots per treatment) in soil treated once with Pyridaben 75 WP at a rate of 0.139 and 0.4634 mg a.s./kg soil

Months after treatment	Variant	Dose (mg a.s./kg soil)	Earthworms/m ^{2(A)}	Deviation from control (%)
16	Control	0	132 (52)	-
	Pyridaben 75 WP	0.139	159 (20)	+20
	Pyridaben 75 WP	0.4634	138 (40)	+4.5

^(A) Standard deviation is given between parentheses

Conclusion

The result is insufficiently reliable (motivation provided above).

Guidelines & Limitations

The study is not acceptable (see motivation provided above).

B.9.6.3 Risk assessment

B.9.6.3.1 Risk assessment of the active substance

Procedures for risk assessment were in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002). Pyridaben is considered persistent since the maximum DT₉₀ (persistence) of pyridaben is 424 days in the field and hence >1 year (Section B.8.1). Therefore, the maximum plateau soil

concentration should be used rather than the initial concentration in soil. For the proposed uses, the plateau soil PEC have been determined according to the criteria detailed in Section B.8.3 of the Environmental Fate and Behaviour Section, taking into account 80% and 70% crop interception for tomato and citrus, respectively. Acute toxicity studies have been supplied on pyridaben (14-day LC50 38 mg a.s./kg) and Pyridaben 75 WP (LC50 84 mg a.s./kg soil). Risk assessment will be based on the LC50 value for the active substance. Since pyridaben has a logPow of >6.37, it is necessary to reduce the LC50 value by a factor of 2. This is to take account of the relatively high organic matter content of the artificial test soil (10%) compared with field soil (EPPO guidelines). The corrected LC50 is annotated as LC50_{corr}. A chronic toxicity/reproductive study with Pyridaben 75 WP was submitted (NOEC 0.29 mg a.s./kg soil). The NOEC was determined in a soil with 5% organic matter and therefore, the NOEC need not be corrected.

The acute TER is presented in Table B.9.6.3.1-01.

Table B.9.6.3.1-01 Acute risk of pyridaben to earthworms

appln.	dose (kg a.s./ha)	LC50 _{corr} (mg a.s./kg)	PECs (mg a.s./kg)	Acute TER	Annex VI trigger 91/414 EEC
Tomato EU N & S	0.25	19	0.077	247	10
Citrus EU S	0.30	19	0.139	137	10

The TER values in Table B.9.6.3.1-01 are far above the Annex VI 91/414 EEC trigger of 10. Hence the acute risk of pyridaben to earthworms is considered to be acceptable.

The long-term TER is presented in Table B.9.6.3.1-02.

Table B.9.6.3.1-02 Chronic risk of pyridaben to earthworms

appln.	dose (kg a.s./ha)	NOEC (mg a.s./kg)	PECs (mg a.s./kg)	Chronic TER	Annex VI trigger 91/414 EEC
Tomato EU N & S	0.25	0.29	0.077	3.8	5
Citrus EU S	0.30	0.29	0.139	2.1	5

The TER values in Table B.9.6.3.1-02 are below the Annex VI 91/414 EEC trigger of 5, and a long-term risk of pyridaben to earthworms cannot be excluded. According to the Guidance Document on Terrestrial Risk assessment, a field study is required when the TER is below 5. A field study was submitted, but this study was considered to be insufficiently reliable to be taken to the list of endpoints because of the lack of pre-test worm counts, the lack of verification of the applied dose by chemical analysis of the soil, and the lack of a positive control. The notifier is therefore requested to provide additional data for further risk assessment for earthworms (e.g. an acceptable earthworm field study). A new earthworm field study is currently in progress.

B.9.6.3.2 Risk assessment of metabolites

A risk assessment should be performed for soil metabolites formed at a maximum level of at least 10% AR (major metabolites), for metabolites formed at least twice at successive samplings at a level of at least 5% AR, and for metabolites for which an increasing trend of formation is observed in soil degradation studies.

Pyridaben was the main component of the residue in soil. The maximum level of the main degradation products PB-4 and PB-7 in any of the investigated soils was 7.6% and 8.5% AR, respectively. Since levels of >5% AR were found on 2 or more successive samplings, the risk of these metabolites should be considered. In a photodegradation study in soil, PB-22 was found at a maximum level of 13% AR (section B.8.1) and the risk of this metabolite must be considered. Given the low levels of individual metabolites in soil, and the fact that the acute TERs for the parent compound (137-247) were clearly on the safe side, the acute risk of the soil metabolites of pyridaben is considered to be low.

Chronic toxicity/reproductive studies on metabolites of pyridaben were not submitted. Chronic studies are not required when the field DT90 is <100 days (Sanco/10329/2002). Field or lab DT90 values are not available for metabolites. In cases where the DT90 value is >365 days, a sublethal study with earthworms is required. The Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002) does not provide guidance for the case where the DT90 is between 100 and 365 days and the number of applications is less than three. In the update of the HTB (Dutch Manual for Authorisation of Pesticides), the Netherlands will propose to require chronic/reproductive data in cases where the 100 days <field DT90 <365 days, if the TERa >10 and ≤100. Considering the large safety margin calculated for the acute risk of pyridaben (TERa = 137-247), and the much lower levels of metabolites compared to those of the parent compound, it is considered unlikely that the TERa values for the soil metabolites will be ≤100. Therefore, chronic/reproductive data are not required when the DT90 values of the soil metabolites are between 100 and 365 days, but only when the DT90 exceeds 1 year.

PB-7 is a minor (<10%) soil metabolite, which was less toxic than parent pyridaben to fish, daphnia and algae by a factor of at least 28 (section B.9.2.1). In rat, PB-7 was also less toxic by a factor of 2.5-5.4 than parent pyridaben (LD50 for pyridaben in carboxymethylcellulose 570-1100 mg/kg bw; LD50 for PB-7 tested in carboxymethylcellulose 2728-3086 mg/kg bw). Although direct extrapolation from these species to earthworms is not justified, the available toxicity data for PB-7 do not indicate that it has a higher toxicity than pyridaben. The likelihood that PB-7 is more toxic to earthworms than parent pyridaben is considered to be low, and if the long-term risk for pyridaben is found to be acceptable, the risk for PB-7 need not be further tested. The assessment for PB-7 therefore awaits fulfilment of the data requirement for long-term risk for pyridaben.

Furthermore, the notifier is requested to submit additional data for the chronic risk assessment of the soil metabolites PB-4 and PB-22 of pyridaben (e.g. DT90 values, or a field study with pyridaben in earthworms, where the risk of metabolites will be addressed).

B.9.7 Effects on soil non-target macro-organisms (IIIA 10.6.2)

The Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Sanco 10329/2002, rev 2 final) states that testing is required if the field DT90 values in soil are >100 days. Since

the (maximum) field DT90 value for pyridaben is 424 days, hence >1 year, a litter-bag test is required. A litter-bag study was submitted. The litter-bag study showed no inhibitory effects on soil litter degradation at a total measured dose of 0.11 mg a.s./kg soil. This is only slightly lower than the worst case soil plateau maximum PEC (0.139 mg a.s./kg, section B.8.3), and much higher than the average plateau PEC (0.061 mg a.s./kg, section B.8.3).

Also, a collembola test was submitted (section B.9.9.2), in which the NOEC was 3.28 mg a.s./kg soil (2.33% oc). The resulting TER values, based on PECsoil values of 0.077 and 0.139 mg a.s./kg for the application in tomato and citrus, respectively, are 43 and 24 and hence above the trigger value of 5.

Considering these results, the risk of pyridaben for soil non-target macro-organisms is considered to be acceptable.

Metabolites

The need for testing of metabolites depends on the field DT90. DT90 values are however not available for any of the soil metabolites of pyridaben. A litter-bag study was submitted, in which soil litter degradation was studied during 365 days. Since all soil metabolites of pyridaben were formed within 5 months (see section B.8.1) in aerobic degradation studies, these metabolites are considered to have been covered by the litter-bag test. Therefore, the risk of the metabolites for soil non-target macro-organisms is considered to be acceptable.

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

B.9.8.1 Toxicity

B.9.8.1.1 Toxicity of the active substance (IIA 8.5)

No data were submitted.

B.9.8.1.2 Toxicity of metabolites (IIA 8.5)

No data were submitted.

B.9.8.1.3 Toxicity of the plant protection product (IIA 8.5, IIIA 10.7)

Study 1

Characteristics

Reference	:	Gerhardt R. (1989)	test organism	:	Soil microflora
type of study	:	Soil microflora test (respiration)	exposure duration	:	28 days
year of execution	:	1989	nominal	:	0.80 and 8.0 mg a.s./kg dry soil
GLP statement	:	Yes	concentrations	:	
Guideline	:	BBA VI, 1-1 (1987)	dosing method	:	Added directly to soil
test substance	:	BAS 9078 6 I, batch no 89-1 (APE/CF); PCF 00030	acceptability	:	Acceptable
			respiration	:	NOEC 8.0 mg a.s./kg

a.s. content	:	200 g pyridaben/L		
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Methods

Data were submitted from a laboratory study on the effect of BAS 9078 6 I (200 g pyridaben/L) on microbial respiration in a loamy sand and a sandy loam soil.

Batches of a loamy sand soil (5, 8, 18 and 70% particles in the <2, 2-20, 20-200 and >200 µm size class, pH 5.8, 0.6% organic carbon, microbial biomass 200 mg C/kg) and of a sandy loam soil (19, 23, 37 and 21% particles in the <2, 2-20, 20-200 and >200 µm size class, pH 7.2, 1.4% organic carbon, microbial biomass 408 mg C/kg) were pre-incubated at 20-22°C for 48 hours and then treated with the test substance at 0.80 and 8.0 mg a.s./kg d.w. Control soil was left untreated. Aliquots (110 and 58 g d.w. for the loamy sand and the sandy loam soil, respectively) were dispensed into plastic bottles (20 replicates per treatment), which were incubated at 20±2°C. Soil moisture was adjusted to 40% and 55% MWHC for the loamy sand and the sandy loam soil, respectively. On day 0, and after 7, 14, 21 and 28 days, four replicates were removed, amended with glucose (908 and 2144 mg/kg soil for loamy sand and sandy loam, respectively), and O₂ consumption was measured for 12 hours.

According to the OECD 217 guideline, 2000-4000 mg glucose/kg soil d.w. is usually sufficient for sandy soils with 0.5-1.5% organic carbon. In this study, the amount of glucose added to sandy loam soil (2144 mg/kg soil d.w.) complied with this guideline, but the amount added to the loamy sand soil (908 mg/kg soil d.w.) did not. The original report did not contain any information to show that the amount of glucose added was sufficient to elicit an immediate maximum respiratory response in the loamy sand soil. However, the notifier later provided information from the testing facility which demonstrates that glucose consumption by the loamy sand soil was assessed and the maximum glucose amendment appropriate for the study was determined to be 50 - 100 mg per 100 g dry weight soil. Both of these glucose amendment rates resulted in a maximum response equivalent to a microbial biomass of 20.04 mg C/ 100 g dry soil. In the study, 100 mg glucose was selected as the addition rate and was added to 110.1 g loamy sand soil, equivalent to 908 mg glucose/kg soil dry weight. Therefore the results for both soils can be accepted.

Results

Variation (RSD) between replicate control samples was always <15%. The respiration rate in the control loamy sand soil and sandy loam soil were respectively 8.0 and 18 mg O₂/hour/kg soil d.w. on day 0. These rates decreased respectively to 5.9 and 16 mg O₂/hour/kg soil d.w. on day 7 and then increased to 7.4 and 19 mg O₂/hour/kg soil d.w. on day 28. Effect percentages for BAS 9078 6 I, based on the cumulative oxygen consumption during 12 hours, are summarised in Table B.9.8.1.3-01.

Table B.9.8.1.3-01 Effect of BAS 9078 6 I on the respiration rate in a loamy sand soil and a sandy loam soil

Treatment rate (mg a.s./kg soil d.w.)	% deviation from control on day:				
	0	7	14	21	28
Loamy sand soil					
0.80	0	+7.0	+6.2	+4.7	+12
8.0	-5.2	+14	+11	+3.5	+12
Sandy loam soil					
0.80	+0.9	-4.3	-9.0	-4.1	-4.4
8.0	+2.3	+2.1	-8.5	+1.8	-2.7

Short-term respiration of treated loamy sand and sandy loam soil differed from untreated soil by $\leq 12\%$ at all sampling points.

Conclusion

NOEC for effects on short-term respiration 8.0 mg a.s./kg soil for sandy loam soil and loamy sand soil.

Guidelines and limitations

Dose levels, expressed as $\mu\text{l/kg}$ soil d.w. were recalculated to mg a.s./kg soil d.w. using the a.s. content of the formulation. Respiration rates, expressed as mg $\text{O}_2/12$ hour/100 g were recalculated into $\text{O}_2/\text{hour/kg}$ soil by the RMS.

Study 2

Characteristics

reference	:	Dohmen G.P. (1989)	exposure duration	:	28 days
type of study	:	Soil microflora test (nitrification)	nominal	:	0.80 and 8.0 mg a.s./kg dry soil
year of execution	:	1989	concentrations	:	
GLP statement	:	Yes	dosing method	:	Added directly to soil
guideline	:	BBA VI, 1-1 (1987)	acceptability	:	Acceptable for loamy sand, not for sandy loam
test substance	:	BAS 9078 6 I, batch no 89-1	nitrification	:	NOEC 8.0 mg a.s./kg for loamy sand
a.s. content	:	200 g pyridaben/L			
test organism	:	Soil microflora			

Methods

Data were submitted from a laboratory study on the effect of BAS 9078 6 I (200 g pyridaben/L) on microbial nitrification in a loamy sand and a sandy loam soil.

Batches of a loamy sand soil (5, 8, 18 and 70% particles in the <2 , 2-20, 20-200 and >200 μm size class, pH 5.8, 0.6% organic carbon, microbial biomass 200 mg C/kg) and of a sandy loam soil (19, 23, 37 and 21% particles in the <2 , 2-20, 20-200 and >200 μm size class, pH 7.2, 1.4% organic carbon, microbial biomass 408 mg C/kg) were treated with the test substance at 0.80 and 8.0 mg a.s./kg d.w. and amended with ammonium sulfate (860-867 mg/kg soil d.w.). Control soil was left untreated, but part of the control soil was amended with ammonium sulfate. A positive control (nitrapyrin, 1.0 mg/kg soil d.w.) was included. Aliquots (59 and 56 g d.w. for the loamy sand and the sandy loam soil, respectively) were dispensed into 500 ml plastic bottles, which were incubated at $20 \pm 2^\circ\text{C}$. Soil moisture was adjusted to 45% and 60% MWHC for the

loamy sand and the sandy loam soil, respectively. On day 0, and after 7, 14, 20 or 21 and 28 (loamy sand soil only) days, three replicates were removed, extracted with aluminium potassium sulfate, and ammonium and nitrate were measured using ammonia- and nitrate-electrodes.

Results

The positive control gave the expected response.

Ammonium

Ammonium levels in unamended, untreated loamy sand and sandy loam soil, respectively, were 0.80 and 0.40 mg/kg at day 0, and decreased to 0.40 and 0.20 mg/kg after 28 and 21 days, respectively. Ammonium levels in amended soils were corrected for these values. Ammonium levels in amended, untreated loamy sand and sandy loam soil were respectively 201 and 153 mg/kg at day 0, and decreased to 63 mg/kg and <LOD after 28 and 21 days, respectively.

In the treated loamy sand soil, ammonium levels decreased from 205 mg/kg at day 0 to 66 and 75 mg/kg after 28 days at the low and high dose, respectively. In the treated sandy loam soil, ammonium levels decreased from 156-158 mg/kg on day 0 to <LOD after 21 days at both doses.

Nitrate

Nitrate levels in unamended, untreated loamy sand and sandy loam soil were respectively 10.4 and 24.2 mg/kg at day 0, and increased to 20.8 and 31.0 mg/kg after 28 and 21 days. Nitrate levels in amended soils were corrected for these values. Nitrate levels in amended, untreated loamy sand and sandy loam soil were respectively 0.20 and 0.60 mg/kg at day 0, and increased to 119 and 207 mg/kg after 28 and 21 days. The OECD 216 guideline for the nitrification test states that evaluation should be based on nitrate formation rates. In the report, nitrate formation rates were not corrected for earlier measurements (rate was defined as absolute nitrate level divided by the number of days at the sampling time). Nitrate formation rates were recalculated by the reviewer from the reported absolute nitrate levels. Results for BAS 9078 6 I based on nitrate levels and nitrate formation rates are summarised in Tables B.9.8.1.3-02 and B.9.8.1.3-03. The variation (CV) between nitrate levels of control replicates was $\leq 4\%$ at any sampling and for both soils.

The following evaluation of the test results was made by the RMS. The final conclusion reached by the RMS agreed with that by the author of the report.

Nitrification, loamy sand soil

After 28 days, nitrate concentrations in treated soils did not differ from untreated soil by more than 2.4%. Nitrate formation rates after 28 days in treated soils were however much higher (58-172%) than in untreated soil. At both doses, nitrate formation was inhibited during the day 0-7 and day 7-14 intervals (4.7-43% and 2.2-27%, respectively). During the day 14-20 and day 20-28 intervals, however, nitrate formation rates were higher than in untreated soil at both doses (3.5-52% and 58-172%, respectively), and this resulted in comparable nitrate concentrations (2-2.4% difference) in treated and untreated soil on the last study day for

this soil (day 28). The inhibitory effects of the test material were therefore considered to be reversible at both doses and the 28-day NOEC is 8.0 mg a.s./kg.

Nitrification, sandy loam soil

After 21 days, nitrate concentrations in treated soils did not differ from untreated soil by more than 4.8%. Nitrate formation rates after 21 days in treated soils were much lower (15-73%) than in untreated soil. At both doses, nitrate formation was inhibited during the day 0-7 interval (4.8-21%). At the low dose, the nitrate formation rate was similar to that in untreated soil during the 7-14 day interval, but inhibited during the 14-21 day interval (73%). At the high dose, the nitrate formation rate was much higher than in untreated soil during the day 7-14 interval (96%), but lower during the day 14-21 interval (15%). The biological relevance of the recorded inhibition of nitrate formation rate during the day 14-21 interval is questionable, since (1) denitrification occurred during this period in the control soil and the treated soils; (2) there was no dose-response for the effects; (3) absolute nitrate levels at the last study day were comparable to those in untreated soil (0-4.8% difference). Therefore, the inhibitory effects of the test material were considered to be reversible at both doses. The study duration of 21 days however is not long enough for full evaluation of the effect of the test material on soil microflora (see also "Guidelines and Limitations", point 1).

Table B.9.8.1.3-02 Effect of BAS 9078 6 I on nitrification in a loamy sand soil

Treatment rate (mg a.s./kg soil d.w.)	Nitrate levels (mg/kg) on day:				
	0	7	14	20	28
0 (control)	0.20	34.0	87.6	110	119
0.80	0	32.2	84.6	108	122
8.0	0	19.4	58.6	93	116
Nitrapyrin	0	1.2	4.2	8.2	15.6
Treatment rate (mg a.s./kg soil d.w.)	% deviation (nitrate levels) from control on day :				
	0	7	14	20	28
0.80	-100	-5.3	-3.4	-2.0	2.4
8.0	-100	-43	-33	-16	-2.0
Nitrapyrin	-100	-96	-95	-93	-87
Treatment rate (mg a.s./kg soil d.w.)	% deviation (nitrate formation rate) from control on day:				
	0	7	14	20	28
0.80	n.a.	-4.7	-2.2	3.5	58
8.0	n.a.	-43	-27	52	172
Nitrapyrin	n.a.	-96	-94	-82	-14

Table B.9.8.1.3-03 Effect of BAS 9078 6 I on nitrification in a sandy loam soil

Treatment rate (mg a.s./kg soil d.w.)	Nitrate levels (mg/kg) on day:			
	0	7	14	21
0 (control)	0.60	195	234	207
0.80	0	185	224	217
8.0	0	153	230	207
Nitrapyrin	-0.40	67.0	105	131
Treatment rate (mg a.s./kg soil d.w.)	% deviation (nitrate levels) from control on day :			
	0	7	14	21
0.80	-100	-5.1	-4.3	4.8
8.0	-100	-21	-1.7	0
Nitrapyrin	-167	-66	-55	-37
Treatment rate (mg a.s./kg soil d.w.)	% deviation (nitrate formation rate) from control on day:			
	0	7	14	21
0.80	n.a.	-4.8	0	-73
8.0	n.a.	-21	96	-15
Nitrapyrin	n.a.	-65	-4.1	-196

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

Conclusion

NOEC for effects on nitrate formation in loamy sand 8.0 mg a.s./kg soil; results for sandy loam not acceptable.

Guidelines and limitations

1. The test disagreed from the BBA VI, 1-1 (1987) on the following point: the test duration was 21 days for the sandy loam soil. A minimum duration of 28 days is also prescribed by OECD 216. The results for this soil are therefore not accepted. Conditions of storage of soil prior to the test were not reported, but the soils were viable considering the adequate increase in nitrate levels during incubation
2. Dose levels, expressed as µL/kg soil d.w. were recalculated to mg a.s./kg soil d.w. using the a.s. content of the formulation.

Study 3

Characteristics

reference	: Carter J.N. (2004)	exposure duration	: 1 year
type of study	: Litter bag test and earthworm field study	nominal concentrations	: total dose 0.32 mg a.s./kg soil (litter bag test); 104 and 349 g a.s./ha equivalent to 0.139 and 0.4634 mg a.s./kg soil (earthworm field test)
year of execution	: 2003-2004	dosing method	: Sprayed on soil
GLP statement	: Yes	acceptability	: Litter bag test: acceptable
guideline	: EPFES 10 and ISO 11268-3		Earthworm field study: not acceptable
test substance	: Pyridaben 75 WP, batch OAJ 030		
a.s. content	: 76.5%		
test organism	: Soil micro- and macroflora & earthworms		

Methods

Data were submitted from studies on the effect of Pyridaben 75 WP (formulated product containing 76.5% pyridaben) on soil litter degradation and on earthworm populations under field conditions. Both studies were conducted on a field with a loam soil (pH 7.9, 1.5% organic carbon), located in Cambridgeshire, England, which was divided into 5 x 5 m plots.

Litterbag study

On May 28, 2003, the bare soil of the test plots (6 treated and 6 untreated plots) was treated with Pyridaben 75 WP at a nominal dose of rate of 174 g a.s./ha in 500 L water/ha (equivalent to 0.1159 mg a.s./kg soil based on 10 cm incorporation depth and assuming a soil density of 1.5 mg/dm³). Control plots were treated with water. On June 2 (control plots) and June 3 (treated plots), the dose was incorporated into the soil to a depth of 10 cm. In each plot, 34 litter bags with 4 g of straw each were buried on June 9 (control plots) and June 10 (treated plots), 2003, at a depth of 5 cm. On June 11, 2003, the plots received a second treatment with Pyridaben 75 WP at a nominal dose of rate of 301 g a.s./ha in 500 L water/ha (equivalent to 0.20 mg a.s./kg soil based on distribution into the top 10 cm and assuming a soil density of 1.5 mg/dm³). This dose was not incorporated. Control plots were treated with water.

On the day of the first application, and 6 days after the second application, ten 0-10 cm soil cores were removed from each plot and pooled per plot. The cores were analysed for pyridaben by HPLC-MS/MS (method described in section B.5.3, study 1). The method was concurrently validated by analysis of untreated soil samples fortified with pyridaben at 0.01 and 0.2 mg/kg ($n=1$ for each level), mean recovery 80-81%.

Eight litter bags per plot were removed on July 11, 2003 (1 month after burying), September 11, 2003 (3 months after burying), December 11, 2003 (6 months after burying) and June 11, 2004 (12 months after burying). In the laboratory the bags were dried for 2-3 days at room temperature, the straw was separated manually and by dry sieving, the dry weight of the straw was determined and the straw was combusted for 30 min at 600°C. When ashing of the cleaned straw could not be scheduled promptly the cleaned straw was stored in closed containers at approximately 4°C. Prior to ashing the straw was incubated at 30-35°C overnight and then ashed on the following day. The percent degradation of straw was calculated taking into consideration experimentally determined correction factors for loss on ignition of the initial straw sample as well as for soil. The number of days between retrieval of litter bags and completion of the ashing process at each sampling time-point is as follows: 11-13 days for the 12 month sample point; 6-12 days for the 6 month sample point; 13-14 days for the 3 month sample point; and 6-20 days for the 1 month sample point. It is noted that at the 1-month sample point the final ashing of a number of straw samples was performed at > 14 days. It is considered that this did not affect the outcome of the study.

On July 8, 2003, the number and biomass of earthworms per m² was determined in six separate 0.5 m² plots.

Earthworm field study

On June 11, 2003, the bare soil of the test plots (3 plots for each pyridaben treatment and 3 untreated plots) was treated with Pyridaben 75 WP at nominal rates of 104 or 349 g a.s./ha in 500 L water/ha (equivalent to 0.139 and 0.4634 mg a.s./kg soil based on distribution into the top 5 cm and assuming a soil density of 1.5 mg/dm³). Control plots were treated with water. The number of earthworms was determined 1, 6 and 12 months after application in two separate 0.5 m² subplots.

Results

The total amount of precipitation was 427 mm in June-December 2003 and 546 mm in December 2003-June 2004.

Litterbag study

On the day of the first application, the measured residues of pyridaben in soil (range for 6 replicate plots) represented 29-41% (mean 35%) of the nominal value. Six days after the second application, the measured residues of pyridaben in soil (range for 6 replicate plots) represented 24-50% (mean 35%) of the nominal value (0.3159 mg/kg, nominal value estimated as the sum of the soil residue from the two treatments). The measured residues are much lower than the nominal values. The report did not provide an explanation for

the low measured residues. The analytical method is considered to be valid. The rate of degradation of pyridaben in the test soil is unknown. In the opinion of the RMS, the nominal test doses and nominal concentrations in the study conclusions should be corrected by a factor of 0.35, representing the percentage recovery of the test compound relative to the nominal dose.

About 4 weeks after the second pyridaben treatment, earthworm density at the test site was 24/m², and earthworm biomass was 11.7 g/m².

The results for straw degradation are summarised in the table below. One month after treatment, decomposition in the treated plots was 27% higher than in the control plots, and at later samplings differences from the control were 7% or less. None of these differences was statistically significant. The NOEC is a 0.32 mg a.s./kg based on the nominal doses, equivalent to 0.11 mg a.s./kg based on measured concentrations.

Table B.9.8.1.3-04 Degradation of straw in litter bags in soil treated twice with Pyridaben 75 WP at a nominal rate of 0.1159 and 0.2 mg a.s./kg soil

Sampling date	Months after treatment	Variant	Mean mass loss (%) ^(A)	Decomposition (% of control)
11 July 2003	1	Control	12.21 (4.73)	127
	1	Pyridaben 75 WP	15.54 (4.79)	
11 September 2003	3	Control	33.27 (8.32)	94
	3	Pyridaben 75 WP	31.41 (5.91)	
11 December 2003	6	Control	41.46 (9.10)	107
	6	Pyridaben 75 WP	44.36 (5.85)	
11 June 2004	12	Control	67.53 (5.90)	100
	12	Pyridaben 75 WP	67.23 (5.05)	

^(A) Standard deviation is given between parentheses

Earthworm field study

The results for earthworm density are summarised in the table below. Earthworm density tended to be lower in treated plots than in untreated plots after 1 and 12 months in a dose related manner, although these differences were not statistically significant. At 6 months after treatment, earthworm densities in treated and control plots were comparable.

Evaluation of earthworm study by RMS. The efficiency of the method to extract earthworms from soil was not checked (requirement of ISO 11268-3). Worm species and the number of adult and juvenile worms were not identified. Pre-test worm counts were not performed. The applied dose in this study was not verified by chemical analysis of the soil, and no positive control was included to test the sensitivity of the study methods. The statistical power of the study design (i.e. the minimum difference from the control that would be statistically significant) was not reported. The level of replication (3 plots per treatment) is considered to be low, taking into account the large variation in earthworms counts, not only between plots of the same treatment but also between subplots of the same plot. The study result is considered to be insufficiently reliable to be taken to the List of Endpoints.

Table B.9.8.1.3-05 Earthworm density (mean of 3 plots per treatment) in soil treated once with Pyridaben 75 WP at a rate of 0.139 and 0.4634 mg a.s./kg soil

Months after treatment	Variant	Dose (mg a.s./kg soil)	Earthworms/m ² (A)	Deviation from control (%)
1	Control	0	34 (16)	
	Pyridaben 75 WP	0.139	28 (17)	-18
	Pyridaben 75 WP	0.4634	18 (9)	-47
6	Control	0	33 (18)	
	Pyridaben 75 WP	0.139	36 (18)	9.1
	Pyridaben 75 WP	0.4634	35 (16)	6.1
12	Control	0	8 (6)	
	Pyridaben 75 WP	0.139	6 (5)	-25
	Pyridaben 75 WP	0.4634	4 (5)	-50

(A) Standard deviation is given between parentheses

Conclusion

Soil litter degradation was not inhibited by two spray applications at 15-days interval on 28 May and 11 June 2003 of Pyridaben 75 WP at a nominal rate of 174 and 301 g a.s./ha (total measured dose 0.11 mg a.s./kg soil). This result is acceptable.

The result of the earthworm field test is insufficiently reliable (motivation provided above).

Guidelines & Limitations

1. The following information was not available from the original report: the time between sampling of soil litter bags and processing in the laboratory, and the storage conditions of the litter bags prior to analysis. This information was later provided by the notifier in an amendment and thus the results of the litter bag test were accepted.
2. Sampled soil cores were stored frozen for up to 6 weeks prior to analysis for pyridaben, and data on storage stability of pyridaben residues in frozen soil were not submitted with this study. Study 1 in section B.9.2.2.14 provided data on storage stability of pyridaben in frozen aquatic sediment (stable for 12 months). On the basis of this information, pyridaben is assumed to have been stable in frozen soil for 6 weeks.
3. Note: a report describing an additional sampling at 16 months after application is summarised under point B.9.6.2.

B.9.8.2 Risk assessment

B.9.8.2.1 Risk assessment of the active substance

Studies on the toxicity of pyridaben on soil microbial respiration and nitrification were submitted in two soils. The study on effects on respiration was found to be acceptable for both soils (sandy loam, 21% particles >200 µm, 1.4% organic carbon; and loamy sand, 70% particles >200 µm, 0.6% organic carbon), while the study on nitrification was found to be acceptable for one soil only (loamy sand, 70% particles >200 µm, 0.6%

organic carbon). Only the loamy sand soil fully meets the requirement of a sensitive soil laid down in the SETAC document on Procedures for assessing the environmental fate and ecotoxicity of pesticides (1995). The data submitted are considered to be sufficient.

The NOEC for effects on nitrification of a loamy sand soil and on respiration of the sandy loam soil and the loamy sand soil treated with pyridaben was 8.0 mg a.s./kg soil.

The worst case soil plateau maximum PEC of pyridaben (0.139 mg a.s./kg, section B.8.3) is a factor of 58 below the highest test concentration of pyridaben without lasting effects on soil microbial activity (8.0 mg a.s./kg). Furthermore, the litterbag study showed no inhibitory effects on soil litter degradation at a total measured dose of 0.11 mg a.s./kg soil. This is only slightly lower than the worst case soil plateau maximum PEC, and much higher than the average plateau PEC (0.061 mg a.s./kg). Therefore, the risk of pyridaben for soil microflora is considered to be acceptable.

B.9.8.2.2 Risk assessment of metabolites

A risk assessment should be performed for soil metabolites formed at a maximum level of at least 10% AR (major metabolites), for metabolites formed at two successive samplings at a level of at least 5% AR, and for metabolites for which an increasing trend of formation is observed in soil degradation studies.

In laboratory incubated soil, the maximum level of the main degradation products PB-4 and PB-7 in any of the incubations was 7.6% and 8.5% AR, respectively. Since levels of >5% AR were found at two successive samplings, the risk of these metabolites should be considered. In a photodegradation study in soil, PB-22 was found at a maximum level of 13% AR (section B.8.1), and the risk of this metabolite must be considered.

PB-4 and PB-7

No separate studies on the effects of metabolites of pyridaben to soil micro-organisms have been submitted. The maximum percentage of formation of these metabolites in any laboratory incubated soil was reached after 150 and 60 days, respectively. The levels of these two metabolites were however low (mean maximum level for all tested soils 4.6% and 4.3% AR, respectively). Moreover, these metabolites appeared already early during incubation, and the mean maximum level within 29-30 days (2.4% and 3.0% AR respectively) represented 60% and 70% of the mean maximum level observed in soil. Exposure of soil micro-organisms to these metabolites is therefore assumed to have occurred at a sufficient level to conclude that their effects have been covered by studies with parent pyridaben to soil micro-organisms, which were conducted at a 58x overdose compared to the PECsoil. Consequently, the risk of these metabolites to soil micro-organisms is considered to be low.

PB-22

Only in one of five tested soils PB-22 could be identified separately, and in this soil the maximum level was 1.2% AR (PB-22, within 7 days). In a study on four other soils, PB-22 and PB-14 could not be separated by

HPLC, and maximum levels of the cluster of up to 6.2% AR (mean for four soils 3.9% AR) were reached late during the study (after 120-151 days). Since in three of five tested soils the levels of the cluster of PB-14 and PB-22 within 29-30 days were very low (0%, 0.1% and 0.6% AR), whilst the occurrence of relatively high levels of the cluster after 4-5 months (6.2% AR) was also observed, it is uncertain that the effects of PB-22 were covered by the studies on effects of parent pyridaben to soil microflora (respiration and nitrification). However, PB-22 can be assumed to have been formed in the litterbag study. Therefore, the risk of PB-22 to soil micro-organisms is acceptable.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (IIA 8.6, IIIA 10.8)

B.9.9.1 Screening tests

Study 1

Characteristics

reference	:	Elliott D.S. (1992)	exposure duration	:	6-21 days
type of study	:	Seedling emergence	nominal	:	Seedling emergence:
year of execution	:	1992	concentrations	:	0 and 0.56 kg a.s./ha
GLP statement	:	Yes		:	Seed germination: 1.5 mg a.s./L
guideline	:	Subdivision J, 122-1	dosing method	:	Test substance was dissolved in
test substance	:	Pyridaben (BAS 300 I-technical), lot no. 900304		:	water and pipetted in Petri dishes or sprayed on soil
purity	:	99.7%	acceptability	:	Acceptable
species	:	Soybean (<i>Glycine max</i>), lettuce (<i>Lactuca sativa</i>), radish (<i>Raphanus sativus</i>), tomato (<i>Lycopersicon esculentum</i>), cucumber (<i>Cucumis sativus</i>), cabbage (<i>Brassica oleracea</i>), oat (<i>Avena sativa</i>), ryegrass (<i>Lolium perenne</i>), corn (<i>Zea mays</i>) and onion (<i>Allium cepa</i>)	NOEC	:	<0.56 kg a.s./ha (tomato, corn); 0.56 kg a.s./ha (others).

Methods

A greenhouse screening study to determine seedling emergence and an *in vitro* study to determine seed germination was conducted on four monocot and six dicot crops.

In the greenhouse screening study, pyridaben (purity 99.7%) was applied to four monocot and six dicot crops at a dose of 0.56 kg a.s./ha and a negative (water) control. For the *in vitro* seed-germination experiment, seeds (10 per replicate) were added to petri-dishes (5 replicates per treatment) containing 10-12 mL of a solution of pyridaben in water (1.5 mg a.s./L). These dishes were placed in plastic boxes sealed with tightly fitted lids and kept in an incubator at 25±2°C or 20±2°C (lettuce only) for 6 days, after which germination was determined.

For seedling emergence and plant growth, applications were made in 468 L/ha pre-emergence (application to the soil surface within 24 hours from planting the seeds) of target plants using a calibrated laboratory sprayer. Each treatment was tested in 5 replicates containing 10 seeds per pot. Assessments of emergence were made at 10 and 14 days after treatment. Assessments of phytotoxicity were made at 10, 14 and 21 days after treatment by visual observations using a rating scale of 0 to 5 (comparison to controls) where 0 represented no injury/effect, 1 slight plant effect or restricted to one area of the plant, 2 moderate effect (engrossing the whole plant), 3 severe effect with recovery possible, 4 total plant effect and 5 moribund or dead. Seedling survival, plant height and biomass were recorded 21 days after treatment. Test conditions were: temperature 21-29°C, relative humidity 35-97%, light >25000 lux (12-13 hour light period).

Results

It was reported that no phytotoxic effects were observed. Seed germination, seed emergence, plant height and plant biomass are shown in Tables B.9.9.1-01 - B.9.9.1-04. Plant biomass was significantly reduced (22% and 23%) for tomato and corn.

Table B.9.9.1-01 The phytotoxic effects of pyridaben on *in vitro* seed germination of ten plant species

	Germination (%)		Deviation from control (%)
	Control	1.5 mg a.s./L	
Soybean (<i>Glycine max</i>)	88	92	5
Lettuce (<i>Lactuca sativa</i>)	100	96	-4
Radish (<i>Raphanus sativus</i>)	94	96	2
Tomato (<i>Lycopersicon esculentum</i>)	94	98	4
Cucumber (<i>Cucumis sativus</i>)	96	96	0
Cabbage (<i>Brassica oleracea</i>)	92	88	-4
Oat (<i>Avena sativa</i>)	100	94	-6
Ryegrass (<i>Lolium perenne</i>)	86	88	2
Corn (<i>Zea mays</i>)	100	98	-2
Onion (<i>Allium cepa</i>)	84	78	-7

Table B.9.9.1-02 The phytotoxic effects of pyridaben on seedling emergence and survival of ten plant species (greenhouse study)

	Seedling emergence (%)				Survival (%)		Deviation from control (%)		
	+10DAT		+14DAT		+21DAT		+10DAT	+14DAT	+21DAT
Dose (kg a.s./ha)	0	0.56	0	0.56	0	0.56	0.56	0.56	0.56
Soybean	98	98	98	98	98	100	0	0	2
Lettuce	98	100	98	100	100	100	2	2	0
Radish	100	100	100	100	100	100	0	0	0
Tomato	98	98	98	98	100	100	0	0	0
Cucumber	84	98	84	98	100	100	17	17	0
Cabbage	78	78	78	78	100	100	0	0	0
Oat	100	100	100	100	100	100	0	0	0
Ryegrass	84	98*	84	100*	100	100	17	19	0
Corn	96	94	96	94	100	100	-2	-2	0
Onion	84	84	86	84	100	100	0	-2	0

* Significantly different from control at 5% level

Table B.9.9.1-03 The phytotoxic effects of pyridaben on plant height of ten plant species (greenhouse study)

	Plant height (mm)		Deviation from control (%)
	0 kg a.s./ha	0.56 kg a.s./ha	
Soybean (<i>Glycine max</i>)	253	256	1
Lettuce (<i>Lactuca sativa</i>)	93	86	-8
Radish (<i>Raphanus sativus</i>)	132	137	4
Tomato (<i>Lycopersicon esculentum</i>)	141	130	-8
Cucumber (<i>Cucumis sativus</i>)	145	145	0
Cabbage (<i>Brassica oleracea</i>)	112	111	-1
Oat (<i>Avena sativa</i>)	333	333	0
Ryegrass (<i>Lolium perenne</i>)	142	145	2
Corn (<i>Zea mays</i>)	339	317	-6
Onion (<i>Allium cepa</i>)	124	128	3

Table B.9.9.1-04 The phytotoxic effects of pyridaben on plant biomass of ten plant species (greenhouse study)

	Dry weight (g)		Deviation from control (%)
	0 kg a.s./ha	0.56 kg a.s./ha	
Soybean (<i>Glycine max</i>)	2.417	2.528	5
Lettuce (<i>Lactuca sativa</i>)	0.853	0.740	-13
Radish (<i>Raphanus sativus</i>)	2.570	2.882*	12
Tomato (<i>Lycopersicon esculentum</i>)	1.387	1.069*	-23
Cucumber (<i>Cucumis sativus</i>)	3.271	3.573	9
Cabbage (<i>Brassica oleracea</i>)	1.644	1.635	-1
Oat (<i>Avena sativa</i>)	2.253	2.189	-3
Ryegrass (<i>Lolium perenne</i>)	0.334	0.340	2
Corn (<i>Zea mays</i>)	3.078	2.391*	-22
Onion (<i>Allium cepa</i>)	0.132	0.140	6

Conclusion

Based on significant biomass reduction, the NOEC for tomato and corn is <0.56 kg a.s./ha. The NOEC for soybean, lettuce, radish, cucumber, cabbage, oat, ryegrass and onion is 0.56 kg a.s./ha.

Guidelines and limitations

1. In the present study, test concentrations were not analytically confirmed, as required by draft OECD 208 (2004). This requirement however was not laid down in OECD 208 (1984), which is still in force at present.
2. OECD 208 from 1984 (emergence test) states that the test substance should be evenly dispersed through the soil. In the present test the substance was sprayed on the soil, which corresponds with common agricultural practice.

Study 2**Characteristics**

reference	: Blackburn R.A. (1992)	exposure duration	: 21 days
type of study	: Vegetative vigor	nominal	: 0 and 0.56 kg a.s./ha
year of execution	: 1992	concentrations	
GLP statement	: Yes	dosing method	: Test substance was dissolved in water and sprayed on plants
guideline	: Subdivision J, 122-1	acceptability	: Acceptable
test substance	: Pyridaben (BAS 300 I-technical), lot no. 900304	NOEC	: <0.56 kg a.s./ha (soybean, tomato, oat, ryegrass); 0.56 kg a.s./ha (others)
purity	: 99.7%		
species	: Soybean (<i>Glycine max</i>), lettuce (<i>Lactuca sativa</i>), radish (<i>Raphanus sativus</i>), tomato (<i>Lycopersicon esculentum</i>), cucumber (<i>Cucumis sativus</i>), cabbage (<i>Brassica oleracea</i>), oat (<i>Avena sativa</i>), ryegrass (<i>Lolium perenne</i>), corn (<i>Zea mays</i>) and onion (<i>Allium cepa</i>)		

Methods

In a greenhouse screening study, pyridaben (purity 99.7%) was applied to four monocot and six dicot crops at a dose of 0.56 kg a.s./ha and a negative (water) control. Applications were made in 468 L/ha post-emergence (applications to the plant canopy 8-21 days after emergence) of target plants using a calibrated laboratory sprayer. Each treatment was tested in 5 replicates containing 5 seedlings per pot. Assessments of phytotoxicity were made at 7, 14 and 21 days after treatment by visual observations using a rating scale of 0 to 5 (comparison to controls) where 0 represented no injury/effect, 1 slight plant effect or restricted to one area of the plant, 2 moderate effect (engrossing the whole plant), 3 severe effect with recovery possible, 4 total plant effect and 5 moribund or dead. Survival, plant height and biomass were recorded 21 days after treatment. Test conditions were: temperature 21-28°C, relative humidity 45-93%, light >30000 lux (12-13 hour light period).

Results

It was reported that no phytotoxic effects were observed. Survival, plant height and plant biomass are shown in Tables B.9.9.1-05- B.9.9.1-07. Plant height was statistically (but not biologically) significantly reduced in soybean (2%), and biomass was significantly reduced in soybean, tomato, oat and ryegrass (7-18%).

Table B.9.9.1-05 The phytotoxic effects of pyridaben on survival of ten plant species

	Survival (%)		Deviation from control (%)
	0 kg a.s./ha	0.56 kg a.s./ha	
Soybean (<i>Glycine max</i>)	100	100	0
Lettuce (<i>Lactuca sativa</i>)	100	100	0
Radish (<i>Raphanus sativus</i>)	100	100	0
Tomato (<i>Lycopersicon esculentum</i>)	100	100	0
Cucumber (<i>Cucumis sativus</i>)	100	100	0
Cabbage (<i>Brassica oleracea</i>)	100	100	0
Oat (<i>Avena sativa</i>)	100	100	0
Ryegrass (<i>Lolium perenne</i>)	100	100	0
Corn (<i>Zea mays</i>)	100	100	0
Onion (<i>Allium cepa</i>)	100	100	0

Table B.9.9.1-06 The phytotoxic effects of pyridaben on plant height of ten plant species

	Plant height (mm)		Deviation from control (%)
	0 kg a.s./ha	0.56 kg a.s./ha	
Soybean (<i>Glycine max</i>)	359	351*	-2
Lettuce (<i>Lactuca sativa</i>)	126	119	-6
Radish (<i>Raphanus sativus</i>)	132	127	-4
Tomato (<i>Lycopersicon esculentum</i>)	242	235	-3
Cucumber (<i>Cucumis sativus</i>)	205	210	2
Cabbage (<i>Brassica oleracea</i>)	132	134	2
Oat (<i>Avena sativa</i>)	523	513	-2
Ryegrass (<i>Lolium perenne</i>)	198	193	-3
Corn (<i>Zea mays</i>)	480	489	2
Onion (<i>Allium cepa</i>)	213	228	7

* Significantly different from control at 5% level

Table B.9.9.1-07 The phytotoxic effects of pyridaben on plant biomass of ten plant species

	Dry weight (g)		Deviation from control (%)
	0 kg a.s./ha	0.56 kg a.s./ha	
Soybean (<i>Glycine max</i>)	6.232	5.797*	-7
Lettuce (<i>Lactuca sativa</i>)	3.637	3.244	-11
Radish (<i>Raphanus sativus</i>)	3.925	3.777	-4
Tomato (<i>Lycopersicon esculentum</i>)	6.676	5.507*	-18
Cucumber (<i>Cucumis sativus</i>)	6.324	6.890	9
Cabbage (<i>Brassica oleracea</i>)	5.040	4.975	-1
Oat (<i>Avena sativa</i>)	4.451	4.108*	-8
Ryegrass (<i>Lolium perenne</i>)	1.551	1.339*	-14
Corn (<i>Zea mays</i>)	4.200	4.145	-1
Onion (<i>Allium cepa</i>)	0.879	0.866	-1

* Significantly different from control at 5% level

Conclusion

Based on significant biomass reduction, the NOEC for soybean, tomato, oat and ryegrass is <0.56 kg a.s./ha. The NOEC for lettuce, radish, cucumber, cabbage, corn and onion is 0.56 kg a.s./ha.

Guidelines and limitations

In the present study, test concentrations were not analytically confirmed, as required by draft OECD 208 (2004). This requirement however was not laid down in OECD 208 (1984), which is still in force at present.

B.9.9.2 Effects on Collembola

Characteristics

reference	:	Gray J. (2003)	species	:	<i>Folsomia candida</i>
type of study	:	Laboratory reproduction toxicity study with springtails	exposure duration	:	28 days
year of execution	:	2002-2003	nominal concentrations	:	0, 0.82, 1.64, 3.28, 6.56 and 13.12 mg a.s./kg soil d.w.
GLP statement	:	Yes	dosing method	:	Test substance was dissolved in water and mixed into the soil
guideline	:	ISO 11267 (1999) and Willem (1902)	acceptability	:	Acceptable
test substance	:	Pyridaben 75 WP, lot number OAJ 030	NOEC	:	3.28 mg a.s./kg (2.33% oc).
a.s. content	:	76.5% (w/w)			

Methods

In a 28-day reproduction study, springtails (*Folsomia candida*) were exposed to Pyridaben 75 WP (76.5% pyridaben) at 0.82, 1.64, 3.28, 6.56 and 13.12 mg a.s./kg dry soil for 28 days in soil (Lufa 2.2). The test substance was added to the soil as a solution in deionised water. The control was treated with deionised water. A positive control (Danadim, a.s. dimethoate, 13.3 mg a.s./kg soil d.w.) was included. The test was conducted in 120 mL glass vessels (5 per treatment) with plastic lids containing 30 g of moist soil and 10 springtails. Jars were maintained at 20-28°C and 37-100% RH under a 16 h light regime (270-660 lux). Soil moisture was maintained at 49-50% of MWHC. The pH was 6.1-6.2 at the start and 6.5-6.6 at 28 days, when adult survival and the number of juveniles were assessed.

Results

The positive control gave the expected response. Other results are summarised in Table B.9.9.2-01. Survival was reduced by 27% (statistically significant) at 13.12 mg a.s./kg, and the number of offspring was reduced (statistically significant) by 26% and 43% at 6.56 and 13.12 mg a.s./kg. No treatment related effects were recorded at all other dose levels up to and including 3.28 mg a.s./kg (NOEC 3.28 mg a.s./kg).

Table B.9.9.2-01 Effects of Pyridaben 75 WP on *Folsomia candida*

Parameter	Value for parameter at dose (mg a.s./kg soil d.w.)						
	0 (control)	0.82	1.64	3.28	6.56	13.12	Dimethoate, 13.3
Mortality adults [%]	4	4	10	14	12	30*	100*
Corrected mortality ^(A)	-	0	6.3	10	8.3	27	100
Mean (SD) ^(C) no. of juveniles per vessel	477.8 (42)	479.8 (43)	491.4 (49)	471.0 (29)	353.6* (62)	273.2* (62)	0*
Reduction in reproduction (%) ^(B)	-	0	0	1.4	26	43	100

* Significantly different from the control at 5% level

(A) According to Abbott formula

(B) Reduction = $1 - (R_t/R_c) \times 100$, where R_t and R_c are the mean number of juveniles in the treated and control group

(C) Standard deviations for juveniles were calculated by the RMS using the reported raw data

Conclusion

NOEC 3.28 mg a.s./kg (soil with 2.33% oc, see below).

Guidelines and limitations

The organic carbon content of the test soil (Lufa 2.2) was not reported. According to the Lufa-Speyer website, Lufa 2.2. soil has an organic carbon content of 2.33% (SD 0.18%). The study is acceptable.

B.9.9.2 Risk assessment

According to Annex II of 91/414, data from preliminary tests used to assess the biological activity and the possible impact on non-target species, both flora and fauna, should be provided. Since no specific data requirements are given, procedures for risk assessment were conducted in agreement with the recommendations in the Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (Working Document Sanco/10329/2002 rev 2 final, 17 October 2002). According to the Working Document (Sanco/10329/2002 rev 2 final, 17 October 2002) assessment for plants is performed using ecotoxicological relevant endpoints for plants (germination, biomass production and survival) of at least 6 species from different taxa. Data were submitted of Tier I screening laboratory tests on 10 plant species.

In a seedling emergence test and a vegetative vigor test, effects on seedling emergence, survival, plant height and biomass were $\leq 23\%$ for all ten species tested at a dose rate of 0.56 kg a.s./ha. *In vitro* seed germination was not affected by more than 7% in any of the species tested at a concentration of 1.5 mg a.s./L.

A Tier I assessment is performed according to the recommendations in the Working Document (Sanco/10329/2002). A preliminary risk assessment is based on the available data of initial laboratory screening. The risk is considered acceptable if less than 50% effect is observed at the maximum application rate (300 g a.s./ha for the application in citrus). Effects were $\leq 23\%$, hence well below 50%, in all ten plant species at a dose of 560 g a.s./ha, a dose 1.9 times higher than the maximum application rate. Therefore, the risk for plants is considered to be acceptable.

B.9.10 Effects on biological methods for sewage treatment (IIA 8.7)**B.9.10.1 Effects of the active substance****Characteristics**

reference	:	Barnes S.P. (2001)	species	:	Activated sludge
type of study	:	Activated sludge, respiration inhibition test	exposure duration	:	3 hours
year of execution	:	2001	nominal concentrations	:	0, 1.0, 10 and 100 mg/L
GLP statement	:	Yes	dosing method	:	Pyridaben was added directly to the test vessels
guideline	:	OECD 209, EC Directive 88/302	acceptability	:	Acceptable
test substance	:	Pyridaben (NC-129), lot 129T950808	3 hour EC50	:	>100 mg/L
purity	:	99.2%			

Methods

The effect of pyridaben (99.2% purity) on the rate of respiration of bacterial populations from activated sludge was determined after a contact time of 3 hours. Pyridaben was first added to the test vessels (glass that had been treated with a siliconising agent, Surfasil™), dechlorinated water was added next and dissolution and dispersion of the test material was facilitated by ultrasound treatment for 10 minutes. Toxicity was tested at 20±2°C in 1-3 flasks/concentration, with untreated controls in duplicate flasks. The sludge was obtained from a water treatment works containing effluent from predominantly domestic origin (final concentration in test 1.6 g solids/L). The contents of each flask (500 mL) was aerated during the contact period at a rate of 1 L air/minute. At the end of the contact time, oxygen consumption was measured for 10 minutes.

Results

The respiratory rate in the duplicate control flasks was 23.8 and 25.7 mg O₂/g/hour. At 1.0, 10 and 100 mg/L, the inhibition percentages of the respiratory rate were 5, 0 and 6%, respectively. The EC50 is >100 mg/L. The concurrently determined EC50 of the reference product 3,5-dichlorophenol was in the acceptable range of 5 to 30 mg/L.

Conclusion

The EC50 is >100 mg/L.

Guidelines and limitations

The test concentration was well above the water solubility of pyridaben (12 µg/L), but measures were taken (ultrasound treatment, maximum aeration rate) to ensure maximum solubility and dispersion of the test compound under the test conditions. The study was conducted according to OECD 209 and is acceptable.

B.9.10.2 Risk assessment

Given the nature of the proposed uses, it is unlikely that pyridaben will reach sewage treatment works. In any case, this compound caused no inhibition of the bacterial respiratory rate in the study submitted (EC50 >100 mg a.s./L). Consequently the risk to sewage treatment processes is acceptable.

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

B.9.11 References relied on**References for the active substance**

Annex point / reference number	Author(s)	Year	Title, Source, Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N	Owner
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IIA, 8.1.1/02	Grimes, J., Jaber, M.	1987b	NC-129: An acute oral toxicity study with the Mallard [REDACTED] Nissan Chemical Industries, Ltd. Report no. E2 GLP, Unpublished	Y	NCI
IIA, 8.1.2/01	Grimes, J., Hinken, C., Jaber, M.	1987	NC-129: A dietary LC50 study with the Bobwhite [REDACTED] Nissan Chemical Industries, Ltd. Report no. E3 GLP, Unpublished	Y	NCI
IIA, 8.1.2/02	Grimes, J., Jaber, M.,	1987c	NC-129: A dietary LC50 study with the Mallard [REDACTED] Nissan Chemical Industries, Ltd. Report no. E4 GLP, Unpublished	Y	NCI
IIA, 8.1.3/01	Beavers, J. B., Sipler, O., Mitchell, L. R., Jaber, M.	1994a	Pyridaben technical (NC-129): A reproduction study with the Northern Bobwhite [REDACTED] BASF Aktiengesellschaft Report no. E100 GLP, Unpublished	Y	BASF
IIA, 8.1.3/02	Beavers, J. B., Sipler, O., Mitchell, L. R., Jaber, M.	1994b	Pyridaben technical (NC-129): A reproduction study with the Mallard [REDACTED] BASF Aktiengesellschaft Report no. E101 GLP, Unpublished	Y	BASF
IIA, 8.2.1/02	Ward, G. S.	1994a	Pyridaben technical: Acute toxicity to rainbow trout, <i>Oncorhynchus mykiss</i> , under flow-through conditions at [REDACTED] [REDACTED] BASF Aktiengesellschaft Report no E102 GLP, Unpublished	Y	BASF

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IIA, 8.2.1/04	Ward, G. S.	1994b	Pyridaben technical: Acute toxicity to bluegill, <i>Lepomis macrochirus</i> , under flow-through conditions at [REDACTED] [REDACTED] BASF Aktiengesellschaft Report no. E103 GLP, Unpublished	Y	BASF
IIA, 8.2.1/05	Morrow, J. E., Ward, G. S.	1993	Pyridaben technical: Acute toxicity to the sheepshead minnow, <i>Cyprinodon variegatus</i> , under flow-through test conditions [REDACTED] BASF Aktiengesellschaft Report no. E105 GLP, Unpublished	Y	BASF
IIA, 8.2.1/06	Jenkins, C.A.	2003a	PB-7: Acute toxicity to rainbow trout [REDACTED] Nissan Chemical Industries, Ltd. Report no. E302 GLP, Unpublished	Y	NCI
IIA, 8.2.2.3	Rhodes, J. E., Leak, T., Holmes, C.M.	1995	Full life-cycle toxicity of BAS 300 I (Pyridaben) to the fathead minnow (<i>Pimephales promelas</i>) under flow-through conditions [REDACTED] BASF Aktiengesellschaft Report no. E110 GLP, Unpublished	Y	BASF
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IIA, 8.2.3/02	Ohuchiya, N.	1987	Test on bioaccumulation of NC-129 in carp [REDACTED] Nissan Chemical Industries, Ltd. Report no. M14 Non-GLP, Unpublished	Y	NCI
IIA, 8.2.4/02	Graves, W. C., Swigert, J. P.	1993	Pyridaben technical a.i.: A 48-hour flow-through acute toxicity test with the cladoceran (<i>Daphnia magna</i>) Wildlife International Ltd. BASF Aktiengesellschaft Report no. E104 GLP, Unpublished	Y	BASF

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IIA, 8.2.4/04	Ward, G. S.	1994c	Pyridaben (BAS 300 I-tech. a.i.): Acute effect on new shell growth of the eastern oyster, (<i>Crassostrea virginica</i>) Toxicon Environmental Sciences BASF Aktiengesellschaft Report no. E107 GLP, Unpublished	Y	BASF
IIA, 8.2.4/05	Jenkins, C. A.	2003b	PB-7: Acute toxicity to <i>Daphnia magna</i> Huntingdon Life Sciences Ltd. Nissan Chemical Industries, Ltd. Report no. E300 GLP, Unpublished	Y	NCI
IIA, 8.2.5/02	Drott, K. R., Swigert, J. P.	1994	14C Pyridaben: A flow-through life-cycle toxicity test with the cladoceran (<i>Daphnia magna</i>) Wildlife International Ltd. BASF Aktiengesellschaft Report no. E108 GLP, Unpublished	Y	BASF
IIA, 8.2.5/03	Holmes, C. M., Machado, M. W.	1995	14C Pyridaben (BAS 300 I) technical - Chronic toxicity to Mysids (<i>Mysidopsis bahia</i>) under flow-through conditions Springborn Laboratories, Inc. BASF Aktiengesellschaft Report no. E109 GLP, Unpublished	Y	BASF
IIA, 8.2.6/02	Hughes, J. S., Jackson, S. H.	1994	Tier 1 non-target aquatic plant toxicity study on BAS 300 I (Pyridaben) Malcolm Pirnie, Inc. BASF Aktiengesellschaft Report no. J3 GLP, Unpublished	Y	BASF
IA, 8.2.6/03	Jenkins, C.A.	2003c	PB-7: Algal growth inhibition assay Huntingdon Life Sciences Ltd., Nissan Chemical Industries, Ltd. Report no. E301 GLP, Unpublished	Y	NCI
IIA, 8.2.7/02	Albuquerque, R.	2003a	PB-7: Acute toxicity to the midge <i>Chironomus Riparius</i> Huntingdon Life Sciences Ltd. Nissan Chemical Industries, Ltd. Report no. E303 GLP, Unpublished	Y	NCI

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IIA, 8.3.1.1/03	Hinken, C.	1987	NC-129: An acute contact toxicity study with the honey bee Wildlife International Ltd. Nissan Chemical Industries, Ltd. Report no. E11 GLP, Unpublished	Y	NCI
IIA, 8.3.1.2	Halsall, N.	1999	Pyridaben 20 WP: Evaluation of effects of insect growth-regulating insecticides on honey bee (<i>Apis mellifera</i>) colony brood development Huntingdon Life Sciences Ltd. Nissan Chemical Industries, Ltd. Report no. E114WP20 GLP, Unpublished	Y	NCI
IIA, 8.3.2/01	Sharples, A.	2001a	Pyridaben 75 WP: Acute toxicity to <i>Typhlodromus pyri</i> in the laboratory Huntingdon Life Sciences Ltd. Nissan Chemical Industries, Ltd. Report no. E208 GLP, Unpublished	Y	NCI
IIA, 8.3.2/02	Sharples, A.	2001b	Pyridaben 75 WP: Acute toxicity to <i>Aphidius rhopalosiphii</i> in the laboratory Huntingdon Life Sciences Ltd. Nissan Chemical Industries, Ltd. Report no. E207 GLP, Unpublished	Y	NCI
IIA, 8.4.1	Coulson, J. M., Yearsdon, H. A., Edwards, P. J.	1989	NC-129: Investigation of the toxicity to the earthworm <i>Eisenia foetida</i> ICI Agrochemicals Syngenta Report no. E14 GLP, Unpublished	Y	NCI
IIA, 8.5/01	Gerhardt, R.	1989	Effect of BAS 9078 6 I on soil respiration BASF Aktiengesellschaft Report no. E16 GLP, Unpublished	Y	BASF

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IIA, 8.5/02	Dohmen, G. P.	1989	Effect of Pyridaben (BAS 9078 6 I) on nitrification BASF Aktiengesellschaft Report no. E17 GLP, Unpublished	Y	BASF
IIA, 8.6/01	Elliott, D. S.	1992	Tier 1 seed germination/seedling emergence nontarget phytotoxicity study using Pyridaben (BAS 300 I-tech. a.i.) Pan-Agricultural Laboratories, Inc. BASF Aktiengesellschaft Report no. J1 GLP, Unpublished	Y	BASF
IIA, 8.6/02	Blackburn, R. A.	1992	Tier 1 vegetative vigor nontarget phytotoxicity study using Pyridaben (BAS 300 I-tech. a.i.) Pan-Agricultural Laboratories, Inc. BASF Aktiengesellschaft Report no. J2 GLP, Unpublished	Y	BASF
IIA, 8.7	Barnes, S.P.	2001	Pyridaben: Activated sludge – respiration inhibition test Huntingdon Life Sciences Ltd. Nissan Chemical Industries, Ltd. Report no. E38 GLP, Unpublished	Y	NCI

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IIIA, 10.3	Anonymous	2006b	Response document no. 28. Points addressed Volume 3 B.9.1.6.1 Terrestrial mammal long-term risk assessment - dietary.	Y	NCI
IIIA, 10.2.2/01	Rand, G. M., Holmes, C. M.	1995	Pyridaben (BAS 300 11 I) An outdoor aquatic microcosm study [REDACTED] Nissan Chemical Industries, Ltd. Report no. E3WP75 GLP, Unpublished	Y	NCI

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IIIA, 10.2.2/02	Singh, M.	1994	Dissipation of 14C-BAS 300 I in an outdoor confined aquatic ecosystem [REDACTED] BASF Aktiengesellschaft Report no. F6 GLP, Unpublished	Y	BASF
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IIIA, 10.2.1/03	Jenkins, C. A.	2002b	Pyridaben 75 WP: Acute toxicity to <i>Daphnia magna</i> Huntingdon Life Sciences Ltd. Nissan Chemical Industries, Ltd. Report no. E6WP75 GLP, Unpublished	Y	NCI
IIIA, 10.2.1/04	Cunningham, F. J.	1996	Pyridaben: An outdoor 96-hour static acute toxicity test with the saltwater mysid, (<i>Mysidopsis bahia</i>) Toxicon Environmental Sciences BASF Aktiengesellschaft Report no. E4WP75 GLP, Unpublished	Y	BASF
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IIIA, 10.2.2/03	Singh, M.	1995	Confined aquatic dissipation of Pyridaben (BAS 300 I) in a simulated microcosm environment BASF Corporation BASF Aktiengesellschaft Report no. F7	Y	BASF

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IIIA, 10.2	Anonymous	2006d	Response document no. 17 (revised). Point addressed B.9.2.4.1.1 (Acute risk assessment) pages 86-87. Fish acute toxicity risk assessment refinement.	Y	NCI
IIIA, 10.3	Anonymous	2006b	Response document no. 28. Points addressed Volume 3 B.9.1.6.1 Terrestrial mammal long-term risk assessment - dietary.	Y	NCI
IIIA, 10.4.1/01	Gough, H. J., Yearsdon, H. A., Lewis, G. B.	1990b	NC-129: Acute contact and oral toxicity to honey bees (<i>Apis mellifera</i>) ICI Agrochemicals Syngenta Report no. E13 GLP, Unpublished	Y	NCI
IIIA, 10.4.1/02	Halsall, N.	1999a	Pyridaben 20 WP Formulation acute toxicity to honey bees (<i>Apis mellifera</i>) Huntingdon Life Sciences Ltd. Nissan Chemical Industries, Ltd. Report no. E113WP20 GLP, Unpublished	Y	NCI
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IIIA, 10.4.3/02-1 and -2	Stute, K.	1988b	Investigation of toxicity to bees for the registration procedure (Pyridaben 200 g/l EC) BASF Aktiengesellschaft Report no. E18EC Non-GLP, Unpublished	Y	BASF
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IIIA, 10.4, 10.5	Anonymous	2006e	Response document no. 29. Points addressed Volume 3, B.9.4.1.2 Acute toxicity of the plant protection product to bees. Volume 3: B.9.4.3 Tunnel studies with honeybees; B.9.4.4 Field studies with honeybees; B.9.4.5 Bee brood studies with honeybees; B.9.4.6.1 Honeybee higher tier risk assessment; B.9.5.3.1 Risk of the active substance to non target arthropods.	Y	NCI
IIIA 10.5.1/01	Bargen, H.	2002a	Pyridaben 75 WP: Toxicity to the predatory mite, <i>Typhlodromus pyri scheuten</i> (Acari, Phytoseiidae) using an extended laboratory test with aged residues GAB Biotechnologie, Germany Nissan Chemical Industries, Ltd. Report no. E210 GLP, Unpublished	Y	NCI
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IIIA, 10.5.2/03	Muther, J.	2003a	A field study to evaluate the effects of Pyridaben on the non-target arthropod fauna in a citrus orchard in Spain GAB Biotechnologie, Germany Nissan Chemical Industries, Ltd.	Y	NCI

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			Report no. E212 GLP, Unpublished		
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IIIA, 10.6.2/02	Carter, J.N.	2004	Pyridaben 75 WP: Breakdown of organic matter in litter bags Huntingdon Life Sciences Ltd. Nissan Chemical Industries, Ltd. Report no. E11WP75 GLP, Unpublished	Y	NCI