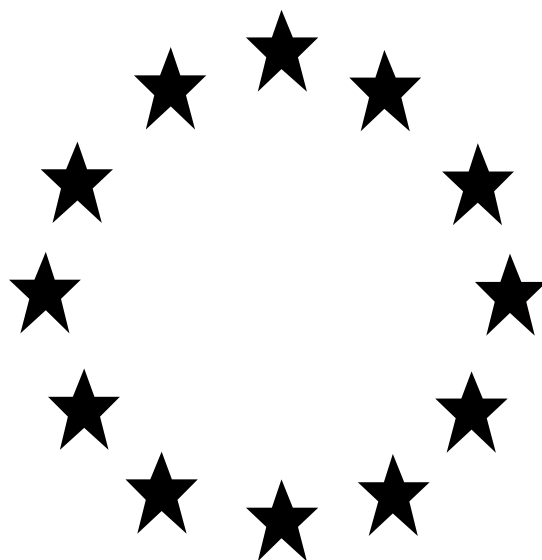




Draft Assessment Report (DAR)

- public version -

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

DICHLOBENIL

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

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B.9 ECOTOXICOLOGY

B.9.1 EFFECTS ON BIRDS

B.9.1.1 Short term oral toxicity

B.9.1.1.1 Acute oral toxicity of dichlobenil technical to mallard duck (IIA 8.1.1/01)

Characteristics of the study

Reference	: Roberts and Hakin (1988)	Duration of study	: 14 d observation after single dose
Year of execution	: 1987 – 1988	Acute oral LD ₅₀ ¹	
Guideline	: EPA FIFRA; section 71-1	males	: > 50 mg/kg bw
GLP	: yes	females	: > 50 mg/kg bw
Test substance	: dichlobenil technical; batch no. FUN7EO8A	NOEL ¹	
		males	: 50 mg/kg bw
		females	: 50 mg/kg bw
Purity	: 99.4%	Acceptable	: yes
Species	: Mallard duck (<i>Anas platyrhynchos</i>)		

¹ Vomiting observed at 100 mg/kg bw and higher

Study design

The acute oral toxicity of dichlobenil technical to the mallard duck was studied; the following study set up was applied:

Age and weight of birds	: > 16 weeks; 900 - 1250 g
housing of birds	: pens of galvanised steel; ca. 1.5 x 1.2m
temperature	: 18 - 20°C
relative humidity	: 85 ± 7%
photoperiod	: 7h light/17h dark
number of birds per group	: 5 males and 5 females
dosing	: single oral administration by gavage
carrier of test substance	: corn oil
dose	: initially 0, 320, 800 and 2000 mg test substance/kg bw additionally (because of vomiting in all initial test groups) 0, 50, 100, 200 mg test substance/kg bw
feeding	: <i>ad libitum</i> with a standard chick diet
type of observations	: daily for mortality and other visual adverse effects macroscopic post-mortem examination of the 200 and 2000 mg/kg bw group
type of measurements	: individual body weight: 14 and 7 days before the start of the test, at start of the test and at t = 7 and 14 days feed consumption per dosage group: periods: -14 to -8, -7 to -1, 1 to 7 and 8 to 14 days temperature and relative humidity: daily

Results

There was no mortality in the control or in any of the treated groups. Vomiting was observed for all birds within half an hour after treatment at 320 mg/kg bw and the higher doses tested. At 100 and 200 mg/kg bw, one and two birds, respectively, vomited within ten minutes. In the control and at 50 mg/kg bw, there were no emetic effects. Body weights were within the normal limits in all treatment groups and there was no effect on feed consumption in any treatment group. Post mortem examination showed no abnormalities.

There was no mortality observed in any of the treatment groups. As the compound was emetic at dose levels of 100 mg/kg and higher, the LD₅₀ is > 50 mg/kg bw for both males and females, the lowest dose where birds did not vomit. The results of the test are summarised in Table B.9.1.1.1a.

Table B.9.1.1.1a Acute oral toxicity of dichlobenil technical to mallard duck

Dose (mg/kg)	No of birds	No. of deaths	Mean body weight (g) at t = 14 days		Mean feed consumption per time period (in days) in g/bird/day (male/female)			
			male	female	-14 to 8	-7 to 1	1 to 7	8 to 14
0	10	0	1108 ± 181	1136 ± 92	83/83	83/97	74/109	60/111
0	10	0	1052 ± 85	954 ± 55	86/71	66/57	71/69	60/89
50	10	0	1062 ± 76	966 ± 92	80/71	63/63	69/74	74/74
100	10	0	1083 ± 102	957 ± 112	83/71	69/63	66/71	86/97
200	10	0	1106 ± 82	988 ± 80	91/91	69/89	69/86	126/134
320	10	0	1132 ± 49	1081 ± 85	83/71	74/74	69/89	80/89
800	10	0	1175 ± 92	1068 ± 86	134/77	89/94	83/77	86/77
2000	10	0	1144 ± 122	1094 ± 110	71/80	63/74	71/100	80/126

Acceptability of the test

The light period was too short (7h instead of 12h – 16h, according to OECD 401). This will not have influenced the results of the test. The test is considered acceptable

Conclusion

For males and females, the oral 14d-LD₅₀ and NOEL of dichlobenil technical to the mallard duck are > 50 and 50 mg/kg bw, respectively.

B.9.1.1.2 Acute oral toxicity of dichlobenil technical to northern bobwhite quail (IIA 8.1.1/02)

Characteristics of the study

Reference	: Johnson (1994)	Duration of study	: 15 d observation after single dose
Year of execution	: 1993 – 1994	Oral 15d-LD ₅₀	:
Guideline	: EPA FIFRA; section 71-1	males	: 698 mg/kg bw
GLP	: yes	females	: 727 mg/kg bw
Test substance	: dichlobenil technical; batch no. FUN87EO8A	NOEL	:
		males	: < 400 mg/kg bw
		females	: < 400 mg/kg bw
Purity	: 98.8%	Acceptable	: yes
Species	: northern bobwhite quail (<i>Colinus virginianus</i>)		

Study design

The acute oral toxicity of dichlobenil technical to northern bobwhite quail was studied; the following study design was applied:

age of birds	: 14 – 15 weeks old
housing of birds	: stainless steel grid cages
temperature	: $21 \pm 3^{\circ}\text{C}$
relative humidity	: $55 \pm 15\%$
photoperiod	: 8h light/16h dark
number of birds per group	: 5 males and 5 females
dosing	: supplied once in gelatine capsules (lubricated with paraffin oil)
dose	0, 400, 800, 1086, 1474 and 2000 mg/kg bw
feeding	: <i>ad libitum</i> with a commercial avian diet; the night before the test, the birds were not fed
type of observations	: mortality and other visual adverse effects; at least once daily post-mortem examination
type of measurements	: individual body weight one day before the start of the test (day -1) on days 1, 8 and 15 feed consumption per dosage group during each week of the study by weighing the residue of a known quantity

Results

Mortality or any other adverse effects were not observed in the control group or the lowest dosage of 400 mg/kg bw. At the higher doses tested, the majority of the birds died. *Ante mortem* effects comprised signs of under-activity cold to touch, ungroomed appearance, plume-erection, little diet eaten, few faeces and feather loss. Some birds treated at 2000 mg/kg bw also had pink staining in the under-trays. In surviving birds, thin body conformation, plume-erection, little diet eaten, few faeces were seen. Feed consumption of birds treated at 400 mg/kg bw was considerably lower than that of the control during the first week of the study. Thereafter the feed consumption recovered. At other doses, surviving birds consumed little diet. Body weight at 400 mg/kg bw was considerably less during the first week of the study, but gained substantially during the second week. Neither the animals that died during the test, nor the surviving birds revealed significant macroscopic lesions. The results of the test are summarised in Table B.9.1.1.2a. The oral 14d-LD₅₀ values of dichlobenil technical to northern bobwhite quail (*Colinus virginianus*) were 698 (488 – 908) mg/kg bw for males and 727 (429 – 1025) mg/kg bw for females.

Table B.9.1.1.2a Acute oral toxicity of dichlobenil technical to northern bobwhite quail (*Colinus virginianus*)

Dose (mg/kg)	No of birds	No. of deaths		Mean feed consumption % of control	
		males	females	males	females
0	10	0	0	100	100
400	10	0	0	55	52
800	10	5	4	-	-
1086	10	3	5	-	-
1474	10	5	5	-	-
2000	10	5	5	-	-

Acceptability of the test

The light period was too short (8 h instead of 12 h – 16 h, according to OECD 401). It is not expected that this has influenced the results of the test. The test is acceptable.

Conclusion

The oral 15d-LD₅₀ values of dichlobenil technical to northern bobwhite quail (*Colinus virginianus*) are 698 (488 – 908) mg/kg bw for males and 727 (429 - 1025) mg/kg for females. The NOEL was < 400 mg/kg bw for both males and females.

B.9.1.2 Dietary toxicity

B.9.1.2.1 Avian dietary toxicity of dichlobenil technical to mallard duck (IIA 8.1.2/01)

Characteristics of the study

Reference	: Roberts and Hakin (1988)	Species	: 99.4%
Year of execution	: 1987 – 1988	Purity	: mallard (<i>Anas platyrhynchos</i>)
Guideline	: EPA FIFRA; section 71-2	Duration of study	: 8 days
		5d-LC ₅₀	: > 5200 mg a.s./kg diet (corresponding with an 8d-LD ₅₀ of > 470 mg a.s./kg bw/day)
GLP	: yes	5d-NOEC	: 1300 mg a.s./kg diet (corresponding with an 8d-NOEL of 280 mg a.s./kg bw/day)
Test substance	: dichlobenil technical; batch no. FUN7E08A	Acceptable	: yes

Study design

The dietary toxicity of dichlobenil technical to the mallard was studied; the following test set up was applied:

age and weight of birds	: 10 days; group mean weight 71 g
housing of birds	: galvanised steel brooders 83 x 48 x 20 cm
room temperature	: 26 – 29°C
relative humidity	: 65%
number of replicates	: 3 (control) and 1 (test groups)
number of birds per group	: 10
dosing	: via diet during 5 days
post treatment period	: 3 days
preparation of spiked feed	: mixing test substance into feed
dose	: 0, 650, 1300, 2600 and 5200 mg/kg feed

feeding	: <i>ad libitum</i> with a standard chick diet
type of observations	: mortality and other adverse effects daily <i>post-mortem</i> examination eight animals of the 5200 mg/kg group and two of the 2600 mg/kg group were macroscopically examined at the end of the study
type of measurements	: body weight; all birds were group weighed 3 days before the start of the test, at the start of the treatment period and on day 5 and 8 of the study feed consumption per dosage group for days -3 to -1, 1 to 5 (daily) and 6 to 8

Results

At 5200 mg/kg, two birds died. There were no other mortalities. During the treatment period, birds at 2600 and at 5200 mg/kg showed adverse effects with respect to behaviour (subdued, huddling together and unsteadiness). At all other exposure groups, the birds were in good health; during the post treatment period, the birds were in good condition. Macroscopically *post-mortem* examination showed no abnormalities in any birds. The results for mortality, body weight gain and feed consumption are given in Table B.9.1.2.1a. It was not possible to determine a LC_{50} value, as only two mortalities occurred at the highest tested dose; the LC_{50} can therefore only be given as > 5200 mg a.s./kg diet. The overall NOEC value is 1300 mg a.s./kg diet.

Table B.9.1.2.1a Dietary toxicity of dichlobenil technical to the mallard (*Anas platyrhynchos*)

Dose (mg/kg feed)	No. of birds exposed	No. of deaths	Mean group body weight gain (g/bird)			Feed consumption (g/bird/day)		
			day -3 to 0	day 0 to 5	day 5 to 8	day -3 to -1	day 1 to 5	day 6 to 8
0	10	0	36	102	44	25	40	52
0	10	0	41	95	47	25	42	58
0	10	0	44	99	49	26	44	58
650	10	0	37	97	52	22	40	60
1300	10	0	41	64	47	25	31	56
2600	10	0	40	7	59	24	18	49
5200	10	2	36	-15	53	24	9	43

According to the guidance in Sanco/4145/2000 toxicity values must be converted to mg test substance/kg body weight/day. A strong avoidance of food was observed at the highest test concentration. Food avoidance may influence the test results as mortalities due to starvation may occur. The LC_{50} was above the highest test concentration and therefore, bias due to starvation is no issue in the present study. RMS calculated the LD_{50} and NOEL value based on the following:

- At 1300 and 5200 mg a.s./kg diet, the mean feed consumption during the treatment period was 31 and 9 g/bird/day, respectively
- Over the exposure period, the average body weight at 1300 and 5200 mg a.s./kg diet was 144 and 99.5 g, respectively
- The LC_{50} value of > 5200 mg a.s./kg diet thus corresponds with a LD_{50} value of $(5200 \times 9)/99.5 = > 470$ mg a.s./kg bw/day

- The NOEC value of 1300 mg a.s./kg diet corresponds with a NOEL value of $(1300 \times 31)/144 = 280$ mg a.s./kg bw/day

Acceptability of the test

The test is considered acceptable.

Conclusions

The 8d-LC₅₀ and NOEC of dichlobenil technical for the mallard (*Anas platyrhynchos*), in a dietary toxicity test are > 5200 mg a.s./kg diet and 1300 mg a.s./kg diet, respectively. This corresponds to a LD₅₀ and NOEC of > 470 and 280 mg a.s./kg bw/day, respectively.

B.9.1.2.2 Avian dietary toxicity of dichlobenil technical to northern bobwhite quail (IIA 8.1.2/02)

Characteristics of the study

Reference	: Roberts and Hakin (1987)	Species	: northern bobwhite quail (<i>Colinus virginianus</i>)
Year of execution	: 1987 – 1988	Duration of study	: 8 days
Guideline	: EPA FIFRA; section 71-2	5d-LC ₅₀	: ca. 5200 mg a.s./kg diet (corresponding to an 8d-LD ₅₀ of ca. 598 mg a.s./kg bw/day)
GLP	: yes	5d-NOEL	: 1300 mg a.s./kg diet (corresponding to an 8d-NOEL of 241 mg a.s./kg bw/day)
Test substance	: dichlobenil technical; batch no. FUN7E08A	Acceptable	: yes
Purity	: 99.4%		

Study design

The dietary toxicity of dichlobenil technical to the northern bobwhite quail was studied; the following test set up was applied:

age and weight of birds	: 10 days; group mean weight 12.6 – 13.0 g
housing of birds	: wooden pens of approximately 83 x 52 x 61 cm
room temperature	: 21 - 25°C
relative humidity	: 67%
number of replicates	: 3 (control) and 1 (test groups)
number of birds per group	: 10
dosing	: via diet during 5 days
post treatment period	: 3 days
preparation of spiked feed	: mixing test substance into feed
dose	: 0, 163, 325, 650, 1300, 2600 and 5200 mg/kg feed
feeding	: <i>ad libitum</i> with a standard chick diet
type of observations	: mortality and other adverse effects daily
	<i>post-mortem</i> examination
	eight animals of the 5200 mg/kg group and two of the 2600 mg/kg group were macroscopically examined at the end of the study
type of measurements	: body weight all birds were group determined 3 days before

the start of the test, at the start of the treatment period and on day 5 and 8 of the study
feed consumption per dosage group on days -3 to -1, 1 to 5 (daily) and 6 to 8

Results

The results of the test with respect to mortality, mean body weight and feed intake are given in Table B.9.1.2.2a. At 5200 mg/kg, five birds died. There was one, no dose related, mortality at 325 mg/kg. Due to the steep mortality curve it was not possible to calculate the LC_{50} value, but this value is estimated to be approximately 5200 mg/kg.

Group mean body weight decreases were observed during the treatment period at 2600 and 5200 mg/kg, while the other groups showed increases. Feed consumption was variable; there was less feed intake at 325, 2600 and 5200 mg/kg during the treatment period. However, at post treatment there were no treatment related effects with respect to feed consumption. Clinical signs of toxicity (unsteadiness) were observed at 2600 and 5200 mg/kg during treatment. There were no abnormalities observed at *post-mortem* examination. When the decreased feed intake at 325 mg/kg is considered as an outlier, the overall NOEC is 1300 mg/kg.

Table B.9.1.2.2a Dietary toxicity of dichlobenil technical to the northern bobwhite quail

Dose (mg/kg feed)	No. of birds exposed	No. of deaths	Mean group body weight gain (g/bird)			Feed consumption (g/bird/day)		
			day -3 to 0	day 0 to 5	day 5 to 8	day -3 to -1	day 1 to 5	day 6 to 8
0	10	0	5	2.9	3.8	3.6	3.6	4.0
0	10	0	5.3	7.6	7.6	4.5	4.7	6.4
0	10	0	4.9	6.5	4.9	4.0	3.3	6.4
163	10	0	5.6	6.0	5.2	4.2	4.1	6.4
325	10	1	4.3	3.5	4.1	3.2	2.8	4.4
650	10	0	4.7	5.5	5.8	3.6	3.9	6.7
1300	10	0	4.9	3.2	5.7	3.7	3.6	4.8
2600	10	0	5.2	-0.8	7.9	3.8	2.6	5.1
5200	10	5	5.3	-1.5	7.8	4.1	2.0	6.3

According to the guidance in Sanco/4145/2000 toxicity values must be converted to mg test substance/kg body weight/day. Conversion of the LC_{50} to a LD_{50} may be unreliable when the LC_{50} is below the top concentration and a distinct food avoidance occurs well below the LC_{50} . Feed consumption was variable but appeared to be slightly reduced at the two highest test concentrations. Further, the LC_{50} was at (and not below) the top concentration. Therefore, the conversion from concentration to daily dose is considered reliable. The calculation was based on the following:

- At 1300 and 5200 mg a.s./kg diet, the mean feed consumption during the treatment period was 3.6 and 2.0 g/bird/day
- Over the exposure period, the average body weight was 19.4 g at 1300 mg a.s./kg diet and 17.4 g at 5200 mg a.s./kg diet

- The NOEC value of 1300 mg a.s./kg diet thus corresponds with a NOEL value of $1300 \times 3.6/19.4 = 241$ mg a.s./kg bw/day
- The LC₅₀ value of 5200 mg a.s./kg diet corresponds with a LD₅₀ value of $5200 \times 2.0/17.4 = 598$ mg a.s./kg bw/d.

Acceptability of the test

The test is considered acceptable.

Conclusions

The 8d-LC₅₀ of dichlobenil technical for the northern bobwhite quail (*Colinus virginianus*), in a dietary toxicity test is approximately 5200 mg/kg diet; the NOEC value is 1300 mg/kg diet. This corresponds to an 8d-LD₅₀ and NOEL value of ca. 598 and 241 mg a.s./kg bw/day, respectively.

B.9.1.2.3 Avian dietary toxicity of the metabolite 2,6-dichlorobenzamide (AE C653711) to northern bobwhite quail (IIA 8.1.2/03)

Characteristics of the study

Reference	: Gallagher <i>et al.</i> (2003)	Species	: northern bobwhite quail (<i>Colinus virginianus</i>)
Year of execution	: 2003	Duration of study	: 8 days
Guideline	: EPA FIFRA section 71-2; OPPTS 850.2200; OECD 205	5d-LC ₅₀ ¹	: 3867 mg metabolite/kg diet
		5d-NOEC	: 1780 mg metabolite/kg diet (corresponding with 876 mg metabolite/kg bw/d)
GLP	: yes		
Test substance	: 2,6-dichlorobenzamide; batch no. I8499A; appearance: powder		
Purity	: 98%	Acceptable	: yes

¹ Due to food avoidance, the conversion of LC₅₀ (in mg/kg diet) to LD₅₀ (in mg a.s./kg bw/day) was unreliable

Study design

The dietary toxicity of 2,6-dichlorobenzamide to the northern bobwhite quail was studied; the following test set up was applied:

weight of birds	: 16 – 23 g at test initiation
housing of birds	: wooden pens of approximately 83 x 52 x 61 cm
room temperature	: $29 \pm 1.1^\circ\text{C}$
relative humidity	: $59 \pm 4\%$
number of replicates	: 2 (control) and 1 (test groups)
number of birds per group	: 5
dosing	: via diet during 5 days
acclimation	: 10 days
post treatment period	: 3 days
preparation of spiked feed	: mixing test substance into feed
dose	: 0, 562, 1000, 1780, 3160 and 5620 mg metabolite/kg feed
feeding	: <i>ad libitum</i> with a standard diet
water supply	: <i>ad libitum</i>
type of observations	: mortality and other adverse effects at the start and end of the

	test and twice daily
	<i>post-mortem</i> examination
	eight animals of the 5200 mg metabolite/kg group and two of the 2600 mg metabolite/kg group were macroscopically examined at the end of the study
type of measurements	: Individual body weight of all birds at the start of the test, on day 5 and at the end of the study (day 8)
	Average feed consumption by pen per dosage group for days on day 1 to 5 and 6 to 8

Results

A treatment related reduction of body weight gain was observed at 562 mg metabolite/kg feed and higher. Feed intake was reduced at 3160 and 5620 mg metabolite/kg feed; the reduced feed intake at the higher concentrations resulted in response data, which are not monotonically increasing with the concentration. One incidental mortality case was observed at 562 mg metabolite/kg. At 3160 mg metabolite/kg five animals died. Signs of toxicity, as wing drop, loss of coordination ruffled appearance and lethargy, were observed at doses of 1000 mg metabolite/kg and higher. The results of the test with respect to mortality are given in Table B.9.1.2.3a, together with those for mean body weight, feed intake and the mean daily dietary dose. According to the authors the dietary LC₅₀ is 3867 (2940 – 5679) mg metabolite/kg feed, which corresponds with a LD₅₀ of 1171 mg metabolite/kg bw/d (confidence intervals could not be calculated). RMS calculated a similar value. The overall NOEC was 1780 mg metabolite/kg feed, corresponding with a NOEL of 876 mg metabolite/kg bw/day.

Table B.9.1.2.3a Dietary toxicity of 2,6-dichlorobenzamide to the northern bobwhite quail

Dose (mg metabolite/kg feed)	No. of birds exposed	No. of deaths after 5 days ¹	Mean body weight (g)			Feed consumption (g/bird/day)		Estimated daily dose (mg metabolite/kg bw/d)
			day 0	day 5	day 8	day 0 to 5	day 6 to 8	
0	30	0	20 ± 2	30 ± 3	40 ± 4	8	10	0
562	10	1	20 ± 2	23 ± 3	33 ± 5	14	11	376
1000	10	0	19 ± 2	20 ± 4	30 ± 7	10	10	528
1780	10	0	20 ± 2	19 ± 3	27 ± 5	9	9	876
3160	10	5	19 ± 2	15 ± 2	22 ± 2	4	11	715
5620	10	7	20 ± 2	16 ± 3	23 ± 6	5	10	1468

¹ there was no further mortality until day 8

Acceptability of the test

The conversion from concentration to daily dose was unreliable at the two highest test concentrations due to a distinct avoidance of food and high mortality. For this reason, the calculation of the LD₅₀ is considered unreliable. The test is considered acceptable.

Conclusions

The 5d-LC₅₀ of 2,6-dichlorobenzamide for the northern bobwhite quail (*Colinus virginianus*), in a dietary toxicity test is 3867 mg metabolite/kg feed. Due to distinct food avoidance and high mortality at the two

highest test concentrations, this value could not be converted to a reliable LD₅₀. In accordance with Sanco/4145/2000 the NOEC could be used instead. The NOEC value is 1780 mg metabolite/kg feed, which corresponds with a NOEL value of 876 mg metabolite/kg bw/d.

B.9.1.3 Long term toxicity

B.9.1.3.1 Long term/reproductive toxicity of the active substance to northern bobwhite quail (IIA, 8.1.3/01)

Characteristics of the study

Reference	: Mitchell <i>et al.</i> (2000)	Species	: northern bobwhite quail (<i>Colinus virginianus</i>)
year of execution	: 1999 – 2000	Duration of study	: 22 weeks
GLP statement	: yes	22wk-NOEC	: 150 mg test substance/kg diet
Guideline	: FIFRA 71-4; OECD 206	reproduction	: (corresponding with a 22wk-NOEL of 15.7 and 14.1 mg test substance/kg bw/day for males and females, respectively)
Test substance	: dichlobenil Technical, Lot No. FUX11000-FUN87E08A, white crystalline solid	Acceptable	: yes
Purity	: 98.5%		

Study design

The purpose of the study was to assess the potential adverse effects of dichlobenil on reproduction when administered to northern bobwhite quail in the diet for a period of 22 weeks. The following test set up was used:

age of birds at the start of test	: 20 weeks
study phases	: acclimation: 6 weeks pre-photostimulation: 7 weeks pre-egg laying (with photostimulation): 3 weeks egg-laying: 11 weeks post-adult-termination: (final incubation, hatching and 14-day offspring rearing period): 6 weeks
husbandry and test conditions	: indoors in batteries of pens; 27 x 51 cm, height 51 cm
temperatures	: 19.4 ± 1.6 °C (study room) 13.2 ± 0.3% (egg storage) 37.5°C (incubator)
relative humidity	: 37 ± 12%
photoperiod	: during acclimation: 8h light/day from beginning of week 8: 16 h light per day
feeding and drinking	: feed and water <i>ad libitum</i> during acclimation and testing
preparation of test diet	: weekly, by mixing dichlobenil into a premix used for preparing the diet
test concentrations	: 0, 150, 300, and 600 mg/kg diet
number of birds per dose	: 16; 8 males and 8 females pair housed
chemical analysis	: concentrations in diet homogeneity in diet stability in diet
chemical analytical method	: GC with ECD
symptoms	
observations (adults)	: daily for signs of toxicity or abnormal behaviour

body weights determination	: at start of treatment and on weeks 2,4, 6, 8 and at adult termination
feed consumption measurement	: once per week during throughout the test
pathology	: all birds in study were subjected to gross necropsy and descriptions of all internal macroscopic abnormalities were recorded
<i>reproduction</i>	
collection of eggs	: daily from all pens, if available
observation on eggs, reported as number per week	: number of eggs per pen, daily number of broken/cracked eggs, daily number of eggs set per pen, recorded and reported weekly weight of eggs laid per pen, recorded and reported weekly number of fertile eggs per pen weekly number of viable 11 and 23-day old embryos weekly number of hatchlings per pen, reported on a weekly base eggshell thickness
<i>incubation of eggs</i>	
storage time	: maximal 7 days
Incubation of new series of eggs	: weekly
maintenance of hatchlings	: 14 days
observation on chicks, reported on weekly basis	: clinical observations daily body weights, individually determined, on day 1 (after removal from the hatcher) and on day 15 (at termination); reported as mean weight per pen

Results

Analysis of the diet

The test substance was homogeneously mixed with the diet (coefficients of variation were 4.42, 1.60 and 7.03% for the 150, 300 and 600 mg/kg diets, respectively. There was no contamination of the control. Mean and standard deviations for the 150, 300 and 600 mg/kg diets were: 146 ± 7.98 , 289 ± 14.7 and 600 ± 40.0 mg/kg diets, respectively and representing 97.3, 96.3 and 100% of the nominal concentrations.

Mortality of adults

In the control, 150, 300 and 600 mg/kg diet groups, 2, 1, 0 and 1 mortalities were observed, respectively. There was thus no treatment-related mortality.

Clinical observations of adults

No overt signs of toxicity were observed except incidental findings, which can be considered as normal in this test and are associated with injuries and pen wear.

Gross necropsy of adults

Minor macroscopic examination *post-mortem* revealed abnormalities in both the control and in the treated groups which could not be attributed to the test substance.

Adult body weight

There were no significant differences between the treatment and control groups for the mean body weight values of hens at any of the body weight intervals. There were slight but statistically significant differences for all intervals at 150 and 600 mg/kg diet and at 300 mg/kg diet in week 8. This significance was primarily caused by the presence of two heavier males in the control group. Since body weight changes over time were equivalent for all groups, the authors did not consider these slight differences in male body weights as biologically relevant. RMS agrees with this.

Adult feed consumption

There were no treatment-related effects with respect to feed consumption.

Reproduction

At 150 mg/kg diet, there were no treatment-related significant effects on reproduction when compared with the control. Slight treatment-related effects on egg production and offspring survival were observed at 300 mg/kg diet. These effects were not statistically significant, but a significant reduction of 14-d old survivors as percentage of the maximum number of eggs set was found. At 600 mg/kg diet, reproduction was significantly less than in the control ($p < 0.01$).

Eggshell thickness

There were no significant treatment-related effects with respect to eggshell thickness at any of the concentrations tested when compared with the control.

Offspring body weight

There were no significant effects on body weight of hatchlings and 14 d old survivors at 150 and 300 mg/kg diet when compared with the control. However, at 600 mg/kg the mean body weight was statistically reduced ($p < 0.01$).

The results of the reproduction test with northern bobwhite quail are summarised in Table B.9.1.3.1a.

Dichlobenil caused effects on reproduction of northern bobwhite quail at a concentration of 300 mg/kg diet. The 22wk-NOEC for reproduction is therefore 150 mg/kg diet.

Table B.9.1.3.1a Results of the reproduction test with northern bobwhite quail exposed to dichlobenil

	0 mg/kg diet	150 mg/kg diet	300 mg/kg diet	600 mg/kg diet
No. of pairs at start of treatment	16	16	16	16
No. of adults at end of treatment	14	15	16	15
Mean body weight at the start of the test (in g)				
- males	203	194*	196	192*
- females	197	195	197	193
Change in body weight at test termination (in g)				
- males	23 ± 15	13 ± 11	17 ± 13	12 ± 11
- females	47 ± 11	54 ± 6	47 ± 15	44 ± 21
Mean feed consumption	21.3 ± 5.5	20.9 ± 4.9	21.0 ± 5.2	21.9 ± 6.4
Total no. of eggs laid	765	734	669	419
No of cracked eggs	3	11	7	1
Mean eggshell thickness (in mm)	0.238 ± 0.017	0.229 ± 0.013	0.231 ± 0.016	0.228 ± 0.012
Total no. of eggs set	689	646	590	350**
No. of viable eggs ¹	640	606	559	328**
No. of live 3-wk embryos	637	603	556	327**
No. of hatchlings	602	586	518	260**
14-d old survivors	580	542	478	194**
No. of eggs laid/hen	55	49	42	26**
No of eggs laid/hen/day	0.54	0.48	0.41	0.26**
No. of 14-d old survivors/hen	41	36	30	12
14-d old survivors/maximum eggs set (%)	62	54	45*	18**
Mean body weight of hatchlings (in g)	6 ± 0	6 ± 0	6 ± 0	5 ± 0**
Mean body weight of 14-d survivors (in g)	26 ± 3	25 ± 2	25 ± 2	19 ± 3**

¹ number of viable embryos at day 11 per number of eggs per pen

* significantly different from the control at p < 0.05

** significantly different from the control at p < 0.01

According to the EU Guidance document on risk assessment (Council directive 91/414/EEC) toxicity values must be converted to mg test substance/kg body weight/day. Therefore the NOEL value was calculated by RMS. This calculation was based on the following:

- At 150 mg test substance/kg diet, the mean feed consumption during the treatment period was 20.9 g/bird/day
- Over the exposure period, the average body weight of male and female birds at 150 mg test substance/kg diet was 200 and 222 g, respectively
- The NOEC value of 150 mg test substance/kg diet thus corresponds with a NOEL value of $(150 \times 20.9) / 200 = 15.7$ mg test substance/kg bw/day for males and $(150 \times 20.9) / 222 = 14.1$ mg test substance/kg bw/day for females.

Acceptability of the test

The test is acceptable.

Conclusion

At 300 mg/kg, dichlobenil caused effects with respect to reproduction. The test substance did not cause significant toxic effects to breeding northern bobwhite quail or to the reproduction of the birds at 150 mg/kg

diet. The 22wk-NOEC for reproduction is therefore 150 mg/kg diet. This corresponds with a NOEL of 15.7 and 14.1 mg test substance/kg bw/day for males and females, respectively.

B.9.1.3.2 Long term/reproductive toxicity of the active substance to mallard duck (IIA, 8.1.3/02)

Characteristics of the study

Reference	: Mitchell <i>et al.</i> (2000)	species	: mallard duck (<i>Anas platyrhynchos</i>)
year of execution	: 1999 – 2000	duration of study	: 21 weeks
GLP statement	: yes	21wk-NOEC	: 300 mg test substance/kg diet
Guideline	: FIFRA 71-4; OECD 206	reproduction	: (corresponding with 35.5 and 36.7 mg test substance/kg bw/day for males and females, respectively)
Test substance	: dichlobenil technical, Lot no. FUX01-1000-FUN87E08A;	Acceptable	: yes
Purity	: 98.5%		

Study design

The purpose of the study was to assess the potential adverse effects of dichlobenil on reproduction when administered to the mallard duck in the diet for a period of 21 weeks. The following test setup was used:

age of birds at the start of test	: 27 weeks
weight range of birds	: 905 – 1373 g
study phases	: acclimation: 13 weeks pre-photostimulation: 10 weeks pre-egg laying (with photostimulation): 0 weeks egg-laying: 10 weeks post-adult-termination: (final incubation, hatching and 14-day offspring rearing period): 6 weeks
temperatures	: 19.3 ± 1.0 °C (study room) 13.2 ± 0.3% (egg storage) 37.2°C (hatching compartment)
relative humidity	: 40 ± 15%
photoperiod	: during acclimation: 8h light/day from beginning of week 11: 17 h light per day (to induce egg laying)
feeding and drinking	: feed and water <i>ad libitum</i> during acclimation and testing
preparation of test diet	: weekly, by mixing dichlobenil into a premix used for preparing the diet
test concentrations	: 0, 150, 300, and 600 mg test substance/kg feed
number of birds per dose	: 16; 8 males and 8 females pair housed
chemical analysis	: concentrations in diet homogeneity in diet stability in diet
chemical analytical method	: GC with ECD
symptoms	
Observations (adults)	: daily for signs of toxicity or abnormal behaviour
body weights determination	: at start of treatment and on weeks 4, 6, 8 and at adult termination
feed consumption measurement	: once per week during throughout the test
pathology	: all birds in study were subjected to gross necropsy
reproduction	
collection of eggs	: daily from all pens, if available

observation on eggs, reported as number per week	: number of eggs per pen, daily number of broken/cracked eggs, daily number of eggs set per pen, recorded and reported weekly weight of eggs laid per pen, recorded and reported weekly number of fertile eggs per pen weekly number of viable 11 and 23-day old embryos weekly number of hatchlings per pen, reported on a weekly base eggshell thickness
<i>incubation of eggs</i>	
Incubation of new series of eggs	: weekly
maintenance of hatchlings	: 14 days
observation on chicks, reported on weekly basis	: clinical observations daily group body weight of surviving hatchlings on day 27 or 28 of incubation and average body weight of ducklings on day 14 of age

Results

Analysis of the diet

The test substance was homogeneously mixed with the diet (coefficients of variation were 4.42, 1.60 and 7.03% for the 150, 300 and 600 mg/kg diets, respectively. There was no contamination of the control. Mean and standard deviations for the 150, 300 and 600 mg/kg diets were: 146 ± 7.98 , 289 ± 14.7 and 600 ± 40.0 mg/kg diets and representing 97.3, 96.3 and 100% of the nominal concentrations, respectively.

Mortality of adults

There was no mortality in the control or in the exposure groups.

Clinical observations of adults

No overt signs of toxicity were observed except incidental findings, which can be considered as normal in this test and are associated with injuries and pen wear.

Gross necropsy of adults

Minor macroscopic examination post-mortem revealed abnormalities in both the control and in the treated groups, which could not be attributed to test substance.

Adult body weight

There were no significant differences between the treatment groups of 150 and 300 mg/kg group and control groups for the mean body weight values of hens at any of the body weight intervals. At 300 mg/kg there were slight and significant greater mean body weights at week 4, 6 and 8 body weight intervals. As the differences were slight and not treatment related, the increase in body weight is considered by the authors as not biological relevant. RMS agrees with this.

Adult feed consumption

There were no treatment-related effects with respect to feed consumption at 150 and 600 mg/kg. At 600 mg/kg there was a significant increase in feed consumption during the first ten weeks of the study; however, there was no corresponding increase in body weight.

Reproduction

At 150 and 300 mg/kg diet, there were no treatment-related significant effects upon reproduction when compared with the control. There were reductions in cracked eggs as percentages of eggs laid at all test concentrations, although the differences were slight. For the other reproductive parameters there were no statically significant differences between the control group and either the 150 or 300 mg/kg group.

At 600 mg/kg diet, there were effects upon egg production, fertility and hatchability (significant for egg production), as also significantly reflected in hatchlings and 14 d old survivors as percentages of eggs set.

Eggshell thickness

There were no significant treatment-related effects with respect to eggshell thickness at any of the concentrations tested when compared with the control.

Offspring body weight

There were no significant effects on body weight of 14-d old surviving hatchlings at 150, 300 and 600 mg/kg diet, when compared with the control.

The results of the reproduction test with the mallard duck are summarised in Table B.9.1.3.2a. At 600 mg/kg, there was a treatment-related increase in feed consumption during the first ten weeks of the study and there were marked effects upon reproduction performance (effects on egg production, fertility and hatchability resulted in reductions in the number of hatchlings and 14-d survivors). The 21wk-NOEC for reproduction is therefore 300 mg/kg diet.

Table B.9.1.3.2a Results of the reproduction test with mallard duck exposed to dichlobenil

	0 mg/kg diet	150 mg/kg diet	300 mg/kg diet	600 mg/kg diet
No. of pairs at start of treatment	16	16	16	16
No. of adults at end of treatment	16	16	16	16
Mean body weight at the start of the test				
- males	1163	1204	1129	1202
- females	1033	1060	1074	1018
Change in body weight at test termination (in g)				
- males	-9	-19	75	16
- females	99	118	108	73
Mean feed consumption g/bird/day	137 ± 39	148 ± 38	138 ± 37	159 ± 33**
Total no. of eggs laid	852	750	745	561**
No. of cracked eggs	33	14*	7**	4**
Mean eggshell thickness (in mm)	0.383 ± 0.022	0.388 ± 0.024	0.379 ± 0.017	0.378 ± 0.022
Total no. of eggs set	741	664	665	485**
No. of viable eggs	643	582	545	347**
No. of live 3-wk embryos	636	582	535	339**
No. of hatchlings	573	538	496	263**
No. of 14-d old survivors	568	536	492	261**
No. of eggs laid/hen	53	47	47	35**
No. of eggs laid/hen/day	0.73	0.64	0.64	0.48**
No. of 14-d old survivors/hen	36	34	31	16**
Mean body weight of hatchlings (in g)	37 ± 3	38 ± 2	37 ± 3	38 ± 2
Mean body weight of 14-d survivors (in g)	260 ± 19	278 ± 20	276 ± 20	256 ± 21

* significantly different from the control at p < 0.05

** significantly different from the control at p < 0.01

According to the EU Guidance document on risk assessment (Council directive 91/414/EEC) toxicity values must be converted to mg test substance/kg body weight/day. Therefore the NOEL value was calculated by RMS. This calculation was based on the following:

- At 300 mg test substance/kg diet, the mean feed consumption during the treatment period was 138 g/bird/day
- Over the exposure period, the average body weight of male and female birds at 300 mg test substance/kg diet was 1167 and 1128 g, respectively
- The NOEC value of 300 mg test substance/kg diet thus corresponds with a NOEL value of $(300 \times 138) / 1167 = 35.5$ mg test substance/kg bw/day for males and $(300 \times 138) / 1128 = 36.7$ mg test substance/kg bw/day for females.

Acceptability of the test

The test is acceptable.

Conclusion

At 600 mg/kg, dichlobenil caused statistically significant effects with respect to reproduction (egg production, fertility and hatchability). The test substance did not cause significant toxic effects to breeding parent mallard duck or to the reproduction of the birds at 300 mg/kg diet. The 21wk-NOEC for

reproduction is therefore 300 mg/kg diet. This corresponds with a NOEL of 35.5 and 36.7 mg test substance/kg bw/day for males and females, respectively.

B.9.1.4 Summary

Mallard duck and northern bobwhite quail were tested with the active substance for oral and dietary acute toxicity. The same two species were also tested in a reproduction test. The metabolite 2,6-dichlorobenzamide was tested in an acute dietary test. The summarised results are given in Table B.9.1.4a.

Table 9.1.4a. Summary of acute toxicity of the active substance dichlobenil and the metabolite 2,6-dichlorobenzamide

Purity	Species	Endpoint	Toxicity value	Test Guideline	Reference
dichlobenil					
Acute oral toxicity					
99.4%	Mallard duck (<i>Anas platyrhynchos</i>)	LD ₅₀ males females	> 50 mg test substance/kg bw > 50 mg test substance/kg bw	EPA FIFRA section 71-1	IIA, 8.1.1/01
		NOEL males females	50 mg test substance/kg bw 50 mg test substance/kg bw		
98.8%	Northern bobwhite quail (<i>Colinus virginianus</i>)	LD ₅₀ males females	698 mg test substance/kg bw 727 mg test substance/kg bw	EPA FIFRA section 71-1	IIA, 8.1.1/02
		NOEL males females	< 400 mg test substance/kg bw < 400 mg test substance/kg bw		
Dietary toxicity					
99.4%	Mallard duck (<i>Anas platyrhynchos</i>)	5d-LC ₅₀ 5d-LD ₅₀ 5d-NOEC 5d-NOEL	> 5200 mg a.s./kg diet > 470 mg a.s./kg bw/day 1300 mg a.s./kg diet 280 mg a.s./kg bw/day	EPA FIFRA section 71-2	IIA, 8.1.2/01
99.4%	Northern bobwhite quail (<i>Colinus virginianus</i>)	5d-LC ₅₀ 5d-LD ₅₀ 5d-NOEC 5d-NOEL	Approx. 5200 mg a.s./kg diet Approx. 598 mg a.s./kg bw/day 1300 mg a.s./kg diet 241 mg a.s./kg bw/day	EPA FIFRA section 71-2	IIA, 8.1.2/02
Reproduction study					
98.5%	Northern bobwhite quail (<i>Colinus virginianus</i>)	22wk-NOEC 22wk-NOEL males females	150 mg test substance/kg diet 15.7 mg test substance/kg bw/day 14.1 mg test substance/kg bw/day	EPA FIFRA section 71-4; OECD 206	IIA 8.1.3/01
98.5%	Mallard duck (<i>Anas platyrhynchos</i>)	21wk-NOEC 21wk-NOEL males females	300 mg test substance/kg diet 35.5 mg test substance/kg bw/day 36.7 mg test substance/kg bw/day	EPA FIFRA section 71-4; OECD 206	IIA 8.1.3/02
2,6-dichlorobenzamide					
Dietary toxicity					
98%	Northern bobwhite quail (<i>Colinus virginianus</i>)	5d-LC ₅₀ ¹ 5d-NOEC 5d-NOEL	3867 mg metabolite/kg bw/day 1780 mg metabolite/kg diet 876 mg metabolite/kg bw/day	EPA FIFRA; section 71-2 OPPTS 850.2200 OECD 205	IIA 8.1.2/03

¹ Due to food avoidance, the conversion of LC₅₀ (in mg/kg diet) to LD₅₀ (in mg a.s./kg bw/day) was unreliable

B.9.1.5 Risk assessment for birds

Procedures for risk assessment for birds must be in agreement with the recommendations in the Guidance Document on Risk Assessment for Birds and Mammals, under Council Directive 91/414/EEC (Draft Working Document SANCO/4145/2000). As recommended in Sanco/4145/2000, also the guidance in the EPPO environmental risk assessment scheme for plant protection products, chapter 11: terrestrial vertebrates (EPPO Bulletin 33, 211-238) is followed. The toxicity values used for the risk assessment for birds are summarised in Table B.9.1.5a.

Table B.9.1.5a. Toxicity values used for the risk assessment for birds.

Study type	Species	Endpoint	Toxicity value
<i>dichlobenil</i>			
acute oral toxicity test	mallard duck (<i>Anas platyrhynchos</i>)	LD ₅₀	> 50 mg/kg bw
	northern bobwhite quail (<i>Colinus virginianus</i>)	LD ₅₀	698 mg test substance/kg bw
dietary toxicity test	mallard duck (<i>Anas platyrhynchos</i>)	LD ₅₀	> 470 mg/kg bw/d
reproduction study	northern bobwhite quail (<i>Colinus virginianus</i>)	NOEL	14.1 mg/kg bw/d
<i>2,6-dichlorobenzamide (BAM)</i>			
dietary toxicity test	northern bobwhite quail (<i>Colinus virginianus</i>)	NOEL ¹	876 mg/kg bw/d

¹ The LD₅₀ could not be accurately calculated because of food avoidance. In accordance with SANCO/4145, the NOEL may then be used as a starting point for the risk assessment.

Routes of exposure

For broadcast spreading of granules, the routes of exposure to birds are considered to be:

- Ingestion of granules by birds seeking grit (acute, short- and long-term);
- ingestion of granules by animals seeking seeds as food;
- ingestion of residues in other food items such as earthworms and fish;
- ingestion of granules with soil, unintentionally ingested with food/water;
- ingestion of residues in drinking water.

It is not usual to assess all these routes in detail. This depends on the risk of achieving a lethal dose by consumption of only one or a few granules, and the resemblance of granules with grit, plant seeds and the bioaccumulation potential of the active ingredient.

The colour of the Casoron granules is grey and the odour is aromatic. The particle size of Casoron granules is 0.5 – 1.0 mm for > 60% and 1.0 – 1.25 mm for < 25%. This means that the granules can be considered as small (< 3.5 mm) and should be taken into account for the risk assessment of small granivorous bird species seeking grit. However the aromatic odour and the taste may prevent the birds for further consumption after one granule has been consumed. Sufficient information on the avoidance of birds with regard to consumption of Casoron granules is not available and therefore acute, short and long term consumption of Casoron granules can not be ruled out at forehand. In case the Casoron granules appear to be not attractive as grit and not be searched for intentionally it is still possible that unintentional consumption of granules together with soil particles and food items occurs. In addition the granules may also resemble plant seeds because some plant seed types may have a comparable size and colour. Therefore the exposure route ingestion of granules by birds seeking seeds as food is also considered below. Residues of the active ingredient and the metabolite BAM are found in surface water (see Section 8.5.1) and may pose a potential threat for birds using surface water for drinking water.

For the exposure routes ingestion of one granule and ingestion of residues in drinking water, only the acute risk is assessed. For the other exposure routes acute, short term and long term risk is considered. The risk methodology in the EPPO environmental risk assessment scheme for plant protection products, Chapter 11: terrestrial vertebrates (EPPO Bulletin 33, 211-238) applies a realistic worst case (rwc) scenario as a first step in each of the three exposure routes and time scales. In case the threshold value for the risk of this rwc is exceeded, a most likely case (mlc) scenario is elaborated as a second step. In the current risk assessment for Casoron G/dichlobenil only the realistic worst case scenario is elaborated. Toxicity-to-exposure (TER) values are calculated and compared with the trigger values according to the Uniform principles (Annex VI 91/414/EEC). It should be noted that the short term, medium term and long term exposure in EPPO Bulletin 33, 211-238) complies with the acute, short-term and long-term risk assessment, respectively, of Sanco/4145 working document.

B.9.1.5.1 Risk of the active substance for birds

Acute risk assessment (standard exposure scenario) (short term in EPPO-scheme)

One granule risk assessment

The one granule criterion is applied as a precautionary warning system for potential high-risk applications for birds and mammals. The following assumptions are made in the calculation of the risk of consumption of one granule. The particle size of Casoron granules is 0.5 – 1.0 mm (> 60%) and 1.0 – 1.25 mm (< 25%). The number of particles per gram product amounts 2500 – 3000. This means that one granule weighs between 0.33 and 0.40 mg. The active ingredient content of the product is to 6.75%, which results

in a content of 0.027 mg dichlobenil per granule. The LD₅₀ values that are available for two bird species are 698 and > 50 mg a.s./kg bw for northern bobwhite quail (males) and mallard duck, respectively. The LD₅₀ > 50 mg dichlobenil/kg body weight is selected for acute risk assessment as a worst case approach.

Because the LD₅₀ is expressed as mg a.s./kg bw, the one-granule-dose (OGD) is adjusted to a body weight of 1 kg by using a short-cut value : $OGD = \text{short-cut value} \times G_{\text{loading}}$. G_{loading} is the amount of the active substance in one granule (0.027 mg). The short-cut value represents the number of granules consumed per kg body weight and amounts to 40 for small birds ingesting small granules (<3.5 mm). This results in an OGD of 1.08 mg/kg body weight.

Table B.9.1.5a. Acute Toxicity Exposure Ratios for exposure of granivorous birds with a body weight of 25 g to granules with dichlobenil applied at vines and non-crops at 5.4 kg a.s./ha. One granule risk assessment.

Exposure route	LD ₅₀ (mg/kg bw)	a.s. in one granule (G _{loading}) (mg)	OGD (mg/kg bw)	TER
One granule	> 50	0.027	1.08	> 46

The TER value listed in table B.9.1.5a is far above 1. It can be concluded that this application can be considered as posing no high risk for birds as a result of the consumption of one granule.

Intentional ingestion of granules as grit

The acute risk assessment was based on the ingestion of granules by birds, using the realistic worst case daily granule dose (DGD) for a representative typical species, using the formula: $DGD = DGI \times (G_{\text{surface}} / SP_{\text{surface}} + G_{\text{surface}}) \times G_{\text{loading}}$ [mg/kg body weight/day].

DGI is daily grit intake amounting for small birds with a body weight of 25 gram (based on EPPO).

G_{surface} is the number of granules at the soil surface (granules/m², based on application rate and content of a.s. in 1 granule);

SP_{surface} is the number of soil particles at the soil surface that are in the same size class as granules (based on EPPO);

G_{loading} is the amount of the active substance in one granule.

In the calculation for dichlobenil the following data is used: DGI = 651, G_{surface} = 20000; SP_{surface} = 15200;

G_{loading} = 0.027.

The granules are not incorporated in the soil.

The short-cut values used in these calculations are taken from the EPPO-scheme. The values for the calculated exposure and TER are presented in Table B.9.1.5b.

Accidental ingestion of granules as grit

The daily dry soil dose (DDSD) for a 25-g bird is calculated with the formula $DDSD = \text{short-cut value} \times \text{application rate in kg a.s./ha}$. Short-cut values are published in the EPPO-scheme. The values for the calculated exposure and TER are presented in Table B.9.1.5b.

Table B.9.1.5b. Acute Toxicity Exposure Ratios for exposure of granivorous birds with a body weight of 25 g to granules with dichlobenil applied at vines and non-crops in applications at 5.4 kg a.s./ha.

Exposure route	LD ₅₀ (mg/kg bw)	Daily dose (mg/kg BW)	TER
Grit, intentional consumption	> 50	10.0	> 5
Grit, accidental consumption	> 50	2.55	> 20

From the acute TER values in Table 9.1.5b it is concluded that dichlobenil can be classified as low risk for acute exposure for accidental grit consumption (TER above the trigger value of 10) and as high risk for acute exposure for intentional grit consumption.

It should be noticed that the toxicological endpoint used in the risk assessment is worst-case because the LD₅₀ is from a study in which no mortality occurred. The 'real' LD₅₀ for the Mallard duck is unknown, due to vomiting at all test doses except for the lowest.

Therefore, the acute TER for the scenario intentional grit consumption is also calculated using the LD₅₀ from the study with Bobwhite quail in which mortality was observed (LD₅₀ 698 mg/kg bw). This should not be seen as a refinement but as a additional information to the TER that was calculated for the Mallard duck. This results in a TER of $698/10 = 70$. This TER is above the trigger value of 10, thus based on the endpoint for Bobwhite quail the risk is acceptable for this scenario.

Taking into account the fact that:

- the TER value is a '>'-value, based on the LD₅₀ for the Mallard duck from a study in which no mortality occurred, while the study with Bobwhite quail showed an LD₅₀ far above 50 mg/kg bw;
- the TER for the one-granule criterion was far above 1 (>46);
- the vomiting of the Mallard duck took place within 10 minutes after exposure, thus before a lethal dose could be reached, indicating the possibility that this could also take place in the field when birds are exposed to Casoron G;

the acute risk to birds as a result from exposure to dichlobenil is considered acceptable.

Ingestion of granules by animals seeking seeds as food

According to the notifier this scenario is irrelevant, because Casoron G consists of anorganic granules and Sanco\4145 only states that granules based on an organic carrier with nutritional value may be mistaken for seeds.

RMS agrees that notifiers remark is in accordance with Sanco\4145, but RMS also used the EPPO-scheme, which states that it is recommended to try and assess whether it is possible that the granules can be mistaken for weed seeds. RMS has consulted several experts of the RIVM¹ by showing them a digital photograph of the granules, which was provided by the notifier. The expert opinion was that the granules were not likely to be mistaken for seeds, not because of the anorganic base, but because of their size and shape. The granules have a grit-like shape of different sizes and according to the experts do not resemble natural seeds.

Based on this expert opinion, in combination with the one-granule risk assessment that showed a TER far above 1 (>46), RMS concludes that the scenario of ingestion of granules by animals seeking seeds as food needs no further assessment and the risk of this route can be considered as low.

Consumption of surface water as drinking water (acute risk assessment, standard exposure scenario)

The acute risk assessment for drinking water was based on a small bird with body weight 15 g, and with daily water intake (DWI) and the daily dose of active substance (ETE) calculated according to Sanco/4145/2000. The selected acute oral LD₅₀ is > 50 mg a.s./kg bw. The estimated theoretical exposure (ETE) for the acute time scale was calculated as PEC_{SW} actual*dwi/bw. The actual PEC_{SW} is the maximum actual PEC_{SW} value, taken from Section B.8.5.1. Residue values in water, ETE and TERa values are presented in Table B.9.1.5c

Table B.9.1.5c. Acute Toxicity Exposure Ratios for exposure of birds to dichlobenil, due to consumption of contaminated drinking water.

Application	dose (kg a.s./ha)	Bird type	body weight (g)	Route	DWI (mL/d)	LD ₅₀ (mg/kg bw)	PEC _{SURFACE WATER} (µg/L)	ETE (mg/kg bw/d)	TERa
Vines	5.4	small bird	15	water	3.5	> 50	240	0.056	> 893

¹ National Institute for Public Health and the Environment in the Netherlands

Non crop	5.4	small bird	15	water	3.5	> 50	53.57	0.012	> 4100
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The TERa values in Table B.9.1.5c for granivorous birds exposed to dichlobenil via drinking (surface) water are above the Annex VI 91/414 EEC trigger of 10. Hence, the acute risk to birds in the field is considered to be acceptable.

Short-term risk assessment (*medium term in EPPO scheme*)

For the short term risk assessment, the exposure estimation as based on the EPPO scheme is in principle the same as for the acute scenario, except for some of the short cut values, which are published in the EPPO scheme. The LD₅₀ value of > 470 mg a.s./kg bw/d obtained for mallard duck is used for risk assessment. The values for the calculated exposure and TER are presented in Table B.9.1.5d.

Table B.9.1.5d. Short term Exposure Toxicity Ratios for exposure of granivorous birds with a body weight of 25 g to granules with dichlobenil applied at vines and non-crops in applications at 5.4 kg a.s./ha.

Exposure route	LD ₅₀ (mg/kg body weight/day)	Daily dose (mg/kg bw/d)	TER
Grit, intentional consumption	> 470	10.0	47
Grit, accidental consumption	> 470	0.51	922

From the TER values in Table 9.1.5d it can be concluded that dichlobenil can be classified as low risk for short term exposure for intentional and accidental grit consumption, because the TER values are above the Annex VI 91/414 trigger value of 10.

Long-term risk assessment

According to the EPPO guideline for birds (2003), long-term risk assessment for granules is often superfluous because granules disintegrate or are covered by soil, or the concentration of active substance in the granule falls by degradation. However, RMS is of the opinion that it cannot be excluded that long term effects (on reproduction) may result from short-term exposure. This is also stated in Sanco/4145 (par. 3.5). Therefore, a long term risk assessment is performed below, using the same method as for the acute and short-term assessment.

Data from two bird reproduction studies are available, namely with Northern bobwhite quail with a 22wk-NOEL of 14.1 mg a.s./kg bw/d and mallard duck with a 21wk-NOEL of 35.5 mg a.s./kg bw/d. The lowest NOEL value is 14.1 and is compared with the exposure. There are no data on degradation of the active

ingredient in the granules, therefore a time weighted average (twa) value is not used. The results are listed in table B.9.1.5e.

Table B.9.1.5e. Long-term Exposure Toxicity Ratios for exposure of granivorous birds with a body weight of 25 g to granules with dichlobenil applied at vines and non-crops in applications at 5.4 kg a.s./ha.

Exposure route	NOEL (mg/kg bw/day)	Daily dose (mg/kg BW)	TER
Grit, intentional consumption	14.1	10.0	1.4
Grit, accidental consumption	14.1	0.51	27.5

The TER value for dichlobenil is above the Annex VI 91/414 EEC trigger value of 5 for long-term exposure for accidental grit consumption and the risk is therefore acceptable. However, the TER value is below the trigger value of 5 for long-term exposure due to intentional grit consumption, indicating an unacceptable risk.

The notifier argues that the long term risk assessment is not relevant, since the granules will dissipate rapidly on contact with moisture and fall apart within one day and thus no long term exposure will occur. Further the notifier states that latent effects (effects on reproduction as a result of acute exposure) are unlikely, since from section B.6.1.4 it appears that the active substance is rapidly excreted from rats and hens (approximately 80% or 65% of the last administered dose is excreted within 24 h after respectively low and high doses). In addition, the notifier also refers to a DEFRA-report (Risk to birds and mammals from solid formulations, DEFRA project code PN0922), in which a DGI of 83 is mentioned for the House sparrow. From the DEFRA-report it remains unclear where this value exactly comes from, it is mentioned without any reference.

RMS does not agree with the argument of rapid dissipation of the granules. Apart from the fact that the time period of dissipation of the granules (within 1 day) as stated by the notifier is not validated with data, the intended uses are not necessarily under moist circumstances, especially as they are also in S-EU. Further, exposure over longer time periods is possible because birds can forage on different fields where intact granules can still be present because applications are made at different time points. Also, it should be noted that the excretion of dichlobenil is not extraordinary rapid. The data from B.6.1.4 show that dichlobenil is of average excretion rate, not different from other substances in general. Both these arguments of the notifier do not address the concern of potential long-term effects caused by short-term exposure, because they do not exclude the possibility of short-term exposure in the field.

Finally, the DGI-value of 83 for the House sparrow is also rejected by RMS, since it is unclear where this value comes from or how it was derived. The value of 651 in the EPPO-scheme was derived from a study with the House sparrow (Luttik & De Snoo, 2004)², in which grit particles in the stomach of 11 House sparrows amounted 64 (size 0.5 to 1 mm) and 155 (size 0.5 to 1.25). Based on the turnover rate from the EPPO scheme (4.2) the daily grit intake amounts 269 or 651. The particle size of Casoron granules is 0.5 – 1.0 mm (> 60%) and 1.0 – 1.25 mm (< 25%). RMS therefore considers the value of 651 for DGI an appropriate value for risk assessment.

Based on the above, the notifier is requested to submit a refined long-term risk assessment for the intended uses of dichlobenil.

Long term risk assessment (bio-accumulation and food chain behaviour)

The long-term risk assessment for secondary poisoning via earthworms is based on a bird weighing 100 g feeding exclusively on earthworms, with a daily food intake (FIR) of 113 g fresh material/day. The BCF for earthworms was calculated as $BCF = (0.84 + 0.01 \cdot \text{Pow}) / (f_{oc} \cdot K_{oc})$, where $K_{oc} = 237 \text{ L/kg}$ and $f_{oc} = 0.02$ (default value). Pow, calculated from $\log \text{Pow} = 2.70$, is 501. The resulting BCF of dichlobenil for earthworms is 1.23 kg earthworm fresh weight per kg soil dry weight. Residue levels in earthworms were calculated from the 21-day TWA PECsoil (6.519 mg/kg dry weight, section B.8.3), as follows:

$$\text{PEC}_{\text{FEED}} = \text{PEC}_{\text{worm}} = \text{PEC}_{\text{soil}} \cdot \text{BCF}_{\text{worm}}$$

The NOEC used for risk assessment is 14.1 mg/kg bw/day. The PEC_{feed} is converted to daily dose by using the multiplication factors from SANCO/4145/2000 (1.1 for worms). Residue levels in earthworms and ETE and TERIt values are presented in the table below.

Long-term Toxicity Exposure Ratios for exposure of birds to dichlobenil due to consumption of contaminated earthworms

application	max. dose (kg as/ha)	NOEC (mg/kg bw/d)	Route	PEC_{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TERIt
Vines and non-crops	5.4	14.1	Earthworms	8.0	8.8	1.6

² Luttik, R. and G.R. de Snoo (2004) Characterisation of grit in arable birds to improve pesticide risk assessment. Ecotoxicology and Environmental Safety 57: 319-329.

The TERIt value for consumption of worms is below the Annex VI 91/414 EEC trigger of 5. Hence, a risk is assumed to be present for the consumption of worms, and a refinement is necessary.

The notifier stated that since the LogPow is < 3 , no risk assessment on secondary poisoning is required for dichlobenil. RMS does not agree, since the risk assessment shows that the trigger value is not met and thus the risk needs to be addressed.

Further the notifier submitted a refined risk assessment based on:

- the use of time-weighted average concentrations in earthworms (based on soil field DT50 values) over a time period comparable to that from the toxicity study in which the endpoint was estimated;
- a proposal to reduce the application rate by 40% to 3.24 kg a.s./ha, because application of the granules is stated not to be applied to the whole area but only to the areas around the vine rows and not to the grass strips (which cover 40% of the area according to the notifier);
- values for earthworm composition of diets for birds representative for vineyards based on two public literature references (not submitted), used for lowering PD to 10%.

RMS agrees that the ETE should not be based on the maximum initial residues, but it is not agreed that the time period from the toxicity study is to be used. Conform the guidance in Sanco/4145 the PECworm was based on the PECsoil twa 21 d, in which degradation is taken into account.

The refinement by reducing the application rate with 40% is not accepted, since the proposed application rate is 5.4 kg a.s./ha. The dose is expressed as an amount of a.s. (granules) per surface area and this ratio does not change with the size of the area that the granules are applied to. If the granules are only applied around the vines, they would still be applied at a dose rate of 5.4 kg a.s./ha (or 0.54 g a.s./m²). If not, RMS considers that the proposed dose should have been lower for vines. Also, this would still not refine the risk for the other intended uses.

The values for earthworm part in the diet of birds representative for vineyards could be acceptable, however RMS did not check the public literature references yet, since they were not submitted by the notifier and there would still be the need for refinement for the other intended uses. In addition, the PD refinement should consist of estimating residue values for all parts of the diet (i.e. sum of PD should be 1), which was not done in the risk assessment of the notifier. Lowering the PD to 0.1 is therefore considered not sufficient for risk refinement. RMS therefore awaits further data, which could be used for refinement of the other intended uses as well.

The notifier is requested to provide a refined risk assessment for earthworm eating birds for the intended uses of dichlobenil. RMS would suggest that data on measured residues in earthworms after exposure to Casoron G according to the appropriate dose rates are submitted for this purpose. Further the notifier is requested, if they still want them to be used, to submit the relevant parts of the public literature references as used in their refined risk assessment*. As one of the references is in French, an English summary should be submitted as well for this reference.

* Reference 1:

Cramp, S et al -1988 – Handbook of the Birds of Europe and the Middle East and North

Africa, The birds of the Western Palearctic Volume V Tyrant Flycatchers to Thrushes – Oxford University Press.

Reference 2:

Géroudet, P. – 1998 – Les Passereaux d'Europe – Tome 1: Des coucous aux Merles and

Tome 2: De la Bouscarle aux Bruants 4th edition, updated by M. Cuisin – Delachaux et Niestlé Ed.

Bio-magnification in terrestrial food chains

As the evaluation of the toxicokinetics studies in the toxicology section (absorption, distribution, excretion and metabolism in mammals) concluded that the potential for bio-accumulation is low it can be assumed that there is no biomagnification along the food chain.

B.9.1.5.2 Risk of metabolites for birds

There are 3 metabolites that need to be considered for the risk assessment of birds. These are the substances 2,6-dichlorobenzamide (BAM), 2-chlorobenzoic acid and 4-chloro-2(3H)benzoxazolone.

2,6-dichlorobenzamide (BAM) is a major metabolite in soil and surface water. Exposure via ingestion of granules is not relevant because this metabolite will probably not be formed within granules. In addition the risk of exposure via contaminated food items such as earthworms can be considered as low, since the logKow of the metabolite is 0.38. The risk of exposure to this metabolite for birds was only determined for the exposure to residues in the surface water by consumption of drinking water. The maximum PEC_{SW} for the metabolite BAM amounts to 72 and 185 µg/L for applications to vines and non crop, respectively (see section B.8.5.1). The PEC_{SW} values are solely based on the metabolite formed from dichlobenil in the soil and entering the surface water by drainage and run-off because the contribution of in surface water formation to the total PEC_{SW} is not quantitatively important. An acute dietary bird toxicity test with this metabolite was performed. Due to food avoidance of the birds in the acute dietary toxicity test a NOEL value was derived instead of a LC₅₀ value. This NOEL value was used in the risk assessment for the use of surface water as drinking water (see Table B.9.1.5.f).

Table B.9.1.5f

Acute Toxicity Exposure Ratios for exposure of birds to 2,6-dichlorobenzamide (BAM), due to consumption of drinking water (based on highest PEC_{sw})

Application	dose (kg a.s./ha)	Bird type	Approx. body weight (g)	Route	DWI (mL/d)	NOEL (mg/kg bw/d)	PEC _{Surface water} (mg/L)	ETE (mg/kg bw/d)	TER
Vines	5.4	small bird	15	Water	3.5	876	0.072	0.0168	52143
Non crop	5.4	small bird	15	Water	3.5	876	0.185	0.0432	20278

The TER value in Table B.9.1.5f, for granivorous birds exposed to 2,6-dichlorobenzamide (BAM) via drinking (surface) water is above the Annex VI 91/414 EEC trigger of 10. Hence, the acute risk to birds in the field is considered to be acceptable.

Birds may be exposed to 2-chlorobenzoic acid and 4-chloro-2(3H)benzoxazolone via drinking water. Although PEC_{SW} values are calculated for these metabolites (see Table B.8.5.1zk), toxicity tests with birds are not available. Therefore the risk can not be calculated for these two metabolites. However, it can be argued that a risk of these two metabolites for birds is unlikely. The PEC_{SW} values for the two metabolites are a factor 10 lower than the PEC_{SW} for BAM (see Table B.8.5.1zk). In addition, the acute toxicity ratios for BAM do not indicate a risk for birds (Table B.9.1.5f). The TER values for BAM are a factor 1000 above the trigger value. It can be argued that the toxicity of the two metabolites should be a factor 10000 higher than the toxicity of BAM in order to lead to exceedance of the trigger value for the two metabolites. This is unlikely, also in view of the molecular structure of the substances under consideration.

B.9.1.6 Risk assessment for mammals

Procedures for risk assessment for mammals were in agreement with the recommendations in the Guidance Document on Risk Assessment for Birds and Mammals, under Council Directive 91/414/EEC (Draft Working Document SANCO/4145/2000). As recommended in Sanco/4145/2000, the guidance in the EPPO scheme for environmental risk assessment scheme for plant protection products, Chapter 11: terrestrial vertebrates (EPPO Bulletin 33, 211 – 238) is followed. The toxicity values used for the risk assessment for mammals are summarised in Table B.9.1.6a.

Table B.9.1.6a. Toxicity values used for the risk assessment for mammals

Study type	Species	Endpoint	Toxicity value	Reference
<i>dichlobenil</i>				
acute oral toxicity test	rat	LD ₅₀	> 2000 mg/kg bw	see section B.6.2.3
two-generation study	rat	NOAEL	4 mg/kg bw/d	see section B.6.6.3
<i>2,6-dichlorobenzamide (BAM)</i>				
acute oral toxicity test	rat	LD ₅₀	500 mg/kg bw	see section B.6.8.8
two-generation study	rat	NOAEL	6.5 mg/kg bw/d	see section B.6.8.8

For the long-term risk assessment, toxicity values from two-generation studies are used. These values are ecotoxicologically the most relevant as they are based on effects on parental body weight, food consumption and/or development of the offspring. For the metabolite 2,6-dichlorobenzamide (BAM), the two-generation study is the only chronic study available, but for dichlobenil a lower toxicity value was found in the chronic oral toxicity and carcinogenicity study in rat (< 3.2 mg/kg bw/d). As the effects observed in this study are not considered relevant for the ecotoxicological risk assessment of mammals, the toxicity value is not used.

Routes of exposure

For broadcast spreading of granules, the routes of exposure to mammals are considered to be:

- ingestion of granules by animals seeking seeds as food;
- ingestion of residues in other food items such as earthworms and fish;
- ingestion of granules with soil, unintentionally ingested with food/water;
- ingestion of residues in drinking water.

It is not usual to assess all these routes for mammals. In contrast with some bird species, mammals do not intentionally ingest grit. Accidental ingestion of a granule as part of soil ingestion may occur. The approach for these risk assessments is highly comparable with the one applied for birds (section B.9.1.5).

B.9.1.6.1 Risk of the active substance for mammals

Acute risk assessment

One granule risk assessment

The one granule criterion is applied as a precautionary warning system for potential high-risk applications for birds and mammals. The method for the calculation of the one granule risk is described in section B.9.5.1. There is one LD₅₀ value available for mammals, namely > 2000 mg a.s./kg bw. This value is used in the risk assessment.

The TER (toxicity-to-exposure ratio) for one granule is listed in Table B.9.1.6b.

Table B.9.1.6b. Acute Exposure Toxicity Ratios for exposure of small mammals with a body weight of 25 g to granules with dichlobenil applied at vines and non-crops at 5.4 kg a.s./ha. One granule risk assessment.

Exposure route	LD ₅₀ (mg/kg bw)	a.s. in one granule (Gloading) (mg)	OGD (mg/kg bw)	TER
One granule	>2000	0.027	1.08	> 1852

The TER value listed in table B.9.1.6b is far above 1. It can be concluded that this application can be considered as posing no high risk for mammals as a result of the consumption of one granule.

Accidental ingestion of granules as part of soil ingestion

The exposure to dichlobenil by accidental ingestion of granules as part of soil ingestion is calculated for a realistic worst case with the formula: DDSD=short-cut value * application rate in kg a.s./ha/d. DDSD is the daily dry soil dose. The short-cut value amounts to 0.113 for small mammals. The dosage is 5.4 kg a.s./ha. The resulting DDSD amounts to 0.61 mg/kg body weight /day. The same LD₅₀ value is taken as for the one granule criterion risk assessment (see sub section above). The TER amounts to > 3278 based on the formula: TER = LD₅₀/DDSD. It can be concluded that there is a low risk for acute exposure (see Table B.9.1.6c).

Table B.9.1.6c. Acute Exposure Toxicity Ratios for exposure of omnivorous mammals with a body weight of 25 g to granules with dichlobenil applied at vines and non-crops in applications at 5.4 kg a.s./ha.

Exposure route	LD ₅₀ (mg/kg bw)	Daily granule dose (mg/kg BW)	TER
Soil ingestion, accidental consumption	> 2000	0.61	> 3278

From the TER value for short-term exposure in Table 9.1.6c and the Annex VI 91/414 EEC trigger of 10, it can be concluded that dichlobenil can be classified as low risk for acute exposure for accidental granule consumption as part of soil ingestion.

Ingestion of granules by animals seeking seeds as food

According to the notifier this scenario is irrelevant, because Casoron G consists of anorganic granules and Sanco\4145 only states that granules based on an organic carrier with nutritional value may be mistaken for seeds.

RMS agrees that notifiers remark is in accordance with Sanco\4145, but RMS also used the EPPO-scheme, which states that it is recommended to try and assess whether it is possible that the granules can be mistaken for weed seeds. RMS has consulted several experts of the RIVM³ by showing them a digital photograph of the granules, which was provided by the notifier. The expert opinion was that the granules were not likely to be mistaken for seeds, not because of the anorganic base, but because of their size and shape. The granules have a grit-like shape of different sizes and according to the experts do not resemble natural seeds.

Based on this expert opinion, in combination with the one-granule risk assessment that showed a TER far above 1 (>1852), RMS concludes that the scenario of ingestion of granules by animals seeking seeds as food needs no further assessment and the risk of this route can be considered as low.

Consumption of surface water as drinking water (acute risk assessment, standard exposure scenario)

The acute risk assessment for drinking water was based on a small mammal with body weight 25 g, and with daily water intake (DWI) and the daily dose of active substance (ETE) calculated according to Sanco/4145/2000. The selected acute oral LD₅₀ is > 2000 mg a.s./kg bw. The estimated theoretical exposure (ETE) for the acute time scale was calculated as $PEC_{SW} \text{ actual} \times dwi/bw$. The actual PEC_{SW} is the maximum actual PEC_{SW} value, taken from Section B.8.5.1. Residue values in water, ETE and TERa values are presented in Table B.9.1.6d.

Table B.9.1.6d. Acute Toxicity Exposure Ratios for exposure of mammals to dichlobenil, due to consumption of contaminated drinking water.

Application	Dose (kg)	Mammal type	body weight (g)	Route	DWI (mL/d)	LD ₅₀ (mg/kg)	PEC _{WATER} (µg/L)	ETE (mg/kg)	TER
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³ National Institute for Public Health and the Environment in the Netherlands

	a.s./ha)					bw)		bw/d)	
Vines	5.4	small mammal	25	water	3.58	>2000	240	0.0344	$> 5.8 \cdot 10^4$
Non crop	5.4	small mammal	25	water	3.58	>2000	53.57	0.0075	$> 2.7 \cdot 10^5$

The acute TER values in Table B.9.1.6d for omnivorous mammals exposed to dichlobenil via drinking (surface) water are above the Annex VI 91/414 EEC trigger of 10. Hence, the acute risk to mammals in the field is considered to be acceptable.

Long-term risk assessment (standard exposure scenario)

Long-term risk assessment is based on the same mammals accidentally consuming granules as part of soil ingestion. This is calculated for a realistic worst case with the formula: DDSD=short-cut value * application rate in kg a.s./ha/ * time weighted average (TWA) factor (mg/kg bw/day). DDSD is the daily dry soil dose. The short-cut value amounts to 0.023 for small mammals. Due to the lack of information on degradation of the active substance in the granules a time weighted average (twa) default value is not used. The dosage is 5.4 kg a.s./ha. The resulting DDSD amounts to 0.12 mg/kg body weight /day.

The toxicity value used for risk assessment is taken from section B.6.6.1.1. The lowest relevant NOAEL was 4 mg as/kg bw/d from a 2-generation rat toxicity study, which is taken to be the ecotoxicologically relevant NOAEL in a First-Tier assessment. The values are presented in Table B.9.1.6e.

Table B.9.1.6e. Long term Exposure Toxicity Ratios for exposure of omnivorous mammals with a body weight of 25 g to granules with dichlobenil applied at vines and non-crops in applications at 5.4 kg a.s./ha.

Exposure route	NOED (mg/kg bw/d)	Exposure via daily dry soil dose (mg/kg bw/d)	TER
Soil ingestion, accidental consumption	4	0.12	32

From the TER values for long term exposure in Table 9.1.6e and the Annex VI 91/414 EEC trigger of 5 it is concluded that dichlobenil can be classified as low risk for long-term exposure for accidental granule consumption as part of soil ingestion.

The notifier disagrees with the use of the NOAEL-value of 4 mg/kg bw/d, but instead is of the opinion that the environmentally relevant NOAEL is 27.9 mg/kg bw/d, on the basis that there were no adverse

effects of dichlobenil on mating performance, pregnancy rate, offspring survival to weaning or the incidence of macroscopically abnormal offspring at this dietary concentration.

RMS does not agree with the notifier and will remain with the 4 mg/kg bw/d NOAEL, since above this NOAEL a significant decrease in body weight for adults and offspring in the F0, F1 and F2 generations was observed and RMS considers effects on body weight as an ecologically relevant parameter.

Long term risk assessment (bioaccumulation and food chain behaviour)

The long-term risk assessment for secondary poisoning via earthworms 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).

The NOEC used for risk assessment is 4 mg/kg bw/day. The PEC_{FEED} is converted to daily dose by using the multiplication factors from SANCO/4145/2000 (1.1 for worms). Residue levels in earthworms and ETE and TERIt values are presented in the table below.

Long-term Toxicity Exposure Ratios for exposure of mammals to dichlobenil due to consumption of contaminated earthworms

application	max. dose (kg as/ha)	NOEC (mg/kg bw/d)	Route	PEC _{FEED} (mg/kg wwt)	ETE (mg/kg bw/d)	TERIt
Vines and non-crops	5.4	4	Earthworms	8.0	8.8	0.45

The TERIt value for consumption of worms is below the Annex VI 91/414 EEC trigger of 5. Hence, a risk is assumed to be present for the consumption of worms, and a refinement is necessary.

The notifier stated that since the LogPow is < 3, no risk assessment on secondary poisoning is required for dichlobenil. RMS does not agree, since the risk assessment shows that the trigger value is not met and thus the risk needs to be addressed.

Further the notifier submitted a refined risk assessment based on:

- the use of time-weighted average concentrations in earthworms (based on soil field DT50 values) over a time period comparable to that from the toxicity study in which the endpoint was estimated;
- a proposal to reduce the application rate by 40% to 3.24 kg a.s./ha, because application of the granules is stated not to be applied to the whole area but only to the areas around the vine rows and not to the grass strips (which cover 40% of the area according to the notifier);

RMS agrees that the ETE should not be based on the maximum initial residues, but it is not agreed that the time period from the toxicity study is to be used. Conform the guidance in Sanco/4145 the PEC_{worm} was based on the PEC_{soil} two 21 d, in which degradation is taken into account.

The refinement by reducing the application rate with 40% is not accepted, since the proposed application rate is 5.4 kg a.s./ha. The dose is expressed as an amount of a.s. (granules) per surface area and this ratio does not change with the size of the area that the granules are applied to. If the granules are only applied around the vines, they would still be applied at a dose rate of 5.4 kg a.s./ha (or 0.54 g a.s./m²). If not, RMS considers that the proposed dose should have been lower for vines. Also, this would still not refine the risk for the other intended uses.

Therefore, the notifier is requested to provide a refined risk assessment for earthworm eating mammals for the intended uses of dichlobenil. RMS would suggest that data on measured residues in earthworms after exposure to Casoron G according to the appropriate dose rates are submitted for this purpose.

Biomagnification in terrestrial food chains

From the evaluation of the toxicokinetic studies in the toxicology section (absorption, distribution, excretion and metabolism in mammals) it is concluded that the potential for bio-accumulation is low. It is therefore assumed that there is no bio-magnification along the food chain.

B.9.1.6.2 Risk of metabolites for mammals

There are 3 metabolites that need to be considered for the risk assessment of mammals: 2,6-dichlorobenzamide (BAM), 2-chlorobenzoic acid and 4-chloro-2(3H)benzoxazolone.

2,6-dichlorobenzamide (BAM) is a major metabolite in soil and surface water. Exposure via ingestion of granules is not relevant in case of the soil metabolite. In addition the risk of exposure via contaminated food items such as earthworms can be considered as low, since the logK_{ow} of the metabolite is 0.38.

The risk of exposure to this metabolite for mammals was therefore only determined for the exposure to residues in the surface water by consumption of drinking water. The total maximum PEC_{SW} for the metabolite BAM amount to 72 and 185 µg/L for applications in vines and non-crop respectively (see section B.8.5.1). For the acute risk assessment the acute oral LD₅₀ in rat of 500 mg/kg bw was used (see Table 9.1.6a). The results are given in Table 9.1.6.f.

Table 9.1.6f Acute Toxicity Exposure Ratios for exposure of mammals to 2,6-dichlorobenzamide (BAM), due to consumption of drinking water.

Application	dose (kg a.s./ha)	Mammal type	Approx. body weight (g)	Route	DWI (mL/d)	LD ₅₀ (mg/kg bw/d)	PEC _{Surface water} (mg/L)	ETE (mg/kg bw/d)	TER _a
Vines	5.4	Omnivorous species	25	Water	3.58	500	0.072	0.0103	48544
Non crop	5.4	Omnivorous species	25	Water	3.58	500	0.185	0.0265	18868

The TER value in Table B.9.1.6f, for omnivorous mammals exposed to 2,6-dichlorobenzamide (BAM) via drinking (surface) water is above the Annex VI 91/414 EEC trigger of 10. Hence, the acute risk to mammals in the field is considered to be acceptable.

Mammals may be exposed to 2-chlorobenzoic acid and 4-chloro-2(3H)benzoxazolone via drinking water. Although PEC_{SW} values are calculated for these metabolites (see Table B.8.5.1zk), toxicity tests with mammals are not available. Therefore the risk can not be calculated for these two metabolites. However, it can be argued that a risk of these two metabolites for mammals is unlikely. The PEC_{SW} values for the two metabolites are a factor 10 lower than the PEC_{SW} for BAM (see Table B.8.5.1zk). In addition, the acute toxicity ratios for BAM do not indicate a risk for mammals (Table B.9.1.5f). The TER values for BAM are a factor 1000 above the trigger value. It can be argued that the toxicity of the two metabolites should be a factor 10000 higher than the toxicity of BAM in order to lead to exceedance of the trigger value for the two metabolites. This is unlikely, also in view of the the molecular structure of the substances under consideration.

B.9.2 EFFECTS ON AQUATIC ORGANISMS

B.9.2.1 Acute toxicity

B.9.2.1.1 Acute toxicity to fish

B.9.2.1.1.1 Acute toxicity of the active substance to fish (IIA 8.2.1/01)

Characteristics of the study

Reference	: Palmer <i>et al.</i> (2003)	Species	: rainbow trout (<i>Oncorhynchus mykiss</i>)
Year of execution	: 2003	Exposure duration and regime	: 96 h, semi-static
Guideline	: OECD 203, OPPTS 850.1075, EC C.1, ASTM E729-88a	96h-LC ₅₀	: 7.2 mg a.s./L
GLP	: yes	96h-NOEC mortality and condition	: 2.6 mg a.s./L
Test substance	: dichlobenil tech; batch no. 42763011; powder	Acceptable	: yes
Purity	: 99.3%		

Study design

The test substance was dosed directly into the dilution water; the nominal concentrations tested were 0.38, 0.75, 1.5, 3.0, 6.0 and 12 mg a.s./L. For each concentration and the control, ten fish were exposed in duplicate. Samples for chemical analysis were taken at t = 0, 24 (prior and after renewal of test medium) and 96 h.

Results

The mean measured concentrations of the test substance varied between 78 and 92% of the nominal values. The low recovery was only found at the highest test concentration, which was supported by the visual observation of undissolved test substance on the surface of the test medium at the start of the test. Therefore, the results of the test are based on mean measured concentrations. The lowest measured dissolved oxygen concentration was 5.2 mg/L, pH values varied between 8.0 and 8.5 and temperature was $12 \pm 2^\circ\text{C}$.

No mortality or other adverse effects were observed in the control groups. Adverse effects (e.g. erratic swimming, loss of equilibrium and lethargy) were observed at 5.5 and 9.4 mg a.s./L. After 24 h of exposure, all fish at the highest test concentration had died. The authors state that the 96h-LC₅₀ and the 96h-NOEC are 7.2 (5.5 – 9.4) mg a.s./L and 2.6 mg a.s./L, respectively (based on measured concentrations). RMS recalculated the LC₅₀ and found a similar result.

Acceptability of the test

In 24-h old test solutions, dissolved oxygen concentrations below 60% of the air saturation level were found. Although the fish appeared normal under these conditions, increased exposure to the test substance may be expected as a result of increased ventilation rates induced by the low oxygen levels. As this represents worst-case conditions, the test is considered acceptable.

Conclusion

The 96h-LC₅₀ and the 96h-NOEC values are 7.2 mg a.s./L and 2.6 mg a.s./L, respectively

B.9.2.1.1.2 Acute toxicity of the active substance to marine fish (IIA 8.2.1/03 and IIA 8.2.1/04))

Characteristics of the study

Reference	: Dunham and Davis (1995)	Species	: sheephead minnow (<i>Cyprinodon variegatus</i>)
Year of execution	: 1995	Exposure duration and regime	: 96 h, flow-through
Guideline	: EPA 72-3	96h-LC ₅₀	: 12.7 mg a.s./L
GLP	: yes	96h-NOEC	: 3.53 mg a.s./L
Test substance	: dichlobenil technical; lot no. FUN87E08A/FUX011000; off-white powder	mortality and condition	:
Purity	: 98.8%	Acceptable	: yes

Study design

The test substance was dosed using dimethylformamide as a solvent; the nominal concentrations tested were 2.59, 4.32, 7.20, 12.0 and 20.0 mg a.s./L. For each concentration and the (solvent) control, twenty fish were exposed. Samples for chemical analysis were taken prior to test initiation and at t = 0 and 96 h.

Results

The mean measured concentrations of the test substance varied between 80 and 89% of the nominal values, which confirms the correct dosing and stability of the test substance. The results of the test are expressed as measured concentrations. The lowest measured dissolved oxygen concentration was 3.1 mg/L, pH values varied between 8.0 and 8.2, temperature was 22.6 ± 0.5°C and salinity was 20‰.

No mortality or other adverse effects were observed in the (solvent) control groups. However, adverse effects (fish at surface and/or loss of equilibrium) were observed at concentrations of 6.44 mg a.s./L and higher. At 10.4 and 16.6 mg a.s./L, 5 and 10% mortality was observed, respectively. The authors state that the 96h-LC₅₀ and the 96h-NOEC are 12.7 (10.4 – 16.6) mg a.s./L and 3.53 mg a.s./L, respectively. RMS recalculated the LC₅₀ and found a similar result.

Acceptability of the test

Dissolved oxygen concentrations below 60% of the air saturation level were determined. This may have resulted into increased ventilation rates leading to increased exposure to the test substance. However this represents worst-case conditions and the test is therefore considered acceptable.

Conclusion

The 96h-LC₅₀ and the 96h-NOEC are 12.7 mg a.s./L and 3.53 mg a.s./L, respectively.

B.9.2.1.1.3 Acute toxicity of the active substance to fish (IIA 8.2.1/05)

Characteristics of the study

Reference	: Gijswijt (1980)	Species	: various freshwater fish species
Year of execution	: not indicated	Exposure duration	: 24, 48 or 96 h
Guideline	: not indicated	96h-LC ₅₀	: generally between 5 and 25 mg a.s./L
GLP	: no	96h-NOEC	: not indicated
Test substance	: dichlobenil	mortality	: and condition
Purity	: not indicated for most of the studies ¹	Acceptable	: no

¹ The results of the test are expressed in a.s.

This report reviews literature and written private communications of dichlobenil to 25 fish species. Both acute and chronic studies are reviewed and discussed. Methods and test substances are not described. Test durations varied between 24 and 96 hours.

Study design

No data available.

Results

96h-EC₅₀ values generally varied between 6.4 and 25 mg a.s./L.

Acceptability of the tests

Raw data on methods, test interpretation or test substances are not given in the report. Chemical analyses of the exposure solutions are not reported and most likely not available (as this was not required according to the international guidelines at the time of the performance of the studies). The various studies are therefore considered as not acceptable.

Conclusion

As there is not sufficient information available, data from this report cannot be used for risk assessment.

B.9.2.1.1.4 Acute toxicity of the active substance to fish (IIA 8.2.1/06)**Characteristics of the study**

Reference	: Mayer, Ellersieck (1986)	Species	: various freshwater species
Year of execution	: not indicated	Exposure duration	: 24 h to 96 h
Guideline	: not indicated	96h LC ₅₀	: not reported
GLP	: no	96h NOEC	: not indicated
		mortality	
Test substance	: dichlobenil	and condition	:
Purity	: not indicated	Acceptable	: no

The report reviews literature and written private communications of 4901 acute toxicity tests with 410 chemicals and 66 species of aquatic animals, including 15 tests with dichlobenil. Methods and test substances are not described. The purpose of the paper was to make taxonomic comparisons using various statistical approaches.

Study design

No information available.

Results

Actual data on dichlobenil are not given.

Acceptability of the tests

Not acceptable as actual data on dichlobenil are not available.

Conclusion

The study is not considered acceptable.

B.9.2.1.1.5 Acute toxicity of the metabolite 2,6-dichlorobenzamide (BAM) to fish (IIA 8.2.1/07)**Characteristics of the study**

Reference	: Wetton and Mullee (2001)	Species	: rainbow trout (<i>Oncorhynchus mykiss</i>)
Year of execution	: 2001	Exposure duration	: 96 h, semi-static
Guideline	: OECD 203, EC C.1, US CFR Title 40 Part 797 Section 1400, EPA 72-4, OPPTS 850.1075	and regime	
GLP	: yes	96h-LC ₅₀	: 240 mg metabolite/L
Test substance	: 2,6-dichlorobenzamide (BAM); batch no. FUX001000/FUN81G02C; off-white powder	96h-NOEC	: 100 mg metabolite/L
		mortality	
		and condition	
Purity	: 99.5%	Acceptable	: yes

Study design

The test substance was dosed directly into the dilution water; the nominal concentrations tested were 100, 180, 320, 560 and 1000 mg metabolite/L. For each concentration and the control, ten fish were exposed in

duplicate. Samples for chemical analysis were taken at t = 0 (fresh media), 24, 48 and 72 (old and fresh media) and 96 h (old media).

Results

The mean measured concentrations of the test substance varied between 97 and 109% of the nominal values, which confirms the correct dosing and stability of the test substance. The lowest measured dissolved oxygen concentration was 7.0 mg/L, pH values varied between 7.5 and 7.9 and temperature was 14.0°C throughout the test.

Mortality or other adverse effects were not observed in the control groups. Adverse effects (swimming at the bottom or surface of the test vessels, loss of equilibrium and presence of moribund fish) were observed at 180, 320 and 560 mg metabolite/L. All fish died at test concentrations of 320 mg metabolite/L and higher. The authors state that the 96h-LC₅₀ and the 96h-NOEC are 240 (180 – 320) and 100 mg metabolite/L, respectively. RMS recalculated the LC₅₀ and found a similar result.

Acceptability of the test

The test is considered acceptable.

Conclusion

The 96h-LC₅₀ and the 96h-NOEC are 240 and 100 mg metabolite/L, respectively.

B.9.2.1.2 Acute toxicity to aquatic invertebrates

B.9.2.1.2.1 Acute toxicity of the active substance to aquatic invertebrates (IIA 8.2.4/01)

Characteristics of the study

Reference	: Kuijpers (1987)	Species	: water flea (<i>Daphnia magna</i>)
Year of execution	: 1987	Exposure duration and regime	: 48 h, static
Guideline	: EPA 72-2	48h-EC ₅₀ mobility	:
		daphnids 16 – 24 h old	> 13 mg a.s./L
		daphnids 0 – 24 h old	16 mg a.s./L
GLP	: yes	48h-NOEC mobility	: 2.4 mg a.s./L
Test substance	: dichlobenil; batch no. FUN87E08A/FUX011000	and condition	:
Purity	: > 99%	Acceptable	yes

Study design

The acute toxicity of the test substance to *Daphnia magna* was tested in two separate experiments; in one test the animals were 16 – 24 hours old, in the other test 0 – 24 hours. The test substance was dosed directly into the dilution water; the nominal concentrations tested were 2.4, 4.0, 6.6, 11 (both tests), 13

(test with 16 – 24 h old animals) and 18 mg a.s./L (test with 0 – 24 h old animals). Samples for chemical analysis were taken at t = 0 and 48 h.

Results

The mean measured concentrations of the test substance varied between 81% and 100% of the nominal values, which confirms the correct dosing and stability of the test substance. The lowest measured dissolved oxygen concentration was 8.3 mg/L; pH values varied between 7.9 and 8.2 and temperature between 19.3 and 21.0°C.

After 48 h, 60% immobility was found at 18 mg a.s./L and reduced mobility was also seen at 4.0 mg a.s./L. The author reports 48-h EC₅₀ values of > 13 and 16 (15 – 18) mg a.s./L. RMS recalculated the EC₅₀ values and found a similar results. The 48h-NOEC for mobility is reported to be 4.0 mg a.s./L. However, effects other than immobility, were reported at that concentration (slower movement); therefore the NOEC value is 2.4 mg a.s./L.

Acceptability of the test

The study was carried out in 1987 and the description of the test is very limited. However, there are no doubts on the reliability of the study. According to RMS, the 48h-NOEC value for mobility and condition is 2.4 mg a.s./L, which differs from the NOEC of 4.0 mg a.s./L that was reported by the author. The test is considered acceptable.

Conclusion

The 48h-EC₅₀ value of dichlobenil for the water flea *Daphnia magna* is between 13 and 16 mg a.s./L; the NOEC value is 2.4 mg a.s./L.

B.9.2.1.2.2 Acute toxicity of the active substance to aquatic invertebrates (IIA 8.2.4/02)

Characteristics of the study:

Reference	Burgess (1987)	Species	: water flea (<i>Daphnia magna</i>)
Year of execution	1987	Exposure duration and regime	: 48 h, flow-through
Guideline	: EPA 72-2	48h-EC ₅₀ mobility ¹	: 6.2 mg a.s./L
GLP	: yes	48h-NOEC mobility ¹ and condition	: 1.0 mg a.s./L
Test substance	: dichlobenil; lot no. FUN87E08A.FUX011000; white powder		
Purity	: > 99%	Acceptable	: yes

¹ RMS assumes that the LC₅₀ and NOEC values determined using mortality as effect parameter can be read as EC₅₀ and NOEC values for mobility

Study design

The test substance was dosed using acetone as a solvent; the nominal concentrations tested were 0.74, 1.2, 2.7, 4.8 and 10 mg a.s./L. Samples for chemical analysis were taken at $t = 0$ and 48 h.

Results

The mean measured concentrations of the test substance varied between 74 and 89% of the nominal values. As the recovery was lower than the 80% outlined by the guideline, the test results were based on measured concentrations. The lowest measured dissolved oxygen concentration was 7.7 mg/L; pH values varied between 8.2 and 8.3 and temperature was 20°C.

No mortality or other adverse effects were seen in the (solvent) control groups. After 24 h and 48 h, 15 and 95% immobility was observed at the highest test concentration, respectively. Adverse effects were observed at 2.0, 3.8 and 8.9 mg a.s./L (daphnids lying on the bottom of the test vessels, floating on the surface, erratic movement, quiescence). The author states that the 24h- and 48h-LC₅₀s are > 8.9 and 6.2 (3.8 – 8.9) mg a.s./L (based on measured concentrations). RMS recalculated the 48h-EC₅₀ and found a similar result. The 48h-NOEC for mortality is 1.0 mg a.s./L.

Acceptability of the test

Mortality is used as effect parameter instead of immobility and the results are expressed as a LC₅₀ and NOEC for mortality. Quiescence is given as an observed effect at 24 h exposure. RMS assumes that this is equal to immobility. At 48 h, however, there were no quiescent animals observed and the use of the mortality parameter does not make any difference for the final conclusion and LC₅₀ can also be read as EC₅₀. The test is considered acceptable.

Conclusion

The 48h-EC₅₀ and 48h-NOEC of dichlobenil to the water flea *Daphnia magna* are 6.2 mg a.s./L and 1.0 mg/l, respectively.

B.9.2.1.2.3 Acute toxicity of the active substance to marine aquatic invertebrates (IIA 8.2.4/04)

Characteristics of the study:

Reference	: Dunham and Davis (1995)	Species	: mysid shrimp (<i>Mysidopsis bahia</i>)
Year of execution	: 1995	Exposure duration and regime	: 96 h, flow-through
Guideline	: EPA 72-3	96h-LC ₅₀	: 2.37 mg a.s./L
GLP	: yes	96h-NOEC mortality and condition	: 1.32 mg a.s./L
Test substance	: dichlobenil technical; lot no. FUN87E08A/FUX011000; off-white powder		
Purity	: 98.8%	Acceptable	yes

Study design

The test substance was dosed using dimethylformamide as a solvent; the nominal concentrations tested were 1.04, 1.73, 2.88, 4.80 and 8.00 mg a.s./L. Samples for chemical analysis were taken prior to test initiation and at $t = 0$ and 96 h. During the test, mysids were fed with live brine shrimp nauplii.

Results

The mean measured concentrations of the test substance varied between 64 and 102% of the nominal values. The low recovery was supported by the finding of undissolved test material. Therefore, the test results were based on measured concentrations.

The lowest measured dissolved oxygen concentration was 7.4 mg/L; pH values varied between 8.2 and 8.4, temperature was $22.4 \pm 0.3^{\circ}\text{C}$, and salinity was 20‰.

No mortality or other adverse effects were observed in the (solvent) control groups. In the treatment groups, mortality ranged from 15% at 0.666 mg a.s./L to 90% at 8.14 mg a.s./L after 96 h of exposure. No other adverse effects were seen in any of the treatments. The authors state that the 96h-LC₅₀ is 2.37 (1.63 – 3.32) mg a.s./L (based on measured concentrations). RMS recalculated the 96h-LC₅₀ and found a similar result. The 96h-NOEC for mortality and condition is 1.32 mg a.s./L.

Acceptability of the test

The test is considered acceptable.

Conclusion

The 96h-LC₅₀ of dichlobenil for the mysid shrimp *Mysidopsis bahia* is 2.37 mg a.s./L and the 96h-NOEC for mortality is 1.32 mg a.s./L.

B.9.2.1.2.4 Acute toxicity of the active substance to marine aquatic invertebrates (IIA 8.2.4/05)

Characteristics of the study:

Reference	Dunham and Davis (1995)	Species	: eastern oyster (<i>Crassostrea virginica</i>)
Year of execution	: 1995	Exposure duration and regime	: 96 h, flow-through
Guideline	: EPA 72-3	96h-LC ₅₀	: > 7.69 mg a.s./L
GLP	: yes	96h-EC ₅₀ shell growth	: 1.92 mg a.s./L
Test substance	: dichlobenil technical; lot no. FUN87E08A/FUX011000; off-white powder	96h-NOEC shell growth and condition	: < 1.00 mg a.s./L
Purity	: 98.8%	Acceptable	: yes

Study design

The test substance was dosed into natural seawater using dimethylformamide as a solvent; the nominal concentrations tested were 1.04, 1.73, 2.88, 4.80 and 8.00 mg a.s./L. Samples for chemical analysis were taken prior to test initiation and at $t = 0$ and 96 h. Oyster juveniles (44 ± 5.8 cm and 1.28 ± 0.68 g wet weight) obtained from a commercial supplier were used. During the test, oysters were fed with a mixture of algae. Effects of the test substance on survival and mean new shell growth were determined.

Results

The mean measured concentrations of the test substance varied between 91 and 105% of the nominal values, although some undissolved test material was observed at the highest test concentrations. The lowest measured dissolved oxygen concentration was 60% of the air saturation level; the pH values varied between 7.9 and 8.2, the temperature was $25.4 \pm 0.8^\circ\text{C}$, and the salinity was 20‰.

No mortality was observed in any of the control and treatment groups, except in the solvent control where 5% mortality was found (this is considered acceptable). Therefore, the 96h-LC₅₀ is > 7.69 mg a.s./L (based on measured concentrations). Relative to shell growth of control oysters, shell growth was significantly reduced at all tested concentrations; from 38% at 1.00 mg a.s./L to 84% at 7.69 mg a.s./L. The authors calculated a 96h-EC₅₀ of 1.92 (1.56 – 2.26) mg a.s./L. RMS recalculated the EC₅₀ and found a comparable result. Since adverse effects were seen in all treatment groups, the NOEC could not be determined (< 1.00 mg a.s./L according to RMS).

Acceptability of the test

For the determination of effects on shell growth, there was no test concentration without effects. As a clear dose-response curve was found and the confidence interval indicates the reliability of the available data, the calculated EC₅₀ value is considered acceptable.

Conclusions

The 96h-LC₅₀ value of dichlobenil for the eastern oyster *Crassostrea virginica* is > 7.69 mg a.s./L. The 96h-EC₅₀ value for shell growth is 1.92 mg a.s./L; the NOEC value for shell growth is less than the lowest concentration tested (< 1.00 mg a.s./L).

B.9.2.1.2.5 Acute toxicity of the metabolite 2,6-dichlorobenzamide (BAM) to aquatic invertebrates (IIA 8.2.4/03)

Characteristics of the study:

Reference	: Wetton and Mullee (2001)	Species	: water flea (<i>Daphnia magna</i>)
Year of execution	: 2001	Exposure duration and regime	: 48 h, static
Guideline	: OECD 202, EC C.2, US CFR Title 40 Part 797 Section 1300, EPA 72-2, OPPTS 850.1010	48h-EC ₅₀ mobility	: 180 mg a.s./L
GLP	: yes	48h-NOEC mobility	: 100 mg a.s./L
Test substance	: 2,6-dichlorobenzamide (BAM); batch no. FUX001000/FUN81G02C; off-white powder		
Purity	: 99.5%	Acceptable	: yes

Study design

The test substance was dosed without using a solvent; the nominal concentrations tested were 10, 18, 32, 56, 100, 180, 320, 560 and 1000 mg a.s./L. Samples for chemical analysis were taken at t = 0 and 48 h.

Results

The mean measured concentrations of the test substance varied between 100 and 112% of the nominal values (calculated by RMS), which confirms the correct dosing and stability of the test substance. The lowest measured dissolved oxygen concentration was 8.0 mg/L; pH values varied between 7.6 and 8.0 and temperature was 21.0°C.

At 180, 320, 560, and 1000 mg a.s./L, 25, 75, 95, and 100% of the daphnids was immobilised after 24 h and this increased to 65, 90, 100, and 100% after 48 h, respectively. The authors report a 48h-EC₅₀ of 180 (150 – 210) mg a.s./L. RMS recalculated the EC₅₀ and found a similar result. The 48h-NOEC based on immobilisation is 100 mg a.s./L.

Acceptability of the test

Information concerning the presence or absence of adverse effects other than immobilisation is not provided. Since this information may only affect the NOEC and not the EC₅₀ which is used for the risk assessment, the test is considered acceptable.

Conclusions

The 48h-EC₅₀ and NOEC values of the metabolite 2,6-dichlorobenzamide for the water flea *Daphnia magna* are 180 and 100 mg a.s./L, respectively.

B.9.2.1.3 Toxicity to algae**B.9.2.1.3.1 Toxicity of the active substance to algae (IIA 8.2.6/01)****Characteristics of the study**

reference	: Giddings (1988)	Species	: green alga (<i>Selenastrum capricornutum</i>)
year of execution	: 1988	Exposure duration and regime	: 120 h, static, cultures were shaken on an orbit shaker
GLP	: yes	72h-EC ₅₀	: > 3.2 mg a.s./L
Guideline	: EPA 540/9-82-020	growth rate and biomass	: > 3.2 mg a.s./L
Test substance	: dichlobenil Technical; lot no. FUN87EO8A FUX 011000; white powder	72h-NOEC	: ≥ 3.2 mg a.s./L
Content of a.s.	: 99%	growth rate	: ≥ 3.2 mg a.s./L
		biomass	: 0.67 mg a.s./L
		Acceptable	: yes

Study design

The following study set up was applied:

inoculum	: 3 x 10 ³ cells/mL
illumination	: 5000 lux for 24 h /d
growth medium	: according to guidelines with slight modifications
cell counts	: using a hemacytometer and microscope
type of test vessel and content	: 125-mL volumetric flasks; 50 mL
number of replicate test vessels	: 3 for control and each test concentration
dosing	: stock solutions in growth medium
exposure concentrations	: nominal: 0, 0.13, 0.25, 0.50, 1.0, 2.0, and 4.0 mg a.s./L measured: 0.085, 0.16, 0.35, 0.67, 1.4 and 3.2 mg a.s./L
type of measurements	: daily cell numbers; temperature, shaking rate of orbit shaker, illumination, pH, conductivity
chemical analysis	: yes, from samples taken before the addition of algae and after filtration (to remove algal cells)
chemical analytical method	: GC equipped with an electron capture detector
sampling times	: t = 0 and 120 h

Results

The mean measured concentrations of the test substance varied between 64 and 79% of the nominal value, which is below the required 80%. Therefore, the test results must be based on mean measured concentrations. pH values varied between 7.2 and 10.6 and temperatures between 23 and 25°C.

The authors report a 72h-EC₅₀ value of 1.1 (0.22 – 5.7) mg a.s./L based on cell densities. RMS used In-transformed cell numbers to calculate biomass as area under the growth curve and growth rate. Biomass was significantly reduced at 1.4 and 3.2 mg a.s./L, resulting in a 72h-NOEC value of 0.67 mg a.s./L. Growth rate was significantly reduced at 1.4 mg a.s./L, when compared with the control; however, one of the triplicates seems to be an outlier. As no significant difference was observed at 3.2 mg a.s./L, this is not considered to be related to the treatment. Therefore, the 72h-NOEC value for growth rate is ≥ 3.2 mg a.s./L. As the percent inhibition of biomass production and growth rate was below 50% at the highest test

concentration, the 72h-EC₅₀ values are > 3.2 mg a.s./L, respectively. The results are presented in Table B.9.2.1.3.1a.

Table B.9.2.1.3.1a Results of the algae growth inhibition test with dichlobenil as calculated by RMS

	72h-EC ₅₀ (mg a.s./L) ¹	72h-NOEC (mg a.s./L) ¹
biomass (area under the growth curve)	> 3.2	0.67
growth rate	> 3.2	≥ 3.2

¹ based on mean measured concentrations

Acceptability of the test

pH values increased in all treatments during the test. Considering the increase in cell numbers in the control treatment, this is not considered to have influenced the results of the test. The test is considered acceptable.

Conclusion

The overall 72h-EC₅₀ for biomass and growth rate of the green alga *Selenastrum capricornutum* is > 3.2 mg a.s./L. The 72h-NOEC is 0.67 mg a.s./L for biomass and ≥ 3.2 mg a.s./L for growth rate.

B.9.2.1.3.2 Toxicity of the active substance to algae (IIA 8.2.6/02)

Characteristics of the study

reference	: Giddings (1988)	Species	: blue-green alga (<i>Anabaena flos-aquae</i>)
year of execution	: 1988	Exposure duration	: 120 h, static, cultures were shaken on an orbit shaker
GLP	: yes	72h-EC ₅₀	
Guideline	: EPA 540/9-82-020	biomass and growth rate	: > 10 mg a.s./L
Test substance	: dichlobenil Technical; lot no. FUN87EO8A, FUX 011000; white powder	72h-NOEC	: ≥ 10 mg a.s./L
Content of a.s.	: 99%	biomass and growth rate	
		Acceptable	: yes

Study design

The following study set up was applied:

inoculum	: 3 x 10 ³ cells/mL
illumination	: 4500 Lux for 24 h /d
growth medium	: according to guidelines with slight modifications
cell counts	: using a hemacytometer and microscope
type of test vessel and content	: 250-mL volumetric flasks; 100 mL
number of replicate test vessels	: 3 for control and each test concentration
dosing	: stock solutions in growth medium
exposure concentrations	: 0, 0.63, 1.3, 2.5, 5.0, and 10.0 mg a.s./L (nominal concentrations)
type of measurements	: chlorophyll a and cell numbers daily ; temperature continuously, shaking rate of orbit shaker daily , light intensity every 24 h , pH and conductivity at the start and end of the test
chemical analysis	: no

Results

During the test, there was no undissolved test material in any of the treatments. Test substance concentrations were not measured and therefore the correct dosing and stability of the test substance cannot be confirmed. The pH values varied between 7.3 and 9.1 and temperatures between 24 and 26°C.

The authors report the results based on cell counts and fluorescence with 72h-EC₅₀ values of 7.1 (0.42 – 580) and 9.6 (4.1 – 17) mg a.s./L, respectively. RMS used In-transformed cell counts from the raw data to calculate 72h-EC₅₀ and -NOEC values for growth rate and biomass (as area under the growth curve). The results are summarised in Table B.9.2.1.3.2a. The 72h-EC₅₀ and -NOEC values are > 10 and ≥ 10 mg a.s./L for both end points.

Table B.9.2.1.3.2a Results of the algae growth inhibition test with dichlobenil as calculated by RMS

	72h-EC ₅₀ (mg a.s./L) ¹	72h-NOEC (mg a.s./L) ¹
biomass (area under the growth curve)	> 10	≥ 10
growth rate	> 10	≥ 10

¹ based on nominal concentrations

Acceptability of the test

Test substance concentrations were not measured, because this was not common at the time the study was performed. According to RMS, the results of the test may be expressed in nominal concentrations, because other aquatic toxicity studies with dichlobenil indicate that the test substance is stable in water. The pH values appeared to be increased at the end of the test (measurements were not carried out during the test). Such a phenomenon can be expected in an algae test after 120 h static exposure. It can be expected that at t = 72 h, pH values were lower. In contrast to the authors, RMS used In-transformed cell counts to calculate 72h-EC₅₀ and NOEC values. The EC₅₀ is > 10 mg a.s./L and the NOEC is ≥ 10 mg a.s./L, both for biomass and growth rate. The test is considered acceptable.

Conclusion

The 72h-EC₅₀ and 72h-NOEC values of dichlobenil for the blue-green alga *Anabaena flos-aquae* are > 10 and ≥ 10 mg a.s./L for both end points, biomass and growth rate.

B.9.2.1.3.3 Toxicity of the metabolite 2,6-dichlorobenzamide (BAM) to algae (IIA 8.2.6/03)**Characteristics of the study**

reference	: Mead and Mullee (2001)	Species	: green alga (<i>Pseudokirchneriella subcapitata</i>)
year of execution	: 2001	Exposure duration	: 96 h, static, cultures were shaken in an incubator
GLP	: yes	72h-EC ₅₀	
Guideline	: OECD 201, EC C.3, US Code of Federal Regulations Part 797 Section 1050, EPA Sub-division J Section 122-2, OPPTS 850.5400	growth rate	: 111 mg metabolite/L
Test substance	: 2,6-dichlorobenzamide (BAM); batch no. FUX001000/FUN81G02C; off-white powder	biomass	: 129 mg metabolite/L
Content of a.s.	: 99.5%	72h-NOEC growth rate and biomass	: 40 mg metabolite/L
		Acceptable	: yes

Study design

The study consisted of a growth-inhibition and a reversibility experiment. In the latter, the algicidal or algistatic effect of the test material was determined by pooling aliquots of each replicate control and test cultures and adding fresh growth medium. The number of cells was counted after 144 h. The following study set up was applied:

inoculum	: 10 ⁴ cells/mL
illumination	: 4500 lux for 24 h/d
growth medium	: according to the guidelines with slight modifications
cell counts	: using a Coulter Counter
type of test vessel and content	: 250-mL conical flasks; 100 mL
number of replicate test vessels	: 3 for control and each test concentration
dosing	: stock solutions in growth medium
exposure concentrations	: 0, 10, 20, 40, 80, and 160 mg metabolite/L (nominal concentrations)
type of measurements	: daily cell numbers; temperature, pH
chemical analysis	: yes, from the control and each test concentration
chemical analytical method	: HPLC
sampling times	: t = 0 and 96 h

Results

The mean measured concentrations of the test substance were > 80% of the nominal value, which confirms the correct dosing and stability of the test substance. pH values varied between 7.0 and 9.8 and temperature was 24 ± 1°C.

The results of the test are given in Table B.9.2.1.3.3a. The authors report a 72h-EC₅₀ and EC₅₀ of 60 (51 – 71) and 120 (confidence limits could not be calculated) mg metabolite/L, respectively, and a 96h-NOEC of 40 mg metabolite/L based on the area under the growth curve. RMS calculated a 72h-EC₅₀ of 129 (114 – 145) mg metabolite/L (based on ln scale for the area under the curve) and a 72h-EC₅₀ of 111 (89 – 138) mg metabolite/L. RMS also calculated 72h-NOECs based on the area under the growth curve and growth rate and agrees with the NOEC of 40 mg metabolite/L as stated by the authors.

Table B.9.2.1.3.3a Results of the algae growth inhibition test with the metabolite 2,6-dichlorobenzamide

	72h-EC ₅₀ (mg metabolite/L) ¹		72h-NOEC (mg metabolite/L) ¹	
	calculated by the authors	calculated by RMS	calculated by the authors	calculated by RMS
biomass (area under the growth curve)	60 (51 – 71)	129 (114 – 145)	40	40
growth rate	120 (-)	111 (89 – 138)		40

¹ based on nominal concentrations

Acceptability of the test

pH values appeared to be increased at the end of the test (measurements were not carried out during the test). Such a phenomenon can be expected in an algae test after 120 h static exposure. It can be expected that at t = 72 h, pH values were lower. The authors calculated biomass as area under the growth curve using untransformed data. RMS recalculated biomass using ln-transformed data and found a 72h-EC₅₀ of 129 mg metabolite/L. The test is considered acceptable.

Conclusion

The 72h-E_rC₅₀ and E_bC₅₀ for the metabolite 2,6-dichlorobenzamide for the green alga *Pseudokirchneriella subcapitata* are 111 and 129 mg metabolite/L. The 72h-NOEC is 40 mg a.s./L for biomass and growth rate.

B.9.2.1.4 Toxicity to aquatic plants

B.9.2.1.4.1 Toxicity of the active substance to aquatic plants (IIA 8.2.8/01)

Characteristics of the study

reference	: Giddings (1987)	Species	: duckweed (<i>Lemna gibba</i>)
year of execution	: 1987 - 1988	Exposure duration and regime	: 14 d; semi-static
GLP	: yes	14d-EC ₅₀	
Guideline	: EPA 540/9-82-020	frond number	: 33 µg a.s./L
		growth rate	: 34.8 µg a.s./L
		biomass	: 27.9 µg a.s./L
Test substance	: dichlobenil Technical; lot no. FUN87EO8A, FUX 011000; white powder	14d-NOEC	
		growth rate	: 6.3 µg a.s./L
		biomass	: 13 µg a.s./L
Content of a.s.	: 99%	Acceptable	: yes, as supplementary information

Study design

In this test, effects with respect to the reduction in frond number, growth rate, biomass and adverse effects with respect to appearance after 14 days of exposure, relative to the control, were studied. The following study set up was applied:

inoculum	: 5 plants with 3 fronds each in each test vessel
Origin of plants	: USDA, Washington DC; USA
exposure temperature	: 25 ± 2°C
illumination	: 5000 lux for 24 h/d

growth medium	: sterilised M-Type Hoagland's medium
type of test vessel and content	: sterilised 250-mL crystallising dishes covered with lass Petri dishes; 100 mL
number of replicate test vessels	: 3
dosing	: stock solutions in growth medium
exposure concentrations	: 0, 6.3, 13, 25, 50, and 100 µg a.s./L (nominal concentrations)
type of observations	: adverse effects on day 3, 6, 9, 12, and 14
type of measurements	: total number of fronds on day 3, 6, 9, 12, and 14, pH, temperature, illumination
chemical analysis	: no

Results

Undissolved test material was not observed during the test. However, as test substance concentrations were not determined, RMS cannot verify the correct dosing and stability of the test substance. The pH varied between 4.5 and 5.9 and the temperature between 22 and 25°C.

The test results are summarised in Table B.9.2.1.4.1a. The condition of the fronds decreased at 13 µg a.s./L and the higher concentrations tested in a dose-dependent way (small and brown fronds, few and brown roots). The authors report a 14d-EC₅₀ value of 33 (15 – 75) µg a.s./L based on frond number. RMS found a similar result for biomass (area under the growth curve). The 14d-NOEC for biomass is 13 µg a.s./L. RMS also calculated a 14d-EC₅₀ and NOEC for growth rate of 34.8 (33 – 37) and 6.3 µg a.s./L, respectively (based on nominal concentrations). The overall NOEC is 6.3 µg a.s./L, based on growth rate and appearance of the fronds.

Table B.9.2.1.4.1a Results of the *Lemna gibba* growth inhibition test with dichlobenil

Endpoint	14d-EC ₅₀ (µg a.s./L) ¹	14d-NOEC (µg a.s./L) ¹
Appearance (visual assessed)		6.3
Frond number	33 (15 – 75)	
Biomass (area under the growth curve)	27.9 (24 – 32) ²	13 ²
Growth rate	34.8 (33 – 37) ²	6.3 ²

¹ based on nominal concentrations

² calculated by RMS

Acceptability of the test

The test duration was 14 d instead of 7 d as stated by the draft OECD guideline. As effects generally deteriorate in time, this is not considered to have influenced the usability of the study. Analytical determination of the test substance concentrations to confirm the correct dosing and stability of the test substance was not required at the time the study was performed. Therefore, the study is considered as supplementary information.

Conclusion

The 14d-EC₅₀ values for frond number, biomass, and growth rate are 33, 27.9, and 34.8 µg a.s./L, respectively. The 14d-NOEC values are 6.3 µg a.s./L for growth rate and appearance and 13 µg a.s./L for biomass.

B.9.2.1.4.2 Toxicity of the metabolite 2,6-dichlorobenzamide to aquatic plants (IIA 8.2.8/02)

Characteristics of the study

reference	: Desjardins <i>et al.</i> (2003)	Species	: duckweed (<i>Lemna gibba</i>)
year of execution	: 2003	Exposure duration and regime	: 7 d; static
GLP	: yes	7d-EC ₅₀	
Guideline	: OPPTS 850.4400; OECD Draft 221	frond number	: 80 mg metabolite/L
		growth rate	: 78 mg metabolite/L
		biomass (area under the curve)	: 75 mg metabolite/L
Test substance	: 2,6-dichlorobenzamide; lot no. I8499A; powder	7d-NOEC	
		frond number	: 50 mg metabolite/L
		growth rate	: 50 mg metabolite/L
		biomass	: 25 mg metabolite/L
Content of a.s.	: 98%	Acceptable	: yes

Study design

In this test, effects of the test substance on frond number, growth rate, biomass and appearance were determined after 7 days of exposure. The following study set up was applied:

inoculum	: 4 plants with 3 fronds each in each test vessel
origin of test organisms	: Wildlife Int. laboratory culture
exposure temperature	: 25 ± 2°C
illumination	: 4500 - 5450 lux for 24 h/d
growth medium	: sterilised 20X AAP medium in accordance with OECD Draft 221
type of test vessel and content	: sterilised 250-mL glass beakers covered with Petri dishes; 100 mL
number of replicate test vessels	: 3
dosing	: stock solutions in growth medium
exposure concentrations	: 0, 13, 25, 50, 100, and 200 mg metabolite/L (nominal concentrations)
type of observations	: adverse effects with respect to appearance (visual observations) on day 3, 5, and 7
type of measurements	: total number of fronds on day 3, 5, and 7, number of plants on day 7, biomass (dw) on day 7, pH, temperature, illumination
chemical analysis	: yes
chemical analytical method	: HPLC with UV detection
sampling times	: t = 0 and 7 d

Results

The mean measured concentrations of the test substance varied between 92 and 102% of the nominal value. This confirms the correct dosing and stability of the test substance. The pH varied between 7.5 and 8.9 and the temperature varied between 24.0 and 25.0°C.

The test results are summarised in Table B.9.2.1.4.2a. Adverse effects with respect to appearance (chlorotic, necrotic, and dead fronds) were observed at 50 mg metabolite/L and higher and increased in a dose-dependent way. RMS found similar 7d-EC₅₀ values as the authors for frond number and dry weight, but calculated a lower EC₅₀ for growth rate (78 instead of 97 mg metabolite/L). RMS also calculated a 7d-EC₅₀ for biomass as area under the growth curve and found a value of 75 mg metabolite/L. 7d-NOEC values were 50 mg metabolite/L for frond density and growth rate and 25 mg metabolite/L for appearance and for biomass (either as dry weight or area under the growth curve).

Table B.9.2.1.4.2a Results of the *Lemna gibba* growth inhibition test with the metabolite 2,6-dichlorobenzamide

Endpoint	7d-EC ₅₀ (mg metabolite/L) ¹		7d-NOEC (mg metabolite/L) ¹	
	calculated by authors	calculated by RMS	calculated by authors	calculated by RMS
Adverse effects with respect to appearance				25
Frond number	80 (73 – 85)	80	50	
Biomass (dw)	80 (68 – 89)	80	25	
Biomass (area under the growth curve)		75 (68 – 84)		25
Growth rate	97 (92 – 102)	78 (70 – 87)	50	

¹ based on mean measured concentrations

Acceptability of the test

Although RMS applied the same method to calculate growth rate, RMS could not reproduce the 7d-EC₅₀ value found by the authors; RMS calculated a 7d-EC₅₀ of 78 instead of 97 mg metabolite/L. However, this deviation is not thought to be relevant as there is a lower EC₅₀ available for biomass. The test is considered acceptable.

Conclusion

The 7d-EC₅₀ for frond number and biomass (as dry weight) is 80 mg metabolite/L. The 7d-EC₅₀ values for biomass (as area under the growth curve) and growth rate are 75 and 78 mg, respectively. The 7d-NOEC values are 50 mg metabolite/L for frond number and growth rate and 25 mg metabolite/L for appearance and biomass (as dry weight and area under the growth curve).

B.9.2.2 Hazard Classification/Labelling

See Volume 3, chapter B.4.

B.9.2.3 Chronic toxicity**B.9.2.3.1 Chronic toxicity to fish****B.9.2.3.1.1 Early-life stage toxicity of the active substance to fish (IIA 8.2.2.2/01)****Characteristics of the study**

Reference	: McAllister (1988)	Species	: rainbow trout (<i>Oncorhynchus mykiss</i> , previously named <i>Salmo gairdneri</i>)
Year of execution	: not indicated	Exposure duration	: 96 d (60-d post hatch), flow through
Procedure	: ASTM E-47.01	and regime	
GLP	: yes	96d-NOEC (overall)	: 0.66 mg a.s./L
Test substance	: dichlobenil technical; batch no. FUN87E08A, FUX011000; white powder		
Purity	: 99.4%	Acceptable	: yes

Study design

The following study setup was applied:

egg stage at the start of the test	: fertilised < 8 h before test initiation
origin of eggs	: fertilisation in the laboratory; eggs were obtained from a commercial hatchery
exposure system	: Mount and Brungs (proportional dilution system)
exposure temperature	: 10°C
photoperiod	: 16 h light/8 h dark
type of dilution water	: aerated well water
total hardness (as CaCO ₃)	: 272 – 306 mg/L
type of test vessel and content	: the eggs were incubated in cups (glass tubing with Nytex® screening silicone glued to the bottom with silicone sealant) which were suspended in the test media after thinning of the sac-fry, in 11.5L glass aquaria covered with stainless steel screens
aeration	: yes
number of replicate test vessels	: 4; 2 aquaria containing two egg-hatching/growth chambers
number of animals per test vessel	: 30 eggs at start of the test; additionally, 50 eggs in separate incubator cups were placed in each control replicate to determine viability; thinned to 15 hatched individuals from day 46
feeding	: at least twice a day with living brine shrimp and commercial fish food, starting on day 50
dosing	: acetone as solvent (103 mg/L)
exposure concentrations	: nominal concentrations; 0, 0 (solvent), 0.312, 0.625, 1.25, 2.50, 5.0 mg a.s./L measured concentrations: 0, 0 (solvent), 0.33, 0.66, 1.2, 2.4 and 5.1 mg a.s./L
type of observations and biological measurements	: egg mortality daily; fertilisation success and embryonic development on day 11; number of eggs hatched in each

	incubation cup until day 41 (when all eggs had either hatched or died); time to hatch, swim-up from day 54; survival, growth (length) and adverse effects on day 70 and 96; growth as standard length and wet weight of each individual fish on day 96
type of further measurements	: dissolved oxygen, temperature, pH, conductivity, hardness, alkalinity, total organic carbon and suspended solids
chemical analysis	: yes
chemical analytical method	: GC-ECD
sampling times	: from test initiation (day 0) weekly until test termination (day 96)

Results

The mean measured concentrations of the test substance varied between 96 and 110% of the nominal values, which confirms the correct dosing and stability of the test substance. The concentrations in the report are expressed in measured concentrations. The authors state that the lowest measured dissolved oxygen concentration was 6.9 mg/L, but in the raw data for the 2.4 mg a.s./L treatment, a value of 3.4 mg/L was reported. The pH varied between 7.9 and 8.3 and the temperature between 9 and 12°C.

Mean viability of eggs in two additional replicates of the control treatment was 96% after 11 days, which indicates a successful fertilisation. Results of the test with the outcome of the statistical analysis are summarised in Table B.9.2.3.1.1a. Hatching occurred from day 39 to 41 and time to hatch did not differ significantly between treatments. Although there was a slight delay in swim-up at 1.2, 2.4, and 5.1 mg a.s./L compared to the control, sublethal effects, such as fry remaining at the bottom or surface of the aquaria, quiescence and vertical swimming, were observed at 2.4 and 5.1 mg a.s./L. No raw data were provided in which the number of fry showing adverse effects was reported. The authors state that the effect on growth determined on day 70 of the test was transitory and may be due to the use of more vulnerable eggs (as shown by a higher egg mortality rate than is usually). Therefore, the authors used the survival data on day 96 and determined a MATC of 0.89 (0.66 and 1.2) mg a.s./L.

Table B.9.2.3.1.1a Results for the various test parameters in the ELS test with dichlobenil

Test parameter	Mean measured concentration dichlobenil in mg a.s./L						
	0	0 (solvent)	0.33	0.66	1.2	2.4	5.1
% egg hatchability on day 41	75	66	73	73	57	63	35
fry survival on day 70	95	93	72	88	80	88	17 ¹
fry survival on day 96	93	93	72	85	73	88	15 ¹
fry growth on day 70 (length in mm)	29	29	27 ¹	26 ¹	24 ¹	24 ¹	17 ¹
fry growth on day 96 (length in mm)	40	40	39	39	36 ¹	34 ¹	21 ¹
fry growth on day 96 (wet weight in mg)	956	974	926	887	711 ¹	584 ¹	100 ¹

¹ significantly different from the control ($\alpha = 0.05$)

Acceptability of the test

The dissolved oxygen concentration was below 60% of the air saturation value at one occasion at the nominal concentration of 2.5 mg a.s./L on day 91. As there was no enhanced mortality at that time, it is assumed that this had no adverse effects on the test

The report does not contain raw data with respect to hatching time and sublethal effects. Adverse effects with respect to growth, measured as length, were found at all test concentrations on day 70 (34 post hatch). However, at day 96 (60 days post hatch), when measured as length or wet weight, there were no significant adverse effects to growth at measured concentrations of 0.33 and 0.66 mg a.s./L. As measuring of length is less accurate than weight and the differences are only minor (2 – 3 mm) it is allowed to use the growth measurements at the end of the test for the estimation of the NOEC (being 0.66 mg a.s./L)

Conclusion

The overall 96d-NOEC of dichlobenil in the early life stage test with the rainbow trout *Oncorhynchus mykiss* is 0.66 mg a.s./L.

B.9.2.3.2 Chronic toxicity to aquatic invertebrates**B.9.2.3.2.1 Chronic toxicity of the active substance to aquatic invertebrates (IIA 8.2.5/01)****Characteristics of the study**

Reference	: Forbis (1988)	Species	: water flea (<i>Daphnia magna</i>)
Year of execution	: 1988	Exposure duration	: 21 d, flow-through
Guideline	: EPA 72-4	and regime	:
GLP	: yes	21d-NOEC	: 1.2 mg a.s./L
Test substance	: dichlobenil; lot no. FUN87EO8A, FUX011000; white powder	reproduction	:
Purity	: > 99%	Acceptable	: yes

Study design

The test substance was dosed from concentrated stock solutions in acetone; the concentrations tested were 0.30, 0.60, 1.2, 2.5, and 5.0 mg a.s./L. Each concentration consisted of four replicates, each containing 10 daphnids. Samples for chemical analysis were taken at t = 0, 4, 7, 14, and 21 d.

Results

The mean measured concentrations of the test substance in the freshly prepared media varied between 83% and 107% of the nominal values, which confirms the correct dosing and stability of the test substance. The lowest measured dissolved oxygen concentration was 98% of the air saturation value, the pH varied between 8.0 and 8.2, and the temperature was 20°C.

The results of the reproduction test with the statistical analysis are summarised in Table B.9.2.3.2.1a. In the control and solvent control group, mortality was below 20%. Mortality was significantly higher at 5.0 mg a.s./L. The reproduction rate and body length of the surviving parent daphnids were significantly reduced compared with the (solvent) control animals at 2.5 and 5.0 mg a.s./L. The authors report an overall MATC value of 1.5 (1.0 – 2.3) mg a.s./L (based on mean measured concentrations). RMS determined 21d-NOEC values for growth, reproduction and survival of 1.2, 1.2, and 2.5 mg a.s./L, respectively (the overall 21d-NOEC is 1.2 mg a.s./L).

Table B.9.2.3.2.1a Results of the *Daphnia* reproduction test with dichlobenil

	dichlobenil in mg a.s./L (nominal concentrations)							
	control	solvent control	0.30	0.60	1.2	2.5	5.0	NOEC according to RMS
% survival on day 21	95	95	95	93 ¹	93 ¹	95	18 ²	2.5
no. of young per reproduction days	5.4	5.4	5.3	5.4	5.2	3.6 ²	0 ²	1.2
body length (mm)	3.8	3.8	3.8	3.9	3.8	3.4 ²	2.1 ²	1.2

¹ calculated by RMS based on the raw data (the authors report 92 and 95% survival at 0.60 and 1.2 mg a.s./L, respectively)

² significantly different from the control ($\alpha = 0.05$)

Acceptability of the test

The test is considered acceptable.

Conclusion

The overall 21d-NOEC of dichlobenil for the water flea *Daphnia magna* is 1.2 mg a.s./L

B.9.2.4 Toxicity to sediment-dwelling organisms

There are no studies available.

B.9.2.5 Bioconcentration**B.9.2.5.1 Bioconcentration of the active substance in fish (IIA 8.2.3/01 and IIA 8.2.3/02)****Characteristics of the study**

Reference	: Forbis (1986); Kampen <i>et al.</i> (1987)	Radiolabelled dichlobenil	: isotope [^{14}C] batch no. AOI86123A
Year of execution	:	Radiochemical purity	: 98.7%
Guideline	: FIFRA 72-6 and 165-4	Chemical purity	: 101.2%
GLP	: yes	Species	: bluegill sunfish (<i>Lepomis macrochirus</i>)
Test substance	: dichlobenil technical; white crystals	Exposure duration and regime	: 28 d exposure, followed by 14 d depuration; flow-through (7.1 x replacement during 24 h)
yes/no	: 98.9%	BCF	: 18 (fish fillet) - 110 L/kg (viscera); depuration $\geq$ 97% after 3 days
		Acceptable	: yes

The following study set up was applied:

size of fish	: 4.0 $\pm$ 1.2 g and 50 $\pm$ 4.6 mm
origin of fish	: commercial supplier
exposure temperature	: 22 $\pm$ 2°C
feeding	: commercial fish food 3% of the body weight
photoperiod	: 16 h daylight photoperiod
type of dilution water	: well water
total hardness (as CaCO_3)	: 225 – 275 mg/L
type of test vessel and content	: 100-L test aquaria
number of replicate test vessels	: 1
aeration	: not indicated
number of animals per test vessel	: 120
dosing	: using dimethylformamide as a solvent
concentration	: 0 and 0.15 mg /L
type of observations	:
sampling of water and fish for measurement radioactivity	: day 0, 1, 3, 7, 14, 21 and 28 during exposure and on day 1, 3, 7 10 and 14 during depuration
metabolite characterisation in fish	: day 21 and day 28 of exposure and on day 14 of depuration
chemical analytical method for water and fish tissue samples	: HPLC

Results

The average water concentration during the uptake phase was 0.14 ± 0.0076 mg/L. Bioconcentration factors ranged from 18 to 32x, 33 to 63x and 48 to 110x for fish fillet, whole fish and viscera, respectively (see Table B.9.2.5.1a). Depuration was quick, being $\geq 96\%$ after 3 days (see also Table B.9.2.5.1a).

Table B.9.2.5.1a Radioactivity (calculated as dichlobenil) in test water and fish tissue

	Water	fillet		whole fish		viscera	
uptake day	mg/L	mg/kg	BCF (L/kg)	mg/kg	BCF (L/kg)	mg/kg	BCF (L/kg)
0	0.14						
0.17	0.12	2.3	18	4.3	33	6.3	48
1	0.14	3.7	28	6.5	50	8.4	65
3	0.13	4.1	32	8.0	62	14	110
7	0.14	3.8	29	8.0	62	11	85
14	0.14	4.0	29	8.8	63	9.4	67
21	0.14	4.4	31	7.6	54	12	86
28	0.14	3.9	28	6.5	46	9.3	66
depuration day	mg/L	mg/kg	depuration %	mg/kg	depuration %	mg/kg	depuration %
1	< LOQ	0.43	89	1.0	85	1.2	87
3	< LOQ	< LOQ	> 96	0.22	97	0.344	96
7	< LOQ	0.27	95	0.27	96	0.26	97
10	< LOQ	< LOQ	> 96	< LOQ	> 97	0.26	97
14	< LOQ	< LOQ	> 96	< LOQ	> 97	0.29	97

Fish extracts (edible fish samples and viscera samples) and aquarium water contained only dichlobenil, identified by GC-MS. Less than 1% could be related to other radio-active contents.

Acceptability of the test

This test was carried out in 1986 and differs from the current guidelines. However, there are no doubts on the outcome of the study and the test is considered acceptable.

Conclusion

The bioconcentration of dichlobenil in the bluegill sunfish *Lepomis macrochirus* is low. The BCF varied between 18 (fish fillet) and 110 L/kg (viscera). Depuration was quick; > 96% (fish fillet) and 97% (viscera) after 14 days.

B.9.2.5.2 Partition coefficient in octanol-water of the active substance and the metabolite 2,6-dichlorobenzamide (IIA 2.8/01)

reference	: Thus (1985)	Purity	: 98.8%
Year of execution	: 1984	Test substance	: 2,6-dichlorobenzamide; batch no., appearance and purity not indicated
Guideline	: OECD 117	Log P _{OW}	: dichlobenil: 2.70
GLP	: no		: 2,6-dichlorobenzamide: 0.38
Test substance 1	: dichlobenil; batch no. FUX003000/FUN82B07A; appearance not indicated	Acceptable	: yes

The log P_{OW} values of dichlobenil and its main metabolite 2,6-dichlorobenzamide were determined by reversed phase HPLC on a phenyl modified silica gel column. The log P_{OW} was 2.70 and 0.38 for dichlobenil and 2,6-dichlorobenzamide, respectively.

Acceptability of the test

The information given about the metabolite was very concise (batch no. and purity were not provided). Chromatograms were not given in the report as well and therefore, RMS cannot rule out the possibility that the interpretation of the chromatograms was hampered due to impurities in the metabolite. The test was not performed according to GLP and the OECD guideline 117 (which was not yet available at the time the study was performed). There are, however, no doubts on the study and therefore the study is considered acceptable.

Conclusions

The log P_{OW} was 2.70 and 0.38 for dichlobenil and the metabolite 2,6-dichlorobenzamide, respectively.

B.9.2.5.3 Partition coefficient in octanol-water of the metabolite 2,6-dichlorobenzamide (IIA 2.8/02)

reference	: Riggs (2000)	Test substance	: 2,6-dichlorobenzamide; lot no. ARS-81C25N; white powder
Year of execution	: 2000	Purity	: 99.7%
Guideline	: OECD 117	Log P _{OW}	: 0.51
GLP	: yes	Acceptable	: yes

The HPLC method was used to determine the log P_{OW} of 2,6-dichlorobenzamide. The log P_{OW} was 0.51.

Acceptability of the test

The test is considered acceptable.

Conclusions

The log P_{OW} of the metabolite 2,6-dichlorobenzamide was 0.51.

B.9.2.6 Summary of aquatic toxicity tests

The toxicity to aquatic organisms is summarised in Table B.9.2.6a.

Table B.9.2.6a Summary of toxicity values of the active substance to aquatic organisms

Purity	Species	Endpoint	Toxicity value in mg a.s./L	Test Guideline	Reference no.
Acute toxicity to fish					
99.3%	rainbow trout (<i>Oncorhynchus mykiss</i>)	96h-LC ₅₀ 96h-NOEC	7.2 2.6	OECD 203, OPPTS 850.1075, EC C.1, ASTM E729-88a	IIA 8.2.1/01
98.8%	sheepshead minnow (<i>Cyprinodon variegatus</i>)	96h-LC ₅₀ 96h-NOEC	12.7 3.53	EPA 72-3	IIA 8.2.1/03 and IIA 8.2.1/04
Acute toxicity to invertebrates					
> 99%	water flea (<i>Daphnia magna</i>)	48h-EC ₅₀ (age 16 – 24 h) (age 0 – 24 h) 48h-NOEC	> 13 16 2.4	EPA 72-2	IIA 8.2.4/01
> 99%	water flea (<i>Daphnia magna</i>)	48h-EC ₅₀ 48h-NOEC	6.2 1.0	EPA 72-2	IIA 8.2.4/02
98.8%	mysid shrimp (<i>Mysidopsis bahia</i>)	96h-LC ₅₀ 96h-NOEC	2.37 1.32	EPA 72-3	IIA 8.2.4/04
98.8%	eastern oyster (<i>Crassostrea virginica</i>)	96h-LC ₅₀ 96h-EC ₅₀ 96h-NOEC	> 7.69 1.92 < 1.00	EPA 72-3	IIA 8.2.4/05
Toxicity to algae					
99%	green alga (<i>Selenastrum capricornutum</i>)	72h-E _r C ₅₀ 72h-E _b C ₅₀ 72h-NOE _r C 72h-NOE _b C	> 3.2 > 3.2 ≥ 3.2 0.67	EPA 540/9-82-020	IIA 8.2.6/01
99%	blue-green alga (<i>Anabaena flos-aquae</i>)	72h-E _r C ₅₀ 72h-E _b C ₅₀ 72h-NOE _r C 72h-NOE _b C	> 10 > 10 ≥ 10 ≥ 10	EPA 540/9-82-020	IIA 8.2.6/02
Toxicity to aquatic plants					
99%	duckweed (<i>Lemna gibba</i>)	14d-EC ₅₀ frond number 14d-E _r C ₅₀ 14d-E _b C ₅₀ 14d-NOE _r C 14d-NOE _b C	0.033 0.0348 0.0279 0.0063 0.013	EPA 540/9-82-020	IIA 8.2.8/01
Chronic toxicity to fish					
99.4%	rainbow trout (<i>Oncorhynchus mykiss</i>)	96d-NOEC	0.66	ASTM E-47.01	IIA 8.2.2.2/01
Chronic toxicity to invertebrates					
> 99%	water flea (<i>Daphnia magna</i>)	21d-NOEC	1.2	EPA 72-4	IIA 8.2.5/01

Table B.9.2.6b Summary of toxicity values of the metabolite 2,6-dichlorobenzamide (BAM) to aquatic organisms

Purity	Species	Endpoint	Toxicity value in mg metabolite/L	Test Guideline	Reference no.
Acute toxicity to fish					
99.5%	rainbow trout (<i>Oncorhynchus mykiss</i>)	96h-LC ₅₀ 96h-NOEC	240 100	OECD 203, EC C.1, US CFR Title 40 Part 797 Section 1400, EPA 72-4, OPPTS 850.1075	IIA 8.2.1/07
Acute toxicity to invertebrates					
99.5%	water flea (<i>Daphnia magna</i>)	48h-EC ₅₀ 48h-NOEC	180 100	OECD 202, EC C.2, US CFR Title 40 Part 797 Section 1300, EPA 72-2, OPPTS 850.1010	IIA 8.2.4/03
Toxicity to algae					
99.5%	green alga (<i>Pseudokirchneriella subcapitata</i>)	72h-E _r C ₅₀ 72h-E _b C ₅₀ 72h-NOE _r C 72h-NOE _b C	111 129 40 40	OECD 201, EC C.3, US Code of Federal Regulations Part 797 Section 1050, EPA Sub-division J Section 122-2, OPPTS 850.5400	IIA 8.2.6/03
Toxicity to aquatic plants					
98%	duckweed (<i>Lemna gibba</i>)	7d-EC ₅₀ frond number 7d-E _r C ₅₀ 7d-E _b C ₅₀ 7d-NOEC frond number 7d-NOE _r C 7d-NOE _b C	80 78 75 50 50 50 25	OPPTS 850.4400; OECD Draft 221	IIA 8.2.8/02

B.9.2.7 Risk assessment

B.9.2.7.1 Acute risk of the active substance dichlobenil

In the acute risk assessment of dichlobenil for fish, invertebrates and algae the following endpoints were used:

- 96h-LC₅₀ for fish is 7.2 mg a.s./L
- 96h-LC₅₀ for mysid shrimp is 2.37 mg a.s./L
- 72h-EC₅₀ for algae is > 3.2 mg a.s./L

The acute TERs for application of granules dichlobenil on vines and non-crops are shown in Table B.9.2.7.1a. PEC values were taken from section B.8.5.1. The PEC_{SW} from the FOCUS Step 3 calculations were selected for these TER calculations because in FOCUS Step 1 and 2 the TER values for dichlobenil were below the trigger values from Annex VI.

Table B.9.2.7.1a. Acute TERs for dichlobenil at different FOCUS step 3 scenarios, following treatment of vines and non-crops

Crop	Scenario	LC/EC ₅₀ (µg a.s./L)			Actual PEC _{SW} (µg a.s./L)	TER		
		Fish	Invertebrates	Algae		Fish	Invertebrates	Algae
Vines	D6 ditch	7200	2370	> 3200	240.47	30	10	>13
	R1 stream	7200	2370	> 3200	16.27	443	146	>197
	R1 pond	7200	2370	> 3200	0.098	73469	24184	>32653
	R2 stream	7200	2370	> 3200	10.557	682	224	>303
	R3 stream	7200	2370	> 3200	49.280	146	48	>65
	R4 stream	7200	2370	> 3200	55.549	130	43	>58
Non-crops	D1 stream	7200	2370	> 3200	39.194	184	60	>82
	D1 ditch	7200	2370	> 3200	53.565	134	44	>60
	D3 ditch	7200	2370	> 3200	0.001	7200000	2370000	>3200000
	D4 stream	7200	2370	> 3200	6.051	1190	392	>529
	D4 pond	7200	2370	> 3200	4.176	1724	568	>766
	D5 stream	7200	2370	> 3200	1.18	6102	2008	>2712
	D5 pond	7200	2370	> 3200	1.237	5821	1916	>2587
	R1 stream	7200	2370	> 3200	36.589	197	65	>87
	R1 pond	7200	2370	> 3200	1.592	4523	1489	>2010

The acute TER for fish is below the Annex VI trigger of 100 for one of the 15 considered scenarios. For invertebrates the TER is below the trigger of 100 for 6 scenarios. For algae the TER values are all above the Annex VI triggers of 10. As a consequence the acute risk from the proposed use is not acceptable for 6 of the 15 scenarios (D6, R3 stream, R4 stream, D1 stream, D1 ditch and R1). Member states for which these scenarios are relevant should use appropriate refinement options or risk mitigating measures.

B.9.2.7.2 Long-term risk of the active substance dichlobenil

The proposed broadcast spreading of granules involves a single application. Dichlobenil degrades in water with a DT₅₀ of 10.9 days. According to the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001), chronic exposure to dichlobenil is therefore possible. In the event of a DT₅₀ < 2 days a long-term/chronic test is not required. Chronic toxicity data for rainbow trout and *Daphnia magna* are available; the NOEC values are 0.66 and 1.2 mg a.s./L respectively. For the aquatic plant duckweed (*Lemna gibba*) a 14d-EC₅₀ (biomass as area under the growth curve) value of 0.0279 mg a.s./L is available. This value is regarded as a chronic toxicity value and used for the long-term risk assessment.

The chronic TERs for application of granules dichlobenil are shown in Table B.9.2.7.2a. PEC_{SW} values were taken from section B.8.5.1. The PEC_{SW} from the FOCUS Step 3 calculations were selected for these TER calculations because in FOCUS Step 1 and 2 the TER values for dichlobenil were below the trigger values from Annex VI.

Table B.9.2.7.2a. Chronic TERs for dichlobenil at different FOCUS step 3 scenarios, following treatment of vines and non-crops

Crop	Scenario	NOEC/14d-EC ₅₀ (µg a.s./L)			Actual PEC _{SW} (µg a.s./L)	TER		
		NOEC	NOEC	14d-EC ₅₀				
		Fish	<i>Daphnia</i>	Aquatic plants		Fish	<i>Daphnia</i>	Aquatic plants
Vines	D6 ditch	660	1200	27.9	240.47	2.7	5.0	0.1
	R1 stream	660	1200	27.9	16.27	41	74	1.7
	R1 pond	660	1200	27.9	0.098	6735	12245	285
	R2 stream	660	1200	27.9	10.557	63	114	2.6
	R3 stream	660	1200	27.9	49.280	13	24	0.6
	R4 stream	660	1200	27.9	55.549	12	22	0.5
Non-crops	D1 stream	660	1200	27.9	39.194	17	31	0.7
	D1 ditch	660	1200	27.9	53.565	12	22	0.5
	D3 ditch	660	1200	27.9	0.001	660000	1200000	27900
	D4 stream	660	1200	27.9	6.051	109	198	4.6
	D4 pond	660	1200	27.9	4.176	158	287	6.7
	D5 stream	660	1200	27.9	1.18	559	1017	24
	D5 pond	660	1200	27.9	1.237	534	970	23
	R1 stream	660	1200	27.9	36.589	18	33	0.8
	R1 pond	660	1200	27.9	1.592	415	754	18

The long-term TERs for *Daphnia* and fish are above the relevant Annex VI trigger of 10 for all scenarios, except for scenario D6. Therefore, the chronic risk as a result of the proposed use is not acceptable for aquatic crustaceans and fish for scenario D6. For all other scenarios the chronic risk from the proposed use is acceptable as far as these taxonomic groups is concerned. For aquatic plants, long-term TER values are below the trigger value of 10 in the case of 10 of the 15 considered scenarios.

Member states for which these scenarios are relevant should use appropriate refinement options or risk mitigating measures.

A study with sediment-dwelling organisms is not required because the NOEC in the chronic *Daphnia* test was 1.2 mg a.s./L, which is above the trigger of 0.1 mg/L.

B.9.2.7.3 Bioaccumulation

The general trigger to require a fish bioconcentration study is $\log P_{OW} > 3$. The $\log P_{OW}$ of dichlobenil is 2.70 which means that a fish bioconcentration study is not required for dichlobenil. However, a bioconcentration study was performed, which revealed that the bioconcentration of dichlobenil in the bluegill sunfish *Lepomis macrochirus* is low. The BCF varied between 18 (fish fillet) and 110 L/kg (viscera). Depuration was quick; > 96% (fish fillet) and 97% (viscera) after 14 days.

B.9.2.7.4 Risk assessment of metabolites

There are 3 metabolites that need to be considered for the risk assessment of aquatic organisms. These are 2,6-dichlorobenzamide (BAM), 2-chlorobenzoic acid and 4-chloro-2(3H)benzoxazolone. PEC_{SW} values are calculated for 2-chlorobenzoic acid and 4-chloro-2(3H)benzoxazolone (see Table B.8.5.1zk) but toxicity tests with aquatic organisms are not available. However, it can be argued that a risk of these two metabolites for aquatic organisms is unlikely. The PEC_{SW} values for these two metabolites are a factor 10 lower than the PEC_{SW} for BAM (see Table B.8.5.1zk). In addition, the acute toxicity ratios for BAM do not indicate a risk for aquatic organisms (see Table B.9.2.7.4 below). Based on this, the risk for the metabolites 2-chlorobenzoic acid and 4-chloro-2(3H)benzoxazolone is considered low.

A risk assessment for aquatic organisms can be carried out for the metabolite 2,6-dichlorobenzamide. This metabolite is formed in the soil at a maximum of 69.41% of the applied dose and can enter the water phase. Also, a small amount can be formed in surface water. Aquatic toxicity tests with 2,6-dichlorobenzamide were carried out with fish, *Daphnia*, algae and aquatic plants. The acute toxicity values are a LC_{50} of 240 mg/L for fish, an EC_{50} of 180 mg/L for *Daphnia* and an EC_{50} of 111 mg/L for algae. One chronic toxicity value is available: a 14d- EC_{50} of 75 mg/L for the aquatic plant *Lemna gibba*.

The acute and chronic TERs for treatment of vines and non-crops with dichlobenil, for the metabolite 2,6-dichlorobenzamide are shown in Table B.9.2.7.4a (acute) and Table B.9.2.7.4b (chronic). PEC_{SW} values for the metabolite were taken from section B.8.5.1 (maximum PEC_{SW} values resulting from the FOCUS Step 3 calculations for 2,6-dichlorobenzamide). The PEC_{SW} values are only based on the metabolite formed from dichlobenil in the soil and entering the surface water by drainage and run-off because the contribution of in surface water formation to the total PEC_{SW} is not quantitatively important.

Table B.9.2.7.4a Acute TERs for the metabolite 2,6-dichlorobenzamide at different FOCUS step 3 scenarios, following treatment of vines and non-crops

Crop	Scenario	LC/ EC_{50} (µg metabolite/L)			Actual PEC_{SW} (µg/L)	TER		
		fish	<i>Daphnia</i>	algae		fish	<i>Daphnia</i>	algae
Vines	D6 ditch	240000	180000	111000	72.034	3332	2499	1541
	R1 stream	240000	180000	111000	1.16	206897	155172	95690
	R1 pond	240000	180000	111000	0.008	30000000	22500000	13875000
	R2 stream	240000	180000	111000	1.816	132159	99119	61123
	R3 stream	240000	180000	111000	7.501	31996	23997	14798
	R4 stream	240000	180000	111000	2.932	81855	61392	37858
Non-crops	D1 stream	240000	180000	111000	137.547	1745	1309	807
	D1 ditch	240000	180000	111000	138.618	1731	1299	801
	D3 ditch	240000	180000	111000	89.744	2674	2006	1237
	D4 stream	240000	180000	111000	93.717	2561	1921	1184
	D4 pond	240000	180000	111000	185.381	1295	971	599
	D5 stream	240000	180000	111000	47.618	5040	3780	2331
	D5 pond	240000	180000	111000	114.400	2098	1573	970
	R1 stream	240000	180000	111000	2.590	92664	69498	42857

	R1 pond	240000	180000	111000	0.121	1983471	1487603	917355
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The acute TERs for fish, *Daphnia* and algae are all above the relevant Annex VI triggers (100, 100 and 10, respectively). Therefore, the acute risk for 2,6-dichlorobenzamide from the proposed use is acceptable.

Table B.9.2.7.4b Chronic TERs for the metabolite 2,6-dichlorobenzamide at different FOCUS step 3 scenarios, following treatment of vines and non-crops

Crop	Scenario	LC/EC ₅₀ (µg metabolite/L)			Actual PEC _{SW} (µg metabolite/L)	TER		
		fish	<i>Daphnia</i>	aquatic plants		fish	<i>Daphnia</i>	aquatic plants
Vines	D6 ditch	n.a.	n.a.	75000	72.034	n.a.	n.a.	1041
	R1 stream	n.a.	n.a.	75000	1.16	n.a.	n.a.	64655
	R1 pond	n.a.	n.a.	75000	0.008	n.a.	n.a.	9375000
	R2 stream	n.a.	n.a.	75000	1.816	n.a.	n.a.	41300
	R3 stream	n.a.	n.a.	75000	7.501	n.a.	n.a.	9999
	R4 stream	n.a.	n.a.	75000	2.932	n.a.	n.a.	25580
Non-crops	D1 stream	n.a.	n.a.	75000	137.547	n.a.	n.a.	545
	D1 ditch	n.a.	n.a.	75000	138.618	n.a.	n.a.	541
	D3 ditch	n.a.	n.a.	75000	89.744	n.a.	n.a.	836
	D4 stream	n.a.	n.a.	75000	93.717	n.a.	n.a.	800
	D4 pond	n.a.	n.a.	75000	185.381	n.a.	n.a.	405
	D5 stream	n.a.	n.a.	75000	47.618	n.a.	n.a.	1575
	D5 pond	n.a.	n.a.	75000	114.400	n.a.	n.a.	656
	R1 stream	n.a.	n.a.	75000	2.590	n.a.	n.a.	28958
	R1 pond	n.a.	n.a.	75000	0.121	n.a.	n.a.	619835

n.a. Not available.

The chronic TERs for aquatic plants are all above the relevant Annex VI trigger of 10. Therefore, the chronic risk for 2,6-dichlorobenzamide from the proposed uses is acceptable.

The chronic TERs for fish and *Daphnia* could not be determined. However, it can be expected that algae and aquatic plants are more sensitive than fish and *Daphnia* towards chronic exposure by 2,6-dichlorobenzamide based on the acute toxicity data in Table 9.2.7.4a. Therefore chronic toxicity tests with fish and *Daphnia* exposed to 2,6-dichlorobenzamide (BAM) are not necessary. The metabolite may enter the sediment compartment via soil. However, toxicity tests with sediment-dwelling organisms are not required as the acute toxicity of the parent to *Daphnia* is 30 times higher than the toxicity of BAM and exposure via sediment is at the most 2 times higher than via water (28-d time-weighted average PEC values).

B.9.3 EFFECTS ON OTHER TERRESTRIAL VERTEBRATES

There are no other studies.

B.9.4 EFFECTS ON BEES

B.9.4.1 Toxicity

There are no studies on the toxicity to bees.

B.9.4.2 Risk assessment

Active substance

The risk for bees could not be assessed as no toxicity studies for bees exposed to dichlobenil are available. However, the risk of dichlobenil to bees is expected to be low because the formulation is applied in a granular form and dichlobenil is non-systemic. It is therefore unlikely that the bees will come into contact with the a.s., either via contact or ingestion.

BAM

The metabolite 2,6-dichlorobenzamide (BAM) was found in grapes at a MRL of 0.3 mg metabolite/kg grapes (see B.7.12.2). When bees are feeding on grapes, oral exposure to BAM can therefore not be excluded. According to RMS's information bees do feed on grapes. This is the case when ripe grapes are damaged, which is caused by foraging wasps, or sometimes mildew in an early stage of the grapes. If this is the case, bees are attracted to the grapes and can come to forage on the grapes in large numbers.

Following SANCO/10329/2002, the risk to bees is considered negligible when the hazard quotient (HQ) (application rate/oral LD₅₀) is below 50. Considering the maximum application rate of dichlobenil in the field of 5400 g a.s./ha, the oral intake of dichlobenil should be below $5400 / 50 = 108 \mu\text{g a.s./bee}$.

Assuming the worst-case situation that all the dichlobenil applied to the field is converted into BAM in grapes and that BAM is equally toxic to the parent compound, the acceptable oral intake of BAM would then be $< 108 \mu\text{g BAM/bee}$. Using the MRL of BAM in grapes (obtained when dichlobenil was applied at the maximum dose), the oral intake of $108 \mu\text{g BAM/bee}$ is reached at a consumption of 360 g grapes per bee, which is very unlikely to occur. Therefore, RMS considers the risk of BAM to bees low.

B.9.5 EFFECTS ON OTHER ARTHROPOD SPECIES

B.9.5.1 Non-target arthropods

Calculation of corrected mortality

In the tests with arthropods the following formula for calculation of corrected mortality percentage was (where applicable):

$$M_{\text{corr}}(\%) = ((M_t - M_c) / (100 - M_c)) * 100\%$$

$M_{\text{corr}}(\%)$ = % corrected mortality, M_t = % mortality of test group, M_c = % mortality of control group

B.9.5.1.1 Laboratory test on effects of the formulation Casoron G on mortality of the carabid beetle *Poecilus cupreus* (IIA 8.3.2/01)

Characteristics of the study

Reference	: Römcke (1989)	Content of a.s.	: 6.75% dichlobenil
Year of execution	: 1989	Species	: carabid beetle (<i>Poecilus cupreus</i>)
GLP	: no	Exposure duration	: 14 d
Guideline	: BBA (1987)	Mortality	: no adverse effects at maximum application rate of ca. 120 kg formulation/ha (equal to ca. 8.1 kg a.s./ha)
Test substance	: Casoron G, batch no. not indicated	Acceptable	: yes

Study design

The purpose of the test was to determine the mortality rate and behavioural changes of the carabid beetle *Poecilus cupreus* exposed to Casoron G for 14 d. The following study set up was applied:

origin of species	: commercial supplier
age organisms at start of test	: 1 – 6 six weeks post-hatch
light intensity; photoperiod	: 16 h light/8 h dark; 500 – 1500 lux
temperature	: 20 ± 2°C
relative humidity	: ca. 85%
test container	: plastic vessel (18.3 x 13.6 x 6 cm) filled with 250 g quartz sand; in the transparent covers lid a hole was cut, covered by a coarse net (mesh size 2 mm)
test substrate	: quartz sand
water content	: ca. 70% of maximum water capacity
no. of larvae per treatment group	: 3 males and 3 females
number of replicates	: 5
number of animals per replicate	: 30
feeding	: 1 fly pupae/surviving animal immediately before application and every two to three days
application	: spraying was performed outdoors; the test substance was sprayed as a granulate on the quartz sand after 2 h, the test containers were placed in a ventilated cabinet; the control was sprayed with water (ca. 400 L/ha)
application rate	: 0 and ca. 120 kg formulation/ha, corresponding to 0 and ca. 8.1 kg a.s./ha

type of observations	: mortality and behavioural changes after 2, 4, and 6 h and 1, 2, 4, 7, 10, and 14 d
type of measurements	: humidity of substrate (by weighing the test containers)
toxic standard	: Afugan (pyrazophos) and E 605 forte (parathion)

Results

The toxic standards gave the expected responses. There were no mortality or other adverse effects in the control groups. Exposure to the test substance resulted in 3.3% mortality. Some beetles were staying in the sand. The mean mortality rates of the control group and treatment groups are given in Table B.9.5.1.1a. As the test was carried out as a limit test, the LR50 can only be given as > 120 kg formulation/ha.

Table B.9.5.1.1a Mortality of *Poecilus cupreus* after 14 d exposure to the formulation Casoron G

Group	Test substance (kg formulation/ha)	
	0	120
Percentage mortality	0	3.3

Acceptability of the test

The test is considered acceptable.

Conclusion

At an application rate of ca. 120 kg formulation/ha (equal to ca. 8.1 kg a.s./ha), the relevant trigger value of 30% for the endpoint mortality in first-tier laboratory testing with the carabid beetle *Poecilus cupreus* was not exceeded.

B.9.5.1.2 Laboratory test on effects of the formulation Casoron G on mortality and food uptake with the wolf spider *Pardosa* spp. (IIA 8.3.2/02)

Characteristics of the study

Reference	: Halsall (2000)	Species	: wolf spider (<i>Pardosa</i> spp.)
Year of execution	: 2000	Exposure duration	: 14 d
GLP statement	: yes	Mortality and food uptake	: no adverse effects at maximum application rate of ca. 120 kg formulation/ha (equal to ca. 8.1 kg a.s./ha)
Guideline	: Wehling and Heimbach (1984); IOBC (Heimbach et al., 2000); Barrett et al. (1994); EU Commission Directive 96/12/EC		
Test substance	: Casoron G; batch no. FLE99B15B; grey granules		
Content of a.s.	: 6.75% dichlobenil	Acceptable	: yes

Study design

The purpose of this 14-d laboratory test was to determine the possible effects of Casoron G, when incorporated into the test substrate, on survival and food uptake of adults of the wolf spider *Pardosa* spp.

The following study design was applied:

origin of species	:	collected in the field
age and weight of test organisms	:	adults; 10.2 – 19.0 mg (males) and 11.1 – 32.8 mg (females)
test environment	:	controlled environment room
photoperiod	:	16 h light/8 h dark; 800 – 1000 lux
temperature	:	18 – 20°C
relative humidity	:	54 – 87%
test cage	:	plastic pots (9 cm diameter x 5 cm depth) closed with ventilated lids; inside walls were treated with Fluon; 2 cm test substrate
test substrate	:	dry sand (ca. 150 g/test cage, washed and graded to be < 1 mm particles)
water content	:	ca. 70% of the water-holding capacity
treatment groups	:	control, test substance and toxic standard
no. of spiders per treatment group	:	30 (15 females and 15 males)
number of replicate cages	:	30
number of animals per cage	:	1
feeding during the test	:	5 live adult pea aphids of a uniform size/spider/feeding time (t = 2 h and 1, 2, 3, 7, 10, and 13 d after application)
application	:	granules were sprinkled onto the surface of the dry sand in the individual pots and then incorporated into the sand with a metal spatula
application rate	:	0.075 g formulation/test cage (ca. 120 kg formulation/ha, corresponding to 0 and ca. 8.1 kg a.s./ha)
type of observations	:	mortality and condition after 2 h and 1, 2, 3, 4, 7, 9, 11, and 14 d after application assessment of food consumption: 1, 2, 3, 7, 10, and 13 d after application
type of measurements	:	temperature, humidity, and light intensity
toxic standard	:	carbosulfan

Results

The toxic standard gave the expected response. Mortality was absent in the control group, while one spider died in the treatment group (3% mortality). With respect to food consumption, the mean number of aphids eaten per spider was significantly lower in the treatment group than in the control group at day 3 and 4 after application. However, no differences were present at the end of the test. The results of the test are given in Table B.9.5.1.2a.

Table B.9.5.1.2a Percentage mortality and food consumption rate of the wolf spider *Pardosa* spp. after 14 days of exposure to Casoron G

Group	Control	Test substance (ca. 8.1 kg a.s./ha)	Toxic standard (carbosulfan)
Percentage mortality after 14 d	0	3	100
Mean food consumption rate (no. of aphids/spider/feeding day) ²	2.1	1.8 ¹ (86)	1.3 (62)

1 Significantly different from the control group at day 3 and 4 after application

2 Percentage of control is given in parenthesis

Acceptability of the test

The spiders were not acclimatised in the test cages before application of the test substance in order to keep the sand in the test cages dry until application. Since the conditions at which the spiders were kept were similar to the test conditions, it is concluded that this has not influenced the outcome of the test. The test is considered acceptable.

Conclusion

At an application rate of ca. 120 kg formulation/ha (equal to ca. 8.1 kg a.s./ha), the relevant trigger value of 30% for the endpoints mortality and food consumption in first-tier laboratory testing with the wolf spider *Pardosa* spp. was not exceeded.

B.9.5.1.3 Laboratory test on effects of the formulation Casoron G on reproduction of the rove beetle *Aleochara bilineata* (IIA 8.3.2/04)

Characteristics of the study

Reference	: Moreth (1992)	Content of a.s.	: 6.75% dichlobenil
Year of execution	: 1991 – 1992	Species	: rove beetle (<i>Aleochara bilineata</i>)
GLP statement	: yes	Test duration	: 29 d exposure of adults to test substance followed by 6 – 8 weeks hatching of beetles
Guideline	: IOBC (Naton, 1989)	Reproduction	: 100% reduction at ca. 120 kg formulation/ha (equal to ca. 8.1 kg a.s./ha)
Test substance	: Casoron G; batch no. not indicated; granules	Acceptable	: yes

Study design

The purpose of the test was to determine the parasitisation success of the rove beetle *Aleochara bilineata* exposed to granules of Casoron G sprayed on a sand substrate. In a 10 – 12 weeks lasting test, the hatching rate of the F1 generation of the beetles and their parasitisation success towards the pupae of the onion fly *Delia antiqua* was investigated (larvae develop in the puparia of dipteran species). The following study design was further applied:

origin of species	:	laboratory culture
age and weight of test organisms	:	3 d old
host organism	:	onion fly <i>Delia antiqua</i> ; ca.500 puparia/test cage were buried in the sand of each replicate on day 8, 16 and 22
test environment	:	no forced ventilation
photoperiod	:	12 h light/12 h dark; subdued light
temperature	:	18°C
relative humidity	:	not indicated
test cage and content	:	Duran glass cylinders (14 cm diameter x 10 cm height) covered with a disc with a 5 cm diameter hole covered with a fine mesh; ca. 4 cm test substrate
test substrate	:	sand
water content	:	mixture of 300 mL air-dried and 30 mL water/cage
treatment groups	:	control and test substance
no. of animals per treatment group	:	60 (30 females and 30 males)
number of replicate cages	:	3
number of animals per cage	:	20 (10 females and 10 males)
feeding during the test	:	a few hours after application and 5 times/week thereafter with <i>Chironomus</i> larvae
application	:	sprayed on the surface of the sand; the beetles were buried in the sand just before application
application rate	:	0 and ca. 120 kg formulation/ha (corresponding to 0 and ca. 8.1 kg a.s./ha)
type of observations	:	number of emerged beetles
type of measurements	:	not indicated
toxic standard	:	no

Results

As no toxic standard was tested, the sensitivity of the test cannot be confirmed. Reproduction data for the control indicates that the test organisms were in good condition. The live and total parasitisation in the exposed group was 100% reduced compared with the control group. The results are given in Table B.9.5.1.3a.

Table B.9.5.1.3a Parasitisation success of the rove beetle *Aleochara bilineata* exposed to Casoron G

Group	Control	Test substance (ca. 8.1 kg a.s./ha)
Mean no. of emerged <i>A. bilineata</i> per replicate (F1 generation) (live parasitisation)	775	0
Total parasitisation per replicate	1030	0

Acceptability of the test

Granules were sprayed on the soil surface, while they should be incorporated into the soil. The exposed and control group each consisted of three replicates, while a minimum of four replicates is required by the IOBC guideline (2000). Little variation was observed between the replicates (coefficient of variation < 15%) and the treatment resulted in 100% inhibition of reproduction in all replicates. It is therefore unlikely

that an extra replicate or a different application method would have changed the outcome of the study. A toxic standard was not included in the test. However, as 100% effect was observed in the test substance group, evidence of the sensitivity of the test is not necessary. The test is considered acceptable.

Conclusion

At an application rate of ca. 120 kg formulation/ha (equal to ca. 8.1 kg a.s./ha), 100% reduction in parasitisation success of the rove beetle *Aleochara bilineata* was observed. Therefore, the relevant trigger value of 30% for the endpoint reproduction in first-tier laboratory testing was exceeded.

B.9.5.1.4 Extended laboratory test on effects of the formulation Casoron G on mortality and reproduction of the rove beetle *Aleochara bilineata* (IIA 8.3.2/05)

Characteristics of the study

Reference	: Taruza (2000)	Species	: rove beetle (<i>Aleochara bilineata</i>)
Year of execution	: 2000	Test duration	: 66 d; 28 d exposure of adults to test substance followed by 38 d hatching of beetles
GLP statement	: yes	Mortality	: 47 – 52% at ca. 120 kg formulation/ha (equal to ca. 8.1 kg a.s./ha)
Guideline	: IOBC (Naton, 1988) ; Barrett et al. (1994); EU Commission Directive 96/12/EC	Reproduction	: 55 - 88% reduction at ca. 120 kg formulation/ha (equal to ca. 8.1 kg a.s./ha)
Test substance	: Casoron G; lot no. FLE99B15B; grey granules		
Content of a.s.	: 6.75% dichlobenil	Acceptable	: yes

Study design

The purpose of this extended laboratory test was to determine survival and parasitisation success of the rove beetle *Aleochara bilineata* exposed to granules of Casoron G incorporated in a sandy soil and a loamy sand soil. Mortality of adults was determined during a 28-d exposure period. Thereafter, adults were removed, the soil was allowed to dry and the host fly pupae were sieved out. The hatching rate of the F1 generation of the beetles and their parasitisation success towards the pupae of the onion fly *Delia antiqua* was investigated during 38 d (larvae develop in the puparia of dipteran species). The following study design was further applied:

origin of species	: commercial supplier
age and weight of test organisms	: adult, 5 – 8 d (physiological age was 3 – 4 d since beetles were stored in a cold environment)
host organism	: onion fly <i>Delia antiqua</i> ; ca.500 puparia/test cage were buried in the sand of each replicate on day 7, 14 and 21
test environment	: controlled environment room
photoperiod	: 16 h light/8 h dark; 1020 lux (day 0 – 28), 1000 lux (day 28 – 66)
temperature	: 20 – 23°C (day 0 – 28), 21 – 25.5°C (day 28 – 66)
relative humidity	: 66 – 87% (day 0 – 28), 60 – 86% (day 28 – 66)

test cage	:	day 0 – 28: plastic flower-pots (23 cm diameter x 24 cm height) covered with ventilated lids; inside walls were treated with Fluon; 1500 g air-dried soil (ca. 5 cm depth) day 28 – 66: pupae were transferred to small plastic pots (9 cm diameter x 5 cm height) with ventilated lids
test substrate	:	LUFA 2.1 (sandy soil, ca. 0.5% organic matter) and LUFA 2.2 (loamy sand soil, ca. 2.2% organic matter)
water content	:	ca. 50% of the water-holding capacity
treatment groups	:	control, test substance, and toxic standard
no. of animals per treatment group	:	60 (30 females and 30 males)
number of replicate cages	:	3
number of animals per cage	:	20 (10 females and 10 males)
feeding during the test	:	immediately after application and every 2 – 3 d thereafter until day 28 with raw minced beef
application	:	sprayed on the soil surface, incorporated in the soil with a metal spatula after which the soil was wetted with tap water
application rate	:	0 and ca. 120 kg formulation/ha, corresponding to 0 and ca. 8.1 kg a.s./ha)
type of observations	:	mortality and condition of adult beetles at day 1, 3, 7, and 28; number of emerged beetles
type of measurements	:	soil water content (by weighing of test cages)
toxic standard	:	Posse G (10% w/w carbosulfan)

Results

The toxic standard gave the expected response. Reproduction data for the control indicates that the test organisms were in good condition. Therefore, the validity criteria of the test were fulfilled. In the exposed group, 47 and 52% mortality (corrected for control mortality) was observed for the sandy soil and the loamy sand soil, respectively. The parasitisation success was 12 and 45% of the control value, i.e. a reduction of 88 and 55% relative to the control. The results are summarised in Table B.9.5.1.4a.

Table B.9.5.1.4a Percentage mortality and parasitisation success of the rove beetle *Aleochara bilineata* after 28 d (adult mortality) and 66 d (parasitisation success) of exposure to Casoron G

	Substrate	Control	Test substance (ca. 8.1 kg a.s./ha)	Toxic standard (carbosulfan)
Percentage mortality after 28 d	LUFA 2.1 (sandy soil)	15	55 (47)	43 (33)
	LUFA 2.2 (loamy sand soil)	23	63 (52)	42 (25)
Mean no. of emerged <i>A. bilineata</i> per replicate (F1 generation) (live parasitisation) ²	LUFA 2.1 (sandy soil)	1604	187 (12)	12 (0.7)
	LUFA 2.2 (loamy sand soil)	2010	909 (45)	620 (31)

1 Mortality corrected for control mortality is given in parentheses.

2 The number expressed as percentage of the control is given in parentheses.

Acceptability of the test

The exposed and control group each consisted of three replicates, while a minimum of four replicates is required by the IOBC guideline (2000). However, the test was performed with two soils and each soil

showed the same response. As it is unlikely that an extra replicate would have changed the outcome of the study, the test is considered acceptable.

Conclusion

At an application rate of ca. 120 kg formulation/ha (equal to ca. 8.1 kg a.s./ha), 47 – 52% mortality and 55 – 88% reduction in parasitisation success was observed in an extended laboratory test with the rove beetle *Aleochara bilineata*. Therefore, the relevant trigger value of 50% for the endpoints mortality and reproduction in an extended laboratory test was exceeded.

B.9.5.1.5 Extended laboratory test on effects of the formulation Casoron G on mortality and reproduction of the rove beetle *Aleochara bilineata* (IIA 8.3.2/06)

Characteristics of the study

Reference	: Taruza (2001)	Species	: rove beetle (<i>Aleochara bilineata</i>)
Year of execution	: 2001	Test duration	: 71 d; 28 d exposure of adults to test substance followed by 43 d hatching of beetles
GLP statement	: yes	Mortality	: 56% at ca. 60 kg formulation/ha (equal to ca. 4.05 kg a.s./ha)
Guideline	: IOBC (Grimm et al. 2000) (Naton, 1988) ; Barrett et al. (1994); EU Commission Directive 96/12/EC	Reproduction	: 50% reduction at ca. 60 kg formulation/ha (equal to ca. 4.05 kg a.s./ha)
Test substance	: Casoron G; lot no. FLE99B15B; grey granules	Acceptable	: yes
Content of a.s.	: 6.75% dichlobenil (nominal); 6.92% (measured)		

The purpose of this extended laboratory test was to determine survival and parasitisation success of the rove beetle *Aleochara bilineata* exposed to granules of Casoron G sprinkled on the surface of a sandy loam soil. Mortality of adults was determined during a 28-d exposure period. Thereafter, adults were removed, the soil was allowed to dry and the host fly pupae were sieved out. The hatching rate of the F1 generation of the beetles and their parasitism success towards the pupae of the onion fly *Delia antiqua* was investigated during 43 d (larvae develop in the puparia of dipteran species). The following study design was applicable:

origin of species	: commercial supplier
age and weight of test organisms	: adult, 4 – 8 d (physiological age was 3 – 4 d since beetles were stored in a cold environment)
host organism	: onion fly <i>Delia antiqua</i> ; ca.500 puparia/test cage were buried in the sand of each replicate on day 7, 14 and 21
test environment	: controlled environment room
photoperiod	: 16 h light/8 h dark; 550 lux
temperature	: 18 – 25°C (day 0 – 28), 19 – 23°C (day 28 – 71)
relative humidity	: 33 – 91% (day 0 – 28), 62 – 91% (day 28 – 71)
test cage and content	: day 0 – 28: plastic flower-pots (25 cm diameter x 27 cm height) covered with ventilated lids; inside walls were treated

		with Fluon; 4000 g air-dried soil (ca. 10 cm depth)
		day 28 – 71: pupae were transferred to small plastic pots (9 cm diameter x 5 cm height) with ventilated lids that were fitted in deeper pots which served as collecting chambers; through a sieve in the base of the test cages, emerging beetles would fall into the collecting chambers
test substrate	:	LUFA 2.2 (sandy loam soil, ca. 2.3% organic matter)
water content	:	40% of the water-holding capacity
treatment groups	:	control, test substance, and toxic standard
no. of animals per treatment group	:	60 (30 females and 30 males)
number of replicate cages	:	3
number of animals per cage	:	20 (10 females and 10 males)
feeding during the test	:	immediately after application and every 2 – 3 d thereafter until day 28 with raw minced beef
application	:	sprinkled onto the soil surface whereafter the soil was wetted with tap water
application rate	:	0 and ca. 60 kg formulation/ha, corresponding to 0 and ca. 4.05 kg a.s./ha)
type of observations	:	mortality and condition of adult beetles at day 1, 7, and 28; number of emerged beetles every 2 – 4 d until day 71
toxic standard	:	Posse 10G (10% w/w carbosulfan)

Results

The toxic standard gave the expected response. Reproduction data for the control indicates that the test organisms were in good condition. Therefore, the validity criteria of the test were fulfilled. In the exposed group, 56% mortality (corrected for control mortality) was observed, which was significantly higher than in the control treatment. The parasitisation success was reduced by 50% relative to the control, but no significant difference was found. The results are given in Table B.9.5.1.5a.

Table B.9.5.1.5a Percentage mortality and parasitisation success of the rove beetle *Aleochara bilineata* after 28 d (adult mortality) and 71 d (parasitisation success) of exposure to Casoron G

	Control	Test substance (ca. 4.05 kg a.s./ha)	Toxic standard (carbosulfan)
Percentage mortality after 28 d ¹	25	67 (56) ³	100 (100)
Mean no. of emerged <i>A. bilineata</i> per replicate (F1 generation) (live parasitisation) ²	2095	1046 (50)	0 (0)

¹ Mortality corrected for control mortality is given in parentheses.

² The number expressed as percentage of the control is given in parentheses.

³ Statistically different from the control treatment ($P < 0.05$).

Acceptability of the test

Granules were sprayed on the soil surface, while they should be incorporated into the soil. The exposed and control group each consisted of three replicates, while a minimum of four replicates is required by the IOBC guideline (2002). According to this guideline, a sandy soil containing less than 1% organic carbon should be used as well. The carbon content of the sandy loam soil used in this test (LUFA 2.2) is too high

(2.2%) and therefore, the test conditions do not represent a worst-case scenario. However, as the reduction in survival and reproduction exceeds the relevant trigger value of 30% for first tier laboratory testing, the results are still of use for the risk assessment. Therefore, the test is considered acceptable.

Conclusion

At an application rate of ca. 60 kg formulation/ha (equal to ca. 4.05 kg a.s./ha), 56% mortality and 50% reduction in parasitisation success was observed in an extended laboratory test with the rove beetle *Aleochara bilineata*. Therefore, the relevant trigger value of 50% for the endpoints mortality and reproduction in an extended laboratory test was exceeded.

B.9.5.1.6 Semi-field test on effects of the formulation Casoron G on reproduction of the rove beetle *Aleochara bilineata* (IIA 8.3.2/07)

Characteristics of the study

Reference	: Vinall (2002)	Species	: rove beetle (<i>Aleochara bilineata</i>)
Year of execution	: 2002	Test duration	: 88 – 95 d; 28 d exposure of adults to test substance followed by 67, 62, and 60 d hatching of beetles in bioassay 1, 2, and 3, respectively
GLP statement	: yes	Reproduction	: 65% reduction at ca. 60 kg formulation/ha (equal to ca. 4.05 kg a.s./ha) up to 28 d post-application
Guideline	: adaptation of IOBC (Grimm et al. (2000)); based on Candolfi et al. (2000)		
Test substance	: Casoron G; lot no. FLE99B15B; grey granules		
Content of a.s.	: 6.75% dichlobenil (nominal); 6.92% (measured)	Acceptable	: yes

The purpose of this semi-field test was to determine the parasitisation success of the rove beetle *Aleochara bilineata* exposed to fresh and field-aged residues (granules) of Casoron G sprinkled on the surface of a sandy loam soil. Half the surface area within the test cages was sprayed, providing the beetles with a refuge. The test was divided in three bioassays, which successively started on day 0, 28, and 96 after application of the test substance by the introduction of adult beetles.

Pupae of the onion fly *Delia antiqua* were buried in the soil (larvae develop in the puparia of dipteran species). After 28 d, the pupae were transferred to the laboratory. The hatching rate of the F1 generation of the beetles and their parasitism success towards the pupae was investigated during 67, 62, and 60 d for the first, second, and third bioassay, respectively. The following study design was applicable:

origin of species	:	commercial supplier
age and weight of test organisms	:	adult, 3 – 12 d (physiological age was < 7 d since beetles were stored in a cold environment)
host organism	:	onion fly <i>Della antiqua</i> ; 800 pupae/test cage (400 in each treated and untreated half of the test cage) were buried in the sand of each replicate on 7, 14 and 21 d after introduction of the beetles; the pupae were contained in bags
test environment	:	day 0 – 28: outside exposed to the elements, except that the beetles were protected from heavy and persistent rain day 28 – test termination: controlled environment room
photoperiod	:	day 28 – test termination: 16 h light/8 h dark; 75 – 720 lux
temperature	:	day 0 – 28: 3 – 30 °C day 28 – test termination: 19 – 22°C
relative humidity	:	day 28 – test termination: 62 – 94%
test cage and content	:	day 0 – 28 after introduction of the beetles: square galvanised-steel barriers (98 cm x 98 cm x 40 cm deep) partly covered with nylon netting; cages were dug into the ground to a depth of ca. 20 cm; 20 cm layer of soil day 28 – test termination: pupae were transferred the laboratory and placed in small plastic pots (9 cm diameter x 5 cm height) with ventilated lids that were fitted in deeper pots which served as collecting chambers; through a sieve in the base of the test cages, emerging beetles would fall into the collecting chambers
test substrate	:	sieved (4 mm) natural sandy loam soil (ca. 3.2% organic matter)
water content	:	40% of the water-holding capacity
treatment groups	:	control, test substance, and toxic standard
no. of animals per treatment group	:	320 (160 females and 160 males) for each bioassay
number of replicate cages	:	4
number of animals per cage	:	80 (40 females and 40 males) for each bioassay
feeding during the test	:	immediately after introduction of the beetles and every 2 – 3 d thereafter with 12 g of raw minced beef pellets
application	:	sprinkled over half of the soil surface area of each treatment test cage; the day before application the soil was moistened with tap water (10L/test cage)
application rate	:	0 and ca. 60 kg formulation/ha, corresponding to 0 and ca. 4.05 kg a.s./ha)
type of observations	:	number of emerged beetles every 2 – 4 d from day 28 until day 67 (bioassay 1), day 62 (bioassay 2), and day 60 (bioassay 3)
type of measurements	:	day 0 – 28 after introduction of the beetles: ambient weather conditions (temperature and rainfall) day 28 – test termination: temperature, relative humidity, light intensity
toxic standard	:	Posse 10G (10% w/w carbosulfan) (reapplied at start of each bioassay)

Results

The toxic standard gave the expected response. Reproduction data for the control indicates that the test organisms were in good condition. Therefore, the validity criteria of the test were fulfilled. The parasitisation success in the untreated part of the cages were not significantly different from that in the

control treatment, independent of the time after application. A significant reduction relative to the control (65%) was seen in the treated part of the test cage with the fresh residues. The parasitisation success in the treated part of the cages with 28d-old residues was reduced by 25%, but differences were not significant. The results are given in Table B.9.5.1.6a.

Table B.9.5.1.6a Percentage parasitisation success of the rove beetle *Aleochara bilineata* after exposure to Casoron G

	Mean no. of emerged <i>A. bilineata</i> per replicate (F1 generation) (live parasitisation) ¹		
	Control	Test substance (4.05 kg a.s./ha)	Toxic standard (carbosulfan)
Bioassay 1 (start 0 DAT)			
Untreated half of test cage	525	474 (10)	503 (4)
Treated half of test cage	434	1532 (65) ²	182 ((96)
Whole test cage	959	6262 (35)	5202 (46)
Bioassay 2 (start 28 DAT)			
Untreated half of test cage	564	624 (-11)	4362 (23)
Treated half of test cage	568	425 (25)	212 (96)
Whole test cage	1132	1049 (7)	4562 (60)
Bioassay 3 (start 96 DAT)			
Untreated half of test cage	431	471 (-9)	396 (8)
Treated half of test cage	464	450 (3)	1732 (63)
Whole test cage	895	921 (-3)	5692 (36)

¹ Percentage reduction relative to the control is given in parentheses.

² Significantly different from the control treatment ($P < 0.05$).

Acceptability of the test

The test is considered acceptable.

Conclusion

At an application rate of ca. 60 kg formulation/ha (equal to ca. 4.05 kg a.s./ha), fresh residues significantly reduced the number of emerged beetles in treated areas (65% reduction). No significant effects on the reproductive potential were found for the 28d- and 96d-old residues.

B.9.5.2 Summary

The summarised results of the tests with non-target arthropods are given in Table B.9.5.2a.

Table B.9.5.2a. Summary of effects of the plant protection product Casoron G on non-target arthropod species during laboratory testing

Species	Stage	Test Substance ¹	Dose [kg a.s./ha]	Endpoint	Adverse effect ²
Laboratory tests					
<i>Poecilus cupreus</i>	adult	Casoron G	8.1	mortality	3.3%
<i>Pardosa</i> spp.	adult	Casoron G	8.1	mortality	3%
<i>Aleochara bilineata</i>	adult	Casoron G	8.1	consumption parasitism	14% 100%
Extended lab tests					
<i>Aleochara bilineata</i>	adult	Casoron G	8.1	sandy soil mortality parasitism	47% 88%
<i>Aleochara bilineata</i>	adult	Casoron G	4.05	loamy sand soil mortality parasitism	52% 55%
<i>Aleochara bilineata</i>	adult	Casoron G	4.05	mortality parasitism	56% 50%
Semi-field tests					
<i>Aleochara bilineata</i>	adult	Casoron G	4.05	parasitism fresh residu 28-d old residu 96-d old residu	65% 25% 3%
¹ Test substance Casoron G = 67.5 g dichlobenil/kg ² Adverse effect means: x % effect on mortality = x % increase of mortality compared to control y % effect on a sublethal parameter = y % decrease of sublethal parameter compared to control (sublethal parameters are e.g. reproduction, parasitism, food consumption)					

B.9.5.3 Risk Assessment

For the proposed broadcast spreading of granules of dichlobenil (maximum application rate 5.4 kg a.s./ha), it is assumed that there will be no interception by the crops and no drift. As the proposed use involves a single seasonal treatment, the maximum recommended dose for glass or sand substrate during laboratory tests will be 100% of the field dose for both foliage and soil dwelling insects when applied in the field (SETAC Guidance Document on Regulatory Testing Procedures for Pesticides with Non-target Arthropods, 1994).

Data have been submitted on three soil dwelling species: carabid beetle (*Poecilus cupreus*), wolf spider (*Pardosa* sp.) and rove beetle (*Aleochara bilineata*). Considering the proposed use, these are considered to be sufficient and acceptable test species. It should be noted that the test dose of 8100 g a.s./ha

exceeded the maximum dose of 5400 g a.s./ha. However, effects from the tests can not be corrected to a dose of 5400 g a.s./ha.

Effects on *P. cupreus* and *Pardosa* sp. were below the trigger value of 30%.

Effects on reproduction of *A. bilineata* as a result of the active substance dichlobenil, applied at doses of 4050 and 8100 g a.s./ha, did exceed the relevant trigger value of 30% for laboratory studies with inert substrate or the relevant trigger value of 50% for extended laboratory studies. In a semi-field test with *A. bilineata* with fresh and aged residues, no adverse effects on reproduction were found as a result of exposure to 96d-old residues. 28d-old residues showed 25% adverse effect on reproduction but this result was not significant. The results from the semi-field study show the potential for recovery in-field within a year and off-field within an ecologically relevant period, as recommended in ESCORTII.

Based on the above, the risk for non-target arthropods is considered to be acceptable.

B.9.6 EFFECTS ON EARTHWORMS

B.9.6.1 Acute Toxicity

B.9.6.1.1 Acute toxicity of the active substance dichlobenil to terrestrial organisms (IIA 8.4.1/01)

Characteristics of the study

Reference	: Sewell (2005)	Purity of a.s.	: 98.6%
Year of execution	: 2004	Species	: earthworm (<i>Eisenia foetida</i>)
GLP	: yes	Exposure duration and regime	: 14 d
Guideline	: OECD 207	14d-LC ₅₀	: 270 mg a.s./kg soil (dw)
		14d-NOEC	: 180 mg a.s./kg soil (dw)
Test substance	: dichlobenil	Acceptable	: yes

Study design

The acute toxicity of dichlobenil (batch no. MI-0512, purity 98.6% w/w) was tested on the earthworm *Eisenia fetida* according to guideline OECD No. 207. The test medium (artificial soil) consisted of quartz sand (70%), kaolinite clay (20%) and sphagnum peat (10%). Calcium carbonate was added to adjust the pH to 6.0 ± 0.5 . The nominal moisture content of the soil was 30%.

Groups of 40 worms (four replicates of 10 worms per concentration, mean body weight of worms 0.40 - 0.46 g) were exposed to nominal concentrations of 100, 180, 320, 560 and 1000 mg a.s./kg of soil for a period of 14 days. Appropriate amounts of test compound were each separately mixed with dry soil and deionised reverse osmosis water. An untreated control was included in the test. A separate study with chloroacetamide was used as a positive control.

The worms were acclimated to the artificial soil approximately 24 hours prior to the start of the test. They received no food during exposure. The test was performed at 20.0 - 21.1 °C under continuous light (681-702 lx).

Mortality and sublethal effects on weight and behaviour were assessed after 7 and 14 days. Body weight of each worm was determined on day 0, of survivors also on day 14. LC₅₀ values were determined using the trimmed Spearman-Kärber method. Statistical analysis of body weight data with the tests of Bartlett and Dunnett, respectively was performed only for worms at the 0, 100 and 180 mg/kg level (because of significant mortalities at higher levels).

Results

The actual moisture content of the soil was 28-29% on day 0 and 23-26% on day 14. The pH on both day 0 and day 14 ranged from 6.0-6.4.

No worms died in the control and no mortalities occurred at concentrations of 100 and 180 mg/kg. After 14 days body weights of worms in the 100 and 180 mg/kg test groups were not significantly different from the control.

Worms at concentrations of 320, 560 and 1000 mg/kg were on the surface of the soil up to day 5 of the test. At the 320 mg/kg test level 26 and 32 worms, respectively were dead after 7 and 14 days (cumulative mortality after 7 and 14 days 65% and 80%). In the 560 and 1000 mg/kg groups cumulative mortality was 100% after 7 days.

The 7-days LC_{50} was reported to be 290 mg/kg (95% C.I. 270-320 mg/kg); the 14-days LC_{50} was 270 mg/kg (95% C.I. 250-290 mg/kg). The NOEC, based on the absence of mortalities and sub-lethal effects, was reported to be 180 mg/kg.

The study with toxic reference chloroacetamide showed an LC_{50} of 28 mg/kg, which is in line with the expected result for this toxic reference.

Acceptability of the test

The study is considered acceptable.

Conclusion

The 14d- LC_{50} and NOEC of dichlobenil to the earthworm *Eisenia fetida* are 270 and 180 mg dichlobenil/kg soil (dw) respectively.

B.9.6.1.2 Acute toxicity of the formulation Casoron G to terrestrial organisms (IIA 8.4.1/02 and IIIA 10.6.1.1/01)

Characteristics of the study

Reference	: Kühner (1991)	Content of a.s.	: 6.75% dichlobenil
Year of execution	: 1990	Species	: earthworm (<i>Eisenia foetida</i>)
GLP	: yes	Exposure duration and regime	: 14 d, static
Guideline	: OECD 207; Verfahrensvorschlag der Adhoc Arbeitsgruppe der Biologischen Bundesanstalt zur Entwicklung Ökotoxikologischer Testverfahren im terrestrischen Bereich	14d- LC_{50}	: > 1000 mg test substance/kg soil (dw) (equal to > 67.5 mg a.s./kg soil (dw)) ≥
Test substance	: Casoron G; identification code SCH 43340 H	14d-NOEC	: ≥ 1000 mg test substance/kg soil (dw) (equal to ≥ 67.5 mg a.s./kg soil (dw))
		Acceptable	: yes

Study design

The following study set up was applied:

age and weight	: adult (ca. 2 months old with clitellum, 250 – 600 mg)
exposure temperature	: 20 ± 2°C
light rhythm	: 24 h light/d (300 – 400 lux)
type of soil	: artificial soil according to OECD 207
soil water content	: 34.2 – 37.0%

type of test vessel and content	: 1 L glass vessels with lids, 500 g soil (dw)
number of replicate test vessels	: 4
number of animals per test vessel	: 10
dosing	: mixed with quartz sand, then mixed with artificial soil and deionised water
treatment groups	: 0 and 1000 mg test substance/kg soil (dw) ¹
type of observations	: survival at day 7 and 14
type of measurements	: individual body weight at the start and end of the study (day 0 and 14, respectively), water content and pH on day 0 and 14
chemical analysis	: no
toxic standard	: no

¹ In the range-finding study, no mortality was observed at concentrations ranging from 0.1 to 1000 mg test substance/kg soil. Therefore, only the highest concentration of 1000 mg test substance/kg soil was tested in the final study.

Results

The pH varied between 5.8 and 6.6. There was no mortality observed in the control or at 1000 mg test substance/kg soil. After 14 d exposure, mean body weight was decreased in the control treatment (7.8 mg/worm) and increased at 1000 mg test substance/kg soil (12.5 mg/worm).

The 14d-LC₅₀ and NOEC for body weight are therefore > 1000 and ≥ 1000 mg test substance/kg soil (dw), which is equal to > 67.5 and ≥ 67.5 mg a.s./kg soil (dw), respectively.

Acceptability of the test

No reference substance was tested and therefore, the sensitivity of the test cannot be confirmed; however, this was no part of the OECD guideline 207. The study is considered acceptable.

Conclusion

The 14d-LC₅₀ and NOEC of Casoron G to the earthworm *Eisenia fetida* are > 1000 and ≥ 1000 mg test substance/kg soil (dw) (equal to > 67.5 and ≥ 67.5 mg a.s./kg soil (dw)), respectively.

B.9.6.1.3 Acute toxicity of the metabolite 2,6-dichlorobenzamide (BAM) to terrestrial organisms (IIA 8.4.1/03)

Characteristics of the study

Reference	: Shacklady (2001)	Species	: earthworm (<i>Eisenia foetida</i>)
Year of execution	: 2001	Exposure duration and regime	: 14 d, static
GLP	: yes	14d-LC ₅₀	: 750 mg metabolite/kg soil (dw)
Guideline	: OECD 207; EEC Commission Directive 87/302/EEC	14d-NOEC	: 320 mg metabolite/kg soil (dw)
Test substance	: 2,6-dichlorobenzamide (BAM); batch no. FUX001000/FUN81G02C; off-white powder		
Purity	: 99.5%	Acceptable	: yes

Study design

The following study set up was applied:

age and weight	: adult (0.40 – 0.42 g)
exposure temperature	: 21°C
light rhythm	: 24 h light/d (694 – 758 lux)
type of soil	: artificial soil according to OECD 207, with a slight modification
soil water content	: 30%
type of test vessel and content	: 1-L glass vessels covered with plastic film, ca. 600 g soil (dw)
number of replicate test vessels	: 4
number of animals per test vessel	: 10
dosing	: mixed with artificial soil and deionised water
treatment groups	: 0, 100, 180, 320, 560, and 1000 mg metabolite/kg soil (dw)
type of observations	: survival and other adverse effects at day 7 and 14
type of measurements	: individual body weight at the start and end of the study (day 0 and 14, respectively), water content and pH on day 0 and 14, temperature and light intensity daily
chemical analysis	: no
toxic standard	: chloroacetamide

Results

The soil water content ranged between 27 and 30%. The pH varied between 5.6 and 6.4. The toxic standard gave the expected response. There was no mortality observed in the control or at concentrations up to and including 560 mg metabolite/kg soil (dw). However, 100% mortality was observed at the highest test concentration of 1000 mg a.s./kg soil (dw). After 14 d, body weights were lower at 560 mg metabolite/kg soil (dw) compared with the control.

The 14d-LC₅₀ and lowest NOEC (for body weight gain) are therefore 750 (560 – 1000) and 320 mg metabolite/kg soil (dw). RMS found a similar results.

Acceptability of the test

The test is considered acceptable.

Conclusion

The 14d-LC₅₀ and overall NOEC are 750 and 320 mg metabolite/kg soil (dw), respectively.

B 9.6.2 Chronic toxicity

B.9.6.2.1 Chronic toxicity of the metabolite 2,6-dichlorobenzamide (BAM) to terrestrial organisms (IIA 8.4.2/01)

Characteristics of the study:

Reference	: Lührs (2003)	Species	: earthworm (<i>Eisenia fetida</i>)
Year of execution	: 2003	Exposure duration	: 56 d (28 d for adults) and another 28 d for juveniles), static
GLP	: yes	28d-NOEC	: ≥ 500 mg metabolite/kg soil (dw)
Guideline	: BBA Part VI 2-2; ISO 11268-2	mortality, body weight gain	
Test substance	: 2,6-dichlorobenzamide (BAM); batch no. 8808018; beige solid	56d-NOEC	: 250 mg metabolite/kg soil (dw)
Purity	: 97.0%	reproduction	
		Acceptable	: yes

Study design

The purpose of the study was to determine the possible effects of the metabolite 2,6-dichlorobenzamide (BAM) on the reproduction of earthworms. Adult animals were exposed for 28 days; thereafter the surviving animals were counted, weighed, and removed. After another 28 days the number of offspring was determined. The following study set up was applied:

age and weight	: adult (ca. 10 months old with clitellum, 350 – 550 mg)
exposure temperature	: 18 – 21°C
photoperiod	: 16 h light/8 h dark (422 – 572 lux)
type of soil	: artificial soil according to OECD 207
soil water content	: 26.2 – 29.7%
type of test vessel and content	: plastic boxes with perforated transparent lids; ca. 500 g soil (dw)
number of replicate test vessels	: 4
number of animals per test vessel	: 10
feeding	: ca. 5 g cattle manure/test vessel/week
dosing	: mixed with quartz sand, then mixed with artificial soil and deionised water
exposure concentrations	: 0, 31, 63, 125, 250, and 500 mg metabolite/kg soil (dw)
type of observations	: mortality and other adverse effects on adults on day 28, number of juveniles on day 56
type of measurements	: individual body weight at test initiation (day 0), mean body weight per test vessel on day 28
	: water content weekly, pH at start (day 0) and end of the test (day 56)
chemical analysis	: no
toxic standard	: Derosal SC 360 (carbendazim)

Results

The toxic standard gave the expected response. In the test with the metabolite, the pH varied between 6.0 and 6.5. At 31 and 250 mg metabolite/kg soil (dw), 2.5 and 5% mortality was observed, but this was not significantly different compared to the control. There were no effects on body weight increase as well. Behavioural abnormalities were not observed, although a slight decrease in feeding activity (not significant) was observed at 500 mg metabolite/kg soil dw. However, reproduction was significantly reduced at 500 mg metabolite/kg soil (dw). RMS reanalysed the reproduction data and found a similar result. The test results are summarised in Table B.9.6.2.1a. Based on the effects on reproduction at the

highest test concentration, the authors determined a 56d-NOEC of 250 mg metabolite/kg soil (dw). The 28d-NOEC for adult mortality is ≥ 500 mg metabolite/kg soil (dw).

Table B.9.6.2.1a. Results of the earthworm reproduction test with 2,6-dichlorobenzamide in a 56-d test

Endpoint	Concentration 2,6-dichlorobenzamide (BAM) in mg metabolite/kg soil (dw)					
	control	31	63	125	250	500
% mortality of adults after 28 days	0	2.5	0	0	5	0
% weight increase of adults after 28 days	37.4	50.8	44.8	44.9	44.9	38.9
mean number of offspring per test vessel ²	281 (23)	259 (25)	239 (21)	274 (37)	257 (51.4)	205 ¹ (72.9)

¹ significantly different from control ($P < 0.05$) using t-test

² standard deviations between brackets

Acceptability of the test

The test is considered acceptable.

Conclusion

The 28d-NOEC for adult mortality and the 56d-NOEC of the metabolite 2,6-dichlorobenzamide for the reproduction of the earthworm *Eisenia fetida* are ≥ 500 and 250 mg metabolite/kg soil (dw), respectively.

B.9.6.3 Risk assessment

For the proposed broadcast spreading of granules of dichlobenil, the initial actual PEC soil has been determined according to the criteria detailed in Section B.8.3 of the Environmental Fate and Behaviour Section. An acute toxicity study has been supplied with the active substance dichlobenil (14d-LC₅₀ 270 mg a.s./kg soil (dw)) and with the formulation Casoron G (14d-LC₅₀ of > 67.5 mg a.s./kg soil (dw)). As the log P_{OW} of dichlobenil is > 2 (2.7), the LC₅₀ was divided by 2 to correct for the difference in organic matter content of the test substrate (10%) and natural soil (5%). The LC₅₀ from the study with the a.s. was used for the risk assessment, since in the study with the formulation no effects were found at the highest test concentration. This LC₅₀ is 135 mg dichlobenil/kg soil (dw) after correction to 5% OM.

B.9.6.3.1 Acute risk

The acute TER is presented in Table B.9.6.3.1a.

Table B.9.6.3.1a Acute risk of dichlobenil to earthworms

Scenario	LC ₅₀ (mg a.s./kg)	PEC _s (mg a.s./kg)	Acute TER	Annex VI trigger 91/414 EEC
Vines and non-crops	135	7.2	19	10

From Table B.9.6.3.1a it can be concluded that the acute risk to earthworms is acceptable, since the TER is above 10.

B.9.6.3.2 Long-term risk

Chronic toxicity data with earthworms are not required for dichlobenil, since the acute trigger value was met and both the (field) DT90 is less than 100 days and the number of applications is less than 3.

B.9.6.3.3 Risk assessment for metabolites

An amount of > 10% the metabolite BAM in soil was detected and therefore the risk of the metabolite to earthworms should be assessed. Toxicity tests for earthworms were performed with this metabolite. The acute 14d-LC₅₀ mortality was 750 mg metabolite/kg soil dw and the chronic NOEC for reproduction was 250 mg metabolite/kg. The initial actual PEC soil is taken from section B.8.3. The results are given in Tables B.9.6.3.3a and b.

Table B.9.6.3.3a. Acute risk of the metabolite 2,6-dichlorobenzamide to earthworms

Scenario	LC ₅₀ (mg metabolite/kg)	PEC _s (mg metabolite/kg)	Acute TER	Annex VI trigger 91/414 EEC
Vines and non-crops	750	5.52	136	10

The acute risk of the metabolite 2,6-dichlorobenzamide to earthworms is acceptable (TER is above 10).

Table B.9.6.3.3b. Long-term risk of the metabolite 2,6-dichlorobenzamide to earthworms

Scenario	NOEC (mg metabolite/kg)	PEC _s (mg metabolite/kg)	Chronic TER	Annex VI trigger 91/414 EEC
Vines and non-crops	250	5.52 (PIEC) 6.88 (PECplateau)	45 36	5

The long-term risk of the metabolite 2,6-dichlorobenzamide to earthworms is acceptable (TER is above 5).

B.9.7 EFFECTS ON SOIL NON-TARGET MACRO-ORGANISMS**B.9.7.1 Acute toxicity**

There are no data available.

B.9.7.2 Chronic toxicity**B.9.7.2.1 Chronic toxicity of the formulation Casoron G to terrestrial organisms (IIA 8.3.2/03)****Characteristics of the study:**

Reference	: Vinall (2003)	Species	: springtail (<i>Folsomia candida</i>)
Year of execution	: 2003	Test duration and regime	: 28 d; static
GLP	: yes	28d-LC ₅₀	: > 1000 mg formulation/kg dw artificial soil (> 67.5 mg a.s./kg dw artificial soil)
Guideline	: ISO 11267	28d-NOEC mortality and reproduction	: ≥ 1000 mg formulation/kg dw artificial soil (≥ 67.5 mg a.s./kg dw artificial soil)
Test substance	: Casoron G; lot no. AM3D23K046; grey granules		
Content of a.s.	: 6.75% 2,6-dichlorobenzonitrile	Acceptable	: yes

Study design

The purpose of the test was to determine the possible effects of the formulation Casoron G on the reproduction of the springtail. The following study set up was applied:

age of test organisms	: juveniles (10 – 12 d old)
test environment	: controlled environment room
photoperiod	: 16 h light/8 h dark; 420 – 720 lux
temperature	: 17 – 23°C
relative humidity	: 57 – 96%
type of soil	: artificial soil (10% sphagnum peat, 20% kaolinite clay, 70% industrial quartz sand), and sufficient CaCO ₃ to bring pH at 6 ± 0.5
soil water content	: 50% of water holding capacity
type of test vessel and content	: lidless clear polystyrene boxes (27 cm x 15 cm x 10 cm deep); inside walls were treated with Fluon; 500 g test substrate to prevent the springtails to climb out
number of replicate test vessels	: 5
number of animals per test vessel	: 10
feeding	: ca. 4 mg of granulated dry yeast/test vessel/week
dosing	: thoroughly mixed with 2400 g dry artificial soil, after which deionised water was added
exposure concentrations	: 0 (control), 62.5, 125, 250, 500, and 1000 mg formulation/kg dw artificial soil
type of observations	: mortality, condition, and number of offspring produced
type of measurements	: pH, soil water content (as weight of test vessel)
chemical analysis	: no
toxic reference	: Betosip 14 (114 g/L phenmediphan)

Results

The toxic standard gave the expected response. The pH varied between 6.73 and 7.44. The variation in the water content of the soil was not indicated. In the control treatment, mortality was 16% and 197 juveniles were produced, which satisfied the validity criteria of the guideline (20% and 100, respectively). The coefficient of variation for reproduction in the control treatment was 8.5% and did not exceed the 30% as required by the guideline. An increased mortality and a reduction in the number of offspring were found in the exposed groups. However, there was no dose-response relationship and statistical analysis did not reveal significant differences with the control group. The results expressed in LC/EC₅₀ and NOEC values are summarised in Table B.9.7.2.1a.

Table B.9.7.2.1a. Results of the springtail reproduction test with Casoron G

	Concentration in mg formulation/kg dw artificial soil		Concentration in mg a.s. /kg dw artificial soil	
	mortality	reproduction	mortality	reproduction
28d-LC/EC ₅₀	> 1000	> 1000	> 67.5	> 67.5
28d-NOEC	≥ 1000	≥ 1000	≥ 67.5	≥ 67.5
Overall 28d-NOEC	≥ 1000		≥ 67.5	

Acceptability of the test

Instead of standard closed jar arenas as described in the guideline, lidless boxes were used to take account of the volatile nature of the test substance. It can not be excluded that this has influenced the results of the test, however it reflects the actual situation in the field. Therefore, the test is considered acceptable.

Conclusions

The overall NOEC is ≥ 1000 mg formulation/kg dw artificial soil; this corresponds with ≥ 67.5 mg a.s./kg dw artificial soil.

B.9.7.3. Summary

A summary of the chronic toxicity of the formulation Casoron G to the springtail is given in Table B.9.7.3a

Table B.9.7.3a Summary of the chronic toxicity of the plant protection product Casoron G to the springtail *Folsomia candida*

% a.s.	Endpoint	Toxicity value (in mg a.s./kg soil dw)	Test Guideline	Reference no.
6.75%	28d-LC ₅₀ 28d-NOEC	> 67.5 ≥ 67.5	ISO 11267	IIA 8.3.2/03

B.9.7.4. Risk assessment on soil non-target macro-organisms**Collembola reproduction**

A chronic toxicity study with a 6.75% formulation is conducted on the springtail *Folsomia candida*, resulting in a 28-d LC₅₀ of > 67.5 mg a.s./kg soil dw and a 28d-NOEC reproduction value of ≥ 67.5 mg a.s./kg soil dw.

The 28d-NOEC value for reproduction was taken as the relevant value for the risk assessment. As the log P_{OW} of dichlobenil is > 2 (2.7), the NOEC was divided by 2 to correct for the difference in organic matter content of the test substrate (10%) and natural soil (5%). The NOEC used for the risk assessment is therefore ≥ 33.8 mg a.s./kg soil (dw).

The PEC soil is taken from section B.8.3. The long-term TER is presented in Table B.9.7.4a.

Table B.9.7.4a Long-term risk of dichlobenil to springtails

Scenario	NOEC (mg a.s./kg)	PEC _s (mg a.s./kg)	Chronic TER	Annex VI trigger 91/414 EEC
Vines and non-crops	≥ 33.8	7.2	≥ 4.7	5

The long-term risk TER dichlobenil to Collembola is ≥ 4.7, which is below the Annex VI trigger value of 5. However, taking into account that no effects were found in the test and the fact that the TER is only slightly below the trigger value, the risk is considered to be acceptable.

Decomposition of organic matter

According to Annex III from Directive 91/414, effects on decomposition of organic matter should be investigated when the DT₉₀field is larger than 1 year. The maximum field DT₅₀ of BAM is estimated to be 315.2 days, the geometric mean of the normalised field DT₅₀ values is 125 days (see section B.8.3).

Based on these data, it can be concluded that the field DT₉₀ is larger than one year. Therefore, the notifier is requested to submit data on the effects of soil metabolite BAM on the decomposition of organic matter in soil (e.g. a litterbag study).

B.9.8 EFFECTS ON SOIL NON-TARGET MICRO-ORGANISMS**B.9.8.1 Effects on carbon mineralisation and on nitrification****B.9.8.1.1 Effects of the formulation Casoron G on microbial mineralisation in soils (IIA 8.5 and IIIA 10.7.1)****Characteristics of the study:**

Reference	: Dijkstra (1989)	Content of a.s.	: 6.75% dichlobenil
Year of execution	: 1988 – 1989	Species	: micro-organisms in two soils
GLP	: no	Exposure duration	: 90 d (soil 1) and 28 d (soil 2)
		carbon mineralisation (dehydrogenase activity)	: 28 days
Guideline	: BBA guideline Part VI (1987)	nitrogen conversion	: not applicable
Test substance	: Casoron G	Final result	: not applicable
		Acceptable	: no

Study design

The purpose of the study was to determine the influence Casoron G on microbial mineralisation in two soil types. The applied amounts are equivalent to 1.5x and 7.5x maximum field rate, corresponding with ca. 120 and 600 kg formulation/ha. One soil (soil 1) has a low organic matter content, the other one (soil 2) is a soil with an average organic matter content and higher microbial activity compared with soil 1. The study covered two aspects, i.e. the influence of Casoron G on carbon mineralisation (dehydrogenase activity) and on nitrogen conversion. For the carbon mineralisation test, samples of incubated soil were taken at several sampling times (see below) and subsequently mixed with glucose. The method for the determination of dehydrogenase activity is not described. For the determination of nitrification, samples of incubated soil were taken at several sampling times (see below) to determine ammonium-N, nitrite-N and nitrate-N. The following study design was applied:

incubation temperature	: 20°C
type of soil	: see Table B.9.8.1.1a.
history of soil	: since 1986 covered with plants, without dunging or application of plant protection products;
soil water content	: 60% of maximum water holding capacity
soil nitrogen content	: 0.090% (soil 1) and 0.240% (soil 2)
soil cation exchange capacity	: not indicated
number of replicate test vessels	: 3 (double determinations)
dosing	: 120 and 600 kg formulation/ha (equal to 8.1 and 40.5 kg a.s/ha)
type of measurements	: short-term on carbon mineralisation activity on day 14, 28, 60, and 90 (soil 1) and day 14 and 28 (soil 2); nitrification on day 7, 14, 21, and 28 (both soils)
chemical analysis of test substance in the soil	: no
toxic standard	: dinoseb acetate

Table B.9.8.1.1a. Soil characteristics

Soil parameter	Soil 1	Soil 2
organic carbon content (%)	0.95	2.42
microbial biomass (?) ¹	244	1011
content of N _{min} (mg/100g of soil)	0.505	2.588
total nitrogen (%)	0.090	0.240
clay	6.9	29.5
silt	39.0	35.9
sand	54.1	34.6
pH	6.8	7.1

¹ unit was not indicated

Results

The toxic standard did not give the expected result for N-mineralisation in soil 2; a difference with the control was not found. The results of the test are given in Table B.9.8.1.1b. After 28 days, less than 25% inhibition of dehydrogenase was observed in soil 2 at both test concentrations. However, more than 25% inhibition of dehydrogenase occurred in soil 1 at both test concentrations and therefore, the formulation is evaluated as having a long-term influence on carbon mineralisation in soils. Less than 25% inhibition of N-mineralisation was observed in both soils and at both test concentrations as well.

Table B.9.8.1.1b Results of the dehydrogenase and N-mineralisation test with the formulation Casoron G in two soils (% of control between brackets)

Group	Incubation time (d)	Control	Test substance (ca. 120 kg formulation/ha)	Test substance (ca. 600 kg formulation/ha)	Toxic standard (dinoseb acetate)	
					1.5x max. appl. rate	7.5x max. appl. rate
Carbon mineralisation (Dehydrogenase determination) (Triphenylformazan/100 g dw soil) ¹						
Soil 1	14	3.135	2.196 (70.05)	2.381 (75.94)	1.616 (51.56)	0.898 (28.64)
	28	2.976	2.085 (69.14)	2.284 (76.72)	1.329 (44.66)	0.477 (16.03)
	60	2.622	1.924 (73.39)	1.796 (68.49)	1.180 (45.01)	0.328 (12.52)
	90	2.617	2.032 (77.65)	1.847 (70.59)	1.386 (52.94)	0.416 (15.88)
Soil 2	14	7.029	5.994 (85.27)	5.852 (83.26)	5.040 (71.71)	2.087 (29.69)
	28	7.792	7.187 (92.28)	6.942 (89.09)	6.566 (84.27)	2.692 (34.55)
N-mineralisation (mg nitrate and nitrite/100 g dw soil) ¹						
Soil 1	7	4.334	3.145 (73)	2.326 (54)	2.927 (68)	1.103 (25)
	14	9.811	9.190 (94)	4.896 (50)	7.836 (80)	1.123 (11)
	21	10.749	10.524 (98)	8.800 (82)	10.861 (101)	1.671 (16)
	28	11.192	10.908 (97)	10.670 (95)	11.172 (100)	2.002 (18)
Soil 2	7	12.223	12.588 (103)	12.672 (104)	12.560 (103)	10.834 (89)
	14	12.609	12.777 (101)	12.693 (101)	12.693 (101)	11.774 (93)
	21	13.016	13.086 (101)	13.030 (100)	13.114 (101)	13.205 (101)
	28	13.549	13.942 (103)	13.479 (99)	13.788 (102)	14.195 (105)

¹ The percentage of the control is given in parentheses

Acceptability of the test

The studies were carried out in 1989; the report does not contain a GLP compliance statement. The test methods are insufficiently described, in particular the determination of dehydrogenase activity is not clear.

The toxic standard gave no inhibition of N-mineralisation in both soils and on carbon mineralisation in soil

2. Therefore, it cannot be determined whether the test substance is really harmless to soil micro-organisms or that the tested soil was not sensitive enough. With respect to carbon mineralisation, a decrease was found for soil 2. The test is considered as not acceptable.

Conclusion

The study is not considered acceptable.

B.9.8.1.2 Effects of a.s. dichlobenil on microbial C-mineralisation in soils (IIA 8.5/01)

Reference	: Mead, C. (2006a)	GLP statement	: yes
Type of study	: soil microflora test (C-mineralisation)	Guideline	: OECD 217
Year of execution	: 2006	Acceptability	: acceptable
Test substance	: dichlobenil, batch no. MI-0779, purity 99.3%		

Soil	Dose	Duration	OC	pH (0.01 M CaCl ₂)	T	Process	Effect at end [%]
	[mg a.s./kg]	[d]	[%]		[°C]		
<i>Loamy sand</i>	7.2	28	1.02	6.2	21	Respiration	-6
	36	28				Respiration	-6

Description

Data were submitted from a laboratory study on the effect of dichlobenil on soil respiration (carbon mineralisation) in a loamy sand.

A loamy sand soil (9.2%, 29.8% and 61.0% particles in the <2, 2-50 and >50 µm size class; pH 6.2; organic carbon: 1.02%; maximum water holding capacity (MWHC): 34.9 g/100 g; CEC: 90 mmol/kg; microbial biomass: 205 mg microb. C/kg) was sampled from the field.

The soil was taken from uncultivated arable land (Offenbach, Rheinland-Pfalz, Germany) on August 23, 2006; no pesticides had been used during the previous 4 years and no mineral or organic fertilizers during the last year. The soil was sieved (2 mm) and had a moisture content of 27% of MWHC; it was stored at 21 °C prior to use. Before use the soil was brought to a final moisture content of 40% of MWHC.

Test substance, dissolved in acetone was added to quartz sand and after evaporation of the acetone the quartz sand was mixed into the wet soil (1.5 kg); final concentrations: 7.2 mg a.s./kg dry soil and 36 mg a.s./kg dry soil. Four treatment groups were tested: 1) control (without quartz sand and acetone), 2) solvent control (quartz sand and acetone), 3) low concentration, 4) high concentration. Each bulksoil (1.5 kg) with or without test substance was split into replicates of 50 g. Incubation was carried out at 21 °C in the dark.

Triplicate soil samples (50 g) were sacrificed after 0, 7, 14 and 28 days for the determination of Substrate (glucose) Induced Respiration. They were placed in 250 mL conical glass flasks after addition of 4000 mg/kg of glucose in sand (10 g/kg) to elicit the maximum respiratory response. The oxygen consumption

was then measured in a CES Respirometer (pressure reduction induced electrolysis) in a temperature controlled water bath (temperature not mentioned) for 12 h after a stabilisation period of 90 min.

Differences between controls and the treated soils after 28 days were tested for significance using one way ANOVA including Bartlett's test for homogeneity and Dunnett's test.

Results

The test generally fulfilled the requirement of less than 15% variation between control replicates (in one case 15.85%).

The average respiration rate in the solvent control was 11.7, 8.34, 8.42 and 7.75 mg O₂/kg/h after 0, 7, 14 and 28 days, respectively.

The respiration rate in the 7.2 mg/kg treatment was 1%, 7% and 6% lower than in the solvent control after 7, 14 and 28 days, respectively (significantly different from solvent control after 28 days). The respiration rate in the 36 mg/kg treatment was 9% and 6% lower than in the solvent control after 14 and 28 days, respectively (significantly different from solvent control after 28 days). No results were obtained after 7 days, due to instrument malfunction.

Acceptability of the test

The study is acceptable.

B.9.8.1.3 Effects of a.s. dichlobenil on microbial N-mineralisation in soils (IIA 8.5/02)

Reference	: Mead, C. (2006b)	GLP statement	: yes
Type of study	: soil microflora test (N-mineralisation)	Guideline	: OECD 216
Year of execution	: 2006	Acceptability	: not acceptable
Test substance	: dichlobenil, batch no. MI-0779, purity 99.3%		

Soil	Dose [mg a.s./kg]	Duration [d]	OC [%]	pH (0.01 M CaCl ₂)	T [°C]	Process	Effect at end [%]
Loamy sand	7.2 36	28 28	1.02	6.2	21	Nitrate formation Nitrate formation	No reliable result No reliable result

Description

Data were submitted from a laboratory study on the effect of dichlobenil on nitrogen mineralisation (nitrate formation) in a loamy sand.

A loamy sand soil (9.2, 29.8 and 61.0% particles in the <2, 2-50 and >50 µm size class; pH 6.2; organic carbon: 1.02%; maximum water holding capacity (MWHC): 34.9 g/100 g; CEC: 90 mmol/kg; microbial biomass: 205 mg microb. C/kg; N_{total}: 1200 mg/kg; nitrate content: 22 mg/kg) was sampled from the field. The soil was taken from uncultivated arable land (Offenbach, Rheinland-Pfalz, Germany) on August 23, 2006; no pesticides had been used during the previous 4 years and no mineral or organic fertilizers during

the last year. The soil was sieved (2 mm) and had a moisture content of 27% of MWHC; it was stored at 21 °C prior to use. Before use the soil was brought to a final moisture content of 40% of MWHC.

Test substance, dissolved in acetone was added to quartz sand and after evaporation of the acetone the quartz sand was mixed into the wet soil (1.5 kg); final concentrations: 7.2 mg a.s./kg dry soil and 36 mg a.s./kg dry soil. The nitrogen source for the N-mineralisation test was lucerne meal (N and C content not given), which was added to a final amount of 0.5%.

Four treatment groups were tested: 1) control (without quartz sand and acetone), 2) solvent control (quartz sand and acetone), 3) low concentration, 4) high concentration. Each bulksoil (1.5 kg) with or without test substance was split into replicates of 50 g. Incubation was carried out at 21 °C in the dark.

Triplicate soil samples (50 g) were sacrificed after 0, 7, 14 and 28 days for the determination of nitrate by reaction with 2,6-dimethylphenol / amidosulphonic acid after extraction with KCl (0.1 mol/L). Differences between controls and the treated soils after 28 days were tested for significance using one way ANOVA including Bartlett's test for homogeneity and Dunnett's test.

Results

The coefficient of variation (CV) in the solvent control was 5.6% after 28 days. It was concluded that this fulfilled the validation requirements of the test.

The nitrate concentrations are given in Table 1. Nitrate levels in the controls and treated soils increased from 51-66 mg nitrate/kg on day 0 to 83-99 mg/kg after 28 days. During the periods 0-7 days and 21-28 days there was a net negative mineralisation (decrease of nitrate concentration), except in the 7.2 mg/kg treatment during days 21-28.

Table 1 Nitrate formation in loamy sand from lucerne meal in the presence of dichlobenil

Concentration dichlobenil [mg/kg]	Average NO ₃ concentration [mg/kg]					Rate of NO ₃ formation (21-28 days)* [mg/kg/d]	Rate of NO ₃ formation (0-28 days)* [mg/kg/d]
	Day 0	Day 7	Day 14	Day 21	Day 28		
0 (control)	50.88	28.44	78.56	113.32	92.22	-3.014	1.476
0 (solvent control)	50.64	44.05	73.08	84.30	83.20	-0.157	1.163
7.2	51.86	20.51	99.30	89.66	91.13	0.210	1.403
36	65.64	55.51	124.05	136.25	98.93	-5.331	1.189

* Calculated by the summariser

In the report the effect of dichlobenil on nitrogen mineralisation in soil was evaluated on the basis of differences in nitrate concentrations in the solvent control and treated samples after 28 days. Since these

differences were < 25%, it was concluded that there was no effect of dichlobenil at 7.2 and 36 mg/kg on soil nitrogen mineralisation.

Acceptability of the test

The coefficient of variation (CV) in the solvent control was 1.5%, 35.7%, 21.5%, 9.4% and 5.6% after 0, 7, 14, 21 and 28 days, respectively. The CV in the control (without acetone and quartz sand) was 4.2%, 39.9%, 34.25%, 8.9% and 4.1% after 0, 7, 14, 21 and 28 days, respectively. The author concluded that the validation requirements of the test were fulfilled because at day 28 the CV of the solvent control was < 15%.

However, the Test Guideline requires that "variation between replicate control samples should be less than $\pm 15\%$ ". No restriction to the 28-day replicates is mentioned in the Guideline. Because on days 7 and 14 the variation was > 15%, the present test does not fulfil the validation requirements of the Test Guideline.

Apart from this, the rate of formation of nitrate during the period 21-28 days was low or negative, which makes the results very difficult to evaluate. The same holds more or less for the period 14-28 days. Also during the period 0-7 days the rate of nitrate formation was negative. This gives an overall inconsistent pattern of nitrate formation.

The Guideline requires the effects to be evaluated on the basis of rates of nitrate formation (mg/kg/d) instead of nitrate concentrations, as done by the author. However, because of the inconsistent pattern of the rate of nitrate formation and the large standard deviation in the measurements, no reliable effect percentages for nitrate formation rate can be calculated.

Based on the above, the study cannot be accepted.

B.9.8.1.4 Effects of the metabolite 2,6-dichlorobenzamide (BAM) on microbial mineralisation in soils (IIA 8.5/03)

Characteristics of the study:

Reference	: Van der Laan-Straathof <i>et al.</i> (1996)	Species	: micro-organisms in sandy soil
Year of execution	: 1996	Exposure duration	: 14 wk
GLP	: yes	Final result	: not applicable
Guideline	: SETAC guideline, Procedures for assessing the environmental fate and ecotoxicology of pesticides, Ch. 4 (1995)		
Test substance	: 2,6-dichlorobenzamide (BAM); batch no. FUX00100/FUN81G02C; white crystals		
Purity	: > 99%	Acceptable	: no

Study design

The purpose of the study was to determine the influence of 0.12 and 6 mg/kg soil 2,6-dichlorobenzamide, corresponding with ca. 0.09 and 4.5 kg metabolite/ha, on glucose-stimulated respiration and on nitrogen mineralisation in a sandy soil. Soil (treated at equivalents to 0.02x and 1x the maximum theoretical concentration of the metabolite) was incubated and samples of soil were taken at the start of the test and at 2-wk intervals, until 14 wk of incubation. To a part of the samples glucose was added and the quantity of released carbon dioxide was measured for 24 h. The other part of the samples was used to determine ammonium-N, nitrate-N and nitrite-N. The following test set up was applied:

incubation temperature	: 20 ± 1°C
light conditions	: darkness
type of test vessel and content	: Erlenmeyer flasks with cotton wool stops; 100 g dw soil, 9.5 g water, and 0.5 g of Lucerne meal pellets as a nitrogen source
type of soil	: see specification below in Table B.9.8.1.2a
number of replicate test vessels	: 24 (3/sampling time)
dosing	: a solution in acetone was incorporated into an aliquot of soil
exposure concentrations	: 0 (solvent control), 0.12, and 6 mg /kg soil corresponding with 0 and ca. 0.09 and 4.5 kg /ha
type of measurements	: short-term soil respiration and nitrification within 6 h and at 2-wk intervals until inhibition is less than 25% of the control or up to a maximum of 100 d; soil water content prior to test initiation; temperature
chemical analysis of test substance in the soil	: yes, of the aliquots of sand, coated with the test substance
reference compound	: dinoseb acetate

Table B.9.8.1.2a Soil characteristics

Soil parameter	Soil type 2.1
Organic matter content (%)	0.59
pH	6.0
Cation exchange capacity (meq/100g)	4
Mechanical analysis (%)	
< 0.002 mm	2.5
0.002 – 0.006 mm	1.7
0.006 - 0.020 mm	2.9
0.020 – 0.062 mm	4.8
0.063 – 0.20 mm	23
0.20 – 0.63 mm	60.7
0.63 – 2.0	4.5
Sand (> 0.063 mm)	88.2
Maximum water capacity (g/100g)	29
Water content	4.975
Classification	sand

Results

The incubation temperature ranged between 18.4 and 24.6°C. The recovery of the test substance in the aliquots to which the test substance was added was 155 and 61% at the low and high dose, respectively.

The actual test concentrations were therefore 0.19 and 0.38 mg metabolite/kg dw soil.

The reference compound gave the expected response in both tests. The results of the soil respiration and nitrogen-mineralisation test are given in Table B.9.8.1.2b. It appeared that the test substance had no influence on soil respiration or nitrogen mineralisation, as the deviation from the control was less than 25% effect. One exception is the amount of nitrite formed (25 – 26% reduction for both test concentrations).

Table B.9.8.1.2b Results of the soil respiration and N-mineralisation test with the metabolite 2,6-dichlorobenzamide¹

Group	Incubation time	Control (solvent control)	Test substance (ca. 0.09 kg/ha)	Test substance (ca. 4.5 kg/ha)	Toxic standard (dinoseb acetate)
<i>Soil respiration</i>					
amount of CO ₂ formed within 48 h (dpm)	< 6 h	2498.3	2416.4 (-3.3)	2503.2 (0.2)	2321.8 (-7.1)
	2 wk	1766.0	1826.5 (3.4)	1797.3 (1.8)	2670.3 (51.2)
<i>N-mineralisation</i>					
ammonium-N (µmol/g dw soil)	< 6 h	0.157	0.164 (4.7)	0.153 (-2.7)	0.205 (30)
	2 wk	0.133	0.127 (-4.6)	0.109 (-18)	1.412 (962)
nitrate-N (µmol/g dw soil)	< 6 h	1.09	1.02 (-5.8)	1.00 (-7.8)	1.10 (1.2)
	2 wk	3.61	3.41 (-5.4)	3.78 (4.7)	1.97 (-45.4)
nitrite-N (µmol/g dw soil)	< 6 h	0.014	0.015 (5.6)	0.016 (8.4)	0.009 (-40)
	2 wk	0.007	0.005 (-26)	0.005 (-25)	0.304 (4296)

¹ Percentage deviation from the control is given in parentheses

Acceptability of the test

The incubation period was 14 d instead of 28 d according to OECD 216 and 217. The variation in control ammonium-N and nitrate-N within 6 h of incubation and control ammonium-N and nitrite-N after 2 weeks of incubation (determined as coefficient of variance) was higher than 15% (the validity limit, given by the guideline). This may have influenced the sensitivity of the test. Therefore the test is considered as not acceptable.

Conclusions

The test does not allow conclusions.

B.9.8.1.5 Effects of the metabolite 2,6-dichlorobenzamide (BAM) on soil respiration (IIA 8.5/04)

Characteristics of the study:

Reference	: Lechelt-Kunze (2004a)	Species	: micro-organisms in loamy sand soil
Year of execution	: 2004	Exposure duration	: 28 d
GLP	: yes		
Guideline	: OECD 217		
Test substance	: 2,6-dichlorobenzamide (BAM); batch no. 08018ET	Final result	: <25% effect after 28 d
Purity	: 96.2%	Acceptable	: yes

Test substance	Soil	Dose [mg/kg]	Duration [d]	OM [%]	pH	T [°C]	Process	Maximal effect [%]	After ... [d]	Effect after 28 d [%]
2,6-dichloro- benzamide (BAM)	<i>Loamy sand</i>	0.09	28	0.7	5.5	20	Soil respiration	-10	14	-8
		0.92						-10	7	-7

Study design

The influence of 2,6-dichlorobenzamide (BAM, a soil metabolite of dichlobenil) on soil microflora was determined in a respiration experiment. The test substance (purity 96.2%; batch no. 08018ET) was applied to a loamy sand soil. The effect on soil respiration was tested according to OECD Guideline 217. The soil was collected on June 22, 2004 at a depth of 0-20 cm from a grassland (Laacherhof, Germany), sieved to 2 mm and stored at 4 ± 2 °C until use. The agricultural history of the soil was described; no pesticides had been used in the previous 13 years and no fertilizers had been used in the previous 8 years. The characterisation of the soil is given in Table B.9.8.1.3a.

Table B.9.8.1.3a Characterisation of the soil used in the respiration test

Soil parameter	Value
Particle size distribution:	
0-2 µm	6.3%
2-63 µm	15.6%
63-2000 µm	78.1%
Soil type	Loamy sand
Organic matter	0.7%
pH (KCl)	5.5
CEC	64 mmol/kg
Nitrogen	0.10%
Microbial biomass	481 mg C/kg (7.4% of total org. C)

The test substance was mixed with the aid of quartz sand into the soil to final concentrations of 0.09 and 0.92 mg/kg dry soil. Three treatment groups were tested: 1) control, 2) low concentration, 3) high concentration. Three replicates of 350 g were incubated in 500 mL brown glass bottles at 20 ± 2 °C and at a moisture content of 40% of the maximum water holding capacity (the absolute moisture content was not reported).

Carbon mineralisation was measured by Substrate Induced Respiration (SIR) rate after 0, 7, 14 and 28 days of incubation. Soil samples (25 g) were mixed with glucose (in a concentration giving the highest respiration rate, i.e. 2000 mg/kg) and the CO₂ formation (mg CO₂/kg/h) measured every hour for at least 12 h. The cumulative CO₂ production per kg soil over 12 h (sum of the measurements of every hour) was used to compare the different treatments.

Statistics: homogeneity of variances was determined by F-test ($p = 0.05$), followed by the appropriate T-test.

Results

In this study the highest coefficient of variation in replicate control samples was 3.0%. Thus the validation criterion of $< 15\%$ was met.

The results are summarised in Table B.9.8.1.3b.

Table B.9.8.1.3b Formation of CO₂ in soil treated with 2,6-dichlorobenzamide (BAM) (average of 3 replicates)

Days of incubation	CO ₂ formation (mg/kg/12 h)		
	Control	0.09 mg BAM/kg	0.92 mg BAM/kg
0	351.2	324.9 (93%)*	328.7 (94%)
7	389.8	361.9 (93%)	351.7 (90%)
14	343.0	307.4 (90%)	320.9 (94%)
28	286.9	263.7 (92%)	267.1 (93%)

* () = percentage of control

Respiration in the 0.09 mg/kg treatment was maximally 10% lower than in the control after 14 days (significant); respiration was maximally 10% lower than in the control in the 0.92 mg/kg treatment after 7 days (significant). After 28 days there were no differences $> 25\%$ in the respiration compared to the control in the two treatment levels.

The most recently performed toxic standard test (sodium chloride, 16 g/kg) gave distinct and long-term (> 28 d) influences on soil respiration. This was reported elsewhere.

Acceptability of the test

The study can be accepted.

Conclusion

After 28 days there were no differences $> 25\%$ in the respiration compared to the control in the two treatment levels.

B.9.8.1.6 Effects of the metabolite 2,6-dichlorobenzamide (BAM) on soil N-mineralisation (IIA 8.5/05)

Characteristics of the study:

Reference	: Lechelt-Kunze (2004b)	Species	: micro-organisms in loamy sand soil
Year of execution	: 2004	Exposure duration	: 28 d
GLP	: yes	Final result	: $< 25\%$ effect after 28 d
Guideline	: OECD 216	Acceptable	: yes
Test substance	: 2,6-dichlorobenzamide (BAM); batch no. 08018ET		
Purity	: 96.2%		

Test substance	Soil	Dose [mg/kg]	Duration [d]	OM [%]	pH	T [°C]	Process	Maximal effect [%]	After ... [d]	Effect after 28 d [%]
2,6-dichloro- benzamide (BAM)	<i>Loamy sand</i>	0.09	28	0.7	5.5- 5.9	20	Nitrate formation (N mineralisation)	-9	14	-2
		0.92						-13	7	-4

Study design

The influence of 2,6-dichlorobenzamide (BAM, a soil metabolite of dichlobenil) on soil microflora was determined in a nitrogen mineralisation experiment. The test substance (purity 96.2%; batch no. 08018ET) was applied to a loamy sand soil. The effect on N mineralisation was tested according to OECD Guideline 216. The soil was collected on June 22, 2004 at a depth of 0-20 cm from a grassland (Laacherhof, Germany), sieved to 2 mm and stored at 4 ± 2 °C until use. The agricultural history of the soil was described; no pesticides had been used in the previous 13 years and no fertilizers had been used in the previous 8 years. The characterisation of the soil is given in Table B.9.81.3c.

Table B.9.81.3c Characterisation of the soil used in the N mineralisation test

Soil parameter	Value
Particle size distribution:	
0-2 µm	6.3%
2-63 µm	15.6%
63-2000 µm	78.1%
Soil type	Loamy sand
Organic matter	0.7%
pH (KCl)	5.5
CEC	64 mmol/kg
Total nitrogen	0.10%
NH ₄ -N	1.13 mg/kg
NO ₃ -N	13.75 mg/kg
Microbial biomass	481 mg C/kg (7.4% of total org. C)

The test substance was mixed with the aid of quartz sand into the soil to final concentrations of 0.09 and 0.92 mg/kg dry soil. Three treatment groups were tested: 1) control, 2) low concentration, 3) high concentration. After addition of the test substance, 0.5% lucerne meal (40.6% C, 2.5% N; C/N 16.2) was also mixed into the soil. Three replicates of 300 g were incubated in 500 mL brown glass bottles at 20 ± 2 °C and at a moisture content of 40% of the maximum water holding capacity (the absolute moisture content was not reported).

Nitrogen mineralisation was measured by determination of the content of ammonium-N, nitrite-N and nitrate-N in the soil after 0, 7, 14 and 28 days of incubation. Soil samples (10 g) were extracted with KCl solution and the extracts analyzed for $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$.

Statistics: homogeneity of variances was determined by F-test ($p = 0.05$), followed by the appropriate T-test.

Results

In this study the highest coefficient of variation in replicate control samples was 8%. Thus the validation criterion of $< 15\%$ was met.

The pH value of the soil increased from 5.5 to 5.9 after 28 days.

Nitrite formation was not detected in any of the soil samples in all test groups.

Ammonium-N concentration in the control decreased from an average 3.37 mg $\text{NH}_4\text{-N/kg}$ on day 0 to an average 1.44 mg $\text{NH}_4\text{-N/kg}$ after 28 days. In the treated samples ammonium-N concentration decreased also and there were no major deviations from the control.

The results of nitrate-N formation are summarised in Table B.9.81.3d. The figures are expressed as rates of nitrate-N formation (mg $\text{NO}_3\text{-N/kg/d}$).

Table B.9.8.1.3d Rate of formation of nitrate-N in soil treated with 2,6-dichlorobenzamide (BAM) (average of 3 replicates)

Time intervals (days)	Rate of formation of nitrate-N (mg $\text{NO}_3\text{-N/kg/d}$)		
	Control	0.09 mg BAM/kg	0.92 mg BAM/kg
0-7	-1.59	-1.64(-3%)*	-1.47(+8%)
7-14	0.79	0.72(-9%)	0.68(-13%)
14-28	1.01	0.99(-2%)	0.97(-4%)

* () = percentage difference from control

The rate of nitrate-N formation in the 0.09 mg/kg treatment was maximally 9% lower than in the control during the 7-14 days period; the rate of nitrate-N formation was maximally 13% lower than in the control in the 0.92 mg/kg treatment during the 7-14 days period. After 28 days there were no differences $> 25\%$ in the rate of nitrate-N formation and total N mineralisation compared to the control in the two treatment levels.

Acceptability of the test

There was no net nitrogen mineralisation during the 0-7 days interval. This can be accepted, because it occurred in one interval only. The study can be accepted.

Conclusion

After 28 days there were no differences > 25% in the N-mineralisation rate compared to the control in the two treatment levels.

B.9.8.2 Risk assessment

Dichlobenil

In the first study (Dijkstra, 1989), with the formulation casoron G, the influence of dichlobenil on soil microflora was determined in short-time respiration experiments and by monitoring of nitrogen transformations. However, the test was considered unacceptable for the nitrification part and it cannot be determined whether the test substance is really harmless to soil micro-organisms or that the test was not sensitive enough.

Two other recent performed studies with dichlobenil were submitted (Mead, 2006a/b).

The study on N-mineralisation was considered not acceptable, due to the high coefficient of variation in the control samples. Therefore, it cannot be concluded that dichlobenil, when applied at the maximum proposed field rate of 5.4 kg a.s./ha, will not affect soil nitrogen transformation.

The study on C-mineralisation was acceptable and conducted with relevant test concentrations (1-5x highest PIECs). Based on this study it can be concluded that dichlobenil causes no effects >25% on soil respiration when applied at at the maximum proposed field rate of 5.4 kg a.s./ha.

2,6-dichlorobenzamide (BAM)

The influence of the soil metabolite of dichlobenil, 2,6-dichlorobenzamide, was determined in an experiment by monitoring of nitrogen transformation. The test was not considered acceptable. Two other tests with BAM are available, one for soil respiration and one for soil N-mineralisation, which were both acceptable. However, the highest test concentration in these tests was 6 times lower than the PEC_{soil} for BAM at the proposed application rate (0.92 mg/kg vs 5.5 mg/kg). Therefore it cannot be determined whether 2,6-dichlorobenzamide has no adverse effects on soil micro-organisms when applied at label recommended doses.

Conclusion

The notifier is requested to provide new data on the effects on soil microflora for dichlobenil (N-mineralisation) and soil metabolite 2,6-dichlorobenzamide (BAM) (C- and N-mineralisation).

B.9.9 EFFECTS ON OTHER NON-TARGET ORGANISMS (FLORA AND FAUNA) BELIEVED TO BE AT RISK (IIA 8.6, IIA 10.8)

B.9.9.1 Toxicity to non-target plants

B.9.9.1.1 Seedling emergence and seedling growth test with the formulation Casoron G (IIA 8.6.01)

Characteristics of the study

Reference	: Spatz <i>et al.</i> (2004)	Content of a.s.	: 6.75% dichlobenil
Year of execution	: 2004	Species	: 6 species from 6 different families
GLP	: yes	Test duration	: 21 days
Guideline	: OECD no. 208 (proposal, July 2000)	Results ¹	
		ER50	: 100.31 g a.s./ha
		NOER	: 32.77 g a.s./ha
Formulation	: Casoron G; batch no. MI-0395	Acceptable	: yes

¹ fresh weight, the most sensitive parameter

Study design

The objective of the study was to test the formulation Casoron G on its influence on seedling emergence and on seedling growth. The following study design was applied:

species	: six plant species; four Dicotyledoneae and two Monocotyledoneae (see Table B.9.9.1.1a)
origin of plant seeds	: not reported
pre-treatment of seeds	: seeds were placed manually onto the soil
application	: one day after sowing
pots	: commercial plastic flower pots; diameter of 14 cm,
soil	: LUFA 2.3 (USDA): sandy loam
exposure temperature	: 17 – 26°C
humidity	: mean: 54% (day) and 64% (night)
light	: 16 h light/8 h dark; mean 10060 lux
preparation of solutions	: the test substance was spread manually on the moist soil surface; the low concentrations were mixed with sand; thus simulating field application
concentrations	: Dicotyledoneae: 81.92, 204.8, 512, 1280 and 3200 g a.s./ha Monocotyledoneae, onions: 13.11, 32.77, 81.92, 204.8, 512 and 1280 g a.s./ha and oats: 81.92, 204.8 and 512 g a.s./ha
exposure	: 4 mL of test solution was added to each Petri dish
number of pots per concentration	: 8
number of seeds per dish	: 5
observations/measurements	: phytotoxicity (visually): after 7, 14 and 21 days germination: after 7, 14 and 21 days mortality: after 21 days fresh weight: after 21 days growth stages: according to BBCH-monograph at application and after 21 days
chemical Analysis	: no

Table B.9.9.1.1a Plant species tested

Class	Family	Species	English name	Plants per pot/pots per treatment	Time of application after sowing (in days)
Monocotyledonae	Poaceae	<i>Avena sativa</i>	Oats	5/8	24
Dicotyledonae	Liliaceae	<i>Allium cepa</i>	Onion	3/14	24
	Solanaceae	<i>Lycopersion esculentum</i>	Tomato		
	Brassicaceae	<i>Raphanus sativus</i>	Radish	3/14	16
	Fabaceae	<i>Pisum sativum</i>	Pea	5/8	19
	Compositae	<i>Helianthus annuus</i>	Sunflower	3/14	16

Results

The analytical recovery of the active ingredient dichlobenil in a 3200 g a.s./ha sample was 88%.

Control plants showed a normal growth throughout the test and the germination was above 65%. The most sensitive species in terms of fresh weight were the two Monocotyledoneae, i.e. *Avena sativa* and *Allium cepa*. The summarised results for the various test parameters are given in Table B.9.9.1.1b. The ER50 (effective rates), based on fresh weight are given in Table B.9.9.1.1c.

Table B.9.9.1.1b Summarised results of plant seedling emergence and seedling growth test with Casoron G

species	Germination	Phytotoxicity	Mortality	Fresh weight	Plant development
<i>Avena sativa</i>	Significantly less at 204.8 g a.s./ha; no germination at 512 g a.s./h	Growth reduction at 13.11 g a.s./ha and higher	Significant at 204.8 g a.s./ha and higher	Significant less at 81.92 g a.s./ha and higher	Delayed at 204.8 g a.s./ha
<i>Allium cepa</i>	Significantly less at 1280 g a.s./ha	Growth reduction at 204.8 g a.s./ha and higher	Significant at 1280 g a.s./ha	Significant less at 81.92 g a.s./ha and higher	Clear delay at 1280 g a.s./ha
<i>Lycopersion esculentum</i>	Significantly less at 1280 g a.s./ha and higher	Growth reduction at 512 g a.s./ha and higher	No mortality	Significant less at 81.92 g a.s./ha and higher	Delayed at 1280 g a.s./ha
<i>Raphanus sativus</i>	Significantly less at 512 g a.s./ha; no germination at 3200 g a.s./ha	Growth reduction at 512 g a.s./ha and higher	No significant mortality	Significant less at 204.8 g a.s./ha and higher	Delayed at 1280 and 3200 g a.s./ha and higher
<i>Pisum sativum</i>	Significantly less at 1280 and 3200 g a.s./ha and higher	Growth reduction at 1280 and 3200 g a.s./ha and higher	No mortality	Significant less at 1280 g a.s./ha and higher	Delayed at 1280 and 3200 g a.s./ha and higher
<i>Helianthus annuus</i>	No effect	Growth reduction at 1280 and 3200 g a.s./ha and higher	No significant mortality	Significant less at 1280 g a.s./ha and higher	No delay

Table B.9.9.1.1c Summary of effective rates, based on fresh weight. results of the terrestrial, non-target, plant test with Casoron G

Plant species	NOER	LOER	Er50
<i>Avena sativa</i>	32.77	81.92	100.31 (84.12 – 120.56)
<i>Allium cepa</i>	32.77	81.92	206.78 (13.46 – 3313.31)
<i>Lycopersion esculentum</i>	81.92	204.8	452.10 (n.d.)
<i>Raphanus sativus</i>	81.92	204.8	659.78 (433.44 – 990.97)
<i>Pisum sativum</i>	512	1280	1249.53 _ 774.94 2024.11
<i>Helianthus annuus</i>	512	1280	1365.04 (1282.08 – 1463.77)

Conclusion

The following conclusions can be drawn:

From the investigated parameters, the fresh weight of the Monocotyledonae *Avena sativa* and *Allium cepa* was the most sensitive. The ER50 values were 100.31 and 206.78 g a.s./ha, respectively, and the NOER value 32.77 g a.s./ha.

Acceptability of the test

The test is considered acceptable.

Conclusions

Avena sativa was the most sensitive species for the formulation Casoron G (6.75% (w/w) dichlobenil); the overall ER50 and NOER values for fresh weight are 100.31 and 32.77 g a.s./ha.

B.9.9.1.2 Toxicity of the formulation Casoron G to non-target plants (IIA 8.6.02)

Characteristics of the study

Reference	: Spatz <i>et al.</i> (2004)	Content of a.s.	: 6.75% dichlobenil
Year of execution	: 2004	Species	: 6 species from 6 different families
GLP	: yes ¹	Test duration	: 21 days
Guideline	: OECD no. 208 (proposal, July 2000)	Result	: lowest overall 21d-NOER (fresh weight <i>Avena sativa</i>) = 7.9 g a.s./ha
Formulation	: Casoron G; batch no. MI-0395; grey granules	Acceptable	: yes

¹ GLP is not claimed for the pre-application period

Study design

The objective of the study was to test the formulation Casoron G for phytotoxic potential. At the 2 – 4 leaves stage, the test formulation was applied to six terrestrial plant species, representing six families. The following test set up was applied:

species	: six plant species; four Dicotyledonae and two Monocotyledonae (see Table B.9.9.1.2a)
origin of plant seeds	: not reported
growth conditions	: exposure in growth chambers
pots	: plastic; 14 cm diameter
exposure temperature	: 18 - 24°C

light regime en intensity	:	16 h day/8 h night (5360 – 19240 lux)
humidity	:	55 – 69%
soil type and parameters	:	LUFA 2.3 (USDA: sandy loam); particle size < 0.2 cm; organic carbon = $1.2 \pm 0.17\%$ and pH = 6.3 ± 0.4
application method	:	different rates of test items were sprayed onto the soil of each pot
application details	:	rates were prepared by mixing the test formulation with sand; an appropriate amount was taken from the mixture and spread onto the soil of each pot
application rates	:	Dicotyledonae 100. 300, 900. 2700, 8100 g a.s./ha (<i>Raphanus sativus</i> , <i>Helianthus annuus</i> and <i>Lycopersicon esculentum</i>) 100. 300, 900. 2700, 8100, 24300 and 72900 g a.s./ha (<i>Pisum sativum</i>) Monocotyledonae 0.49, 1.98, 7.91, 32, 127 g a.s./ha (<i>Avena sativa</i>) 1.98, 7.91, 32, 127, 506 and 2025 g a.s./ha (<i>Allium cepa</i>)
number of plants per treatment	:	8 – 14, depending on the plant class (see Table B.9.9.1.2a)
observations	:	visual assessment for phytotoxicity (chlorosis, necrosis, abnormal growth) 7, 14 and 21 days after application according to EPPO Standard PP 1/135
measurements	:	fresh weight determination of all plants (living and dead), 21 days after application
statistics	:	Kolmogoroff-Smirnov for normality of fresh weight data Homogeneity with Cochran-Test, Bonferroni-U-test, Dunnett-test or William test (depending whether the data were normally distributed, homogeneous and monotonous in- or decreasing) Fisher Exact-test for mortality The significance level for all tests was 0.05.
Chemical Analysis	:	Analytical verification of one sample of 8100 g a.s./ha

Table B.9.9.1.2a Plant species used in the test and application time after sowing

Class	Family	Species	English name	Plants per pot/pots per treatment	Time of application after sowing (in days)
Monocotyledonae	Liliaceae	<i>Allium cepa</i>	Onion	5/8	24
Dicotyledonae	Solanaceae	<i>Lycopersicon esculentum</i>	Tomato	3/14	24
Monocotyledonae	Poaceae	<i>Avena sativa</i>	Oat	5/8	19
Dicotyledonae	Fabaceae	<i>Pisum sativum</i>	Pea	3/14	19
	Compositae	<i>Helianthus annuus</i>	Sunflower	3/14	16
	Brassicaceae	<i>Raphanus sativus</i>	Radish	3/14	16

Results

The analytical recovery of the active ingredient dichlobenil in a 8100 g a.s./ha sample was 107% and thus confirmed the correct dosing.

Control plants showed a normal growth throughout the test and there was no mortality in the control.

Phytotoxicity was observed for the various plant species at the following concentrations:

- *Allium cepa*: Chlorosis and growth reduction at 506 g a.s./ha and higher
- *Lycopersicon esculentum*: Growth reduction at 900 g a.s./ha, chlorosis and growth reduction at 2700 g a.s./ha and chlorosis, necrosis and growth reduction at 8100 g a.s./ha
- *Avena sativa*: Slight growth reduction at 32 and 127 g a.s./ha
- *Pisum sativum*: Chlorosis at 8100 g a.s./ha; chlorosis and growth reduction at 24.300 and 72.900 g a.s./ha
- *Helianthus annuus*: Chlorosis and growth reduction at 2700 and at 8100 g a.s./ha

Further results of the test with respect to mortality, fresh weight and growth are given in Tables B.9.9.1.2b and B.9.9.1.2c.

Table B.9.9.1.2b Results of the (non-target) plant test with CasoronG (in g a.s./ha)

	Treatment	Mortality	Fresh weight (g)	St dev	Effect %	Phytotoxicity (%)			Growth stage (BBCH)	
						7 d	14 d	21 d	at application	21 d
<i>Allium cepa</i>	control	0	14.63	± 1.36		0	0	0		15
	1.98	0	12.25	± 1.98	- 16.25	0	0	0	12	15
	7.91	0	12.35	± 1.59	- 15.59	0	0	0		15
	32	0	12.61	± 1.96	- 13.79	0	0	0		15
	127	0	12.08	± 2.16	- 17.46 ²	1	0	0		15
	506	13	10.87	± 3.53	- 25.67 ²	3	16	17		14-15
	2025	73 ¹	2.39	± 2.61	- 83.66 ²	11	76	80		14
<i>Lycopersicon esculentum</i>	control	0	40.82	± 2.37		0	0	0		17
	100	0	41.10	± 5.11	- 0.69	0	0	0	14	17
	300	0	40.70	± 5.70	- 0.28	0	0	0		17
	900	0	34.93	± 2.96	- 14.43 ³	0	3	2		17
	2700	0	27.30	± 3.49	- 33.12 ³	6	14	18		16-17
	8100	0	9.69	± 4.25	- 76.25 ³	25	45	74		15-16
<i>Avena sativa</i>	control	0	31.04	± 3.43		0	0	0		16
	0.49	0	30.27	± 3.90	- 2.50	0	0	0	13	16
	1.98	0	32.24	± 1.62	3.85	0	0	0		16
	7.91	0	30.05	± 3.16	- 3.22	0	0	0		16
	32	0	25.32	± 2.44	- 18.42 ²	0	0	1		16
	127	0	25.10	± 2.89	- 19.13 ²	0	3	2		16
<i>Pisum sativum</i>	control	0	17.38	± 1.62		0	0	0		70
	100	0	14.80	± 1.60	- 14.84 ³	0	0	0	14	70
	300	0	14.22	± 2.69	- 18.20 ³	0	0	0		70
	900	0	12.84	± 2.05	- 26.10 ³	0	0	0		70
	2700	0	17.09	± 1.34	- 1.67	0	0	1		70
	8100	0	14.59	± 2.39	- 16.07 ³	0	5	3		67
	24300	0	9.55	± 3.40	- 45.04 ³	0	12	31		67
<i>Raphanus sativus</i>	control	0	11.35	± 1.82		0	0	0		15
	100	0	11.33	± 1.63	- 0.16	0	0	0	13 - 14	15
	300	0	10.40	± 1.39	- 8.33	0	0	0		15
	900	0	10.94	± 1.95	- 3.63	0	9	0		15
	2700	0	8.96	± 2.36	- 21.03 ³	4	9	10		14-15
	8100	0	9.35	± 3.60	- 17.61	1	19	40		14-15
<i>Helianthus annuus</i>	control	0	40.83	± 2.90		0	0	0		19
	100	0	39.18	± 3.06	- 4.04	0	0	0	14	19
	300	0	37.60	± 2.00	- 7.89	0	0	0		19
	900	0	36.25	± 2.19	- 11.21	0	3	0		18-19
	2700	0	33.86	± 1.81	- 17.06 ⁴	0	3	1		18-19
	8100	0	30.52	± 3.68	- 25.25 ⁴	7	18	24		18-19

1 significant mortality; p = 0.05 (Fischer Exact test)

2 multiple comparison; p = 0.05 (Williams test)

3 multiple comparison; p = 0.05 (Bonferroni-t-Test)

4 multiple comparison; p = 0.05 (Dunnett-test)

Table B.9.9.1.2c Summary of results of the terrestrial, non-target, plant test with Casoron G (concentrations in g a.s./ha) based on fresh weight.

Plant species	Highest tested concentration	NOER	LOER	Er50
<i>Allium cepa</i>	2025	32	127	821.07
<i>Lycopersicon esculentum</i>	8100	300	900	3914.05
<i>Avena sativa</i>	127	7.9	32	4406.52 ¹
<i>Pisum sativum</i>	72900	< 100	100	32308.33 ¹
<i>Raphanus sativus</i>	8100	≥ 8100	> 8100	258027.45 ¹
<i>Helianthus annuus</i>	8100	100	300	136379.00 ¹

¹ extrapolated value

Fresh weight appeared to be the most sensitive parameter. The most sensitive species was *Allium cepa* (ER50 value is 821.07 g a.s./ha, followed by *Lycopersion esculentum* (ER50 value is 3914.05 g a.s./ha). The ER50 values for the other tested species were above the highest tested rate. Taking into account the NOER value, however, *Avena sativa* was the most sensitive species (the NOER value was 7.9 g a.s./ha)

Acceptability of the test

The test is considered acceptable

Conclusions

Avena sativa was the most sensitive species for the formulation Casoron G (6.75% (w/w) dichlobenil); the overall NOER value for fresh weight was 7.9 g a.s./ha).

B.9.9.1.3 Seed Germination test with the metabolite 2,6-dichlorobenzamide (II A 8.6.03)

Characteristics of the study

Reference	: Balk and Tikovsky (1992)	Purity	: 99.4%
Year of execution	: not indicated	Species	: three Monocotyledonae and two Dicotyledonae species
GLP	: no	Test duration	: five days
Guideline	: no	Lowest EC ₅₀	: 29 mg/L
		(initial growth of seedling)	
Test substance	: Dichlorobenzamide; charge no. FUX001000, cask 1; sample no. FUN81G02C	Acceptable	: yes

Study design

The objective of the study was to test the influence of the metabolite 2,6-dichlorobenzamide (BAM) on seed germination and initial growth of pre-germinated seeds. The results of the test were compared with those of an initial growth test, carried out in a similar way with dichlobenil by Eussen (1989). As there are no raw data for the seed germination test with dichlobenil to which was referred in the study with BAM, this part of the report is not summarised. The following test set up for the initial growth test with 2,6-dichlorobenzamide was applied:

species	: five plant species Monocotyledonae Family Graminae: <i>Avena sativa</i> , <i>Triticum aestivum</i> , <i>Zea mais</i> Dicotyledonae Family Cucurbitacea <i>Cucumis sativas</i> Family Solonaceae <i>Lycopersicum esculentum</i>
origin of plant seeds	: not reported
pre-treatment of seeds	: for initial growth test pre-germinated in demineralised water
growth conditions	: growth box of metal and plexi-glass with a layer of moistened cotton on the bottom, placed in a dark climatic room

pots	:	Petri dishes with a diameter of 5 cm, supplemented with two filter papers
exposure temperature	:	20 ± 0.5°C
preparation of solutions	:	a stock solution of 1000 mg/L in demineralised water, from this solution the test solutions were prepared
concentrations	:	initial growth of seedlings: 1, 3, 10, 30, 100 and 300 mg/L were prepared
exposure	:	4 mL of test solution was added to each Petri dish
number of dishes per concentration	:	10
number of seeds per dish	:	10
observations/measurements	:	measuring of initial growth, as root and shoot length, 5 days after planting the pre-germinated seeds
positive control	:	thiram
chemical analysis	:	no

Results

Inhibition of radicle emergence of seeds appeared to be species dependent. As large differences in the length of the radicle were observed, the test parameter initial growth of pre-germinated seeds was preferred. The results for this parameter are summarised in Table B.9.9.1.3a; the EC₅₀ values are given in Table B.9.9.1.3b. A considerable difference in the sensitivity between species was observed.

Table B.9.9.1.3a Effects on initial growth of seedlings (length of roots and shoots in cm and concentrations in mg/L)

Plant species		0	3	10	30	100	300	1000
<i>Avena sativa</i>	root	10.97	10.49	10.31	9.02	1.84	0.74	0.79
	shoot	6.89	6.97	7.21	6.76	6.43	1.21	0
<i>Triticum aestivum</i>	root	12.66	12.41	11.41	11.47	3.58	0.73	0.50
	shoot	6.53	6.93	6.41	7.15	6.68	1.98	0.15
<i>Zea mais</i>	root	9.9	10.25	9.98	10.23	8.27	1.80	0.77
	shoot	5.23	5.42	4.99	5.1	4.94	3.46	0.50
<i>Cucumis sativus</i>	root	10.68	10.52	10.68	10.62	2.78	0.74	0.56
	shoot	4.41	4.25	4.31	4.31	2.88	0.43	0.04
<i>Lycopersicum esculentum</i>	root	0.98	0.8	0.93	0.33	0.10	0.10	0.11
	shoot	1.49	1.65	1.69	1.45	0.19	0.19	0.17
	root	1.56	1.78	2.22	0.92	0.09	0.09	-
	shoot	1.99	1.8	2.44	2.07	0.19	0.19	-

Table B.9.9.1.3b EC₅₀ values for initial growth of seedlings (concentrations in mg/L)

Plant species	Root	Shoot
<i>Avena sativa</i>	54	185
<i>Tricum aestivum</i>	67	225
<i>Zea mais</i>	180	460
<i>Cucumis sativus</i>	68	140
<i>Lycopersicum esculentum</i>	29 ¹	69 ²

1 mean of 23 and 36 mg/L

2 mean of 70 and 68 mg/L

Acceptability

The test is considered acceptable.

Conclusion

The lowest EC₅₀ value for initial growth of seedlings was 29 mg/L (root growth of *Lycopersicum esculentum*).

B.9.9.2 Risk assessment

The exposure assessment for terrestrial plants is based on emission via drift. However, dichlobenil enters the terrestrial compartment via drainage and run-off. Therefore, the risk assessment of dichlobenil for terrestrial plants is not relevant.

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.9.10 EFFECTS ON BIOLOGICAL METHODS FOR SEWAGE TREATMENT PLANT**B 9.10.1 Effects of the active substance to bacteria (IIA 8.7/01)****Characteristics of the study:**

Reference	: Mead (2002)	Exposure duration and regime	: 3 h ; static
Year of execution	: 2002	Species	: mixed population of micro-organisms (activated sludge)
GLP	: yes	3h-EC ₅₀	: > 21 mg a.s./L
Guideline	: OECD 209; EEC Commission Directive 87/302/EEC; OPPTS 850.6800		
Test substance	: dichlobenil; batch no. 42763011/MI-0246; white solid		
Purity	: 99.3%	Acceptable	: yes

The following test design was applied:

origin of activated sludge	: from a waste water treatment plant treating predominantly domestic sewage
exposure temperature	: 21°C
photoperiod	: normal laboratory lighting
physicochemical oxygen consumption	: not determined
type of dilution water	: dechlorinated and partly softened tap water (ca. 100 mg CaCO ₃ /L)
type of test vessel and content	: 500-mL conical flasks; 500 mL test solution for the incubation period; 250-mL BOD bottles for measurement of the respiration rate
number of replicate test vessels	: 2 (control), 1 (21 mg a.s./L), and 3 (1000 mg a.s./L)
concentration activated sludge	: 4.0 g suspended solids/L; 200 mL in 500 mL dilution water
aeration	: yes
dosing	: dispersed in water; synthetic sewage was added as a respiratory substrate (16 mL in 500 mL water)
exposure concentrations	: limit test: 0, 21 (limit of water solubility), and 1000 mg a.s./L
type of measurements	: respiration rate at t = 30 min and 3 h; pH
chemical analysis	: no
reference substance	: 3,5-dichlorophenol

Results

The toxic standard gave the expected response. The pH varied between 8.1 and 8.5. The differences between the control replicates were less than 15%. The tested concentration was above the water solubility limit of ca. 21 mg a.s./L, which was confirmed by the observation of undissolved test substance at both test concentrations. Therefore, RMS sets the 3h-EC₅₀ at the water solubility level, i.e. > 21 mg a.s./L.

Acceptability of the test

The study is considered acceptable.

Conclusion

The 3h-EC₅₀ for respiration inhibition of activated sludge was > 21 mg a.s./L (i.e. water solubility level).

B.9.10.2 Risk assessment

Dichlobenil did not cause inhibition of the bacterial respiratory rate at concentrations of 21 mg a.s./L (aqueous solubility level), the 3h-EC₅₀ was > 21 mg a.s./L. Due to the intended use, there is no risk of exposure.

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

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