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Report prepared in the context of the application for first inclusion of dodine in Annex I of the Council Directive 91/414/EEC

DODINE

Volume 3-5 rev.1

Annex B

Section B9

Summary, evaluation and assessments of the data.

List of tests and studies relied upon

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B.9.1.1 Acute Oral Toxicity (Annex IIA 8.1.1)

The Acute oral toxicity (LD50) of dodine to the Mallard Duck

Hakin B. (1990a); Huntingdon Research Centre Ltd, England; Unpublished report # CMK 40/881487.

Guidelines and GLP:

The study was conducted according to US EPA FIFRA Subdivision 71-1 (1982) Guidelines. Principles of GLP were complied with.

Test species and methods:

Test material: ISO common name: dodine; IUPAC name: 1-dodecylguanidinium acetate; Technical substance: batch 92/88/2, purity 95.3%.

Test Animal: Mallard duck (*Anas platyrhynchos* L.); total number of birds used: 2 males and 4 females in range-finding, 30 males and 30 females in the main study (5 males and 5 females per dose level).

Age of birds at start of treatment: 8 months.

Body weight range at start of treatment: 955 - 1245 g

Administration (once on day 1) by oral gavage at dose levels 0 (control), 100, 200, 400, 800 and 1600 mg s.a./kg body weight.

Control birds were administered corn oil, which was the vehicle also in dosing with dodine. A prior dose range finding study used dose levels of 250, 500 and 1000 mg/kg body weight with each one bird (male and female) per level except for the 250 mg/kg dose group were 2 females only were used.

Observation of birds after dosing (main study) for 14 days, including:

Mortality: daily;

Body weights: on Days -14, -7, 0 (prior dosing), 7 and 14;

Food consumption: over days -14 to -8, -7 to -1, 1 to 7, 8 to 14;

Symptoms/toxic effects/clinical signs: daily

At termination: all birds were sacrificed and subjected to gross necropsy.

Processing of results: body weight and food consumption data were analysed statistically, LD50 was calculated using the method of Probit Analysis[1] and using Maximum Likelihood Program[2].

Findings:

Several mortalities occurred in the 3 highest dose groups.

The results obtained are summarised in the table below.

[1] Finney, D. J. (1978) Statistical method in Biological Assay, Griffin & Co., London

[2] Ross, G. J. S. (1080) Maximum Likelihood Program, Rothams Experimental Station, Harpenden, U.K.

Table B.9.1.1.1 - Acute oral toxicity of dodine in mallard duck

Dose level (mg/kg b.w.)	Toxicol. result (mort./clin. signs/total nº)	Onset on death on day	Dose level (mg/kg b.w.)	Toxicol. result (mort./clin. signs/total nº)	Onset on death on day
Males			**Females**		
Group 1: 0	0/0/5	-	0	0/0/5	-
Group 2: 100	0/0/5	-	100	0/0/5	-
Group 3: 200	0/0/5	-	200	0/0/5	-
Group 4: 400	0/1/5	-	400	1/1/5	5
Group 5: 800	3/5/5	1-1-1	800	1/5/5	1
Group 6: 1600	4/5/5	1-1-2-10	1600	5/5/5	1-1-2-2-2

Food consumption results were very variable and there was no clear evidence of a dose-related response, although consumption appeared depressed in Group 4 females (400 mg/kg) and was particularly low in surviving female birds in Group 6 (1600 mg/kg) in the 7-day period after dosing. There was evidence of a treatment-related effect on bodyweights in Groups 4, 5 and 6.

No clinical findings were observed in Group 1, 2 and 3. In the higher doses groups 4, 5 and 6, several birds appeared to vomit shortly after dosing: 2, 2 and 5 animals out of 10 respectively in group 4, 5 and 6. However, there was no evidence of a relationship between vomiting and the incidence or timing of death. This would confirm that the birds which vomited must have ingested most or the entire test compound.

Macroscopic post-mortem examination of all animals that died during the study and the animals that were sacrificed at study termination did not reveal any abnormalities.

The acute oral **$LD_{50\ male\ and\ female}$** value of dodine in the mallard duck was found to be **857 mg/kg bw** with 95% confidence limits of 625 – 1234 mg/kg. The **NOEL** was found to be **200 mg/kg bw** and the lowest effect level, based on clinical signs, bodyweight and food consumption was 400 mg/kg bw.

The Acute oral toxicity (LD50) of dodine to the bobwhite quail

Hakin B. (1990b); Huntingdon Research Centre Ltd, England; Unpublished report # CMK 41/881123.

Guidelines and GLP:

The study was conducted according to US EPA FIFRA Subdivision 71-1 (1982) Guidelines. Principles of GLP were complied with.

Test species and methods:

Test material: ISO common name: dodine; IUPAC name: 1-dodecylguanidinium acetate; Technical substance: batch 92/88/2, purity 95.3%.

Test Animal: Bobwhite quail (*Colinus virginianus*); total number of birds used: 5 males and 5 females per dose level.

Age of birds at start of treatment: 18 weeks.

Body weight range at start of treatment: 170 - 233 g

Administration (once on day 1) by oral gavage at dose levels 0 (control), 400, 600, 900, 1350 and 2025 mg as/kg body weights. Control birds were administered corn oil, which was the vehicle also in dosing with dodine.

Observation of birds after dosing for 21 days, including:

Mortality: daily;

Body weights: on Days -14, -7, -1, 0 (prior dosing), 7, 14 and 21;

Food consumption: over days -14 to -8, -7 to -1, 1 to 7, 8 to 14, 15 to 21;

Symptoms/toxic effects/clinical signs: daily

All birds which died during the study were examined post-mortem for gross pathological changes. At termination: 10 birds from the highest dose groups were they were survivors were sacrificed and subjected to macroscopic examination.

Processing of results: body weight and food consumption data were analysed statistically, LD_{50} was calculated using the method of Probit Analysis and using Maximum Likelihood Program.

Findings:

Several mortalities occurred in the 3 highest dose groups.

The results obtained are summarised in the table below.

Table B.9.1.1.2 - Acute oral toxicity of dodine in bobwhite quail

Dose level (mg/kg b.w.)	Toxicol. result (mort./clin. signs/total nº)	Onset on death on day	Dose level (mg/kg b.w.)	Toxicol. result (mort./clin. signs/total nº)	Onset on death on day
Males			**Females**		
Group 1: 0	0/0/5	-	0	0/0/5	-
Group 2: 400	0/5/5	-	400	0/5/5	-
Group 3: 600	0/5/5	-	600	0/5/5	-
Group 4: 900	1/5/5	3	900	3/5/5	1-2-3
Group 5: 1350	5/5/5	2-2-2-5-6	1350	4/5/5	2-2-3-11
Group 6: 2025	5/5/5	2-2-2-3-13	2025	5/5/5	1-2-2-2-7

During the 7-day period after dosing, there was an indication of a treatment-related reduction in food consumption in groups 2, 3 and 4; this effect was more marked in groups 5 and 6 whereas food consumption in the control group 1 was similar to that recorded in the pre-treatment period. Food consumption in surviving birds in Groups 2-6 increased again from day 8 and was higher than the control. There was evidence of a treatment-related effect on bodyweights in all groups dosed with dodine.

No clinical findings were observed in the control Group 1. All birds from dose group 2 and 3 appeared subdued from Day 1 to Day 7 and recovered after day 8. At 900 mg/kg, 1350 mg/kg and 2025 mg/kg clinical signs of toxicity included subdued appearance, unsteadiness and ruffled feathers and several of them died.

Macroscopic post-mortem examination of all animals that died during the study and the animals that were sacrificed at study termination did not reveal any abnormalities.

The acute oral **$LD_{50\ male\ and\ female}$** value of dodine in the bobwhite quail was found to be **981 mg/kg bw,** with 95% confidence limits of 824 – 1156 mg/kg. The **NOEL** and the lowest effect level were found to be **< 400 mg/kg bw**.

B.9.1.2 Avian dietary toxicity (5day) (Annex IIA 8.1.2)

The Dietary Toxicity (LC50) of dodine to the bobwhite quail

Hakin B. (1990a); Huntingdon Research Centre Ltd, England; Unpublished report # CMK 39/881199.

Guidelines and GLP:

The study was conducted according to US EPA FIFRA Subdivision 71-2 (1982) Guidelines. Principles of GLP were complied with.

Test species and methods:

Test material: ISO common name: dodine; IUPAC name: 1-dodecylguanidinium acetate; Technical substance: batch 92/88/2, purity 95.3%.

Test Animal: Bobwhite quail (*Colinus virginianus*); total number of 90 birds not sexed (10 for each of the 6 treatment groups, 30 in 3 control groups).

Age of birds at start of treatment: 11 days.

Test design: Administration *ad libitum* with the diet for 5 days (free access to water) at dose levels 0 (control), 162.5, 325, 650, 1300, 2600 and 5200 mg dodine/kg diet. Control birds were fed the diet without test item.

Chemical analysis of the diet was performed in term of stability and homogeneity.

Study duration: 3 day pre-treatment period (Day -3 until Day -1); 5 day treatment period (Day 1 to Day 5) and 3 day post-treatment period (Day 6 to Day 8)

Observations of birds included:

- Mortality: daily;

- Clinical signs: daily;

- Body weights: on Days -3, 0 (prior dosing), 6 and 8;

- Food consumption: from day -3 to -1, from days 1 to 5 (daily), from days 6 to 8.

Pathology: at termination of the study, macroscopic examination was carried out on ten birds from the highest dose level groups in which there were survivors. Birds which died during the study were also examined.

Findings:

The chemical analysis of the diet proved homogeneity and accuracy. Found concentrations agreed with the nominal values within 4%. The overall dietary intake of test item is calculated in the following table:

Table B.9.1.2.1 - Calculation of the dietary intake by the quail

Dose Group (mg as/kg diet)	Mean body weight over the 5 days period (g/bird)	Mean food consumption (g/bird/day)	Calculated dietary intake (mg as/kg bw/day)
Group 1/2/3:0	20.93	4.5	-
Group 4: 162.5	21.35	4.7	36
Group 5: 325	21.35	4.5	68
Group 6: 650	20.50	4.5	143
Group 7: 1300	21.85	4.9	292
Group 8: 2600	20.95	4.5	558
Group 9: 5200	17.05	3.2	976

Only two mortalities occurred during the study, both in Group 9. One bird died on day 3 and the other on Day 4 of the treatment period. No clinical signs of toxicity were recorded in any birds.

Birds remained in good health, except the two birds which died. Birds from the 3 control groups and birds from group 4 to 8 showed continued bodyweight increases and normal food consumption and there was no evidence of any treatment-related effect. In the highest dose group 9, however, there was evidence of a marked reduction in bodyweight increase and food consumption during the treatment period. This was followed by a marked mean bodyweight increase during the post-treatment period.

No abnormalities were detected in either of the birds in Group 9 which died during the study. The surviving eight birds in group 9 and two birds in group 8 were examined macroscopically at termination and no abnormalities were detected in any birds.

As only two mortalities occurred in the test groups, LC50 could not be calculated. The **LC50** value for dodine in the bobwhite quail **is therefore > 5200 mg/kg (which correspond to 976 mg dodine/kg bw/day**). The **5-day NOEL** was found to be **2600 mg/kg (558 mg dodine/kg bw/day**) and the 5-day LOEL was also 5200 mg/kg.

The Dietary Toxicity (LC50) of dodine to the mallard duck

Hakin B. (1990b); Huntingdon Research Centre Ltd, England; Unpublished report # CMK 38/881122.

Guidelines and GLP:

The study was conducted according to US EPA FIFRA Subdivision 71-2 (1982) Guidelines. Principles of GLP were complied with.

Test species and methods:

Test material: ISO common name: dodine; IUPAC name: 1-dodecylguanidinium acetate; Technical substance: batch 92/88/2, purity 95.3%.

Test Animal: Test Animal: Mallard duck (*Anas platyrhynchos*); total number of birds used: 90 birds, not sexed (10 for each of the 6 treatment groups, 30 in 3 control groups).

Age of birds at start of treatment: 8 days.

Test design: Administration *ad libitum* with the diet for 5 days (free access to water) at dose levels 0 (control), 162.5, 325, 650, 1300, 2600 and 5200 mg dodine/kg diet. Control birds were fed the diet without test item.

Chemical analysis of the diet was performed in term of stability and homogeneity.

Study duration: 3 day pre-treatment period (Day -3 until Day -1); 5 day treatment period (Day 1 to Day 5) and 3 day post-treatment period (Day 6 to Day 9).

Observations of birds in main study included:

- Mortality: daily;

- Clinical signs: daily;

- Body weights: on Days -3, 0 (prior dosing), 5, 8 and 9;

- Food consumption: from day -3 to -1, from days 1 to 5 (daily), from days 6 to 8, day 9.

Pathology: at termination of the study, macroscopic examination was carried out on ten birds from the highest dose level groups.

Findings:

The chemical analysis of the diet proved homogeneity and accuracy. Found concentrations agreed with the nominal values within 4%. The overall dietary intake of test item is calculated in the following table:

Table B.9.1.2.2 - Calculation of the dietary intake by the mallard

Dose Group (mg as/kg diet)	Mean body weight over the 5 days period (g/bird)	Mean food consumption (g/bird/day)	Calculated dietary intake (mg as/kg bw/day)
Group 1/2/3:0	102	33	-
Group 4: 162.5	98.5	27	44
Group 5: 325	113	34	98
Group 6: 650	136	25	119
Group 7: 1300	111	18	211
Group 8: 2600	79.5	7	229
Group 9: 5200	62	5	419

Several mortalities occurred in the 2 highest dose groups. The results obtained are summarised in the table below:

Table B.9.1.2.3 - Dietary toxicity (5-day) of dodine in mallard

Dose level (mg/kg b.w.)	Toxicol. result (mort./clin. signs/total nº)	Onset on death on day
Males		
Group 1/2/3: 0	1/0/30	1
Group 4: 162.5	0/0/10	-
Group 5: 325	0/0/10	-
Group 6: 650	0/0/10	-
Group 7: 1300	0/10/10	-
Group 8: 2600	7/10/10	2-3-5-5-5-6-6
Group 9: 5200	10/10/10	2-2-3-3-3-3-4-5-6-6

There was evidence of a depression of food consumption and a decrease in body weight during the treatment period in group 6 to 9. From the group 7 onwards, the animals appeared subdued after administration or died.

From the animals that died, 3 from group 8 and 4 from group 9 were found to be emaciated during gross necropsy. No abnormalities were detected in any other birds which died. At termination, the three surviving birds in Group 8 and seven surviving birds in group 7 were examined macroscopically. No abnormalities were detected.

The **dietary LC50** value for dodine in the Mallard duck **was found to be 2263 mg/kg (which corresponds to 280 mg dodine/kg bw/day**) with 95% confidence limits of 1772 – 2890 mg/kg. The **5-day NOEL was found to be 325 mg/kg (which corresponds to 98 mg dodine/kg bw/day**), and on the basis of bodyweight and food consumption data, the 5-day LOEL was found to be 650 mg/kg (119 mg dodine/kg bw/day).

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

Dodecylguanidine acetate (dodine) technical grade: six-week dietary toxicity and reproduction pilot study in bobwhite quail.

Pedersen, C. A. & Mumper J. A. (1993a); Bio-Life Associates Ltd, USA, report # BLAL Nr 119-006-05.

Guidelines and GLP:

The study was conducted according to Range-finding based on US EPA FIFRA Subdivision 71-4 Guidelines. Principles of GLP were complied with.

Test species and methods:

This study was performed to determine treatment levels for a reproduction study and to assess the relative toxicity of Dodecylguanidine Acetate (dodine)

Test material: ISO common name: dodine; IUPAC name: 1-dodecylguanidinium acetate; Technical substance: Batch APA303/90: 94% (HPLC), 97.6 (titration).

Test Animal: Bobwhite quail (Colinus virginianus); total number of 60 birds were used (5 males and 5 females, paired, for each of the 5 treatment groups and the control group).

Age of birds at start of treatment: 49 weeks; weight: 220-300g, all birds from the same hatch.

Test design: Administration *ad libitum* with the diet for 6 weeks (free access to water) at dose levels 0 (control), 75, 150, 300, 750 and 1500 mg dodine/kg diet. Control birds were fed the diet without test item.

Chemical analysis of the diet was performed in terms of stability and homogeneity.

Study duration: 33 days pre-treatment period (acclimatation) 6 weeks treatment period (day 1 to 42).

Photoperiod during test period: 17 hours light / 7 hours dark

Observations of birds in the study included:

- Mortality: daily;
- Clinical signs: twice daily;
- Body weights: on Days 1 (prior dosing) then weekly;
- Food consumption: weekly.
- Egg collection: daily

Pathology: at test termination, macroscopic examination was carried out on 4 birds (2 males and 2 females) from each dose group and included: GI tract, liver, kidney, heart, reproductive organs, spleen, subcutaneous fat and muscle.

Statistics: body weight and feed consumption data using SPSS[3] software.

Findings:

The chemical analysis of the diet proved homogeneity and accuracy. Percent recoveries from the diets varied from 81 % to 125%. The overall dietary intake of test item is calculated in the following table:

Table B.9.1.3.1 - Calculation of the dietary intake by the bird

Dose Group (mg as/kg diet)	Mean body weight over the 6 weeks period (g/bird)	Mean food consumption (g/bird/day)	Calculated dietary intake (mg as/kg bw/day)
Group 1: 0	250	21.3	-
Group 2: 75	239	23.9	7.5
Group 3: 150	233	21.2	13.6
Group 4: 300	231	20.5	26.6
Group 5: 750	243	22	68.0
Group : 1500	223	20.1	135.2

One bird from the control group died on day 7. No other mortality was observed during the 6 weeks administration period. No statistically significant differences in body weights or feed consumption were noted. All feed consumption values in the test groups were considered to be normal.

During the pre-administration period, the total number of eggs produced per group ranged from 54 to 107. During the test period, the total number of eggs produced per group ranged from 86 to 166 (23.0%, 46.0%, 19.8%, 37.2%, 33.5%, 19.1 % increase for group 1 to 6 respectively). Egg production increases during the test period in the test groups were considered to be normal when compared to the control group.

Gross pathology examinations of 24 arbitrarily selected survivors on test day 42 revealed a mottled liver in one female at 750 ppm. No abnormal findings were noted for the other 23 birds that were examined.

The 6-week NOEL for bobwhite quail was found to be the highest dose tested: 1500 mg/kg (135 mg as/kg bw/day).

Dodecylguanidine acetate (dodine) technical grade: toxicity and reproduction study in bobwhite quail.

Pedersen, C. A., (1994a); Bio-Life Associates Ltd, USA, report # BLAL Nr 119-008-07.

Guidelines and GLP:

The study was conducted according to US EPA FIFRA Subdivision 71-4 (a) and OECD 206 Guidelines. Principles of GLP were complied with.

[3] SPSS – PC^{+TM}, Ver. 4.0, SPSS Inc., 444 N. Michigan Avenue, Chicago, IL 60611

Test species and methods:

Test material: ISO common name: dodine; IUPAC name: 1-dodecylguanidinium acetate; Technical substance: Batch APA303/90: 94% (HPLC), 97.6 (titration).

Test Animal: Bobwhite quail (Colinus virginianus); total number of 128 birds were used (16 males and 16 females, paired, for each of the 3 treatment groups and the control group).

Age of birds at start of treatment: 20 weeks, weight: 150-250g at study initiation, all birds from the same hatch, treated prophylactically with virginiamycin and wormed with Tramisol.

Test design: Administration *ad libitum* with the diet for 24 weeks (free access to water) at dose levels 0 (control), 200, 600 and 1000 mg dodine/kg diet. Control birds were fed the diet without test item.

Chemical analysis of the diet was performed in term of stability and homogeneity.

Study duration: 41 days pre-treatment period (quarantine); 24 weeks FO treatment period; 13 weeks F1 generation and 2 weeks growth period

Photoperiod during test period: 7 hours light / 17 hours dark until week 9; 17 hours light / 7 hours dark from week 9.

Observations of birds in the study included:

- Mortality and clinical signs: daily;

- Body weights: on Days 1 (prior dosing) then biweekly through test week 10 and at study termination;

- Food consumption: biweekly.

- Egg collection: daily, numbers of egg, numbers of normal eggs, number of abnormal egg, number of cracked and broken egg, number of defective egg were recorded.

- Hatchability after incubation: number of infertile egg, fertility and early death, embryo survival, number of midterm egg, number of full term egg, number hatched

- Eggshell thickness at equatorial circumference

- F1 generation: individual weight of chick on day 1 and 14, feed consumption for the first and second week after hatching, survivability, gross pathology of selected chicks

- Pathology: at termination of the study, macroscopic examination was carried out on all parent birds.

- Statistics: variance homogeneity, one way analysis of variance with Dunnett's test, non parametric analysis of variance.

Findings:

The chemical analysis of the diet proved homogeneity and accuracy. The average percent recoveries from the diets varied from 102.4% to 109.3% during test weeks 1, 5, 10, 15 and 20 for all dose groups. The overall dietary intake of test item is calculated in the following table:

Table B.9.1.3.2 - Calculation of the dietary intake by the F0 quails during reproduction study.

Dose Group (mg as/kg diet)	Mean body weight over the 24 weeks period (g/bird)	Mean food consumption (g/bird/day)	Calculated dietary intake (mg as/kg bw/day)
Group 1: 0	228	20.7	-
Group 2: 200	230	20.4	17.7
Group 3: 600	225	21.0	56.0
Group 4: 1000	220	21.0	95.5

F0 generation: a statistically significant difference in body weight was noted during test week 24 for the 1000 mg/kg females only. This finding was not considered to be treatment-related since it occurred during only one weighing interval out of seven.

Therefore, no significant differences were noted for body weight or food consumption in the F0 generation. Several mortalities were noted during the study and are summarized in the following table:

Table B.9.1.3.3 - Mortality of the F0 quails during the reproduction study

Dose Group (mg as/kg diet)	Number of males deaths (%)	Number of females deaths (%)	Mean (%)
Group 1: 0	1/16 (6.3%)	4/16 (25%)	16%
Group 2: 200	0/16 (0%)	0/16 (0%)	0%
Group 3: 600	2/16 (12.5%)	1/16 (6.3%)	10%
Group 4: 1000	1/16 (6.3%)	3/16 (18.8%)	13%

Although mortality in females in the control group and high dose group was relatively high, reassignment of surviving animals allowed remaining with at least 12 pens (according to the guideline minimal recommendation) without interruption of egg collections and incubation of those eggs. Due to the inconsistency and lack of dose-related gross pathological observations in birds found dead or sacrificed during the study or at termination, the gross pathologic observations noted were attributed to factors other than the test material.

F1 generation: The reproductive parameters are presented in the following table:

Table B.9.1.3.4 - Reproduction study in bobwhite quail - reproductive parameters

Reproductive Parameter	Group 1: 0 mg/kg	Group 2: 200 mg/kg	Group 3: 600 mg/kg	Group 4: 1000 mg/kg
Eggs laid per hen in 12 weeks	63 (± 24)	66 (± 18)	65 (± 11)	59 (± 22)
% Eggs cracked or broken of eggs laid	3 (± 3)	3 (± 4)	1 (± 2)	1 (± 1)
% viable of embryos of eggs set	95 (± 8)	98 (± 3)	89 (± 20)	93 (± 5)
% live 17-day embryos of viable embryos	99 (± 1)	99 (± 1)	99 (± 1)	99 (± 1)
% normal hatchlings of live 17-days embryos	87 (± 8)	88 (± 6)	88 (± 8)	91 (± 7)
% 14-day-old survivors of normal hatchlings	89 (± 8)	82 (± 9)	88 (± 7)	**79*** (± 16)
Mean body weight of 1-day-old survivors (g)	6.7 (± 0.5)	6.7 (± 0.4)	6.7 (± 0.4)	6.7 (± 0.4)
Mean body weight of 14-day-old survivors (g)	29.2 (± 2.3)	**26.4*** (± 2.3)	**27.3*** (± 2.1)	28.1 (± 1.9)
14-day-old survivors per hen	41 (± 19)	40 (± 11)	36 (± 18)	35 (± 13)
Mean shell thickness	0.231	0.229	0.229	0.236

* statistically significant (Dunnett's test; 95% confidence level)

Statistically significant differences in mean body weights for offspring were noted on day 14 for the 200 (low) and 600 mg as/kg (intermediate) dose group. However, since the high dose group (1000 mg as/kg) was not affected this finding was not considered treatment-related. No effects were seen on the feed consumption.

Statistically significant differences in survival rate for offspring were noted on day 14 in the high dose group only. A reduced survival rate was also observed in the low dose group even if not statistically significant. Considering that this is a rarely affected parameter and is probably more indicative of the conditions under which the chicks were reared in battery brooders than the chemical to which the adults were exposed it was concluded that this finding was not considered treatment-related.

2 young birds died during the 14 day period (1 in the low dose group and 1 in the intermediate dose group). No other clinical sign of toxicity or abnormal behaviour was noted in the offspring. Due to the lack of dose-related gross pathologic observations in birds sacrificed on day 14, the observations noted were not attributed to the test substance.

The 24-week reproductive NOEL on the bobwhite quail was established as the highest dose tested: 1000 mg/kg (95 mg as/kg bw/day).

Avian reproductive toxicity study with Dodecylguanidine acetate (dodine) technical in bobwhite quail.

Pedersen C. A. (1999). Bio-Life Associates Ltd, USA, report # BLAL Nr 108-029-07.

Guidelines and GLP:

The study was conducted according to US EPA Guideline 71-4 (a) and OECD 206 Guidelines. Principles of GLP were complied with.

Test species and methods:

Test material: ISO common name: dodine; IUPAC name: 1-dodecylguanidinium acetate; Technical substance: batch 3044, purity 96.7%.

Test Animal: Bobwhite quail (*Colinus virginianus*); total number of birds used: 128 birds (16 males and 16 females, paired, for each of the 3 treatment groups and the control group). Age of birds at start of treatment: 21 weeks, weight: approx. 200g at study initiation, all birds from the same hatch, treated prophylactically with amprolium and wormed with fenbendazole.

Test design: Administration *ad libitum* with the diet for 21 weeks (free access to water) at dose levels 0 (control), 75, 150 and 300 mg as/kg diet. Control birds were fed the diet without test item.

Chemical analysis of the diet was performed in term of stability and homogeneity.

Study duration: 23 days pre-treatment period (quarantine) 21 weeks F0 treatment period 11 weeks F1 generation and 2 weeks growth period.

Photoperiod during test period: 17 hours light / 7 hours dark.

Observations of birds in the study included:

- Mortality and clinical signs: daily;

- Body weights: on Days 1 (prior dosing) then biweekly through test week 10 and at study termination;

- Food consumption: weekly;

- Egg collection: daily, numbers of egg, numbers of normal eggs, number of abnormal egg, number of cracked and broken egg, number of defective egg were recorded;

- Hatchability after incubation: number of nonviable egg, viability and early death, embryo survival, number of midterm egg, number of full term egg, number of full term piped, number hatched;

- Eggshell thickness at equatorial circumference;

- F1 generation: individual weight of chick on day 1 and 14, feed consumption for the first and second week after hatching, survivability over 14 days, gross pathology of selected chicks;

Pathology: at termination of the study, macroscopic examination was carried out on all parent birds: muscle, liver and fat were frozen.

Statistics: Levene's median test, variance homogeneity, one way analysis of variance with Dunnett's test, non parametric analysis of variance, Kruskal-Wallis One-way analysis of variance.

Findings:

The chemical analysis of the diet proved homogeneity and accuracy. The percent recoveries from the diets varied from 83.8% to 133% of the nominal concentrations. The overall dietary intake of test item is calculated in the following table:

Table B.9.1.3.5 - Calculation of the dietary intake by the F0 quails during reproduction study.

Dose Group (mg as/kg diet)	Mean body weight over the 21 weeks period (g/bird)	Mean food consumption (g/bird/day)	Calculated dietary intake (mg as/kg bw/day)
Group 1: 0	212	19.1	-
Group 2: 75	213	19.5	6.9
Group 3: 150	214	19.7	13.8
Group 4: 300	214	19.3	27.1

F0 generation: a statistically significant difference in body weight was noted for the 150 and 300 mg/kg females during test week 21. This finding was not considered to be treatment-related since it occurred during only one weighing interval out of seven and consisted in an increase of weight. Additionally, a statistically significant difference in feed consumption was noted for the 150 mg/kg treatment group during test week 1 to 2 only. This finding was not considered to be treatment-related and the overall feed consumption values for all of the groups were comparable.

Therefore, no significant differences were noted for body weight or food consumption in the F0 generation.

Only 2 animals died during the study, one female in the control group on week 10 and one female in the low dose group on week 21. Due to the inconsistency and lack of dose-related gross pathological observations in birds found dead or sacrificed at termination, the gross pathologic observations noted were attributed to factors other than the test material.

F1 generation: The reproductive parameters are presented in the following table:

Table B.9.1.3.6 - Reproduction study in bobwhite quail - reproductive parameters

Reproductive Parameter	Group 1: 0 mg/kg	Group 2: 75 mg/kg	Group 3: 150 mg/kg	Group 4: 300 mg/kg
Eggs laid per hen in 10 weeks	45 (± 20)	51 (± 12)	45 (± 18)	59 (± 11)
Normal eggs laid per hen	42 (± 19)	48 (± 12)	43 (± 17)	46 (± 11)
% normal eggs of eggs laid	92 (± 13)	94 (± 8)	95 (± 6)	95 (± 10)
% eggs cracked or broken per hen	2 (± 2)	3 (± 3)	2 (± 3)	1 (± 1)
% eggs cracked or broken of eggs laid	8 (± 13)	5 (± 6)	4 (± 5)	2 (± 2)
% viable of embryos of eggs set	89 (± 23)	90 (± 21)	95 (± 8)	86 (± 26)
% live 18-day embryos of viable embryos	99 (± 1)	97 (± 5)	99 (± 1)	99 (± 3)
% normal hatchlings of eggs set	84 (± 23)	81 (± 22)	89 (± 9)	82 (± 27)
% normal hatchlings of viable embryos	94 (± 6)	88 (± 12)	94 (± 4)	94 (± 8)
% normal hatchlings of live 18-days embryos	95 (± 6)	91 (± 9)	95 (± 5)	95 (± 6)
14-day-old survivors per hen	32 (±17)	35 (±14)	36(± 15)	33 (± 14)
% 14-day-old survivors of eggs laid	67 (± 28)	68 (± 21)	79 (± 12)	68 (± 25)
% 14-day-old survivors of eggs set	74 (± 31)	78 (± 23)	87 (± 9)	76 (± 25)
% 14-day-old survivors of normal hatchlings	89 (± 26)	92 (± 17)	98 (± 3)	94 (± 10)
Mean body weight of 1-day-old survivors (g)	7.35 (± 0.39)	7.24 (± 0.46)	7.33 (± 0.49)	7.16 (± 0.44)
Mean body weight of 14-day-old survivors (g)	34.83 (± 1.64)	33.87 (± 2.43)	34.81 (± 2.31)	35.25 (± 1.7)
Mean shell thickness	0.241	0.243	0.241	0.244

No statistically significant differences were noted (Dunnett's test; 95% confidence level)

No statistically significant differences in the reproductive parameters, survivability, body weight or feed consumption of the offspring were noted in any of the treatment groups. No abnormal treatment-related gross observations were noted on the chicks that were found dead or sacrificed.

The 21-week reproductive NOEL was determined to be 300 mg as/kg (27 mg as/kg bw/day) (highest concentration tested) for parental systemic toxicity, reproductive effects and for the F1 generation in the bobwhite quail.

Dodecylguanidine acetate (Dodine) technical grade: six-week dietary toxicity and reproduction pilot study in mallard ducks.

Pedersen C.A. & Mumper J. A. (1993b). Bio-Life Associates Ltd, USA, report # BLAL Nr 119-007-06.

Guidelines and GLP:

The study was conducted according to US EPA Guideline 71-4. Principles of GLP were complied with.

Test species and methods:

Test material: ISO common name: dodine; IUPAC name: 1-dodecylguanidinium acetate; Technical substance: Batch APA303/90: 94% (HPLC), 97.6 (titration)

Test Animal: Mallard duck (*Anas platyrhynchos*); total number of birds used: 60 birds (5 males and 5 females, paired, for each of the 5 treatment groups and the control group).

Age of birds at start of treatment: 110 weeks, weight: 1000-1400g.

Test design: Administration *ad libitum* with the diet for 6 weeks (free access to water) at dose levels 0 (control), 75, 150, 300, 750, and 1500 mg as/kg diet. Control birds were fed the diet without test item.

Chemical analysis of the diet was performed in term of stability and homogeneity.

Study duration: 32 days pre-treatment period (quarantine) and 6 weeks treatment period (day 1 to 42).

Photoperiod during test period: 17 hours light / 7 hours dark

Observations of birds in the study included:

- Mortality: daily;

- Clinical signs: twice daily;

- Body weights: on Days 1 (prior dosing) then weekly;

- Food consumption: weekly;

- Egg collection: daily.

Pathology: at termination of the study, macroscopic examination as carried out on 4 birds (2 males and 2 females) from each dose group and on all animals that died during the study and included: GI tract, liver, kidney, heart, reproductive organs, spleen, subcutaneous fat and muscle.

Statistics: body weight and feed consumption data using one way analysis of variance.

Findings:

The chemical analysis of the diet proved homogeneity and accuracy. Percent recoveries from the diets varied from 81 % to 138.7%. The overall dietary intake of test item is calculated in the following Table:

Table B.9.1.3.8 - Calculation of the dietary intake by the ducks.

Dose Group (mg as/kg diet)	Mean body weight over the 6weeks period (g/bird)	Mean food consumption (g/bird/day)	Calculated dietary intake (mg as/kg bw/day)
Group 1: 0	1188	96.1	-
Group 2: 75	1234	90.3	5.5
Group 3: 150	1156	82.2	10.6
Group 4: 300	1183	95.4	24.2
Group 5: 750	1188	79.1	49.9
Group 6: 1500	939*	58.5*	93.4

* statistically significant difference (95% confidence interval)

Five birds died in the high dose group (1500 mg as/kg) between days 29 and 41. No other mortalities were observed during the 6 weeks administration period. Statistically significant differences in body weights or feed consumption were noted in males and females from the high dose group.

From test day 33 through day 37, at the highest dose level two birds appear lethargic and were found dead one by day 38 and the other by day 41.

During the pre-administration period, the total number of eggs produced per group ranged from 6 to 32. During the test period, the total number of eggs produced per group ranged from 1 to 59 (+67.8%, -64.3%, -16.7%, +48.7%, -96.9% and -75.0% percentage of eggs produced for group 1 to 6 respectively). The only groups that experienced an increase in percent production were the control and the Group 4. Since egg production was so sporadic, no real conclusions may be drawn from these data.

Gross pathology examinations of the five birds that died during the study revealed abnormal findings such as empty intestines and gizzards or emaciation. Additionally, gross pathology of 24 arbitrarily selected survivors on test day 42 revealed abnormal findings in 8 birds such as dilated

intestinal vessels or enlarged liver in two birds from group 4, dilated intestinal vessels or resorbed egg matter in two birds from group 5, presence of feathers in the gizzard or dilated intestinal vessels in 5 birds from group 6. No abnormal findings were noted for the other 16 birds that were examined.

The **6-week NOEL for mallard duck was found to be: 750 mg as/kg (50 mg as/kg bw/day)** based on adverse effects on body weight, feed consumption, clinical signs and survival noted at 1500 mg as/kg (94 mg as/kg bw/day).

Dodecylguanidine acetate (Dodine) technical grade: toxicity and reproduction study in mallard ducks.

Pedersen C.A. (1994b). Bio-Life Associates Ltd, USA, report # BLAL Nr 119-009-08.

Guidelines and GLP:

The study was conducted according to US EPA Guideline 71-4 (b). Principles of GLP were complied with.

Test species and methods:

Test material: ISO common name: dodine; IUPAC name: 1-dodecylguanidinium acetate; Technical substance: Batch APA303/90: 94% (HPLC), 97.6 (titration)

Test Animal: Mallard duck (*Anas platyrhynchos*); total number of birds used: 128 birds (16 males and 16 females, paired, for each of the 3 treatment groups and the control group).

Age of birds at start of treatment: 24 weeks old, weight: approx. 1200g at study initiation, all birds from the same hatch, treated prophylactically with P.A.Bacterin.

Test design: Administration *ad libitum* with the diet for 20 weeks (free access to water) at dose levels 0 (control), 200, 600 and 1000 mg as/kg diet. Control birds were fed the diet without test item.

Chemical analysis of the diet was performed in term of stability and homogeneity.

Study duration: 43 days pre-treatment period (quarantine); 20 weeks FO treatment period; 11 weeks F1 generation and 2 weeks growth period.

Photoperiod during test period: 7 hours light/17 hours' dark during quarantine and until test period week 8, 17 hours light / 7 hours dark from test period week 9.

Observations of birds in the study included:

- Mortality and clinical signs: daily;

- Body weights: on Days 1 (prior dosing) then biweekly through test week 10 and at study termination;

- Food consumption: bi-weekly;

- Egg collection: daily, numbers of egg, numbers of normal eggs, number of abnormal egg, number of cracked and broken egg, number of defective egg were recorded ,

- Hatchability after incubation: number of infertile egg, fertility and early death, embryo survival, number of midterm egg, number of full term egg, number hatched;

- Eggshell thickness at equatorial circumference;

- F1 generation: individual weight of chick on day 1 and 14, feed consumption for the first and second week after hatching, survivability over 14 days, gross pathology of selected chicks.

Pathology: at termination of the study, macroscopic examination was carried out on all parent birds and on all birds that died during the study.

Statistic analysis: Levene's test, variance homogeneity, one way analysis of variance with Dunnett's test, non parametric analysis of variance.

Findings:

The chemical analysis of the diet proved homogeneity and accuracy. The percent recoveries from the diets varied from 102.4% to 109.3% of the nominal concentrations. The overall dietary intake of test item is calculated in the following table:

Table B.9.1.3.8 - Calculation of the dietary intake by the F0 mallard duck during reproduction study.

Dose Group (mg as/kg diet)	Mean body weight over the 20 weeks period (g/bird)	Mean food consumption (g/bird/day)	Calculated dietary intake (mg as/kg bw/day)
Group 1: 0	1200	126.2	-
Group 2: 200	1204	120.4	20.0
Group 3: 600	1182	113.1*	57.4
Group 4: 1000	1061*	109.6*	103.3

* statistically significant difference (95% confidence interval)

F0 generation: a statistically significant difference in body weight was noted for the high dose group (1000 mg/kg) in males and females. This finding was considered to be treatment-related. A statistically significant difference in feed consumption was noted for the intermediate dose group (600 mg/kg) and the high dose group (1000 mg/kg). This finding was considered to be treatment-related.

Therefore, significant differences were noted for body weight and food consumption in the F0 generation.

Only 2 animals died during the study, one female in the intermediate dose group on week 20 and one male in the high dose group on week 11. Due to the inconsistency and lack of dose-related gross pathological observations in birds found dead or sacrificed at termination, the gross pathologic observations noted were attributed to factors other than the test material.

F1 generation: The reproductive parameters are presented in the following table:

Table B.9.1.3.9 - Reproduction study in mallard duck - reproductive parameters

Reproductive Parameter	Group 1: 0 mg/kg	Group 2: 200 mg/kg	Group 3: 600 mg/kg	Group 4: 1000 mg/kg
Eggs laid per hen in 10 weeks	43 (± 15)	40 (± 19)	**22*** (± 15)	**6*** (± 8)
Eggs laid per hen per day	0.60 (± 0.20)	0.55 (± 0.27)	**0.31*** (± 0.21)	**0.08*** (± 0.11)
% eggs cracked or broken of eggs laid	4 (± 8)	2 (± 2)	6 (± 7)	32 (± 40)
% viable of embryos of eggs set	92 (± 10)	88 (± 21)	**72*** (± 24)	**64*** (± 29)
% live 21-day embryos of viable embryos	91 (± 10)	86 (± 25)	**57*** (± 23)	**69*** (± 25)
% normal hatchlings of live 21-days embryos	84 (± 17)	86 (± 8)	**49*** (± 34)	**81*** (± 26)
14-day-old survivors per hen	26 (±10)	25 (±14)	5 (± 6)	1 (± 2)
% 14-day-old survivors of eggs set	70 (± 17)	66 (± 25)	**24*** (± 22)	**31*** (± 19)
% 14-day-old survivors of normal hatchlings	99 (± 2)	99 (± 2)	100 (± 0)	100 (± 0)
Mean body weight of 1-day-old survivors (g)	36.2 (± 2.5)	34.6 (± 3.6)	**29.2*** (± 3.9)	**28*** (± 4.2)
Mean body weight of 14-day-old survivors (g)	261.6 (± 18.5)	266.8 (± 18.9)	265.2 (± 26.9)	237.7 (± 66.7)
Mean shell thickness	0.391	0.402	0.67	**0.311***

* Statistically significant (Dunnett's test; 95% confidence level)

Statistically significant differences were observed on several reproductive parameters from the intermediate (600 mg/kg) and high dose group (1000 mg/kg) and were clearly treatment-related.

Mean body weight of hatchlings from the intermediate and the high dose group was significantly lower than the control group at day 1 but not at day 14. No abnormal behavioural reactions or clinical signs of toxicity were noted in any of the hatches. Gross pathology in birds sacrificed at day 14 did not reveal any treatment-related abnormalities.

The **20-week reproductive NOEL was determined to be 200 mg/**kg (20 mg as/kg bw/day) for parental systemic toxicity, reproductive effects and for the F1 generation in the mallard duck. **The LOEL was 600 mg/kg (57.4 mg as/kg bw/day).**

B.9.1.4 Supervised cage or field trials (Annex IIIA 10.1.2)

No data presented and data for this point are not required.

B.9.1.5 Acceptance of bait, granules or treated seeds by birds (palatability test) (Annex IIIA 10.1.3)

Dodine as the plant protection product "SYLLIT SC" is intended for spray application and therefore data for this point are not required.

B.9.1.6 Effects of secondary poisoning (Annex IIIA 10.1.4)

In accordance with the Guidance Document on Risk Assessment for Birds and Mammals, substances with a log P_{ow} greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains. Since log P_{ow} of dodine was less than 3 (log P_{ow} = 0.9) was not necessary to address the risk of dodine to earthworm and fish-eating birds and biomagnification in terrestrial food chains following the proposed use of SYLLIT SC.

B.9.1.7 Supplementary studies to birds and mammals(Annex IIIA 10.1.5)

A study to evaluate residues of dodine 400 SC in *Tenebrio molitor* L. as feed source for wild birds and mammals following an application.

Hirth, N. (2005). GAB Biotechnologie GmH & GAB Analytik GmbH, Niefern-Öschelbfronn, Germany report Nº 20051278/01-NHTm.

Guidelines and GLP:

The study was conducted according to SANCO 4145/2000 and SANCO 3029/99-rev:4/2000 and EC 9188/VI/97. Principles of GLP were complied with.

Test species and methods:

Dodine, formulated as Dodine 400 SC (= Syllit 400 SC): formulation batch 164503, content: 400.0 g/L dodine, were used to assess the amount of residues of Dodine 400 SC in larvae and beetles of *Tenebrio molitor* following an application onto the test organisms.

The actual applied rate of Dodine was 1.9 L/ha in a spray volume of 200 L/ha and 3.8 L/ha in a water volume of 400 L/ha. A control with test organisms exposed to untreated quartz sand was also performed. For application the test organisms were randomly exposed under bean plants (*Vicia faba*) and on quartz sand without bean plants. Directly after application the treated living test organisms were collected from the exposure units to determine the amounts of Dodine 400 SC on larvae and beetles. Larvae and beetles exposed under bean plants were used only for determination of the initial (C_0).

After an ageing period of 1, 2, 4, 8 and 16 days test organisms, larvae and beetles exposed on quartz sand units, were sampled. Ageing period was carried out under semi-field conditions at the outside area of the testing facility (rain protected under an UV-permeable roof).

For each sampling, living beetles/larvae were collected from quartz sand and were kept deep-frozen (≤ -18ºC) until analysis. Each sampling comprised four replicates of ≥ 2.0 g biomass.

Calibration functions were calculated and plotted by linear regression using external standards. The correlation coefficients of the calibration curves were above 0.9984. Evaluation of the final extracts was performed against the regression curve.

Findings:

Dodine residues were determined using methodology validated at PTRL Europe in the course of this study. Acceptable recoveries were obtained for fortified control larvae and beetle specimens processed concurrently with the field specimens.

Average residue data for dodine in larvae and beetles specimens are summarised in the following table.

Table B.9.1.7 – Summary of residue results.

Sampling date	Treatment	Type	Mean residue (n = 4) mg/kg	Type	Mean residue (n = 4) mg/kg
0	Control	Larvae	< LOQ	Beetle	< LOQ
0	T1	Larvae	29*	Beetle	34*
0	T2	Larvae	23	Beetle	23*
1	T2	Larvae	10	Beetle	8.7
2	T2	Larvae	9.6	Beetle	8.0
4	T2	Larvae	8.1	Beetle	6.7
8	T2	Larvae	5.9	Beetle	5.0
16	T2	Larvae	1.3	Beetle	4.3

T1: Larvae or beetle exposed under bean plants; T2: Larvae or beetle exposed on quartz sand; LOQ = 0.3 mg/kg.

***** This value is determine for double applied field rate (3.8 L/ha), which is not in accordance with the study plan

The initial concentration C_0 for larvae and beetles was 23 mg/kg, when applied directly on quartz sand.

The initial concentration C_0 for larvae and beetles when exposed under bean plants during application was 29 mg/kg and 34 mg/k, respectively. However, this value did not reflect the initial concentration for field rate. Due to a mistake at the application and preparation of the spray solution a double concentration of the field rate (3.8 L/ha) was applied in T1.

Based on the data of the analytical part the 1st order kinetic Model DT_{50} and DT_{90} of dodine could be determined to be 2.6 and 8.64 days for larvae and 1.55 and 5.14 days for beetles, respectively. The coefficient of variance was $r^2 = 0.809$ for larvae and $r^2 = 0.819$ for beetle which are higher than 0.7.

In conclusion, no dodine (< LOQ) was observed in untreated control specimens of beetle and larvae obtained from the semi-field study. Dodine residues in treated beetle and larvae specimens showed a decline over the sampling period (0, 1, 2, 4, 8 and 16 DAA).

The initial concentration C_0 for larvae and beetles, when applied directly on quartz sand was 23 mg/kg.

B.9.1.8 Summary of effects on birds

The results of acute, short-term and long-term studies conducted with dodine technical are summarised in Table B.9.1.8.1

The acute LD_{50} values for species tested were between 857 and 981 mg/kg. The lowest value was 857 mg/kg obtained for Mallard duck (Hakin, 1990b) and the highest being 981 mg/kg obtained for bobwhite quail (Hakin, 1990a).

Two dietary LC_{50} studies have been reported. Those studies were conducted with mallard duck and bobwhite quail indicated LC_{50} values of 2263 and 5200 ppm respectively.

The lowest NOEL values for reproduction studies conducted with mallard duck and bobwhite quail were 200 and 300 ppm respectively.

Table B.9.1.8.1 - Summary of toxicological endpoints on birds dosed with dodine technical

Species	Study type	Findings	GLP status	Reference
Bobwhite quail	Acute oral LD_{50}	21-day LD_{50} 981 mg/kg	GLP	Hakin, 1990a
Mallard duck	Acute oral LD_{50}	21-day LD_{50} 857 mg/kg	GLP	Hakin, 1990b
Bobwhite quail	Sub-chronic LC_{50}	8 day LC_{50} > 5200 ppm (976 mg as/kg/day)	GLP	Hakin, 1990a
Mallard duck	Sub-chronic LC_{50}	8 day LC_{50} 2263 ppm (280 mg as/kg/day)	GLP	Hakin, 1990b
Bobwhite quail	Sub-chronic (Range finding)	6-week NOEL 1500 ppm (135 mg as/kg/day)	GLP	Peterson & Mumper, 1993a
Bobwhite quail	Reproduction	24-week NOEL reproduction 1000 ppm (95 mg as/kg/day)	GLP	Peterson, 1994a
Bobwhite quail	Reproduction	21-week NOEL reproduction 300 ppm (27 mg as/kg/day)	GLP	Peterson, 1999
Mallard duck	Sub-chronic (Range finding)	6-week NOEL 750 ppm (50 mg as/kg/day)	GLP	Peterson & Mumper, 1993b
Mallard duck	Reproduction	20-week NOEL reproduction 200 ppm (20 mg as/kg/day)	GLP	Peterson, 1994b

For the refinement of the risk assessment for birds a residue study of dodine in insects -*Tenebrio molitor* L. has been presented.

Based on the data of the analytical part the DT_{50} of dodine could be determined to be 2.6 days for larvae and 1.55 days for beetles, according to 1st order kinetic Model and the initial concentration C_0 for larvae and beetles, when applied directly on quartz sand was 23 mg/kg.

B.9.1.9 Exposure and Risk Assessment to Birds (Annex IIIA 10.1)

Avian risk assessment is a sequential process, initially the acute short- and long-term toxicity/exposure ratios (TER_a TER_{st} and TER_{lt}) have been calculated using a worst case scenario.

Toxicity Exposure Ratio (TER) and Estimated Theoretical Exposure (ETE) were calculated based on the methods, i.e. equations and tabulated values described in the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (Sanco/4145/2000 of 25 September 2002).

A realistic approach will take into account real food ingestion of birds in orchards and for this scenario an insectivorous bird (a 10g wren) will be considered as the standard species. Exposure of birds to dodine contained in the plant protection product SYLLIT 400 SC is considered to arise mainly from feeding dodine-contaminated insects present in treated orchards.

The scenarios considered for the Risk Assessment included the consumption of contaminated food (insects) and the uptake of contaminated water. The risk assessments were conducted in a stepwise approach in accordance with the model proposed in the document "Guidance document on risk assessment for birds and mammals[4]".

The key toxicological endpoints used for the Risk Assessment Calculation are the following:

Table B.9.1.9.1 - Key toxicological endpoints

More sensitive specie	Endpoint	Reference
Mallard duck	21-day LD50 = 857 mg as/kg	Hakin, 1990b
Mallard duck	8-day LC50 = 2263 ppm corresponding to 280 mg as/kg bw/day	Hakin, 1990b
Mallard duck	20-week NOEL = 200 ppm corresponding to 20 mg as/kg bw	Peterson, 1994

The worst-case scenario is as follows: peach, 5 x 900 g as/ha with 7 days interval between applications.

4 SANCO/4145/2000 "Guidance document on risk assessment for birds and mammals under Council Directive 91/414/EEC", Draft Working Document, September 25th 2002.

Table B.9.1.9.2 - Calculated 1st Tier TER values for acute, short-term, subchronic and reproductive risk for birds.

Type	Indicator Specie	Scenario	FIR/bw	RUD	f_{twa}	AR	PT	C	ETE	Toxicity	TER
Tier 1											
Acute	Insectivorous bird (passerines)	Small insects	1.04	52	1	0.9	1	46.8	48.7	857	17.6
Short-term			1.04	29	1	0.9	1	26.1	27.1	280	10.3
Long-term			1.04	29	1	0.9	1	26.1	27.1	20	**0.7**

FIR = Food intake rate; bw = Bodyweight; RUD = Residue per unit dose; Ftwa = Time weighted average factor; AR = Application rate; PT = Fraction of diet obtained in treated area; C = Concentration in diet = RUD * AR; ETE = Estimated theoretical exposure = C*FIR/bw*Ftwa*PT; TER = Toxicity exposure ratio.

The resulting TER values for acute, short- and long-term sub-chronic exposures were respectively 17.6, 10.3 and **0.7**. The TER_{lt} are below the trigger value of 5 for long-term toxicity. In conclusion, the 1st risk assessment indicates an unacceptable risk to birds from long-term exposure.

Refinement of the long-term exposure is possible by using a lower PT value. On a long-term basis, a PT value of 1 is not realistic since birds will gather their food also in untreated areas. With reference Crocker et al. (1998) with a PT value of 0.61 for small insectivorous bird (blue tits) which spends less than 61 % of their foraging time among orchards. With a more realistic PT = 0.61, the TER_{lt} = **1.2** (ETE = 27.1 x 0.61 = 16.5) which is however still below the trigger value of 5.

Further refinement is needed. According to SANCO/4145/2000 (25/09/2006), the long-term exposure assessment employs time-weighted-average residues rather than initial residues and if data show that the DT50 is shorter than 10 days which is used as a default value in tier 1 then f_{twa} should be recalculated.

Taking into account the degradation of dodine in insects described in the residue study (Hirth, N., 2005), the a DT50 of 2.6 days for larvae of *T. molitor* sprayed on sand at the normal field application rate without plant cover could be considered to refine f_{twa} factor.

Based on these results, the twa factor could be refined as follows:

$f_{twa} = (1-e^{-kt})/kt$

$k = \ln 2/DT50$

t = average time

With a DT50 in insects of 2.6 days and a default averaging time of 21 days for long term exposure, the refined twa factor is 0.178.

Considering PT and f_{twa} refinement the ETElt will be 2.95 (= 1.04 x 26.1 x 0.178 x 1 x 0.61 = 2.95) and the consequent TERlt = 6.78 which is higher than the trigger value of 5.

Based on the assessment described above, it can therefore be concluded that the **risk to insectivorous birds from the use of dodine is acceptable**.

Exposure via Drinking Water

Birds that frequent open water bodies can also be exposed to dodine via drinking water, if they ingest residues of a.s. that reach water for example via spray drift from treated fields. The exposure concentration in this case is equal to PECsw, obtained from the environmental fate section (B.8.6.1), regarding the worst case application scenario, i.e. 5 early applications of 900 g a.s/ha in orchards, with 7 days interval and interception of 40% (average crop cover).

For the estimation of exposure via drinking water (SANCO/4145/2000) it was considered that birds obtain all their water demand directly from puddles with contaminated water. Table B.9.1.8.5 summarises the evaluation of exposure via drinking water.

Table B.9.1.9.3 – Daily Water Intake by different types of birds as a function of their body weight and TERa for different types of birds consuming contaminated water.

Bird Type	Approx. body weight (kg)	Total water ingestion rate (l/day)	PECsw (µg/l)	DWI (dm^3/day)	TERa
			orchards	orchards	orchards
large bird (e.g. goose)	3.0	0.123	87.64	$3.6x10^{-3}$	$>10x10^4$
medium bird (e.g. duck)	1.0	0.059		$5.2x10^{-3}$	
medium bird (e.g. pigeon)	0.30	0.026		$7.8x10^{-3}$	
small bird (e.g. wren, tit)	0.010	0.003		$23.6x10^{-3}$	

The resulting TER values for acute exposures were for all bird types greater than 10^4. These TERs are all higher the trigger value of 10 for acute toxicity. The conclusion is that all results indicate an acceptable risk to birds expose to contaminate water via drinking water.

B.9.2 Effects on aquatic organisms (fish, aquatic invertebrates, algae) (Annex IIA 8.2; Annex IIIA 10.2)

B.9.2.1 Acute Toxicity of the active substance and metabolites, degradation or reaction products to fish (Annex IIA 8.2.1)

Dodine: Determination of acute toxicity (LC50) to rainbow trout (96h, semi-static) (EPA)

Caley, C. Y., Cameron, B. D., Chapleo, S. and Knight, B. (1990a). Inveresk Research Int., UK; unpublished report # 7068

Guidelines and GLP:

The study was conducted according to OECD Guideline No 203 (1984) and EPA FIFRA 72-1 (1982). Principles of GLP were complied with.

Test species and methods:

Technical substance: batch 92/88/2, purity 95.3%; Test animal: Rainbow trout (*Oncorhynchus mykiss*) from Cloan Hatchery, Auchterarder; mean length: 4.3 ± 0.17 cm, mean weight: 1.045 ± 0.106 g.

The study was performed over 96h at 5 dose groups (control, 0.625, 1.25, 2.5, 5 and 10 mg as/L) with 20 fish each. No toxic reference was used. Test solutions with test item were daily renewed. The test system consist of a photoperiod of 16 hours per day, continuous aeration, temperature of 14°C and no feeding 24 hours prior to test start.

Sampling and analysis of test item concentration occurred at start 0 h, 24 h (before 1st renewal), 72h (0h after 3rd renewal) and 96h (24h after 3rd renewal).

Measurements and recordings/observations: mortality/other, sublethal effects at 3, 6, 24, 48, 72 and 96 hours.

Statistic analysis: The LC50 values at 24, 48, 72 and 96 h were determined using an arcsine transformation[5] as the data did not fit the probit model sufficiently well to calculate 95% confidence limits. Results were calculated using means of the measured concentrations at 0 h and 24 h for the first and last batches of tanks prepared, since the measured concentrations of dodine fell out with ± 20% of nominal in several cases.

Findings:

During the study, no mortalities occurred in the control. Actual concentrations were below the acceptable ±20% of nominal.

Recovery from quality control samples varied between 95.8% and 117% confirming the validity of the analytical method used.

The mortality data of the test with dodine are presented in the following table:

Table B.9.2.1.1 - Incidence and total mortality of trout exposed to dodine.

Dose Group (mg as/L) nominal/actual	Nº of fish	Cumulative mortality						Total mortality (%)
		3h	6h	24h	48h	72h	96h	
0	20	0	0	0	0	0	0	0
0.625/0.34	20	0	0	0	0	0	0	0
1.25/0.79	20	0	0	0	2	2	2	10
2.5/1.66	20	0	0	0	0	2	3	15
5/2.50	20	0	0	5	15	19	20	100
10/6.29	20	0	0	20	20	20	20	100

At 96h, all fish were dead at 5 and 10 mg/L, several fish were darkened in appearance at 2.5mg/L. No other unusual characteristics were observed during the test.

The LC_{50} of dodine for rainbow trout in a 96h-exposure test was shown to be 1.5 mg/L with 95% confidence limits of 0.882 and 2.616 mg/L. The value of NOEC was 0.335 mg/L.

Dodine: Determination of acute toxicity (LC50) to bluegill sunfish (96h, semi-static) (EPA)

Caley, C. Y., Cameron, B. D., Chapleo, S. and Knight, B. (1990b). Inveresk Research Int., UK; unpublished report # 7071

Guidelines and GLP:

The study was conducted according to OECD Guideline No 203 (1984) and EPA FIFRA 72-1 (1982). Principles of GLP were complied with.

Test species and methods:

Technical substance: batch 92/88/2, purity 95.3%; Test animal: Bluegill sunfish (*Lepomis macrochirus*) from Monkfield aquatics, Cambridge; mean length: 4.4 ± 0.5 cm, mean weight: 2.296 ± 0.855 g.

The study was performed over 96h at 5 dose groups (control, 0.625, 1.25, 2.5, 5 and 10 mg/L) with 20 fish each. A dosage group in the study consisted of 2 x 10 fish per 20L of medium (tap water) each. No toxic reference was used.

[5] Debanne S M, Haller H S {1985), Toxicology and Applied Pharmacology, **79**, 274-282.

The test system consisted of a photoperiod of 16 hours per day, continuous aeration, temperature of 22°C and no feeding 24 hours prior to test start.

Sampling and analysis of test item concentration occur at start 0 h, 24 h (before 1st renewal), 72h (0h after 3rd renewal) and 96h (24h after 3rd renewal).

Measurements and recordings/observations: mortality/other, sublethal effects at 3, 6, 24, 48, 72 and 96 hours in all vessels.

Statistic analysis: The LC50 values at 96 h were determined using probit analysis[6]. Where possible LC50 values were determined at other time points. Results were calculated using means of the measured concentrations at 0 h and 24 h for the first and last batches of tanks prepared, since the measured concentrations of dodine fell out with ± 20% of nominal in several cases.

Findings:

During the study, no mortalities occurred in the control. Actual concentrations were below the acceptable ± 20% of nominal.

Recovery from quality control samples varied between 97.8% and 109.6% confirming the validity of the analytical method used.

The mortality data of the test with dodine are presented in the following table:

Table B.9.2.1.2 - Incidence and total mortality of bluegill exposed to dodine.

Dose Group (mg as/L) nominal/actual	Nº of fish	Cumulative mortality						Total mortality (%)
		3h	6h	24h	48h	72h	96h	
0	20	0	0	0	0	0	0	0
0.625/0.49	20	0	0	1	1	1	2	10
1.25/1.13	20	0	1	14	17	18	19	95
2.5/1.65	20	4	17	20	20	20	20	100
5/3.33	20	20	20	20	20	20	20	100
10/7.09	20	20	20	20	20	20	20	100

At 10 mg/L, the test material was visible in suspension in water showing that the solubility limit was reached. At 96h, all fish were dead at 2.5, 5 and 10 mg/L and 95% were dead at 1.25 mg/L, several fish were noted to have red `blood-like' regions at the base of the dorsal fin at 72h at 0.625 mg/L. No other unusual characteristics were observed during the test.

The LC_{50} of dodine for bluegill sunfish in a 96h-exposure test was shown to be 0.7 mg/L. with 95% confidence limits of 0.589 and 0.842 mg/L.

Dodine technical: Acute toxicity to sheepshead minnow (*Cyprinodon variegates*) under flow-through conditions.

Bettencourt, C. M. J. (1992). Springborn Lab., USA; unpublished Report # 92-9-4416

[6] Finney, D.J. (1971), 'Probit Analysis', 3rd Edition, Cambridge University Press.

Berkson, J. (1953), Journal of American Statistical Association, **48**, 565-599.

Guidelines and GLP:

The study was conducted according to US EPA FIBRA 72-3 Guidelines. Principles of GLP were complied with.

Test species and methods:

Technical substance: batch 303/90, purity 94.07%, radiolabelled dodine: batch 920511, >99%

Test animal: Sheepshead minnow (*Cyprinodon variegatus*) from SP Inc., Salem, Massachusetts; mean length: 2.4 cm, mean weight: 0.31 g.

The study was performed over 96h at the same 5 dose groups (solvent control (methanol), control, 0.97, 1.6, 2.7, 4.5 and 7.5 mg as/L) with 20 fish each. A dosage group in the study consisted of 2 x 10 fish per 11 L of medium (seawater) each. No toxic reference was used.

Test solutions with test item were continuously renewed (flow-through design, 50m1/minute corresponding to 6.5 volume replacements per aquarium every 24hours).

The test system consisted of a photoperiod of 16 hours per day, no aeration, temperature of 22°C and no feeding 48 hours prior to test start.

Sampling and analysis of test item concentration by LSC occur at start 0 h and at the end (96h) of the study.

Measurements and recordings/observations: mortality/other, sublethal effects at 3, 6, 24, 48, 72 and 96 hours in all vessels.

Statistic analysis: The mean measured concentrations tested (based on 0- and 96-hour analyses) and the corresponding mortality data derived from the definitive toxicity test were used to estimate the LC50 and 95% confidence intervals at each 24-hour interval of the exposure period. If at least one test concentration caused mortality of greater than or equal to 50% of the test population, then a computer program (Stephan[7], 1977,1982) was used to calculate the LC50 values and 95% confidence intervals.

Three statistical methods were available in the computer program: moving average angle analysis, probit analysis, and nonlinear interpolation with 95% confidence intervals calculated by binomial probability. Moving average angle and probit analyses yield statistically sound results only if at least two concentrations produce a mortality of between 0 and 100% of the test organism population. The selection of reported LC50 values and 95% confidence intervals was based upon an examination of the data base and the results of the computer analysis. Selection criteria included the establishment of a concentration-effect relationship (mortality), the number of concentrations causing partial responses, and the span of responses bracketing the LC50 value. If two or more statistical methods produced acceptable results, then the method which yielded the smallest 95% confidence interval was selected. The No Observed Effect Concentration (NOEC) during the 96-hour exposure period was also determined. The NOEC is defined as the highest concentration tested at and below which there were no toxicant-related mortalities or physical and behavioral abnormalities (e.g.; lethargy, loss of equilibrium, darkened pigmentation), with respect to the control organisms.

Findings:

[7] Stephan, Charles. 1977. U.S. EPA; Environmental Research Laboratory, Duluth, Minnesota. Personal communication.

Stephan, Charles. 1982. U.S. EPA, Environ~nental Research Laboratory, Duluth, Minnesota. Personal communication to Dr. Lowell Bahner, Chairrnan ASTM Task Group on Calculating LC50's.

Analysis of the stock solution (75 g as/L) by LSC at 0 and 96h resulted in mean measured concentrations of 73.5 and 78.7 g as/L, respectively. Analysis of the same stock by HPLC-RAM established measured concentration of 65.9 and 77.8 g as/L for the 0 and 96 hours intervals respectively. Comparison of these results demonstrated relatively good agreement between the two types of measurements. HPLC-RAM analysis indicated that the C14-measurements were a reliable indication of the concentration of parent dodine present in the exposure aquarium.

During the study, no mortalities occurred in the solvent control, the control and in the actual 1.4, 2.1 and 2.9 mg as/L dose groups. Darkened pigmentation was observed in the actual 1.4, 2.1 and 2.9 mg as/L dose group. At 96h, 100% mortality was reached in the actual 4.8 and 7.0 mg as/L dose groups.

The mortality data of the test with dodine are presented in the following table:

Table B.9.2.1.3 - Incidence and total mortality of sheepshead minnow exposed to dodine.

Dose Group (mg as/L) nominal/actual	Nº of fish	Cumulative mortality (%)			
		24h	48h	72h	96h
Solvent control	20	0	0	0	0
0	20	0	0	0	0
0.97/1.4*	20	0	0	0	0
1.6/2.1*	20	0	0	0	0
2.7/2.9*	20	0	0	0	0
4.5/4.8*	20	35	75	100	100
7.5/7.0*	20	65	100	100	100

* C14-dodine analysed by LSC

The LC_{50} of dodine for sheepshead minnow in a 96h-exposure test was shown to be 3.7 mg as/L seawater g/L with 95% confidence limits of 2.9 and 4.8 mg/L. The NOEC was determined to be 1.4 mg as/L.

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

Dodine technical: the toxicity to fathead minnow (*Pimephales promelas*) during an early life stage exposure.

Sousa, J. V., (1995). Springborn Lab, USA unpublished report # 95-10-6126.

Guidelines and GLP:

The study was conducted according to US EPA FIFRA 72-4 (1982) Guidelines. Principles of GLP were complied with.

Test species and methods:

Technical substance: batch DA717, purity 98.6%, radiolabelled dodine: batch 950214, >99%

Test animal: Fathead Minnow (*Pimephales promelas*), eggs obtained from own brood stock at Springborn Lab/USA; mortality of the adult brood fish was <0.5% during the 14-day period prior to study initiation.

The test was performed at nominal concentrations of 22, 44, 87, 170 and 350 µg as/L, a control group, a dilution water control and a solvent control containing methanol (0.01 ml/L).

The test item was continuously freshly dosed by means of a flow-through design which allowed renewal of 6.3 aquarium volumes per 24 hours. A dosage group consisted of 2 replicates of each 60

eggs (<24h old) per incubation cup, with a minimum of 40 viable embryos per replicate transferred to each test vessel of 15L (medium: tap water), max of 0.5 grams fish/l/24h.

The test system consisted of a photoperiod of 16 hours per day, with continuous aeration from day 20 and temperature of 25°C. Fish were feed *ad libitum* three times daily with nauplii (*Artemia salina*) twice daily on weekend, stopped 24h before study termination

Sampling and analysis of test item concentration by LSC occur on days 0, 5, 12, 19, 26, 33 and 35 in all treatment vessels and additionally by HPLC-RAM in the highest dose group to verify the identity of the radiolabelled compound.

Measurements and recordings/observations:

- Eggs: number of live and dead daily, hatchability;

- Hatchlings: number of live, deformed, dead twice weekly, behaviour, appearance. Fish size and length: all surviving fish at termination (day 30 post hatch).

- Data handling: chemical analytical method is shown to be appropriate and validated; embryo hatching success, percentage of embryos that produced live normal fry, total length and weight were subject to statistical analysis.

Statistical analysis: Analyses were performed using the mean organism response in each treatment group rather than individual response values. All statistical analyses were conducted at the 95% level of certainty except in the case of Shapiro-Wilks and Bartlett's Tests (Weber[8], *et al*., 1989), in which the 99% level of certainty was applied. The 99% level of certainty is preferred for qualifying tests. The following procedures were used:

1) Significant differences in the percent survival were determined after transformation (e.g. arcsine square-root percentage) of the data.

2) Student's T-test (Sokal and Rohlf,[9] 1981) was conducted for each endpoint (survival at hatch, larval survival and larval growth) to compare the performance of the dilution water control organisms with that of the solvent control. For this study, analyses demonstrated no significant difference between dilution water control and solvent control data for all endpoints, therefore, all subsequent statistical analyses for these parameters were performed using pooled (control and solvent control) data.

3) As a check on the normality, data for each endpoint were analyzed using the Chi Square test (Horning and Weber[10], 1985) and Shapiro-Wilks Test.

4) As a check on the assumption of homogeneity of variance, implicit in parametric statistics, data for each endpoint were analyzed using Bartlett's Test (Horning and Weber, 1985).

5) For each endpoint, the performance of organisms exposed to each treatment level of the test material was compared with the performance of the pooled control or solvent control data using the William's test (Williams[11], 1971, 1972).

[8] Weber, C.I. et al. 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 2nd edition. EPA/600/4-89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

[9] Sokal, R.R. and F.J. Rohlf. 1981. Biometry. 2nd Edition. W.H. Freeman and Company, New York. 859 pp.

[10] Homing, W.B. and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-85/014. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.

6) Organism survival at hatch and larval survival data were analyzed before larval growth (length and weight); treatment levels which caused significant survival effects were excluded from further statistical analysis for treatment effects on growth.

A computer program (Gulley[12], et al, 1994) was used to perform the statistical computations. The theoretical threshold concentration expected to produce no deleterious effects at the 95% level of certainty was estimated as the Maximum-Acceptable-Toxicant Concentration (MATC). The MATC is equal to the geometric mean of the limits set by the lowest mean between LOEC (Lowest-Observed-Effect Concentration) and NOEC (No-Observed-Effect Concentration) the highest mean measured test concentration that showed no statistically significant difference between the exposed organisms and the control Based on these data, the MATC was estimated. Determination of these levels is based on the most sensitive of the performance criteria evaluated (e.g., organism survival at hatch, larval survival and growth at study termination).

Findings:

The results of the measurements made during this study established that water quality parameters maintained throughout the 35-day exposure remained within acceptable ranges for the promotion of fathead minnow embryo hatchability, larval survival and growth. The results obtained prove the concentrations at the sampling points to be within a range of 114-124% of nominal.

HPLC-RAM analyses of the highest treatment level defined the mean measured concentration to be 90% of the nominal which was in agreement with the measured concentrations by LSC. It was therefore determined that the C14-residues used to quantify the exposure concentrations of dodine were associated with the parent material. It was concluded that exposure conditions, as defined by the LSC analysis, accurately represented the concentration of parent material.

Survival at hatch and survival, total length and wet weight of fathead minnow larvae after 30-day exposure to dodine technical are given in the following table:

Table B.9.2.2.1 - Survival at hatch and survival, total length and wet weight of fathead minnow larvae after 30-day exposure to dodine technical

	Nominal/Mesured concentration of dodine (µg/L)					
	Pooled control	22/26	44/55	87/99	170/200	350/400
Survival at hatch (%)	84	83	76	73	83	75
Survival after 30-day exposure (%)	91	94	94	96	94	40*
Mean total length (mm)	31.5	31.7	31.3	31.2	30.1*	25.0**
Mean wet weight (g)	0.300	0.307	0.289	0.275	0.267*	0.162**

* significantly different ($p < 0.05$);
** excluded from statistical analyses due to a significant effect on larval survival at this treatment level.

[11] Williams, D.A. 1971. A test for differences between treatment means when survival dose levels are compared with a zero dose control. Biometrics **27**: 103-117.

Williams, D.A. 1972. A comparison of several dose levels with a zero control. Biometrics: **28** 519-531.

[12] Gulley, D.D., Boetler, A.M. and Bergman, H.L. 1994 Toxstat Release 3.4. University of Wyoming, Laramie, Wyoming.

Based on the statistically significant reduction on larval length and weight following 30 days post-hatch exposure, the **NOEC 30-day, was determined to be 0.099 mg as/L**. The LOEC was determined to be 0.20 mg as/L.

Utilizing these data, the MATC for dodine technical and fathead minnow embryos and larvae was determined to be **higher than 0.099 mg as/L and less than 0.200 mg as/L** (Geometric Mean **MATC = 0.140 mg as/L**).

B.9.2.3 Bioaccumulation potential in fish (Annex IIA 8.2.3)

Not applicable and/or necessary for the active substance since the log Pow = 0.96.

B.9.2.4 Summary of effects on Fish

Dodine was of hight acute toxicity to fish with a LC_{50} for the most sensitive species of 0.7 mg/l (blueguill sunfish). In an early life stage study, no effects on hatchability or survival of fathead minnows were observed at 0.026, 0.055, 0.099 and 0.20 mg as/L. The mean total length of larvae and mean wet weight of larvae exposed to 0.20 mg as/L and above were statistically less than that of the pooled controls. The overall NOEC was 0.099 mg/L.

Table B.9.2.4 - Summary of the effects of dodine on fish species

Test	Species	Parent/ metabolite	Result	Reference
Acute, 96 hr (daily renewal)	Rainbow trout	dodine	LC50 = 1.5 mg as/L	Caley, *et al* 1990a
Acute, 96 hr (flow-through)	Sheepshead minnow	dodine	LC50 = 3.7 mg as/L seawater	Bettencourt, 1992
Acute, 96 hr (daily renewal)	Blueguill sunfish	dodine	LC50 = 0.7 mg as/L	Caley, *et al* 1990b
Early life stages (flow-through)	Fathead minnow	dodine	NOEC = 0.099 mg as/L	Sousa, 1995

B.9.2.5 Acute toxicity to invertebrates (Annex IIA 8.2.4)

B.9.2.5.1 Acute toxicity to invertebrates with the active substance (Annex IIA 8.2.4)

Dodine technical: Acute Toxicity daphnids (*Daphnia magna*) under flow-through conditions.

Putt, A. E. (1992). Springborn Lab./ USA, unpublished report # 92-4-4245.

Guidelines and GLP:

The study was conducted according to US EPA FIFRA 72-2. Principles of GLP were complied with.

Test species and methods:

Technical substance: batch 303/90, purity 94% HPLC - 97.6% titration.

Test species: *Daphnia magna*, the culture medium was specially prepared with tap water. For the test young daphnia with age <24 hours were selected.

Test solutions with test item were continuously renewed (flow-through design, 6 volume replacements per aquarium of 1.8L every 24hours).

The study was performed over 48h at 5 dose groups (solvent control (methanol), control, 6.2, 10, 17, 29 and 48 µg as/L) with 20 daphnia each. A dosage group in the study consisted of 2 x 10 daphnia per 1.8L of medium per replicate.

The test system consists of a photoperiod of 16 hours per day, with no aeration and no feeding. Sampling and analysis of test item concentration occur at start 0 h and at the end (48h) of the study in duplicate.

Measurements and recordings of immobility (incl. mortality) occur at 24 and 48 hours in all vessels.

Statistical analysis: The mean measured concentrations (0- and 48-hour analyses) and corresponding effect (immobilization) data derived from the toxicity test were used to estimate 24- and 48-hour (EC50) and corresponding 95% confidence intervals. A computer program (Stephan, 1977, 1982, personal communication) was used to calculate the EC50 values and 95% confidence intervals.

Findings:

In the study, water quality parameters remained within acceptable ranges for the survival of daphnia magna. Based on mean measured concentrations of dodine technical, the exposure concentrations averaged 66% of the nominal treatment levels and defined the test concentrations as 36, 19, 8.9, 5.3, and 5.4 µg as/L.

The biological observations are summarised below:

Table B.9.2.5.1.1 - Acute Immobilisation of *Daphnia magna* at 24 and 48 hours.

Dose group (µg/l). nominal/actual	Initial Nº of daphnia	Cumulative mortality (%)	
		24 h	48 h
Solvent control	20	0	0
0	20	0	0
6.2/5.4	20	0	5
10/5.3	20	0	0
17/8.9	20	0	0
29/19	20	0	55
48/36	20	0	100

Following 48 hours of exposure, immobilization of 55 and 100% was observed among daphnids exposed to the two highest dose groups. Immobilization of 5% was observed in the lowest dose group, however in view of low incidence (<10%, one daphnia) and lack of similar observations in the next two higher concentrations, this was considered incidental and unrelated to treatment.

Under the conditions of the test (flow-through), the **48h-EC_{50} for immobilisation of daphnia was found to be 18 µg/L with 95% confidence interval of 8.9 – 36 µg/L. The 48h-NOEC value was 8.9 µg/L**.

Dodine: Determination of Acute Toxicity (LC50) to daphnia (*Daphnia magna*) (48h, semi-static) (EPA).

Caley, C. Y., (1989). Inveresk Research / UK, unpublished report # 7069

Guidelines and GLP:

The study was conducted according to US EPA FIFRA 72-2. Principles of GLP were complied with.

Test species and methods:

Technical substance: batch 92/88/2, purity 95.3%

Test species: *Daphnia magna*, in-house culture; for the test young daphnia with age < 24 hours were selected.

Test solutions with test item were renewed after 24h (semi-static design).The study was performed over 48h at 5 dose groups (control, 62.5, 125, 250, 500 and 1000 µg as/L) with 20 daphnia each. A dosage group consisted of 4 x 5 daphnia per 100 ml of medium per replicate.

The test system consists of a photoperiod of 16 hours per day, with no aeration and no feeding. Sampling and analysis of test item concentration occur at start 0 h and at the end (48h) of the study in duplicate.

Measurements and recordings of immobility (incl. mortality) occur at 24 and 48 hours in all vessels.

Statistical analysis: The median lethal concentrations (LC50) at 24 h and 48 h were determined using probit analysis (Finney, 1971 and Berkson, 1953). Results were calculated using means of the estimated measured concentrations in test solutions for the initial and final batches of tanks prepared since the measured concentrations of dodine were out with ± 20% of nominal in several cases.

Findings:

In the study, water quality parameters remained within acceptable ranges for the survival of daphnia magna. Measured concentrations in the stock solutions were out with the 20% of nominal during the test. The concentration of dodine analysed in the first stock solution (0-24h) decreased from 117% of nominal at 0h to 76% of nominal at 24h. The concentration of dodine analysed in the second stock solution (24-48h) decreased from 126% of nominal at 24h to 40% of nominal at 48h. Dodine was shown to be "instable" at this dose level (10 mg as/L). The method of analysis used has been validated prior the start of the study but it should be noted that it does not differentiate between chemical degradation and the possibility of adsorption (onto glass for example) at these dose levels.

The biological observations are summarised below:

Table B.9.2.5.1.2 - Acute Immobilisation of *Daphnia magna* at 24 and 48 hours.

Dose group (µg/l). nominal/actual	Initial Nº of daphnia	Cumulative mortality (%)	
		24 h	48 h
0	20	0	5
62.5/56.4*	20	25	55
125/112.3*	20	70	95
250/242*	20	100	100
500/484	20	100	100
1000/967.5	20	100	100

* calculated based on the measured concentration of stock solution

Following 48 hours of exposure, immobilization of 95 and 100% was observed among daphnids exposed to the four highest dose groups (112.3 – 967.5 µg as/L). Immobilization of 55% was observed in the lowest dose group. There was no concentration tested which did not cause any immobilization within the test period.

Under the conditions of the test (semi-static), the **48h-LC50 for immobilisation of daphnia was found to be 52.9 µg as/L with 95% confidence interval of 28.5 – 65.8 µg/L**. No NOEC could be defined.

Acute Toxicity Study in *Daphnia magna* with Dodine in a water-sediment system (Static).

Migchielsen, M. H. J. (2002). Notox / NL, unpublished report # 354825.

Guidelines and GLP:

The study was conducted according to OECD Nº 202, Part C (1984). Principles of GLP were complied with.

Test species and methods:

Technical substance: batch S01L01, purity 98.5%, radiolabelled dodine: batch 011009 (Source: ARC / USA), radiochemical purity >97.9%

Test species: *Daphnia magna*, in-house culture; for the test young daphnia with age < 24 hours were selected.

Test solutions with test item (dissolved in methanol) were not renewed (static design). Sediments were added to the test vessels in order to represent a more realistic scenario since water/sediment studies showed that the active substance is readily adsorbed onto the sediment.

The study was performed with natural sediment over 48h at 5 dose groups (control+methanol, 51, 86, 160, 265 and 477 µg as/L with 20 daphnia each. A dosage group in the main study consisted of 4 x 5 daphnia per 270 ml of medium per replicate.

The ratio overlying medium: sediment was ca. 4 : 1. Sediments were obtained from a natural pool situated near Leerdam (NL) and sieved over a 2mm sieve. The sediment was fully characterized under GLP at the Cranfield University. The report includes the results of a reference test with potassium dichromate to validate the sensitivity of the test organisms.

The test system consists of a photoperiod of 16 hours per day, with no aeration and no feeding. Samples for determination of total radioactivity (LSC) and radiochemical purity (TLC) were taken from all concentrations and the treatment control at start (t=0 h) and at termination (t=48h).

Measurements and recordings of immobility (incl. mortality) occur at 24 and 48 hours in all vessels.

Statistical analysis: The EC50-value was calculated at 24 and 48 hours of exposure from the probits of the percentages of affected daphnia and the logarithms of the corresponding test substance concentrations using the maximum likelihood estimation method (Finney, D.J., 1971).

Findings:

In the study, water quality parameters remained within acceptable ranges for the survival of daphnia magna. Measured concentrations at the start of the test were 50, 83, 146, 281 and 497 µas/L (all >90% of nominal concentrations). After 48 hours, these concentrations had decreased by approx. 60% below initial, the decrease proved to be mainly due to degradation to CO2, while adsorption to the sediment layer was negligible. TLC analysis showed that at least 98% of the remaining activity was still identified as the parent compound dodine.

The biological observations are summarised below:

Table B.9.2.5.1.3 - Acute Immobilisation of *Daphnia magna* at 24 and 48 hours in a water/sediment static system.

Dose group (µg/l). nominal/actual	Initial Nº of daphnia	Cumulative mortality (%)	
		24 h	48 h
0	20	0	0
51/50	20	0	0
86/83	20	0	0
160/146	20	5	75
265/281	20	85	95
477/497	20	100	100

Following 48 hours of exposure, immobilization of 95 and 100% was observed among daphnids exposed to the two highest dose groups. Immobilization of 75% was observed in the dose group at a nominal concentration of 160 µg as/L. No immobilization was observed in the two lowest dose groups.

Under the conditions of the test (static + sediment), the **48h-EC50 for immobilisation of daphnia was found to be 146 µg as/L based on nominal concentrations with a 95% confidence interval between 112 and 203 µg as/L**. **A 48h-NOEC of 86 µg as/L could be defined based on nominal concentrations.**

Dodine technical: Acute toxicity to mysid shrimp (*Mysidopsis bahia*) under flow-through conditions.

Bettencourt, M. J. (1992). Springborn Lab / USA, unpublished report # 92-9-4401.

Guidelines and GLP:

The study was conducted according to EPA FIBRA 72-3. Principles of GLP were complied with.

Test species and methods:

Technical substance: batch 303/90, purity 94.07%, and radiolabelled dodine: batch 920511, >99%.

Test species: Mysid shrimp (*Mysidopsis bahia*); obtained from own culture at Springborn Lab./USA. For the test, mysids with less than 24h age, reproductively immature, were selected. The medium/dilution water was on natural filtered seawater (salinity: 32‰). The test occurs at 25°C under flow-through conditions with 6.5 volume replacements per aquarium of 11 L every 24 hours.

The study was performed over 96h at 5 dose groups (solvent control (0.1 ml methanol/l), control, 97, 160, 270, 450 and 750 µg as/L) with 20 mysid shrimp each. A dosage group in the study consisted of 2 x 10 mysid shrimp per 11 L of medium per replicate.

Photoperiod: 16 hours per day; constant temperature in water bath at 25±1 °C.

Sampling and analysis of test item concentration occur at start (t=0 h), and at termination (t=96h) in each replicate and was done by (LSC) in all treatment vessels and additionally by HPLC-RAM in the stock solution (7.5 g as/L) to verify the identity of the radiolabelled compound.

Statistical analysis: The median lethal concentration (LC50) was calculated by moving angle analysis.

Findings:

Mean measured concentrations of C14-dodine averaged 101% of nominal. Analysis of the stock solution (7.5 g as/L) by LSC at 0 and 96h of the exposure period resulted in mean measured concentrations of 7.74 and 7.85 g as/L respectively. Analysis of the same stock by HPLC-RAM

established a mean measured concentration of 7.7 g as/L at each interval. Comparison of these results demonstrated relatively good agreement between the two types of measurements. HPLC-RAM analysis indicated that the C14-measurements were a reliable indication of the concentration of parent dodine present in the exposure aquaria.

The cumulative mortality data are presented in the following table:

Table B.9.2.5.1.4 - Cumulative mortality data of mysids exposed to dodine.

Dose group (µg/l) nominal/actual	Initial Nº of daphnia	Cumulative mortality (%)				
		0 h	24 h	48 h	72 h	96 h
Solvent control	20	0	0	0	0	0
Control	20	0	0	0	0	0
97/100	20	0	0	0	0	0
160170	20	0	0	0	0	0
270/260	20	0	0	15	15	20
450/450	20	0	0	20	35	45
750/740	20	0	0	35	60	100

Mortality of 100%, 45% and 20% was observed in the 3 highest dose groups i.e. 740, 450 and 260 µg as/L respectively. In addition, sublethal effects (e.g., erratic swimming behaviour, lethargy) were observed among all of the surviving mysids exposed to the 450 µg as/L treatment level and among several of the surviving mysid shrimp exposed to the 260 µg as/L treatment level. No mortality or sublethal effects were observed in the lower dose groups tested (170 and 100 µg/L).

The 96 h LC_{50}, value for mysid shrimps exposed to dodine was determined as 390 µg as/L with a 95% confidence interval of 350 – 450 µg as/L. **The 96 h NOEC established was 170 µg as/L.**

Dodine technical: acute toxicity to eastern oyster (*Crassostrea virginica*) under flow-through conditions

Dionne, E. (1992). Springborn Lab./ USA, unpublished report # 92-9-4404

Guidelines and GLP:

The study was conducted according to EPA FIBRA 72-3. Principles of GLP were complied with.

Test species and methods:

Technical substance: batch 303/90, purity 94.07%, and radiolabelled dodine: batch 920511, >99%.

Test species: Eastern oyster (*Crassostrea virginica*); obtained from P. Cummins Oysters Co, Pasadena, Maryland/USA. The acclimation period was 16 days prior to study initiation. For the test oysters of similar age, reproductively immature with mean valve height of 37±5 mm were selected. The medium/dilution water was on natural unfiltered seawater (salinity: 31‰). The test occurs under flow-through conditions with 96 hours of exposure in glass aquaria (18L vol. with approx. 6 daily vol. replacements).

The study was performed over 96h at 5 dose groups (solvent control (0.08 ml methanol/L), control, 39, 65, 110, 180 and 300 µg as/L) with 40 oysters each. A dosage group consisted of 2 x 20 oysters per 18L of medium per replicate.

Photoperiod: 16 hours per day; circulation by pumping (approx. 5l/oyster and hour). Oysters were feed with algae.

Sampling and analysis of test item concentration occur at start (t=0 h), and at termination (t=96h) in each replicate and was done by LSC in all treatment vessels and additionally by HPLC-RAM in the stock solution (7.5 g as/L) to verify the identity of the radiolabelled compound.

Statistical analysis: The 96-hour EC50 value and 95% confidence limits were determined by fitting the untransformed and transformed (i.e., growth data as percent reduction transformed to probit, concentrations transformed to log concentration) data to a best fit linear regression curve based on least squares. The regression equation was applied to calculate the EC50 and its 95% confidence limits, using the method of inverse prediction (Sokal and Rohlf, 1969). Results reported are based on the mean measured concentrations of the test material.

The NOEC during the 96-hour exposure period was also determined. This was determined using Williams' Test (Williams, 1971, 1972) coupled with Bartlett's test for determination of homogeneity of variances and Shapiro-Wilks test for normality (Weber, *et. al*., 1989).

Findings:

Mean measured concentrations of ^{14}C-dodine averaged 47% of nominal when analysed by LSC. Since recoveries of approx. 100% were seen in previous marine using filtered seawater, this suggests adsorption of dodine to the high density of organics present in unfiltered seawater under conditions maintained during this oyster study. However, measured concentrations achieved during the exposure of oysters were sufficient to elicit a biological response and define the acute toxicity of dodine. Analysis of the stock solution (3.59 g as/L) by HPLC-RAM at 0 and 96h of the exposure period resulted in mean measured concentrations of 3.23 and 3.48 g as/L confirming stability of dodine in water at this dose level. HPLC-RAM analysis indicated that the C14-measurements were a reliable indication of the concentration of parent dodine present in the exposure aquaria.

The average growth of control oysters was determined as 2.5 mm shell deposit, thus fulfilling the EPA requirement of minimum growth of 2.3 mm new shell (historical Springborn data covering 1987 to 1991 demonstrate a range from 0.9 to 3.8 mm new shell).

The results of new shell growth at the different exposure concentrations are presented in the following table:

Table B.9.2.5.1.5 - Mean shell deposit I growth in Eastern oysters exposed to different concentrations of dodine for 96 hours.

Nominal Conc. µg as/L (actual)	Control (0)	Solvent control (0)	39 (18	65 (39	110 (46	180 (84)	300 (120)
Shell deposit (mm)	2.5	1.3	1.3	1.2	1.2	0.6	0.6
% Growth reduction vs. solvent control	NA	NA	1.5	11	12	53*	58*

* significantly different ($p < 0.05$)

No mortality was observed in any of the dose groups or the controls. Reduced feeding and significant reduction in growth shell was observed in the two highest dose groups (84 and 120 µg as/L).

The 96-hour EC50 for eastern oyster was calculated by linear regression to be 98 µg as/L with 95% confidence interval of (53 – 230 µg as/L), the NOEC was concluded as 46 µg as/L.

B.9.2.5.2 Acute toxicity to invertebrates with the formulation (Annex IIA 10.2.4)

Acute toxicity study in *Daphnia magna* with Dodine 400 SC (Semi-static).

Migchielsen, M. (2004). Notox/ NL, unpublished report # 413213.

Guidelines and GLP:

The study was conducted according to OECD Guideline 202 (1984). Principles of GLP were complied with.

Test species and methods:

Test material: dodine, formulated as a 400 g/L SC, batch 164503, purity 405.4 g/L. Dodine was applied as a blank formulation (batch 081604) + radiolabelled dodine: batch 011009, radiochemical purity >97.9%.

Test species: *Daphnia magna*, Crustacea; in house culture; for the test young daphnia with age < 24 hours were selected. Test solutions with test item were renewed after 24h (semi-static design).

The study was performed after mixing the blank formulation with radiolabelled dodine (to facilitate analytical work) over 48h at 6 dose groups: control, 14, 20, 29, 40 and 60 µg equivalent as/L, equivalent to 36, 50, 72, 100 and 150 µg Syllit 400 SC/L, with 20 daphnia each. A dosage group consisted of 4 replicates of 5 daphnia per 100 ml of medium each.

The report includes the results of a reference test with potassium dichromate to validate the sensitivity of the test organisms.

Photoperiod: 16 hours per day with no aeration and no feeding.

Samples for determination of total radioactivity (LSC) and radiochemical purity (TLC) were taken from all concentrations and the treatment control at start (t=0 h), at 24h (before and after renewal) and at termination (t=48h).

Measurements and recordings of immobility at 24 and 48 hours occur daily in all vessels.

Statistical analysis: The EC50-value was calculated at 48 hours of exposure from the probits of the percentages of affected daphnia and the logarithms of the corresponding test substance concentrations using the maximum likelihood estimation method (Finney, D.J., 1971: Probit analysis, Cambridge University Press, Cambridge, U.K., 3rd edition).

Findings:

Analysis of the samples showed that recoveries for ^{14}C-dodine in the freshly prepared solutions (t=0 and t=24 hours) ranged between 69 and 90% of nominal. The concentrations remained stable within 80% during the 24-hour refreshment periods, with an average decrease of less than 10%, except for the lowest concentration during the second renewal. The concentrations measured in the freshly prepared solutions at 24 hours of exposure showed that the procedure of preparation was repeatable. Based on these results, average exposure concentrations were calculated.

The biological observations are summarised below:

Table B.9.2.5.2 - Acute immobilisation of Daphnia magna at 24 and 48 hours in a semi-static system exposed to Syllit 400 SC.

Dose group (µg as equiv./L) nominal/actual	Initial Nº of daphnia	Cumulative mortality (%)	
		24 h	48 h
Control	20	5	5
14/10	20	0	0
20/16	20	0	0
29/25	20	0	0
40/35	20	0	0
60/52	20	15	65

Following 48 hours of exposure, 65%immobilization was observed among daphnids exposed to the highest dose group. No immobilization was observed in the other dose groups.

Under the conditions of the test (semi-static, formulation), **the 48h EC50 for immobilisation of daphnia was found to be 123 µg Syllit 400 SC/L (equivalent to 49 µg as/L)** (95% confidence interval between 115 and 136 µg/L) based on actual concentrations.

A 48h-NOEC of 88 µg Syllit 400 SC/L (equivalent to 35 µg as/L) could be defined based on actual concentrations.

B.9.2.6 Chronic toxicity to aquatic invertebrates (Annex IIA 8.2.5)

Dodine technical: The chronic toxicity to *Daphnia magna* under flow-through conditions.

Putt, A. E. (1995). Springborn Lab./USA, unpublished report # 95-10-6162.

Guidelines and GLP:

The study was conducted according to EPA FIBRA 72-4. Principles of GLP were complied with.

Test species and methods:

Dodine technical: batch DA717, purity 98.6%, radiolabelled dodine: batch 950214, >99%

Test species: *Daphnia magna*, in-house culture. The culture medium was prepared with tap water based on the formula for hard water (EPA 1975). For the test young daphnia with age less than 24 hours were selected.

Test design: flow-through system (with 6 volume replacement per day) in 4 x 1.4 L glass vessels (with 10 daphnia) per concentration. The test exposition time was 21 days with feeding of alga (Ankistrodesmus falcatus) daily.

Test concentrations were: control, solvent control (methanol), 3.1, 6.3, 13, 25 and 50 µg as/L with 40 daphnids per concentrations (4 replicates of 10). Test temperature vary between 19 and 21ºC.

Photoperiod: 16 hours per day at approx. 320-540 lux.

Sampling and analysis of test item concentration occur at start (day 0), on day 7, on day 14 and at termination (day 21) in 2 replicates by LSC in all treatment groups and additionally by HPLC-RAM in the highest dose group (50 µg as/L) to verify the identity of the radiolabelled compound.

Biological recordings/observations: number of immobilized F1 daphnids and abnormal behaviour on day 1, 2, 4, 7, 9, 11, 14, 16, 18 and 21, offspring production on day 7 and then 3 times per week until termination on day 21, number of immobilized offspring and the time to first brood release were recorded, length and dry weight of each survival adult daphnid at termination (day 21).

Data obtained on organism survival, reproduction and growth were statistically analysed.

Statistical analysis: Data obtained on organism survival, reproduction and growth (mean total length and dry weight) were statistically analysed to establish significant treatment level effects. Analyses were performed using the mean organism response in each replicate vessel rather than individual response values. All statistical conclusions were made at the 95% level of certainty except in the case of the Shapiro-Wilks Test and the Bartlett's Test, in which the 99% level of certainty was applied.

The 21-day EC50 value and 95% confidence interval was calculated using a computer program (Stephan, 1982, personal communication).

Findings:

Measured concentrations of C14-dodine in the test vessels ranged from 85% to 120% of nominal when analysed by LSC. HPLC-RAM analysis of the highest concentration indicated that the C14-measurements were a reliable indication of the concentration of parent dodine present in the exposure aquaria.

The biological observations are summarised below:

Table B.9.2.6.1 - Survival of F0 of *Daphnia magna* in chronic exposure to dodine.

Dose group (µg as equiv./L) nominal/actual	Initial Nº of daphnia	Mean % survival of F0 daphnids									
		1	2	4	7	9	11	14	16	18	21
Pooled control	80	100	100	98	96	96	96	95	95	94	94
3.1/4.4	40	98	95	95	95	95	95	95	95	95	93
6.3/7.3	40	100	100	100	98	98	98	98	98	98	98
13/13	40	98	98	98	95	95	95	95	93	93	88
25/25	40	100	70	68	68	65	65	63	63	63	60
50/52	40	88	35	33	30	25	25	25	23	20	**20***

*significantly different ($p < 0.05$) when compared to control

The reproduction yielded the following mean numbers of offspring/female:

Table B.9.2.6.2 - Cumulative mean number of offspring/female

Dose group (µg as equiv./L) nominal/actual	Mean cumulative number of offspring/female						
	7	9	11	14	16	18	21
Pooled control	1	0	7	25	39	47	64
3.1/4.4	0	1	9	29	55	58	75
6.3/7.3	0	0	5	24	38	41	**53***
13/13	0	0	3	18	32	36	**43***
25/25	0	0	4	9	14	17	**24***
50/52	0	0	0	0	0	0	**0****

*significantly different ($p < 0.05$) when compared to control

**excluded fro, statistics due to effect on survival

Control and solvent control were pooled together since no statistically differences were observed between both control groups. At termination, a significant reduced survival (20%) was observed in the highest dose group (52 µg as/L) and this dose group was therefore excluded for further reproduction parameter analyses.

The number of offspring released was significantly reduced in all dose groups except the lowest dose group of 4.4 µg as/L. First brood release in both controls and lowest dose group (4.4 µg as/L) was on day 8. It occurred one day later at 13 and 25 µg as/L and two days later at 7.3 µg as/L. With the exception of three immobilized young daphnids in the 25 µg as/L treatment level on day 9, all offspring released during this test appeared normal. No statistical differences were noted for mean body length and mean dry weight of parental daphnids in any of the groups compared to the control.

The 21-day EC50 for parental immobility was found to be 30 µg as/L whereas the overall LOEC was 7.3 µg as/L and the overall NOEC was 4.4 µg as/L. Based on data the value of MATC is 5.7 µg as/L (geometric mean between NOEC and LOEC).

B.9.2.7 Summary of Effects on Aquatic Invertebrates

Dodine exhibited a high level of acute toxicity to *Daphnia* with an acute EC_{50} of 0.018 mg/L. In a chronic toxicity study, for the parental generation the 21 day EC_{50} (immobilisation) was 0.030 µg/l and the overall NOEC and LOEC was 0.0044 mg/L and 0.0073 mg/L, respectively. The Syllit 400 SC form. exhibited an acute toxicity identical to the parent compound with an EC_{50} of 0.049 mg as/L.

Table B.9.2.7 - Summary of the effects of dodine and Syllit 400 SC on aquatic invertebrates.

Test	Species	Test Material	Result (a.s.)	Reference
Acute, 48 hr (flow-through)	*D. magna*	dodine	LC50 = 0.018 mg/L	Putt, 1992
Acute, 48 hr (daily renewal)	*D. magna*	dodine	LC50 = 0.053 mg/L	Caley, 1989
Acute, 48 hr (static + sediment)	*D. magna*	dodine	LC50 = 0.146 mg/L	Migchielsen, 2002
Acute, 48 hr (semi-static)	*D. magna*	Syllit 400 SC	LC50 = 0.123 mg form./L (0.049 mg as/L)	Migchielsen, 2004
Chronic, 21 days (flow-through)	*D. magna*	dodine	NOEC = 0.0044 mg/L	Putt, 1992
Acute, 96 hr (flow-through)	*C. virginica*	dodine	EC50 = 0.098 mg/L	Dionne, 1992
Acute, 96 hr (flow-through)	*M. bahia*	dodine	LC50 = 0.390 mg/L	Bettencourt, 1992

B.9.2.8 Effects on algal growth (Annex IIA 8.2.6)

Dodine: Toxicity to the freshwater green alga, *Selenastrum capricornutum*.

Hoberg, J.R. (1993). Springborn Lab / USA, unpublished report # 92-12-4550

Guidelines and GLP:

The study was conducted according to EPA FIFRA 122-2 and 123-2 (1982). Principles of GLP were complied with.

Test species and methods:

Dodine technical: batch 303/90, purity 94.07% and radiolabelled dodine: batch 920511, >99% was used in the study.

Test species: *Selenastrum capricornutum*, (unicellular green alga), strain 1648. The study occur under static conditions with 120 hours of exposure in 50 ml medium which was prepared with

sterile deionised water in glass vessels; initial cell density was0.3x104 cells/ml; test vessels were continuously illuminated at 3500 to 4600 lux; algal cells were kept in suspension by continuous shaking at 25°C.

Test concentrations were: 0.091, 0.19, 0.38, 0.74, 1.5, 3.0, 6.0 and 12 µg as/L, in 3 replicates per test concentration and 6 replicates of blank control (3 control + 3 solvent control).

Sampling for analysis of test item concentration occured at start (t=0h) and at termination (t=120h, composite sample of all 3 replicates).

Measurements and recordings of cell densities: at start, at 24, 48, 72, 96 and 120 hours (microscopically by counting) and observations of cell health.

Statistical analysis: Based on the results of statistical analyses performed for 120-hour cell density and maximum growth rate data, the highest test concentration that caused no statistically significant reduction (No Observed Effect Concentration, NOEC) was determined using Williams' Test (Williams, 1971, 1972). The data were first checked for normality using the Shapiro-Wilks Test (Weber et al., 1989) and for homogeneity of variance using Bartlett's Test (Horning and Weber, 1985).

EC50 values were calculated based on cell density after 48, 72, 96 and 120 hours of exposure. The EC values and their 95% confidence limits were determined by linear regression of response (percent reduction of cell density or growth rate as compared with pooled control data) vs initial measured exposure concentration over the range of test concentrations where a clear exposure-response relationship was observed. This regression equation was then applied to estimate the EC values and their 95% confidence limits, using the method of inverse prediction (Sokal and Rohlf, 1981).

Findings:

During the test, the initial concentration measured by LSC at t=0 ranged from 89.4 to 102% of nominal concentration. Measured concentrations obtained at 120 hours ranged from 20 to 30% of nominal value. The recovery of 5 of the six QC samples ranged from 94.4 to 105% of nominal concentration and demonstrated that appropriate precision was achieved during these analyses. Based on the observed decrease in test material concentration between test initiation and test termination, the results of this test are based on the initial measured concentrations.

The biological recordings regarding cell growth together with derived data are summarised below:

Table B.9.2.8.1 - Mean cell densities of *Selenastrum capricornutum* after exposure to dodine.

Initial conc. of dodine (µg as/L)	Mean cell densities during exposure (x10^4 cells/ml)				
	24 h	**48h**	**72h**	**96h**	**120h**
Pooled control	2	3	34	73	111
0.082	2	2	33	64	113
0.17	2	4	32	46	**89***
0.36	1	3	32	50	**75***
0.66	1	2	31	48	**61***
1.4	1	1	29	43	**49***
2.9	1	1	28	33	**33***
6	1	< 1	6	15	**12***
12	0	0	0	2	**4***

*statistically significant ($p < 0.05$) William's test

Table B.9.2.9.2 - Calculated growth rates per day of *Selenastrum capricornutum* exposed to dodine.

Initial conc. of dodine (µg as/L)	Observation intervals in hours				
	24-48 h	48-72 h	72-96 h	96-120 h	Maximum
Pooled control	na	na	na	na	2.299
0.082	0.332	2.607	0.648	0.651	2.607
0.17	0.726	2.115	0.365	0.759	2.115
0.36	1.047	2.305	0.453	0.466	2.305
0.66	0.323	2.858	0.423	0.272	2.858
1.4	0.347	2.932	0.379	0.163	2.932
2.9	0.247	2.988	0.276	-0.024	2.988
6	-0.657	2.518	1.045	-0.245	2.518
12	na	na	na	1.025	**1.025 ***

- not calculated due to the lack of cell growth
* significant reduction ($p<0.05$ Williams test) when compared to pooled control
na : not applicable

Cell densities followed the established concentration gradient, decreasing with increasing test concentration. The control and the solvent control group were pooled since no statistical differences were noted between both groups.

Statistically significant reduction in cell density and cell fragments were observed in all dose groups except in the lowest dose group (0.082 µg as/L). Maximum growth rate was significantly reduced in the highest dose group (12 µg as/L) only.

Based on cell density, the 5-day EC50 on *Selenastrum capricornutum* was calculated to be 0.95 µg as/L. The 5-day NOEC was determined to be 0.082 µg as/L.

Based on maximum growth rate, the 5-day EC50 was calculated to be 11 µg as/L with a 95% confidence limits of 6.5 – 18 µg as/L and the 5-day NOEC was determined to be 6 µg as/L.

Dodine - Toxicity to the freshwater green alga, *Selenastrum capricornutum* during a 15-day partial renewal test.

Hoberg J.R. (1995). Springborn Lab./ USA, unpublished report # 95-10-6147

Guidelines and GLP:

The study was conducted according to OECD Nº 201 (1984) and based on guidance obtained from the US EPA and protocol acceptance by dutch authorities (Ctb). Principles of GLP were complied with.

Test species and methods:

Radiolabelled dodine batch 950214, >99% was used in the study.

Test species: *Selenastrum capricornutum*, (unicellular green alga), strain 1648.

Test design: this study consist on a limited microcosm study simulating 3 field applications with 5 days interval between sprayings and followed by a recovery period to estimate potential regeneration of the algal population remaining in the treated water: semi-static for 15 days (renewal on day 5 and 10). Recovery phase of 4 days for the highest dose level in the same test vessels and recovery period of 5 additional days for subcultures (dilution 1:10) transferred from the highest dose level vessels on recovery day 4. Initial cell density at each renewal: 0.3 x 104 cells/ml. Test medium was prepared with sterile deionised water. Vessels were continuously illuminated: at 3200 to 5400 lux and algal cells were kept in suspension by continuous shaking at 24°C.

Test concentrations: solvent control (methanol), control, 0.05, 0.16, 0.54, 1.8, 6 and 20 µg as/L in 3 replicate / concentration.

Renewal on day 5 and 10 were done at higher concentrations corresponding to initial nominal concentration + measured 5-day old concentration to take into account possible accumulation of dodine after each spraying).

Analysis by LSC for all treatments and HPLC-RAM on the secondary stock solution for confirmation of radiochemical identity.

Recordings: cell densities at start, on day 1, 2, 3, 4, 5, 8, 10, 13 and 15 (microscopically by counting), observations of cell health.

Statistics: Evaluation of algal growth and cell densities were conducted using William's test preceded by Shapiro-Wilks and Bartlett's tests for normality and homogeneity of data sets.

Findings:

The results of this study are expressed by mean measured concentrations (N=6) which provide a conservative estimate of exposure levels by incorporating the maximum and minimum concentrations for each 5-day exposure period. Based on mean measured concentrations, treatment level: ranged from 66 to 120% of the nominal concentrations with the highest measured concentration of 45 µg as/L after second renewal in the highest dose group. The recovery of 15 of the 18 QC samples demonstrated that appropriate precision was achieved during these analyses. The analyses of the secondary stock solution (0.2 g as/L) averaged 0.21 and 0.27 g as/L at study initiation and study termination respectively showing stability of dodine in the stock solution at this concentration.

The biological recordings regarding cell growth together with derived data are summarised below:

Table B.9.2.8.3 - Mean cell densities (x 10^4 cells/ml) of *Selenastrum capricornutum* after exposure to dodine.

Nominal/actual conc. of dodine (µg as/L)	Days											
	1	**2**	**3**	**4**	**5**	**a)**	**8**	**10**	**b)**	**13**	**15**	**c)**
Solvent control	< 1	2	7	40	232	-	26	218	-	13	D	
Control	2	5	9	48	187	-	46	236	-	62	334	
Pooled control								227	-			
0.05/0.06	1	1	7	36	253	-8.8	19	**208***	8.5	22	431	-29
0.16/0.15	2	2	6	45	**179***	23	18	**205***	9.7	14	436	-30
0.54/0.42	1	2	8	32	**246***	-5.7	11	**191***	16	15	**208***	38
1.8/1.4	1	1	6	26	**156***	33	8	**137***	40	8	**108***	68
6.0/3.9	1	2	3	9	**51***	78	3	**18***	92	6	**83***	75
20/20	< 1	1	2	1	**2***	99	1	**1***	99	2	**8***	98

*statistically significant ($p < 0.05$) William's test
a) % reduction on day 5 as compared to solvent control values
b) % reduction on day 10 as compared to pooled control values
c) % reduction on day 15 as compared to control values
d) excluded due to contaminations with test item

Table B.9.2.8.4 - Mean cell densities (x 10^4 cells/ml) of *Selenastrum capricornutum* after exposure to dodine.

Nominal/actual conc. of dodine (µg as/L)	Observation intervals in day*s								
	0-1	0-2	0-3	0-4	0-5	5-8	5-10	10-13	10-15
Solvent control	0.305	0.805	1.096	1.237	1.364	1.515	1.393	1.281	**d**
Control	1.837	1.334	1.165	1.282	1.319	1.715	1.410	1.843	1.477
Pooled control							1.402		
0.05/0.06	1.356	0.754	1.104	1.205	1.382	1.414	1.383	1.489	1.530
0.16/0.15	1.728	0.908	1.033	1.267	1.311	1.371	1.380	1.340	1.533
0.54/0.42	0.935	0.919	1.150	1.179	1.376	1.235	1.365	1.346	**1.377[c]**
1.8/1.4	0.957	0.722	1.025	1.122	**1.283[a]**	1.101	1.295	1.121	**1.237[c]**
6.0/3.9	0.908	0.784	0.745	0.830	**1.050[a]**	0.700	**0.801[b]**	1.016	**1.184[c]**
20/20	0.179	0.203	0.593	0.093	**0.356[a]**	0.357	**0.299[b]**	0.655	**0.671[c]**

*growth rate calculated from initial cell density of each renewal period
a) significant reduction ($p < 0.05$ William's test) when compared to solvent control
b) significant reduction ($p < 0.05$ William's test) when compared to pooled control
c) significant reduction ($p < 0.05$ William's test) when compared to control
d) excluded due to contaminations with test item

On day 5, cell density was significantly reduced in all dose groups except the lowest - 0.059 µg as/L. Growth rate was significantly reduced in the 3 highest dose groups (1.4, 3.9 and 20 µg as/L).

On day 10, cell density was significantly reduced in all dose groups. Growth rate was significantly reduced in the 2 highest dose groups (3.9 and 20 µg as/L).

On day 15, cell density was significantly reduced and cells fragments and bloated cells were noted in all dose groups except the two lowest at 0.06 and 0.15 µg as/L. Growth rate was significantly reduced in the 3 highest dose groups (0.42, 1.4, 3.9 and 20 µg as/L).

The results of the recovery period are given in the following table:

Table B.9.2.8.5 - Results of the algal growth recovery phase subsequent to the 15-day partial renewal exposure of *Selenastrum capricornutum* to dodine.

Mean measured concentration (µg as/L)	Cell density (x10^4 cells/ml) post exposure (days)				
	2	4	6	8	9
20	4.9	4.7	-	-	-
2	-	-	3.1	91	177

On day 2 and 4 of the recovery period, the cell density closely approximated the average density observed at the termination of the exposure phase (8 x 10^4 cells/ml). Since no growth was observed in these cultures at this point and the concentration of dodine four days prior (1.5 µg as/L) was known to be less toxic to *Selenastrum*, the potential for nutrient limitation within the recovery cultures was investigated. On day 4 of the recovery phase, the medium in the recovery cultures had aged for nine days since the previous renewal on exposure day 10. The amount of aged medium which was transferred on exposure days 5 and 10 was calculated. Based on the cell density present on exposure day 5, 17% of the newly prepared 20 µg as/L solution prepared on day 5 were aged solution from test initiation. The exposure day 10, newly prepared 20 µg as/L solution contained 24% aged solution. The concentration of aged solution in all other treatments 0and the controls at each renewal period was less than 2% due to the relatively high cell densities throughout the exposure.

Consequently, there was reason to believe that nutrient limitation may be a limiting factor for cell growth in the recovery phase.

To investigate this factor, an aliquot of the recovery solution was taken and diluted by a factor of 10 in freshly prepared algal medium. After 5 days (recovery day 9) the cell density in these subcultures averaged 177 x 10^4 cells/ml and the subcultures were terminated. This method is a standard technique required by the US EPA to distinguish between "algistatic" or "algicidal" effects of a test substance. The average concentration of dodine measured in the subcultures at termination was 0.071 µg as/L.

The above data suggest that the growth potential of *Selenasturm* during the recovery phase was reduced due to nutrient limitation rather than the presence of residual dodine. This conclusion is supported by the following information:

1) a substantial increase in cell density (4.7 x10^4 to 177 x10^4 cells/ml) was observed within five days of the addition of fresh growth medium to the subcultures

2) it is unlikely that residual dodine present in the initial recovery cultures was inhibiting algal growth since at termination of exposure phase the concentration of dodine has already declined to a subtoxic level (1.5 µg as/L). A cell density of 83 x10^4 cells/ml was observed in the second highest test concentration at termination of the exposure phase which contained 1.1 µg as/L. Additionally, during the exposure phase, the cell density observed in the highest test concentration continued to increase (1 to 8 x10^4 cells/ml) even though the measured concentration of dodine was increased according to the study design from 20 to 45 µg as/L. Since no growth was observed in the undiluted recovery cultures during the initial four days, and the dodine concentration was < 1.5 µg as/L which was demonstrated to be relatively non-toxic, it is concluded that the observed lack of growth was due to nutrient limitation and not dodine concentration.

Based on cell density of *Selenastrum capricornutum*, the following toxicity values were found:

5-day EC50 = 2.5 µg as/L - 5-day NOEC = 0.059 µg as/L.

10-day EC50= 0.75 µg as/L - 10-day NOEC < 0.059 µg as/L.

15-day EC50= 1.6 µg as/L - 15-day NOEC = 0.15 µg as/L.

Based on growth rate of *Selenastrum capricornutum*, the following toxicity values were found:

5-day EC50= 9.1 µg as/L - 5-day NOEC = 0.42 µg as/L

10-day EC50= 7.4 µg as/L - 10-day NOEC = 1.4 µg as/L

15-day EC50= 22 µg as/L - 15-day NOEC = 0.15 µg as/L

The 5-day EC50 for cell density (2.5 µg as/L) determined for this study closely approximates the 5-day EC50 (0.95 µg as/L) determined during the acute exposure test (Hoberg, 1993). Based on the results of this 15-day exposure phase, it is evident that multiple dodine treatments (resulting in initial exposure levels of 20 to 45 µg as/L) did not demonstrate a cumulative effect on cell growth.

Based on the results of the recovery phase conducted subsequent to the 15-day exposure phase, it was concluded that *Selenastrum capricornutum* has the ability to recover from exposure to dodine concentrations as high as 45 µg as/L (leading to 99% reduction of cell density), when sufficient nutrients are available. Dodine proved to be algistatic rather than algicidal.

Fresh water algal growth inhibition test with Dodine 400 SC.

Migchielsen, M. H. J. (2004).Notox/ NL, unpublished report # 413224.

Guidelines and GLP:

The study was conducted according to OECD 201 (1984) and EEC 92/69, part C.3 (1992). Principles of GLP were complied with.

Test species and methods:

Dodine formulated as a 400 g/L SC, was applied in the study as a blank formulation (batch 081604) + radiolabelled dodine: batch 011009, radiochemical purity >97.9%.

Test species: *Selenastrum capricornutum*, (unicellular green alga), strain NIVA CHL 1, in house laboratory culture.

Test study occur under static test conditions with 72 hours of exposure in 50 ml medium in glass vessels; initial cell density: $1x10^4$ cells/ml. The vessels were continuously illuminated at 61 to 84 $\mu E.m^{-2}.s^{-1}$; algal cells were kept in suspension by continuous shaking at 23°C.

Test concentrations were performed after mixing the blank formulation with radiolabelled dodine (to facilitate analytical work) over 72h at 6 dose groups: control, 15, 33, 73, 160 and 350 µg form. 400 SC/L (corresponding to 6, 13, 29, 64 and 140µg as/L), in 3 replicates per test concentration, 6 replicates of blank control, 2 replicates of the highest concentration without algae (to assess eventual adsorption of dodine onto algae) and 1 or 2 extra replicates of each concentration for sampling purposes.

Sampling for analysis of test item concentration occur at start (t=0h), at t=24h and at termination (t=72h).

Measurements and recordings of cell densities at start (microscopically by counting), at 24 (spectrophotometric measurement), 48 and 72 hours.

Findings:

Analysis of the samples taken during the final test showed that recoveries for ^{14}C-dodine at the start of exposure ranged between 73 and 86% of nominal. The concentrations decreased significantly during the first 24-hour test period, but remained fairly stable during the remainder of the test period. Based on the fact that the decrease in concentration was mainly observed during the first 24-hour test period and the fact that the results at the highest concentration with and without algae were comparable, it could be stated that adsorption of dodine to algae was not the main reason for the observed decrease. Adsorption to glass and/or degradation was the most likely causes for the observed decrease. Based on these results, average exposure concentrations were calculated.

The biological recordings regarding cell growth together with derived data are summarised below:

Table B.9.2.8.6 - Mean cell densities of *Selenastrum capricornutum* after exposure to Syllit 400 SC.

Dose group (µg as equiv./L) nominal/actual	Mean cell densities during exposure (x 10^4 cells/ml)			
	0 h	24 h	48 h	72 h
Control	1.0	5.2	22.9	68.6
6/2.8	1.0	5.1	22.5	64.9
13/4.8	1.0	4.9	19.3	57.5
29/7.6	1.0	3.1	4.5	4.3
64/22	1.0	2.1	2.2	2.0
140/66	1.0	1.0	1.2	1.0

Table B.9.2.8.7 - Growth reduction and growth inhibition of *Selenastrum capricornutum* after exposure to Syllit 400 SC.

Dose group (µg as equiv./L) nominal/actual	Growth reduction (%)			Growth inhibition (%)
	0 - 24 h	0 - 48 h	0 - 72 h	0 - 72 h
Control	na	na	na	
6/2.8	0.9	0.6	1.2	3.9
13/4.8	2.9	5.4	4.1	15.7
29/7.6	30.9	51.9	**65.3***	**87.9***
64/22	53.8	74.9	**84.1***	**95.3***
140/66	100.0	95.2	**99.2***	**99.7***

* statistically significant (Tukey test, 0.05)

Inhibition of cell growth increased with increasing concentration of Dodine 400 SC from 2.8 µg as/L upwards resulting in more than 95% inhibition at and above an average exposure concentration of 22 µg as/L. Statistically significant inhibition of cell growth was found at average exposure concentrations of 7.6 µg as/L and higher.

Growth rates were in the range of the controls at average exposure concentrations of 2.8 and 4.8 µg as/L during the 72-hour test period, whereas the growth rate of algae exposed to 7.6 µg as/L and above were increasingly reduced. Reduction of growth rate increased as exposure progressed at 7.6 and 22 µg as/L. Statistically significant reduction of growth rate was found at 7.6 µg as/L and above.

Based on cell growth inhibition and on the actual concentrations, the **72h-EbC50** on *Selenastrum capricornutum* was calculated to be **14 µg form. 400 SC/L (equivalent to 5.6 µg as/L**) with 95% confidence interval ranging from 7.9 to 24 µg form. 400 SC/L. Based on growth rate reduction and on the actual concentrations, the **72h-ErC50** on *Selenastrum capricornutum* was calculated to be **22 µg form. 400 SC/L (equivalent to 8.8 µg as/L**) with 95% confidence interval ranging from 9.4 to 53 µg form. 400 SC/L.

For both cell growth inhibition and growth rate reduction, the **72h-NOEb,rC** was determined to be **12 µg Syllit 400 SC/L (equivalent to 4.8 µg as/L**).

B.9.2.9 Summary of Effects on Algal growth

Dodine was of relatively high toxicity to algae with an EbC_{50} (5 days) value of 0.95 µg/L to the specie *S. capricornutum.* The Syllit 400 SC form. exhibited an acute toxicity identical to the parent compound with an EbC_{50} of 0.0056 mg as/L.

Table B.9.2.9 - Summary of the effects of dodine and Syllit 400 SC on green algae.

Test	Species	Test Material	Result (µg as/L)	Reference
Acute, 120 hr (static)	*S. capricornutum*	dodine	EbC50 = 0.95 ErC50 = 11	Hoberg, (1993)
Sub cronic (15d semi-static)	*S. capricornutum*	dodine	EbC50 = 1.6 ErC50 = 22 NOEb,rC = 0.15	Hoberg, (1995)
Acute, 72 hr (static)	*S. capricornutum*	dodine 400 SC	EbC50 = 5.6 ErC50 = 8.8	Migchielsen, 2004

B.9.2.10 Effects on sediment dwelling organisms (Annex IIA 8.2.7)

Sediment water chironomid toxicity test using water spiked with dodine technical.

Desmares-Koopmans, M. J. E., (2002). Notox./ NL, unpublished report # 327127.

Guidelines and GLP:

The study was conducted according to OECD Guideline 219 (draft February 2001). Principles of GLP were complied with.

Test species and methods:

Dodine technical: batch S01106, purity 96.2%, Radiolabelled material received from ARC/ USA; Batch number: 011009, radiochemical purity >97.9%.

Test species: *Chironomus riparius*, (freshwater chironomid) were cultured in the laboratory. The study was conducted under static conditions with 28 days exposure after application of the test substance involving spiking of the water column. Artificial sediment was used according to the OECD guideline 207 (5% sphagnum, 20% kaolin clay, 75% industrial sand) with a final organic carbon content of 1.6% at 20°C under continuous aeration and 16 hours daily photoperiod. Ratio sediment/water was 1 : 4. Larvae at the first larval stage (2-3 days old) were introduced into the vessels 1 day before treatment. Midge larvae were fed with artificial feed (Trouvit) from day -1 to 27. Treatment was performed by spiking the water layer just below the surface without disturbing the sediment.

Test concentrations were: 320, 560, 1000, 1800 and 3200 µg as/L, in 4 replicates per test concentration and 2 replicates of blank control (1 control + 1 solvent (methanol) control). The highest concentration tested was 3200 µg as/L as a consequence of the poor solubility of dodine.

Sampling for analysis of test item concentration (320, 1000 and 3200 µg as/L only)occur at start (t=0h), at day 1, day 7 and at termination (day 28) in three fractions (water, pore water and sediment).

Analysis by LSC (water) and combustion (sediment).

Measurements and recordings: Number of emerged male and female midges (daily), number of pupae (day 28), egg packets, behaviour (3 times a week) and mortality (day 28).

Findings:

For each concentration, the actual applied radioactivity was in the range of 101.1 to 108.7% of nominal concentration and distribution of the test item was found to be homogenous in the radiolabelled stock solution and in the spiking solutions as well as in the test vessels at the start of the study (89.0 to 98.8% of nominal).

Upon addition of dodine technical to the sediment-water system, a fast transfer of the substance towards the sediment was observed followed by mineralization to CO_2. The activity recovered in the overlying water 20 minutes after spiking was in the range of 71.9% to 81.9% of the applied activity. This value dropped to 10.6 - 41.2% in the next 7 days and disappeared almost completely from the water layer at the end of the test (1.7-4.2%). The activity in the pore water was < 2% of applied for all time points and at all concentrations. The activity in the dry sediment was 4% at the start of the test, increased to 39.7-58.1 % after one day and then decreased again to 11.2-12.3% at the end of the test. Mass balance at the beginning was in the range 75.7% - 86.1 % but decreased to 13.8-16.6% at the end. Losses were most probably due to the metabolism and mineralization of dodine in the sediment-water systems (loss of $C0_2$).

The test can be considered as valid since:

- the mortality in the control did not exceed 30% at the end

- on day 24, 74% of the midges emerged in the blank-control and 78% in the solvent control (acceptability criteria : 50-70% emergence in the control).

- oxygen concentrations were always at least 60% of the air saturation value and adequate temperature was maintained.

A comparison with the controls showed no statistically significant effect on emergence rate and development time/rate at any of the concentrations tested.

The **EC50** for emergence of midges and for development rate/time **and the LOEC were above 3.2 mg as/L. The NOEC was 3.2 mg as/L.**

B.9.2.11 Effects on aquatic plants (Annex IIA 8.2.8)

According to Directive 91/414/EEC, this study is not required since dodine is not used as herbicide but as fungicide.

B.9.2.12 Microcosm and mesocosm study (Annex IIIA 10.2.2)

No data presented.

B.9.2.13 Residue data in fish (Annex IIIA 10.2.3)

Based on the outcome of the toxicity studies in fish, as well as based on the lack of bioaccumulation, this requirement is regarded to be non relevant.

B.9.2.14 Supplementary studies of toxicity to fish and aquatic invertebrates (Annex IIIA 10.2.4)

Studies of toxicity performed with the preparation to aquatic invertebrates and algae have already been described under B.9.2.6 and B.9.2.8.

B.9.2.15 Exposure and risk assessment for aquatic organisms (Annex IIIA 10.2)

The proposed uses for dodine are presented on Table below:

Table B.9.2.15.1 - Proposed use pattern for dodine.

Crop	Application (maximum frequency)	Max. application rate per season (kg as/ha)
Orchards/peach	5	5 x 0.900 = 4.5
cherry	5	3 x 0.800 = 2.4

Regarding exposure for aquatic organisms the PEC_{SW} and PECsed values for dodine were calculated using the FOCUS scenarios and the four-steps approach as recommended in the European Guidance doc. on Focus surface water scenarios (SANCO/4802/2001-rev.2 final May 2003) as described on section B.8.6.1. These PEC values will be used for the risk assessment. Table B.9.2.15.1 summarised the worst case PECsw and PECsed values.

Table B.9.2.15.1 - PECsw and PECsed values calculated using the FOCUS scenarios

Step		PECsw initial (µg/L)	PECsw TWA 4d (µg/L)	PECsw TWA 21d (µg/L)	PECsed initial (µg/kg)	PECsed TWA 21d (µg/kg)
Step 1		87.64	10.97	2.09	0.225	172.61
Step 2	North./EU	69.35	9.61	1.83	618.9	40.95
	South EU	69.35	9.61	1.83	1220.0	60.47
Step 3 (R3 Stream)		59.30	4.15	2.255	12.71	4.75
Step 4 - 10m		24.149 (R3 Stream)	1.75 (R3 Stream)	0.960 (R3 Stream)	10.977 (R4 Stream)	1.527 (R4 Stream)
Step 4 - 20m		5.887 (R3 Stream)	0.511 (R3 Stream)	0.250 (R3 Stream)	10.976 (R4 Stream)	1.527 (R4 Stream)
Step 4- 30m		1.981 (R3 Stream)	0.245 (R3 Stream)	0.097 (R3 Stream)	10.976 (R4 Stream)	1.527 (R4 Stream)
Step 4- 35m		1.309 (R3 Stream)	0.200 (R3 Stream)	0.072 (R3 Stream)	10.976 (R4 Stream)	1.527 (R4 Stream)
Step 4- 40m		0.914 (R3 Stream)	0.173 (R3 Stream)	0.056 (R3 Stream)	10.976 (R4 Stream)	1.527 (R4 Stream)

The endpoints from selected studies to the aquatic organisms were summarised in the Table below.

Table B.9.2.15.2 - Toxicity endpoints used in the risk assessment.

Time -scale	Species	Toxicity test	Endpoint (mg as/L)	Reference
Acute	Fish (Bluegill sunfish)	LC50 96h (daily renewal)	0.7	Caley *et al*, 1991
	Daphnia	EC50 48h (flow through)	0.018	Putt, 1992
	Daphnia	EC50 48h (static + sediment)	0.146	Migchielsen, 2002
	S. capricornutum	EbC50 120h ErC50 120h (static)	0.00095 0.011	Hoger, 1993
Chronic	Fish (Fathead minnow)	NOEC 30d (flow through; Early Life Stage)	0.099	Sousa, 1995
	Daphnia	NOEC 21d (flow through)	0.0044	Putt, 1992
	S. capricornutum	NOEb,rC 15d (semi-static)	0.00015	Hoger, 1995

The acute toxicity end point for bluegill sunfish and for Daphnia was taken as a worst case. For Daphnia, the result of the higher tier static + sediment study was also used. This more realistic approach was necessary because of the high sensitivity of daphnia to dodine.

Acute risk

In order to calculate TER values for acute risk, the initial PEC_{SW} of dodine were used.

The following TER for fish, daphnia and algae were obtained:

Table B.9.2.15.2 - Acute risk assessment to aquatic organisms.

Species	End point (mg/l)	PEC initial (mg/L)		TER	Trigger value
Bluegill sunfish	0.7	Step 1	0.08764	**7.99**	100
		Step 2	0.06935	**10.09**	
		Step 3	0.05993	**11.80**	
		Step 4 – 10m	0.02415	**28,99**	
		Step 4 – 20m	0.00588	119,05	
		Step 4 – 30m	0.00198	353,54	
		Step 4 – 35m	0.00131	534,35	
		Step 4 – 40m	0.00091	769,23	
Daphnia	0.018 (0.146)	Step 1	0.08764	**0,21 (1.67)**	100
		Step 2	0.06935	**0,26 (2.11)**	
		Step 3	0.05993	**0,30 (2.46)**	
		Step 4 – 10m	0.02415	**0,75 (6.05)**	
		Step 4 – 20m	0.00588	**3,06 (24.83)**	
		Step 4 – 30m	0.00198	**9,09 (73.74)**	
		Step 4 – 35m	0.00131	**13,74** (111.45)	
		Step 4 – 40m	0.00091	**19,78** (160.44)	
S. capricornutum	0.00095	Step 1	0.08764	**0,01084**	10
		Step 2	0.06935	**0,013699**	
		Step 3	0.05993	**0,01602**	
		Step 4 – 10m	0.02415	**0,039337**	
		Step 4 – 20m	0.00588	**0,161565**	
		Step 4 – 30m	0.00198	**0,479798**	
		Step 4 – 35m	0.00131	**0,725191**	
		Step 4 – 40m	0.00091	**1,043956**	

For **fish** a safe TER is reached with a buffer zone of **20 meters**.

Since dodine as a high adsorption potential with a mean Koc > 423.6 x 10^4, the toxicity study performed with sediment reflects a more realistic exposure.

For **Dapnhia** values in brackets correspond to the study performed with sediment and it can be concluded that for a **35m buffer zone** safe use was identified.

Regarding algae no safe use could be derived, if we consider PECinitial. Since dodine has a DT50water value < 1 day, it will be more realistic to compare the toxicity value (EbC50 120h) with TWA PEC values, as recommended on Higher-tier Aquatic Risk Assessment Guidance Doc.[13]. The following table summarises the 4 days TER values to algae.

[13] Campbell PJ, Arnold DJS, Brock TCM, Grandy NJ, Heger W, Heimbach F, Maund SJ, Streloke M (1999) Guidance document on higher-tier aquatic risk assessment for pesticides (HARAP). Lacanau Ocean, France: Setac-Europe Publication. 178 pp.

Table B.9.2.15.3 - Refined acute risk assessment to algae.

Species	End point (µg/l)	PEC TWA 4 days (µg/L)		TER	Trigger value
S. capricornutum	0.95	Step 1	10.97	**0.086**	10
		Step 2	9.61	**0.099**	
		Step 3	4.15	**0.229**	
		Step 4 – 10m	1.75	**0.543**	
		Step 4 – 20m	0.511	**1.859**	
		Step 4 – 30m	0.245	**3.877**	
		Step 4 – 35m	0.200	**4.75**	
		Step 4 – 40m	0.173	**5.491**	

It is evident that a safety margin of 10 is not granted. However, one higher Tier study for 15 days has been submitted (Hoger, 1995).

However risk assessment for algae will be further discussed under the chronic risk assessment.

Chronic risk

The first stage chronic risk assessment was based on the initial PEC values as a worst case. This first approach leads to TERlt below the relevant triggers. In a second step, the risk assessment was refined as suggested in the guidance document on aquatic ecotoxicology (Sanco/3268/2001 rev.4). Indeed, it is appropriate to refine the risk assessment using PEC_{twa} values if an unrealistic exposure regime prevailed in the relevant toxicity test. This is the case for dodine: chronic toxicity studies on fish and daphnia were performed using a flow-through system which maintained the level of concentration of dodine constant during all the study. This type of studies are quite unrealistic and represent a worst case knowing the very rapid dissipation of dodine from the water phase (DT50 water=0.37).

Table B.9.2.15.4 – Long-term risk assessment to aquatic organisms (1st Tier).

Species	End point (mg/l)	PEC initial (mg/L)		TERlt	Trigger value
Fathead minnow	0.099	Step 1	0.08764	**1.13**	10
		Step 2	0.06935	**1.43**	
		Step 3	0.05993	**1.67**	
		Step 4 – 10m	0.02415	**4.10**	
		Step 4 – 20m	0.00588	16.84	
		Step 4 – 30m	0.00198	50.00	
		Step 4 – 35m	0.00131	75.57	
		Step 4 – 40m	0.00091	108.79	
Daphnia	0.0044	Step 1	0.08764	**0.05**	10
		Step 2	0.06935	**0.06**	
		Step 3	0.05993	**0.07**	
		Step 4 – 10m	0.02415	**0.18**	
		Step 4 – 20m	0.00588	**0.75**	
		Step 4 – 30m	0.00198	**2.22**	
		Step 4 – 35m	0.00131	**3.36**	
		Step 4 – 40m	0.00091	**4.84**	
S. capricornutum	0.00015	Step 1	0.08764	**0.002**	10
		Step 2	0.06935	**0.002**	
		Step 3	0.05993	**0.003**	
		Step 4 – 10m	0.02415	**0.006**	
		Step 4 – 20m	0.00588	**0.026**	
		Step 4 – 30m	0.00198	**0.076**	
		Step 4 – 35m	0.00131	**0.115**	
		Step 4 – 40m	0.00091	**0.165**	

Table B.9.2.15.5 - Long-term risk assessment to aquatic organisms (2nd Tier).

Species	End point (mg/l)	PEC TWA 21d (mg/L)		TER	Trigger value
Fathead minnow	0.099	Step 1	0.00209	47.37	10
		Step 2	0.00183	54.10	
		Step 3	0.00233	43.81	
		Step 4 – 10m	0.00096	103.13	
		Step 4 – 20m	0.00025	396	
		Step 4 – 30m	0.000097	1020.62	
		Step 4 – 35m	0.000072	1375	
		Step 4 – 40m	0.000056	1767.86	
Daphnia	0.0044	Step 1	0.00209	**2.12**	10
		Step 2	0.00183	**2.40**	
		Step 3	0.00226	**1.95**	
		Step 4 – 10m	0.00096	**4.58**	
		Step 4 – 20m	0.00025	17.60	
		Step 4 – 30m	0.000097	45.36	
		Step 4 – 35m	0.000072	61.11	
		Step 4 – 40m	0.000056	78.57	
S. capricornutum	0.00015	Step 1	0.00209	0.072	10
		Step 2	0.00183	0.082	
		Step 3	0.00233	0.067	
		Step 4 – 10m	0.00096	0.156	
		Step 4 – 20m	0.00025	0.6	
		Step 4 – 30m	0.000097	1.546	
		Step 4 – 35m	0.000072	2.083	
		Step 4 – 40m	0.000056	2.679	

Regarding the long-term risk assessed using PEC_{twa} (more realistic approach), no buffer zone is necessary for fish whereas a buffer zone of 20 meters is necessary for daphnia.

For algae no safe use could be identified; however in the 15 day study it is observed that:

- multiple dodine treatments (resulting in initial exposure levels of 20 to 45 µg as/L) did not demonstrate a cumulative effect on cell growth

- based on the results of the recovery phase conducted subsequent to the 15-day exposure phase, it was concluded that *Selenastrum capricornutum* has the ability to recover from exposure to dodine concentrations as high as 45 µg as/L (leading to 99% reduction of cell density), when sufficient nutrients are available. Dodine proved to be algistatic rather than algicidal.

In conclusion, a sufficient margin of security is obtained for fish and daphnia using a buffer zone of 35 meter.

Since dodine partitions into the sediment, a particular concern can be raised regarding exposure of sediment dwelling organisms. In order to assess exposure of midges, TER was calculated using (1) toxicity end point obtained in the water spiked 28 days test on *Chironomus riparius* and (2) initial Step4 PECsw value calculated considering 10m buffer zone.

Table B.9.2.15.6 - Risk assessment to sediment dwelling organisms.

Species	Test type	Endpoint (µg as/l)	PECsw initial 10m (µg/l)	TER	Trigger value
Chironomus riparius	Acute, 28-day, water spiked (static)	EC50 > 3200	24.149	132	100

For sediment dwelling organisms a safety margin of 10 meters is sufficient for safe uses.

B.9.3 Effects on other terrestrial vertebrates (Annex IIIA 10.3.1)

B.9.3.1 Exposure and risk assessment to terrestrial vertebrates other than birds

Mammals may be exposed to dodine mainly through the consumption of contaminated food following application of Syllit 400 SC to orchards.

For this evaluation the data from the following mammalian toxicity studies have been used:

Risk evaluation	Toxicity study endpoint	Result	Reference
Acute	Acute rat oral toxicity (LD_{50})	851 mg as/kg bw/d	Kern, T. G., 1999
Long-term	Rat, 2-Generations (NOAEL)	13.14 mg as/kg bw/d	Henwood, S. M., 1996
	Rat, Teratogenicity (NOAEL)	45 mg as/kg bw/d	Hazelden, K.P. *et al*, 1989b

Toxicity Exposure Ratio (TER) and Estimated Theoretical Exposure (ETE) were calculated based on the methods, i.e. equations and tabulated values described in the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (Sanco/4145/2000 of 25 September 2002).

A realistic approach will take into account real food ingestion of small mammals in orchards. Exposure of small mammals to dodine contained in the plant protection product Syllit 400 SC is considered to arise mainly from feeding on contaminated short grasses present in treated orchards. One small herbivorous mammal (a 25g vole) will be considered as the standard species.

The worst-case scenario is as follows apples/pears/peach, 5 x 900 g as/ha with 7 days application interval.

Estim. Theor Exposure (ETEa) = (FIR/BW) * C * AV * PT * PD

(mg/kg bw/d)

In the 1st Tier calculations, the AV (Avoidance factor), PT (fraction of diet obtained in treated area) and PD (fraction of food in diet) factors were considered to be 1 (worst case) both for acute and for long term risk assessment.

The Table below summarises the acute risk assessment.

Table B.9.3.1.1 - Calculated 1st and 2nd Tier TER values for acute risk for mammals.

Type	Indicator Species	Scenario	FIR/bw	RUD	MAF	AR	C	ETE	Toxicity	TER
Orchards										
Acute 1st Tier	Small herbivorous mammal	Short grass	1.39	85	1.9	0.9	145.4	202	851	**4.2**
			1.39	42.5	1.9	0.9	38.25	101		**8.4**
Acute 2nd Tier	Small herbivorous mammal	Residue data	1.39	-			4.50	6.25		136

FIR = Food intake rate; bw = Bodyweight; RUD = Residue per unit dose; Ftwa = Time weighted average factor; AR = Application rate; PT = Fraction of diet obtained in treated area; C = Concentration in diet = RUD* AR; ETE = Estimated theoretical exposure = C*FIR/bw*Ftwa*PT; TER = Toxicity exposure ratio.

From the Table above it's possible to conclude that an insufficient margin of safety exists in the worst case scenario.

The C value used was estimated based on the standard RUD value for orchard given in the guidance document SANCO/4145 (25/9/2002) based on generic data obtained from Fletcher et al. 1994 and Fisher and Bowers 1997. It may be used for a first Tier assessment but refinement is possible regarding that the application of the active substance dodine will occur in later stages were deposition will be lower (30%), considering that the RUD value will be 42.5. Using this refinement TERa value will be 8.4 wish is still below the trigger value.

For a 2nd Tier risk assessment calculations the data obtained from residue trials which already include a sum up of residue after multiple applications at exaggerated rates (no MAF factor required in this case) could be used.

On apple, the highest residue level found 2 hours after the 12th application (exaggerated number of application) was 3.4 mg/kg (see B.7.). On pear, the highest residue level found 2 hours after the 4th application was 3.5 mg/kg (see B.7). On cherry, the highest residue level found 2 hours after the 3rd application was 4.5 mg/kg (see B.7).

Based on these results, it sounds reasonable to use a revised máx. C value of 4.5 mg/kg from residue trials on cherry, no deposition factor will be used to convert residue from foliage to short grass.

With a more realistic exposure value the acute TER value was 136, which is higher than the trigger value of 10, safe uses could be identified to small herbivorous mammals following applications of dodine in orchards.

For the long-term toxicity exposure ratio (TERlt) to small mammals, the worst-case scenario was the use in peach, 5 x 900 g as/ha spaced 7 days.

Table below summarises the long term risk assessment.

Table B.9.3.1.2 - Calculated 1st Tier TER values for long term risk for mammals.

Type	Indicator Species	Scenario	FIR/bw	RUD	MAF	AR	C	Ftwa	ETE	Toxicity	TER
Orchards											
Long-term 1st Tier	Small herbivorous mammal	Short grass	1.39	46	2.4	0.9	99.4	0.53	73.2	13.14	**0.2**
										45	**0.6**

FIR = Food intake rate; bw = Bodyweight; RUD = Residue per unit dose; Ftwa = Time weighted average factor; AR = Application rate; PT = Fraction of diet obtained in treated area; C = Concentration in diet = RUD* AR; ETE = Estimated theoretical exposure = C*FIR/bw*Ftwa*PT; TER = Toxicity exposure ratio.

Insufficient margin of safety is concluded for mammals for the first Tier long term risk assessment. Therefore a refined long-term risk assessment is needed.

A refined long-term mammalian risk assessment has been conduced according to SANCO/4145/2000.

It is unlikely that mammal species would be feeding exclusively on treated grass. The high food intake rate (FIR) of 1.39 employed in the 1st Tier SANCO/4145/2000 is representative of small herbivorous mammals but most species, such as the wood mouse (*Apodemus sylvaticus*), harvest mouse (*Micromys minitus*), common dormouse (*Muscardinus avellanarius*) and bank vole (C*lethrionomys glareolus*) are largely omnivorous, taking a range of food items including insects, fruit and seeds plus mixed vegetation.

Regarding the fact that wood mice are found in hedgerows in cultivated land all year round and further enhanced by the fact that wood mice are found almost everywhere in central Europe, the refined long-term risk assessment for mammals will consider the wood mice as a relevant species.

For assessing the exposure of wood mice feeding on a mixed diet, the calculation of food intake rates was performed according to SANCO/4145/2000 using information indicated by Croker *et al*. (2002) on Daily Energy Expenditure (DEE), energy and moisture contents of different food types and on assimilation efficiencies for mammals. A mean body weight of 18 g was assumed for the wood mouse (Guerney, *et al*., 1998).

The log (DEE) was estimated according to the following equation using values for other eutherians as indicated by Croker *et al*. (2002):

$$\text{Log (DEE)} = 0.8459 + 0.7050 * (\log \text{bw (g)}) = 0.8459 + 0.7050 * \log 18 = 1.731$$

$$\text{DEE} = 53.8 \text{ kJ/d}$$

In accordance with Croker *et al*. (2002) the average daily food intake was then estimated according to the following equation for four food types: short grass, small seeds, large insects and earthworms:

$$\text{Daily Food Intake (FIR) (wet weight)} = \frac{\text{Daily Energy Expenditure (DEE) (kJ)}}{\text{Energy in Food (kJ/g) * (1- Moisture)* Assimilation Efficiency}}$$

Values for energy and moisture content as well as for assimilation efficiency of the separate food items were drawn from SANCO/4145/2000 as indicated in Table below. The resulting food intake rates were related to the average body weight of wood mice (18 g) to estimate FIR/g bw values. As these values are based on the assumption that wood mice feed exclusively on the separate food items, they are weighted for ETE calculation by PD values.

Table B.9.3.1.3 – Daily energy expenditure (DEE), energy and moisture content, assimilation efficiencies and resulting food intake rates (FIR and FIR/g bw) for different food types for a small mammal weighing 18 g.

Food type	DEE (kJ/g)	Energy content (kJ/g)	Moisture content	Assimilation efficiency	FIR (g/d)	FIR/g bw
Short grass	53.8	18.0	0.764	0.46	27.5	1.53
Small seeds	53.8	21.0	0.119	0.83	3.2	0.19
Large insects	53.8	21.9	0.705	0.88	9.5	0.53
Earthworms	53.8	19.3	0.846	0.88	20.6	1.14

For estimating the residues in different food types, for the long-term exposure, the default values (SANCO/4145/2000) for residues in short grass of 76 mg as/kg and for small seeds of 40.2 mg as/kg were considered. However, a deposition factor of 0.3 was applied, considering that by the time of application orchards foliage were at development foliage phase. The resulting RUD values will be 22.8 mg as/kg for short grass and 12.1 mg as/kg for small seeds.

For insects, the default residue value of 5.1 mg as/kg for long-term exposure will be used in the risk assessment.

Regarding earthworms the residue of dodine in those organisms are based on the consideration that dodine is not expected to accumulate in earthworms due to the low log Pow of 0.96 and accordingly, the PEC for soil represents the worst case for residues. The PEcsoil for an application rate of 1 kg as/ha, 30% deposition and a soil layer of 5 cm depth with a soil bulk density of 1.5 g/cm^3 is 0.4 mg as/kg, representing the worst case RUD value for earthworms.

For estimating residue decline in insects the DT_{50} of 2.6 day from the laboratory study with *T. molitor* (Hirth, 2005) was used and the resulting f_{twa} will be 0.2 . Regarding earthworms, f_{twa} was determined based on the mean $DT_{50field}$ in soil of 13 days (mean value of all soil degradation studies under field conditions) and the resulting f_{twa} will be 0.83.

F_{twa} values calculated above were based on a minimum interval between applications of 7 days and the correspondent DT_{50} values for insects and earthworms.

For short grass and small seeds the default DT_{50} value of 10 days was considered and consequently the f_{twa} long-term used for risk assessment will be 0.53.

Considering the different food types the MAF factor will be the following:

Table B.9.3.1.3 - Calculated MAF values for long term risk for mammals.

Food type	Interval between applications	DT50	MAF
Short grass	7 days	10	2.4
Small seeds		10	2.4
Large insects		2.6	1.2
Earthworms		13	2.7

In addition and according to radio-tracking data obtained by the Central Science Laboratory[14] (2003), it may be expected that wood mice do not satisfy their entire food demand in the treated area. Accordingly, a refined PT value can be used in the risk assessment. Observations by the Central Science Laboratory (2003) were based on 58 wood mice that were caught and radio-tagged in cereal fields in autumn and winter and in potato fields in summer (median contact of 6 hours 50 min). According to these observations, the average value for the proportion of active time that was spent in cultivated areas is 26%, corresponding to a PT value of 0.26 for long-term exposure.

In refining PD, data from stomach contents, faecal analysis, and pellet analysis can be used to determine likely food consumption. Therefor the following considerations will be used for the refinement of PD.

Data on the different food types in the diet of wood mice was collected by Pelz (1989, as cited by Guerney *et al*., 1998) on arable farms in the Rhineland, Germany. Data (volume percent of stomach contents) were collected from 346 individuals trapped over a seven year period on a monthly base. In view of the expected time of application of dodine, diet composition data for the months of March to July were taken into consideration for the mammalian risk assessment (based on a total of 146 individuals). The different food types encountered by Pelz (1989, as cited by Guerney *et al*., 1998) were: cereal grain (ranging from 5 to 48% of stomach contents over the period in question), vegetative plant material (8 to 24%), dicotyledonous seeds (0-25%), insect larvae (10 to 45%) and earthworms (9 to 40%). Although proportions are indicated as volume percent, it is evident from this data that all food items indicated above contribute to a large but varying extent of the diet of wood mice.

Depending upon availability, the diet of the wood mouse representative for a 3-week period considered in the long-term risk assessment may thus be expected to be composed of all of the food items indicated above. As wood mice are not expected to find cereal seeds within pome fruit orchards, cereals are not included in the risk assessment calculations. Approximate PD values of 0.25 are thus assigned to the different food type categories.

Calculation of the refined ETE value for long-term exposure is summarised below.

[14] Central Science Laboratory (2003): Improving estimates of wildlife exposure to pesticides in arable crops. Final report, DEFRA project code PN0915 (available via the DEFRA website www.defra.gov.uk as at December 2005)

Table B.9.3.1.4 - Calculated 1st Tier TER values for long term risk for mammals.

Indicator Species	FIR/ g bw	Food type	RUD	AR	MAF	PT	PD	Ftwa	ETE
Wood mouse	1.53	Short grass	22.8	0.9	2.4	0.26	0.25	0.53	2.60
	0.19	Small seeds	12.1		2.4	0.26	0.25	0.53	0.171
	0.53	Insects	5.1		1.2	0.26	0.25	0.20	0.038
	1.14	Earthworms	0.4		2.7	0.26	0.25	0.83	0.060
								Overall (Sum):	**2.869**

FIR = Food intake rate; bw = Bodyweight; RUD = Residue per unit dose; Ftwa = Time weighted average factor; AR = Application rate; PT = Fraction of diet obtained in treated area; ETE = Estimated theoretical exposure = FIR/bw*RUD*AR*MAF*PT*PD*Ftwa.

The long-term TER was calculated by relating the mammalian toxicity endpoints to the overall theoretical exposure value as summarised below:

Table B.9.3.1.5 - Calculated Refined TER values for long term risk for mammals.

Risk evaluation	Toxicity study endpoint	Endpoint	Overall ETE	TERlt
Long-term	Rat, 2-Generations (NOAEL)	13.14 mg as/kg bw/d	2.869	4.6
	Rat, Teratogenicity, (NOAEL)	45 mg as/kg bw/d		15.7

Based on the assessment described above and considering both long-term studies the overall risk for **mammals from the use of dodine is acceptable.** However for safety precautions the use of dodine during breeding season should be restricted.

B.9.4 Effects on bees (Annex IIA 8.3.1; Annex IIIA 10.3.2)

B.9.4.1 Acute toxicity to bees (Annex IIA 8.3.1.1)

Determination of contact and oral acute toxicity of dodine to honey bees (*Apis mellifera*)

Servajean, E. (2004). Phytosafe/ France, unpublished report # 04-99-037-ES.

Guidelines and GLP:

The study was conducted according to OECD 213 and 214 (January 2000). Principles of GLP were complied with.

Test species and methods:

Dodine: batch S01 L01, purity 98.5% and dimethoate (99.9%) was used as toxic standard (positive control) at 3 concentrations (between 0.1 and 0.3 µg/bee) in this study. A negative control, treated with the solvent used for the dilution of dodine was also used and served as solvent control group.

Test species: honey bee (*Apis mellifera caucasica* triple Hybrid). Test was performed with bees collected the day before the application, directly out from the frames, by opening the hive, without distinction of sex and age. The colony was received in June 2004.

Bees were kept in round cages (20-25 bees per cage = 1 replicate group). Males and moribund females were discarded.

The oral toxicity test was divided on two different tests:

- 1st test (limit dose): three replicates of each 19-24 bees at nominal concentrations of 100.7 µg dodine/bee; each bee receiving 10 µL of a dodine suspension in a sucrose solution.

- 2nd test (5 concentrations): three replicates of 16-24 bees at nominal concentrations of 19, 34.3, 61.8, 111.3 and 200.5 µg dodine/bee; each bee receiving 10 µL of a dodine suspension in a sucrose solution.

In the contact toxicity test 3 replicates with 20 bees each were exposed to 1 µL of test solution (dodine diluted in water acidified with acetic acid 0.1 %) on the thorax at nominal concentrations of 9.9, 17.6, 31.5, 56.2 and 100.4 µg dodine/ bee. After dosing/application, the bees were fed 50% aqueous sucrose solution.

The observations during the study were: mortality at 24, 48, 72, 96, 120 (oral only) and 144 (oral only) hours; post treatment feeding dynamics of a sucrose solution *ad libitum* after 24, 48, 72, 96 hours were assessed. Repellent effect and bee behaviour was also assessed.

Temperatures varied between 22°C and 26°C.

Findings:

Oral: No repellent effect was noted in any of the tests, the bees accepted to feed the dodine treated syrup. After oral exposure in the **1st limit dose** test at 100.7 µg/bee, the percent mortality ranged between 10 and 20.8% 24 hours after treatment and between 15 and 31.6% after 48 hours. **Oral LD50 value was higher than 100.7 µg/bee**.

The post-treatment feeding dynamics was reduced to 63% of the control after 48 hours. In the **2nd** test, the percent mortality was low in the dodine treated groups after 24 hrs. Then after, the mortality was increased, but remained lower than 50% for every treated groups after 48 hrs exposure (**LD50oral > 200 µg/bee**). According to the OECD guideline, the observation period was prolonged (up to 6 days) after treatment as percent mortality was increased by more than 10% in the three highest groups (61.8, 111.3 and 200.5 µg/bee). The induced mortality exhibited a regular increase throughout the additional observation period for every treatment doses.

Regression analysis of data did not give satisfying results as the dose-response was not clear: the bees appeared similarly affected for every treatment doses. The relevance of this finding in practical field conditions is doubtful since the mortality of bees kept in small cages is known to increase normally with time especially after a prolonged period of time. The only conclusion was that the **LD50oral > 200 µg/bee.**

The post-treatment feeding dynamics was observed throughout the 96-hour period. Ingestion of syrup was higher or similar to the control for every treatment dose except the 200.5 µg/bee group. Nevertheless, the surviving bees appeared weak in treated groups ranging between 61.8 and 200.5 µg/bee: moving and reactivity of the bees were reduced.

Table B.9.4.1 - Oral exposure - Induced mortality 120 h and 144 h after the treatment application

Dose (µg/bee)	Replicate	% mortality	
		120 h	144 h
control	1	13.0	17.4
	2	5.3	5.3
	3	13.6	18.2
	Mean	**10.7**	**13.6**
19.0	1	25.0	25.0
	2	38.1	42.9
	3	18.8	50.0
	Mean	**27.3**	**39.3**
34.3	1	27.8	33.3
	2	31.6	31.6
	3	20.0	35.0
	Mean	**26.5**	**33.3**
61.8	1	36.8	42.1
	2	52.4	57.1
	3	33.3	41.7
	Mean	**40.9**	**47.0**
111.3	1	23.8	42.9
	2	47.4	47.4
	3	17.4	26.1
	Mean	**29.5**	**38.8**
200.5	1	30.0	35.0
	2	50.0	72.7
	3	60.0	90.0
	Mean	**46.7**	**65.9**

As can be seen from Table above, the induced effect of dodine still increased 6 days after the treatment application for every treatment doses. The dose-effect relationship was not clear: mean corrected percent mortality was about 40 % in the 61.8 µg/bee treated group, 60% in the 200.5 µg/bee treated groups, and 20-30 % only in every other case (19.0, 34.3 and 111.3 µg/bee).

Contact: After 24 hours, no additional mortality as compared to control was observed in any of the dodine treated groups. Then, 48 hours after the treatment, some replicates exhibited abnormally high percent mortality (20 to 35%) without clear dose related effect (LD50contact > 100 µg /bee). The bee behaviour was considered as normal except in the 31.5 µg/bee treated group were moving and flying were reduced within the first 48h. The observation period was prolonged for 2 days, mortality exhibited a normal and regular increase throughout those two days and the test was not prolonged further. The post-feeding dynamics was observed throughout the 96 hour-period. Ingestion of syrup was higher or similar to the control for every treatment dose.

The results allow the conclusion that dodine in bees has a **48-h LD50oral > 200 µg as/bee, as well as a 48-h LD50contact > 100 µg as/bee**.

B.9.4.2 Bee brood feeding test (Annex IIA 8.3.1.2)

Dodine is not an insect growth regulator and as such data for this point are not required.

B.9.4.3 Acute toxicity of the preparations to bees (Annex IIIA 10.4.1)

Determination of contact and oral acute toxicity of Syllit 400 SC to honey bees (*Apis mellifera*)

Servajean, E. (2004). Phytosafe/ France, unpublished report # 04-99-038-ES.

Guidelines and GLP:

The study was conducted according to OECD 213 and 214 (January 2000). Principles of GLP were complied with.

Test species and methods:

Dodine formulated as a 400 g/L SC, product batch 164503 and purity: 405.4 g/L and dimethoate (99.9%) was used as toxic standard (positive control) at 3 concentrations (between 0.1 and 0.3 µg/bee) in this study. A negative control lot, treated with the solvent used for the dilution of dodine was also used and served as solvent control group.

Test species: honey bee (*Apis mellifera caucasica* triple Hybrid). The test was performed with bees collected the day before the application, directly out from the frames, by opening the hive, without distinction of sex and age. The colony was received in June 2004.

Bees were kept in round cages (20-25 bees per cage = 1 replicate group). Males and moribund females were discarded.

The oral toxicity test has performed with (5 concentrations) and three replicates of 15-24 bees at nominal concentrations of 18.9, 34.0, 61.3, 110.5 and 199 µg Syllit 400 SC/bee; each bee receiving 10 µL of a dodine suspension in a sucrose solution.

In the contact toxicity test of 3 replicates with 20 bees each were exposed to 1 µL of test solution (Syllit 400 SC diluted in water/ethanol 75/25 /v carrier) on the thorax at nominal concentrations of 9.5 17.2, 31.0, 55.8 and 100.6 µg Syllit 400 SC/bee. After dosing/application, the bees were fed 50% aqueous sucrose solution.

The observations during the study were: mortality at 24, 48, 72 and 96 (oral only) hours; post treatment feeding dynamics of a sucrose solution *ad libitum* after 24, 48, 72, 96 (oral only) hours were assessed. Repellent effect and bee behaviour was also assessed.

Temperatures vary between 22°C and 26°C.

Findings:

Oral: No repellent effect was noted in any of the tests, the bees accepted to feed the Syllit 400 SC treated syrup.

Percent of mortality ranged from 13.9 to 49.9% and exhibited a clear dose-related effect within the first 48 hours but remained lower than 50% in every Syllit treated groups with a 48-hours calculated **LD50oral value of 153 µg Syllit 400 SC**.

According to the OCDE guideline, the observation period was prolonged (up to 96 hours) after treatment as percent mortality was increased by more than 10% in the three lowest groups (18.9, 34.0 and 61.3 µg/bee). The induced mortality exhibited a regular increase throughout the additional observation period for treatment doses ranging between 18.9 and 61.3 µg/bee.

For the two highest treatment doses (110.5 and 199.0 µg/bee), the induced effects tended to stabilize around 50-55% mortality. The post-treatment feeding dynamics was observed throughout the 96-hour period. Ingestion of syrup was reduced up from day 2 for treatment groups at 61.3 µg/bee and above and surviving bees appeared weak in those groups.

Below that, post-treatment feeding dynamics was similar or higher than the control.

Contact: After 24, 48 and 72 hours, no significant additional mortality as compared to control was observed in any of the dodine treated groups (**48-hours LD50contact > 100 g Syllit 400 SC/bee**). Bee behaviour was considered as normal after contact exposure. The post-feeding dynamics was observed throughout the 72 hour period. Ingestion of syrup was slightly lower than the control for every treatment dose (ranging from 63 to 88% of control without clear dose related effect).

The results allow the conclusion that Syllit 400 SC in bees has a 48-hours LD50oral of 153 µg Syllit 400 SC/bee (⇔ 61.2 µg as/bee), as well as a 48-hours LD50 contact >100 µg Syllit 400 SC/bee (⇔ > 40 µg as/bee).

B.9.4.4 Effects on bees of residues on crops (Annex IIIA 10.4.2)

No data has been submitted.

B.9.4.5 Cage tests (Annex IIIA 10.4.3)

No data has been submitted

B.9.4.6 Field tests to investigate special effects (Annex IIIA 10.4.4)

No data has been submitted.

B.9.4.7 Tunnel testing to investigate effects of feeding on contaminated honey (Annex IIIA 10.4.5)

No data has been submitted.

B.9.4.8 Summary of Effects on Bees

The acute toxicity of dodine technical and Syllit 400 SC was been evaluated (Servajean, 2004a and b). With Syllit 400 SC the 48 hr oral and contact LD_{50} expressed as dodine was 61.2 µg as/bee > 40 µg as/bee, respectively. It seems that Syllit was more toxic to bees than the active. The LD_{50} values obtained with the a.s. were higher than the ones obtained from the study with the preparation (48 hr oral LD_{50} > 200 µg/bee, contact LD_{50} > 100 µg/bee). Since mortality didn't stabilise during the 48 hr observation period the two oral acute studies were prolonged till 96 hr, however the oral LD_{50} was still > 200 µg/bee. These values are summarised in the Table below.

Table B.9.4.8 - Honeybees toxicity studies

Test species	Test material	Results	References
Honeybee *(Apis mellifera* L*)*	a.s.	LD_{50} (48h) contact > 100 µg a.s./bee LD_{50} (48h) oral > 200 µg a.s./bee	Servajean, 2004a
Honeybee *(Apis mellifera* L*)*	Syllit 400 SC	LD_{50} (48h) contact > 40 µg a.s./bee LD_{50} (48h) oral = 61.2 µg a.s./bee	Servajean, 2004b

B.9.4.9 Exposure and risk assessment for bees (Annex IIIA 10.4)

For bees, TER values are superseded by the expression of the hazard quotient (HQ) which is defined as the ratio of application rate and LD_{50} oral and contact with the LD_{50} in µg a.s./bee and the application rate in g a.s./ha eventually corrected by a multiple application factor (MAF).

The following hazard quotients were obtained with the maximum application rate of 900 g dodine/ha and a MAF factor of 1.9 (DT50 7 days: spray interval 7 days; ESCORT 2)

Table B.9.4.9 - Risk to bees from exposure to dodine

Test substance	LD50 (µg/bee)	Application rate (g as/ha)	Hazard quotient	Trigger
dodine	Contact > 100	1.9 x 900 = 1710	17.1	< 50
	Oral > 200		8.55	
Syllit 400 SC	Contact = 40		42.75	
	Oral = 61.2		27.9	

Both the hazard quotients are less than 50, indicating that dodine pose a negligible acute risk to adult bees.

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

B.9.5.1 Effects of the active substance and formulations on non-target terrestrial arthropods

EXP 10343A : acute toxicity to the predatory mite, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in the laboratory.

Kühner, C. (1997a).GAB-IFU / Germany, unpublished report # 97123/02-NLTp.

Guidelines and GLP:

The study was conducted according to Louis/Ufer[15] (1995) and Guidance document ESCORT (1994). Principles of GLP were complied with.

Test species and methods:

Dodine, formulated as EXP 10343A (= Syllit 400 SC, Dodine 400 SC): formulation batch OP960466, content: 405 g/L dodine and the toxic standard: Perfekthion containing nominally 37.4% dimethoate were used to assess the effects on protonymph of *Typhlodromus pyri* 1 day old.

Test treatments consisted of: 1 tap water (control), toxic standard and 2 concentrations of Syllit: 2.23 kg Syllit 400 SC per ha (= approx. 900 g as/ha) and 4.46 kg Syllit 400 SC per ha (=approx. 1800 g as/ha). This corresponds to 1-fold and 2-fold maximum recommended single field application rate.

Five replicates per dose level of 20 protonymphs each (100 mites/concentration) on glass plates placed on wet filter paper; mites were fed with pollen of *Vicia fabae*, *Corylus avellana*, *Helianthus tuberosus* were exposed for14 days. Observations of mortality and escape rate occurred on days 3 and 7 of exposure. Sex ratio of the surviving mites was observed at day 7 of exposure. Counting of eggs and all other juvenile stages occurred 4 times, between day 7 and 14 and on day 17 for treatment done with 900 g as/ha.

Environmental conditions: temperature: 25 ± 3 °C; relative humidity: 70% HR; photoperiod: 16 h light - 8 h darkness. Test was performed under ventilated room conditions.

Findings:

Based on toxic standard and control mortality, as well as reproductive performance in the control, the test can be regarded as valid and acceptable.

[15] LOUIS, F., UFER, A., 1995: Methodical improvements of standard laboratory tests for determining the side-effects of agrochemical on predatory mites (Acari: Phytoseiidae). Anz. Schadlingskde., Pflanzenschutz, Umweltschutz 68, 153-154

Mortality findings were corrected using the Schneider-Orelli[16] (1947), fecundity and beneficial capacity were calculated acc. to guideline. The results obtained are summarised in the following tables.

Table B.9.5.1.1 - Mortality of *T. pyri* protonymphs on exposure day 7.

Treatment g/ha	Mortality in replicates (number)					Observed mortality (mean)	Corrected mortality
	1	2	3	4	5		
Control	4/20	3/20	5/20	0/20	2/20	14%	
900 g as/ha	12/20	10/20	10/20	11/20	4/20	47%	38.4%
1800 g as/ha	18/20	14/20	19/20	16/20	17/20	83%	80.2%
Toxic standard	19/20	19/20	15/20	18/20	16/20	87%	84.9%

In the control group, the surviving adults were determined as 37 females and 49 males. Surviving adults were determined as 14 females/39 males and 1 female/16 males in the group treated with 900 and 1800 g as/ha respectively. In the toxic standard group, surviving adults were 3 females and 10 males.

The reproductive phase resulted in the following rates and numbers:

Table B.9.5.1.2 - Reproductive performance of *T. pyri*.

Treatment g/ha	R	Egg production per female					Eggs/female (mean)
		1	2	3	4	5	
Control	-	6.9	6.2	7.8	7.5	6.6	7.0
900 g as/ha	0.43	-	1.3	-	-	4.7	3.0
900 g as/ha*	0.53	-	2.3	-	-	5.0	3.7
Toxic standard	0.57	4.0	-	-	-	-	4.0

R: reproduction factor; * egg production until day 17

Due to the low number of females in the 1800 g as/ha group, fertility was not assessed in this group.

Reduction in beneficial capacity was calculated (according to Overmeer and Van Zon[17], 1982) to be 73.5%, 67.4% and 91.4% in the 900, 900 (egg collection until day 17) g as/ha and toxic standard treatment levels respectively.

In conclusion, a **reduction of beneficial capacity of 73.5%** was calculated at 900 g as/ha (2.23 g Syllit 400SC/ha). According to the IOBC classification, **Syllit 400 SC has to be considered as "harmful"** (Class 4 - reduction in beneficial capacity 31 - 80 %) to *Typhlodromus pyri* on glass plates.

Determination of LD50 of Syllit on protonymphs of the predatory mite, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) on detached bean leaves in the laboratory.

Jansen, J. -P. (2001a). Lab. d'Ecotoxicologie, Gembloux/ Belgium, unpublished report #TE 02/2001

[16] SCHNEIDER-ORELLI, O. (1947): Entomologisches Praktikum. Aarau, 2. Auflage.

[17] OVERMEER, W.P.J., VAN ZON, A.Q., 1982: A standardized method for testing the side effects of pesticides on the predacious mite *Amblyseius potentillae*. (Acarina: Phytoseiidae). Entomophaga. 27: 357 - 363.

Guidelines and GLP:

The study was conducted according to Overmeer[18] (1988), Samsoe-Petersen[19] (1983) and SETAC Guidance. Principles of GLP were complied with.

Test species and methods:

Dodine, formulated as Syllit 400 SC: formulation batch S01D02, content: 389 g/L dodine no toxic standard was used to assess the effects on protonymph of *Typhlodromus pyri* 2-3 days old.

Test treatments consisted: on 1 tap water (control) and 5 concentrations of Syllit: 0.625, 1.25, 2.5, 5.0 and 10 L Syllit 400 SC per ha (250, 500, 1000, 2000, 4000 g as/ha) sprayed in 200 L/ha; dilution rate for test: 0.33, 0.66, 1.25, 2.5, 5 % w/v.

Three replicates per dose level of 20 protonymphs each (60 mites/concentration) were placed on bean leaves, underside up in Petri dishes on wet cotton pads; mites were fed with pollen of broad bean and exposed for 7 days. Observations of mortality and escape rate were made on day 7 of exposure for determination of the LD50.

Environmental conditions: temperature: 25°C; relative humidity: 70% HR; photoperiod: 16 h light - 8 h darkness.

Findings:

Mortality findings were corrected using the Abbot[20] formula (1925). The results obtained are summarised in the following table.

Table B.9.5.1.3 - Mortality of *T. pyri* protonymphs on exposure day 7 on bean leaves

Treatment	Mortality in replicates (number)			Observed mortality (mean)	Corrected mortality
	1	2	3		
Control	4/20	3/20	3/20	16.7%	-
250 g as/ha	4/20	2/20	4/20	16.7%	0.0%
500 g as/ha	3/20	1/20	4/20	13.3%	-4.0%
1000 g as/ha	3/20	4/20	4/20	18.3%	2.0%
2000 g as/ha	5/20	7/20	3/20	25.0%	10.0%
4000 g as/ha	7/20	8/20	10/20	41.7%	30.0%

The **LD50 > 4000 g as/ha** (>10 L Syllit 400 SC/ha) for *Typhlodromus pyri* on natural substrate (bean leaves). **Syllit 400 SC can be considered as "harmless"** to *Typhlodromus pyri* under extended laboratory conditions.

EXP 10343A: Acute toxicity to the aphid parasitoid, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) in the laboratory.

Kühner, C. (1997b).GAB-IFU/Germany, unpublished report # 97123/02-NLAp.

[18] Overmeer, W. {1988). Laboratory method for testing side-effects of pesticides on the predacious mites *Typhlodromus pyri* and *Amblyseius potentillae*. IOBC/WPRS Bulletin, 1988/XI/4 :65-70.

[19] Samsoe-Petersen, L. (1983) Laboratory method for testing side-effects of pesticides on juvenile stages of the predatory mite, *Phytoseiulus persimilis* (Acarina, Phytoseiidae). Entomophaga, 28(2) :167-178.

[20] ABBOTT, W. S., (1925): A method of computing the effectiveness of an Insecticide. 3. of Econ. Entomol. 18; 265 - 267

Guidelines and GLP:

The study was conducted according to Polgar[21] (1988), Mead-Briggs[22] (1992) and Guidance document ESCORT (1994). Principles of GLP were complied with.

Test species and methods:

Dodine, formulated as EXP 10343A (= Syllit 400 SC, Dodine 400 SC): formulation batch OP960466, content: 405 g/L dodine and the toxic standard: Perfekthion containing nominally 37.4% dimethoate were used to assess the effects on adults of *Aphidius rhopalosiphia* maximum of 48 hr old.

Test treatments consisted of: 1 tap water (control), toxic standard and 2 concentrations of Syllit: 2.23 kg Syllit 400 SC per ha (= approx. 900 g as/ha) and 4.46 kg Syllit 400 SC per ha (=approx. 1800 g as/ha). This corresponds to 1-fold and 2-fold maximum recommended single field application rate.

Ten adults (5 male + 5 female) per exposure unit (glass plates and aluminium frame) with 4 replicates per treatment (40 adults/treatment) during 48 hours of exposure, followed by fertility assessment phase of 11 days were used in the study.

Observations of mortality and other effects occurred at 0.5, 2, 24 and 48 hours.

The fertility performance phase was assessed by transferring surviving females (up to 10 per treatment) for 24 hours to individual cages containing young oat seedlings infested with 50-100 aphids than the aphid mummies were counted 11 days after the 24h-parasitising phase.

Environmental conditions: temperature: 20 ± 3 °C; relative humidity: 50 - 85% HR; photoperiod: 16 h light - 8 h darkness during fertility test and light intensity: 1000 lux

Findings:

Based on toxic standard and control mortality, as well as reproductive performance in the control, the test can be regarded as valid and acceptable.

Mortality findings were corrected using the Schneider-Orelli formula (1947), reduction in reproduction rate R (foll. Abbot, 1925) and beneficial capacity (foll. Overmeer and Van Zon, 1982) were calculated acc. to guideline. The results obtained are summarised in the following tables.

Table B.9.5.1.4 - Mortality of adults of *Aphidius rhopalosiphi* after 48 hours of exposure to treated glass plates.

Treatment g/ha	Mortality in replicates (number)				Observed mortality (mean)	Corrected mortality
	1	2	3	4		
Control	0/10	1/10	1/10	0/10	2.5%	
900 g as/ha	2/10	1/10	1/10	1/10	10.0%	7.7%
1800 g as/ha	2/10	3/10	4/10	3/10	22.5%	20.5%
Toxic standard	10/10	10/10	10/10	10/10	100%	100%

[21] POLGAR, L. (1988): Guideline for testing the effect of pesticides on *Aphidius malricariae* Hal./Hym., Aphidiidae. Bulletin SORP/WPRS Bulletin, 1988/XI/4, 29-33

[22] MED-BRIGGS, M. A. (1992): A laboratory method for evaluating the side effects of pesticides on the cereal aphid parasitoid *Aphidius rhopalosiplii* (DeStefani-Perez). Aspects if Applied Biology 31, 179-189

Table B.9.5.1.5 - Reproduction performance (R) of *Aphidius rhopalosiphi* (24h egg-laying) and reduction in beneficial capacity (E).

Treatment g/ha	Number of female	Total number of mummies	Mean mummies/female	Reduction of reproduction rate (R)	Reduction beneficial capacity (E)
Control	20	159	8.0	-	-
900 g as/ha	18	108	6.0	24.5%	30.3%
1800 g as/ha	12	88	7.3	7.8%	26.7%

In conclusion, the **LD50 > 1800 g as/ha** for *A. rhopalosiphi*. The combination of the corrected pre-imaginal mortality of the test organisms with the R factor resulted in a **reduction of beneficial capacity of 30.3% and 26.7%** was calculated at 900 and 1800 g as/ha, respectively. According to the IOBC classification, **Syllit 400 SC can be considered as "slightly harmful"** (Class 2) to *A. rhopalosiphi* on glass plates.

Side effects of Syllit on larvae of the green lacewing *Chrysoperla carnea* (Neuroptera; Chrysopidae) on detached bean leaves in the laboratory.

Jansen, J. -P. (2001b). Lab. d'Ecotoxicologie, Gembloux/Belgium, unpublished report #CCE.01/2001.

Guidelines and GLP:

The study was conducted according to Biggler[23] (1988) and SETAC guidance document (1994). Principles of GLP were complied with.

Test species and methods:

Dodine, formulated as Syllit 400 SC: formulation batch S01D02, content: 389 g/L dodine and a toxic standard (Karate 25 EC containing nominally 25 g lambdacyhalotrine/L) were used to assess the effects on larvae of *Chrysoperla carnea* Steph. 2-3 days old.

Test treatments consisted: on 1 tap water (control), 1 toxic standard at 40 ml/ha (= 1 g lambdacyhalotrine/ha) sprayed in 200 L/ha; dilution rate for test: 4 ml in 200m1 water and then 2 ml of this solution in 200m1 water and 2 concentrations of Syllit: 2.25 L Syllit 400 SC per ha (= approx. 900 g as/ha, 1x maximum field rate) and 4.5 L Syllit 400 SC per ha (=approx. 1800 g as/ha) sprayed in 200 I/ha; dilution rate for test: 1.1 or 2.2% Syllit 400 SC w/v.

Forty larvae per treatment group and control and 20 larvae for toxic standard, confined individually were exposed in exposure units (one treated bean leave placed on wet cotton pad, underside up and ring of perspex). Larvae were fed 5x a week with *E. kuehniella* sterilised eggs, until pupation.

Larval mortality was assessed daily. After pupation, pupae were carefully transferred to plastic petri dishes for adult emergence. After emergence, observed pre-imaginal mortalities were calculated and corrected with the value of the corresponding control with the help of the Abbott formula (1925).

When they emerged, adults were transferred in to fertility assessment units and egg production was followed during a 4-week period. Mean number of viable eggs/female/day was used as a measure of reproductive performance.

[23] Biggler, F. (1988). A laboratory method for testing side-effects of pesticides on larvae of the green lacewing, *Chrysoperla carnea* Steph. (Neuroptera, Chrysopidae). IOBCIV1/PRS Bulletin, 11(4) :71-77.

Environmental conditions: temperature: 20±3°C; relative humidity: 70% HR; photoperiod: 16 h light - 8 h darkness; light intensity > 3000 lux during exposure.

Findings:

Based on toxic standard and control mortality, as well as reproductive performance in the control, the test can be regarded as valid and acceptable.

Mortality findings were corrected using the Abbott formula (1925), reproductive ratio R and beneficial capacity were calculated acc. to guideline. The results obtained are summarised in the following tables.

Table B.9.5.1.6 - Mortality of *C. carnea* larvae at the end of the study (31 days) on detached bean leaves

Treatment	Dead larvae	Dead pupae	Adults alive	Observed mortality (%)	Corrected mortality (%)
Control	5/40	1	34	15	-
900 g as/ha	8/40	0	32	20	5.9
1800 g as/ha	9/40	2	29	27.5	14.7
Toxic standard	20/20	-	-	100	100

The reproductive assessment resulted in the following rates and numbers:

Table B.9.5.1.7 - Reproductive performance of *C. carnea* over 4 weeks after 19-days exposure to dodine on detached bean leaves

Treatment	Total eggs	Hatching rate (%)	Viable eggs/female	Reproduction ratio (R)	Reduction beneficial capacity (E)
Control	3882	68.8	188.1(6.72/day)	-	-
900 g as/ha	3371	75.7	183.0 (6.54/day)	0.97	8.5%
1800 g as/ha	3180	72.6	186.7 (6.67/day)	0.99	15.3%

A **reduction of beneficial capacity of 8.5% and 15.3% was calculated at 900 and 1800 g as/ha respectively.** According to the IOBC classification, **Syllit 400 SC can be considered as "harmless" (Class 1)** to *Chrysoperla carnea* under extended laboratory conditions.

Side effects of Syllit on larvae of the ladybird *Coccinella septempunctata* (Coleoptera; Coccinellidae) on detached bean leaves in the laboratory.

Jansen, J.-P. (2001c). Lab. d'Ecotoxicologie, Gembloux/Belgium, unpublished report #CSE.01/2001.

Guidelines and GLP:

The study was conducted according to Biggler (1988) and SETAC guidance document (1994). Principles of GLP were complied with.

Test species and methods:

Dodine, formulated as Syllit 400 SC: formulation batch S01D02, content: 389 g/L dodine and a toxic standard (Karate 25 EC containing nominally 25 g lambdacyhalotrine/L) were used to assess the effects on larvae of *Coccinella septempunctata* 2-3 days old.

Test treatments consisted: on 1 tap water (control), 1 toxic standard at 40 ml/ha (= 1 g lambdacyhalotrine/ha) sprayed in 200 L/ha; dilution rate for test: 4 ml in 200m1 water and then 2 ml of this solution in 200m1 water and 2 concentrations of Syllit: 2.25 L Syllit 400 SC per ha (= approx. 900 g as/ha, 1x maximum field rate) and 4.5 L Syllit 400 SC per ha (=approx. 1800 g as/ha) sprayed in 200 I/ha; dilution rate for test: 1.1 or 2.2% Syllit 400 SC w/v.

Forty larvae per treatment group and control and 20 larvae for toxic standard, confined individually were exposed in exposure units (one treated bean leave placed on wet cotton pad, underside up and ring of perspex). Larvae were fed daily with aphids.

The exposure period was 21 days (until emergence of last adult). Mortality, pupation and hatching were observed daily until termination.

Larval mortality was assessed daily. After pupation, pupae were carefully transferred to plastic petri dishes for adult emergence. After emergence, observed pre-imaginal mortalities were calculated and corrected with the value of the corresponding control with the help of Abbott formula (1925).

When they emerged, adults were transferred in fertility assessment units and egg production was followed during a 4-week period. Mean number of viable eggs/female/day was used as a measure of reproductive performance.

Environmental conditions: temperature: 20±3°C; relative humidity: 70% HR; photoperiod: 16 h light - 8 h darkness; light intensity > 3000 lux during exposure.

Findings:

Based on toxic standard and control mortality, as well as reproductive performance in the control, the test can be regarded as valid and acceptable.

Mortality findings were corrected using the Abbott formula (1925), reproductive ratio R and beneficial capacity were calculated acc. to guideline. The results obtained are summarised in the following tables.

Table B.9.5.1.8 - Mortality of *C. septempunctataa* larvae at the end of the study (30 days) on detached bean leaves

Treatment	Dead larvae	Dead pupae	Adults alive	Observed mortality (%)	Corrected mortality (%)
Control	4/40	1	35	12.5	-
900 g as/ha	5/40	1	34	15.0	2.5
1800 g as/ha	8/40	2	30	25.0	14.3
Toxic standard	20/20	-	-	100	100

The reproductive assessment resulted in the following rates and numbers:

Table B.9.5.1.9 - Reproductive performance of *C. septempunctata* over 4 weeks after 21-days exposure to dodine on detached bean leaves

Treatment	Total eggs	Hatching rate (%)	Viable eggs/female	Reproduction ratio (R)	Reduction beneficial capacity (E)
Control	1759	59.6	106.6	-	--
900 g as/ha	1511	58.5	100.5	0.94	5.7%
1800 g as/ha	1472	57.0	101.65	0.95	18.2%

A **reduction of beneficial capacity of 5.7% and 18.2% was calculated at 900 and 1800 g as/ha respectively.** According to the IOBC classification, **Syllit 400 SC can be considered as "harmless" (Class 1)** to *Coccinella septempunctata* under extended laboratory conditions.

Side effects of Syllit on protonymph of the anthocorid bug *Orius insidiosus* (Heteroptera; Anthocoridae) on detached bean leaves in the laboratory.

Jansen, J. -P. (2002). Lab. d'Ecotoxicologie, Gembloux/Belgium, unpublished report #OI.01/2001.

Guidelines and GLP:

The study was conducted according to Wesiak and Neumann (unpublished) and SETAC guidance document (1994). Principles of GLP were complied with.

Test species and methods:

Dodine, formulated as Syllit 400 SC: formulation batch S01D02, content: 389 g/L dodine and a toxic standard (Karate 25 EC containing nominally 25 g lambdacyhalotrine/L) were used to assess the effects on protonymphs of *Orius insidiosus ata* 5 days old.

Test treatments consisted: on 1 tap water (control), 1 toxic standard at 40 ml/ha (= 1 g lambdacyhalotrine/ha) sprayed in 200 L/ha; dilution rate for test: 4 ml in 200m1 water and then 2 ml of this solution in 200m1 water and 2 concentrations of Syllit: 2.25 L Syllit 400 SC per ha (= approx. 900 g as/ha, 1x maximum field rate) and 4.5 L Syllit 400 SC per ha (=approx. 1800 g as/ha) sprayed in 200 I/ha; dilution rate for test: 1.1 or 2.2% Syllit 400 SC w/v.

One hundred protonymphs per treatment group and control (20 exposure units of 5 protonymphs per each group) were exposed in exposure units. Exposure units consisted of a foliar disk of a broad bean leaf placed on agar solution. Protonymphs were fed with eggs of *E. kuhniella*.

The exposure period was 10 days followed by 10 days reproduction phase in untreated units.

Mortality was assessed 3 days and 10 days after treatment. Mean mortality in each group was corrected with the value observed in the control according to Abbott (1925).

At day 10, surviving adults of *O. insidiosus* were harvested and 5 replicates per treatment each containing 3 males and 3 females were selected randomly in each group for assessment of reproductive performance. These couples were released in 5 untreated units, similar to those used for exposure, and egg production was counted at day 13.

Surviving adults were thus transferred in new untreated units at day 13, 15 and 17 and egg production assessed in these units at day 15, 17 and 20, respectively. Mean egg production/female was calculated for each object.

Environmental conditions: temperature: 25±2°C; photoperiod: 16 h light - 8 h darkness.

Findings:

Based on toxic standard and control mortality, as well as reproductive performance in the control, the test can be regarded as valid and acceptable.

Mortality findings were corrected using the Abbott formula (1925), reproductive ratio R and beneficial capacity were calculated acc. to guideline. The results obtained are summarised in the following tables.

Table B.9.5.1.10 - Mortality of *O. insidiosus* protonymphs at the end of the 10 day exposure period on detached bean leaves

Treatment	Number of dead	Observed mortality (%)	Corrected mortality (%)
Control	14/100	14.0	-
900 g as/ha	22/100	22.0	9.3
1800 g as/ha	32/100	32.0	20.9
Toxic standard	94/100	94.0	93.0

The reproductive assessment resulted in the following rates and numbers:

Table B.9.5.1.11 - Reproductive performance of *O. insidiosus* over 10 days after 10 days exposure to dodine on detached bean leaves

Treatment	Viable eggs produced/female	Reproduction ratio (R)	Reduction beneficial capacity (E)
Control	14.20	-	-
900 g as/ha	12.67	0.89	19.1
1800 g as/ha	14.13	0.99	21.3

A **reduction of beneficial capacity of 19.1% and 21.3% was calculated at 900 and 1800 g as/ha respectively.** According to the IOBC classification, **Syllit 400 SC can be considered as "harmless" (Class 1)** to *Orius insidiosus* under extended laboratory conditions.

B.9.5.2 Summary of effects on other arthropod species

The results of studies conducted with non-target arthropod species are summarised in Table B.9.5.2.

All studies presented were conducted to GLP.

Regarding the two standard non-target arthropod species, *Typhlodromus pyri* was the most sensitive with a reduction of beneficial capacity of 73.5%. For *Aphidius rhopalosiphi* the toxicity was considerably lower with a reduction of beneficial capacity of 30.3%. In laboratory conditions but with natural substrate (bean leaves), *Typhlodromus pyri*, exhibit a LR50 > 4000 g/ha (maximum application rate) and can be concluded that dodine was harmless for *T. pyri*.

Regarding the other species tested, *C. carnea*, *C. septempunctata* and *O. insidiosus* in the laboratory, the results obtain for all of them indicate that dodine could be classified as harmless when applied at the maximum application rate of 1800 g as/ha.

Table B.9.5.2 - Results of Tier I LR50 studies with Diazol 60EC

Species	Test material	Conditions and Results	Reference
T.pyri	Syllit 400 SC	**Laboratory study** **Artificial substrate** (glass plates) Max. applic. rate: 900 g as/ha **Reduction of beneficial capacity of 73.5%** Harmful = Class 4	Kühner, 1997a
T.pyri	Syllit 400 SC	**Laboratory study** **Natural substrate** (bean leaves) Max. applic. rate: 4000 g as/ha **LR50 > 4000 g as/ha** Harmless = Class 1	Jansen, 2001a
A. rhopalosiphi	Syllit 400 SC	**Laboratory study** **Artificial substrate** (glass plates) Max. applic. rate: 1800 g as/ha **LR50 > 1800 g as/ha** **Reduction of beneficial capacity of 30.3%** Slightly Harmful = Class 2	Kühner, 1997b
C. carnea	Syllit 400 SC	**Laboratory study** **Natural substrate** (bean leaves) Max. applic. rate: 1800 g as/ha **Reduction of beneficial capacity of 15.3%** Harmless = Class 1	Jansen, 2001b
C. septempunctata	Syllit 400 SC	**Laboratory study** **Natural substrate** (bean leaves) Max. applic. rate: 1800 g as/ha **Reduction of beneficial capacity of 18.2%** Harmless = Class 1	Jansen, 2001c
O. insidiosus	Syllit 400 SC	**Laboratory study** **Natural substrate** (bean leaves) Max. applic. rate: 1800 g as/ha **Reduction of beneficial capacity of 21.3%** Harmless = Class 1	Jansen, 2002

In conclusion, on artificial substrate, *Typhlodromus pyri* was shown to be severely affected following exposure to dodine. Therefore, the test was repeated on natural substrate resulting in a reduced effect of the preparation. Syllit 400 SC was shown to be of low toxicity to non-target arthropods.

B.9.5.3 Exposure and risk Assessment on other arthropod species (Annex IIIA 10.5)

Terrestrial non-target arthropods will be exposed to residues of Syllit 400 SC both in-field, and in off-field environments as a result of spray drift. In evaluating the effects of the preparation Syllit 400 SC to non-target arthropod species, a tiered testing approach was adopted as recommended in the ESCORT 2 workshop (Candolfi *et al.* 2001)

Initial evaluations of the toxicity of Syllit 400 SC were conducted under worst case laboratory conditions to determine LR_{50} values with the most sensitive indicator species *Typhlodromus pyri*. The result of this study is summarised in the Table below.

Table B.9.5.3.1 - Results of Tier I LR50 study with Syllit 400 SC

Species	Test material	Result	Reference
T.pyri	Syllit 400 SC	**Laboratory study** LR_{50} > 4000 g as/ha.	Jansen, 2001a
A. rhopalosiphi	Syllit 400 SC	**Laboratory study** LR_{50} > 1800 g as/ha.	Kühner, 1997b

The following hazard quotients are obtained with maximum application rate of 900 g dodine/ha and a MAF factor of 1.9 (DT50 7 days: spray interval 7 days and 5 applications; ESCORT 2).

The drift factor used, was based on the overall 72nd percentile for 5 late season applications of the product in orchard crops, when there will be maximal intercept (6.59%drift/100 = 0.0659)

Both vegetation distribution factor and correction factor are set at 10. Hence, these parameters cancel each other out.

Table B.9.5.3.2 - Hazard quotients for Syllit 400 SC

Species	LR50 (g as/ha)	Application rate (g as/ha)	MAF	HQ Orchard use (5 late season applications)	
				HQ in-field	HQ off-field
T.pyri	4000	900	1.9	0.43	0.028
A. rhopalosiphi	1800	900	1.9	0.95	0.063

Hazard quotient is less than 2, indicating that dodine poses a negligible risk to terrestrial non-target arthropods both in field and off field.

B.9.6 Effects on earthworms (Annex IIA 8.4; Annex IIIA 10.3.6)

B.9.6.1 Acute toxicity to earthworms (Annex IIA 8.4.1)

Dodine: Acute toxicity (14 day) to earthworms (*Eisenia foetida*). Artificial soil method.

Suteau, P. (1995). Rhone-Poulenc/France, unpublished report # SA 95248.

Guidelines and GLP:

The study was conducted according to OECD Nº 207 (1984) and Dir. 87/302/EEC part C. Principles of GLP were complied with.

Test species and methods:

Dodine technical: batch 1174 (=DA717), purity 98.6% was used to assess acute effects to the earthworm *Eisenia foetida*, at least 2 months old with clitellum and wet mass of 300 to 364 mg. The earthworms were kept in a humidified substrate (18% organic material and 65% vegetal matter) at pH 6.0 ± 0.5. Worms were feed with pelleted rabbit diet.

The test was performed over 14 days, in test vessels with 1.5 L capacity. Test medium consist on artificial soil acc. to the guideline.

Four replicates per dose group of 10 earthworms per vessel (= 10 per approx. 500 g dry weight artificial soil, total of 40 earthworms/concentration), acclimatized for 24 h prior to start.

Test concentrations: 0, 95, 171, 309, 556 and 1000 mg as/kg dry soil (nominal), plus a blank control.

Temperature and light were recorded daily. Temp. of climatic chamber was 20°C.

Biological observations and measurements as mortality and other effects were assessed on days 7 and 14 (termination). Earthworm mass, cumulative mass in all replicates at test initiation and at termination were also recorded.

Statistical analysis: The method of LITCHFIELD and WILCOXON (1949) was used to performed LC50 calculations.

Findings:

Biological findings are presented in the following table:

Table B.9.6.1 - Mortality data and mean body weight change of earthworms exposed to dodine.

Nominal conc. (mg as/kg dry soil)	Mortality (%)		Mean weight change on day 14 compared to day 0 (%)
	Day 7	Day 14	
Control	0	0	-1.89
95	0	0	-2.44
171	0	0	-2.69
309	0	15	-14.04*
556	13	55	-20.46*
1000	58	85	-47.77*

* significantly different (p = 0.05) from control

Following 7 days of exposure, no mortality was observed up to 309 mg as/kg dry soil, whereas the concentrations of 556 and 1000 mg as/kg dry soil induced percentages of mortality of 13 and 58% respectively.

Following 14 days of exposure, no mortality occurred in the control and at 95 and 171 mg as/kg dry soil. Mortality percentages of 15, 55 and 85% were recorded for the concentrations of 309, 556 and 1000 mg as/kg dry soil. The validation criterion of less than 10% mortality in the control was respected. A significant decrease in mean body weight was observed at concentrations of 309, 556 and 1000 mg as/kg dry soil.

Lethargy was observed in the surviving earthworms at concentrations of 556 and 1000 mg as/kg dry soil after 7 and 14 days.

The test is valid and acceptable as shown by mortality and wet mass loss in the control.

Under the conditions of this test, the **14-day acute NOEC is concluded to be 171 mg as/kg dry soil with a 14-day acute LC50 of 547 mg as/kg dry soil with 95% confidence limits of (435 – 687 mg/kg)**.

B.9.6.2 Sublethal effects on earthworms (Annex IIA 8.4.2)

No data submitted and at this stage of evaluation no more data is necessary.

B.9.6.3 Acute toxicity of the formulations to earthworms (Annex IIIA 10.6.1.1)

No study was submitted.

B.9.6.4 Sublethal effects of the formulation on earthworms (Annex IIIA 10.6.1.2)

No study was submitted.

B.9.6.5 Field tests - residue content of earthworms (Annex IIIA 10.6.1.3)

No study was submitted.

B.9.6.6 Summary of effects on earthworms

Dodine is slightly toxic to earthworms with a LC_{50} (14 days) of 547 mg/kg. No major soil metabolites were identified on soil degradation studies of dodine.

Therefore, dodine is considered to be of no ecotoxicologically of concern in the soil environment to earthworms.

Table B.9.6.6 - Summary of effects on soil non-target organisms with dodine.

Test conditions	Findings	References
Earthworm – acute	LC_{50} = 547 mg/kg (14 day) NOEC = 171 mg/kg	Suteau, 1995

B.9.6.7 Exposure and Risk assessment for earthworms (Annex III, 10.6.1.1)

The predicted maximum point soil PECs for the proposed uses of Syllit 400 SC, assuming 80% is deposited on soil in orchard are presented in Table B.8.25 (see point B.8.3). From the acute earthworm toxicity study with dodine technical a 14 day LC_{50} value was determined to be 547 mg/kg soil (Suteau, 1995). Using this data the TERa value has been calculated and are summarised in Table B.9.6.7.2.

Table B.9.6.7.2 - Acute TER values based on worst case maximum point soil concentrations

Species & Test type	PECinitial[a)]	Ecotox. Endpoint[b)]	TERa	Trigger value
Earthworm 14 days acute Test	3.04 mg/kg	LC50 = 316.18 mg/kg	104.0	10
		NOEC = 98.84 mg/kg	32.5	

a) Multiple application actual PECinitial. Allowance has been made for the degradation of dodine between successive applications, the DT_{50} used for PEC calculations were 18.6 days.
b) Toxicity values were corrected for organic matter content of the soil (1.73)

This assessment indicates that dodine is of low risk to earthworms for all the proposed uses, with TER values well in excess of the Annex VI trigger of 10.

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

No data submitted. However laboratory soil degradation studies with dodine showed that the geometric mean DT_{90} was 60.08 days, accordingly, no further testing is required. Studies conducted with earthworms and soil micro-organisms (see point B.9.6 and B.9.8) show low risk to soil organisms following exposure to dodine. Effects on organic matter breakdown were not investigated, as the DT_{90} in soil was less than 1 year.

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

B.9.8.1 Impact of the active substance on soil microbial activity (Annex IIA 8.5)

Assessment of the side effects of Dodine 400 SC on the activity of the soil microflora.

Kolzer, U. (2002). GAB-IFU / Germany, unpublished report # 20011182/01-ABMF.

Guidelines and GLP:

The study was conducted according to OECD Nº 216 - 217 Guidelines. Principles of GLP were complied with.

Test species and methods:

Dodine as applied as a formulated product Dodine 400 g/I SC: batch 40830, purity 385.9 g/L to two different soil types. A reference substance (toxic standard) Dinoterb, Batch 69115; purity: 250 g/I formulated product was also used to test de sensitivity of the test.

Test soils: soil 1 (loamy sand soil (obtained through, and characterised by LUFA, Speyer, Germany); soil 2 (silt soil (characterised by GAB-IFU, taken from permanent grassland not treated with pesticides within one year before sampling)

Test study was performed to assessed Nitrogen turnover and Short-time respiration. Three treatment groups (3 replicates each) for each soil type were prepared: untreated soil (control) with lucerne meal; soil treated with 3.13 mg dodine 400 SC/kg dry soil corresponding to 900 g as/ha) with lucerne flour; soil treated with 31.3 mg dodine 400 SC/kg dry soil corresponding to 9000 g as/ha) with lucerne flour and soil treated with 53.3 mg dinoterb/kg soil.

Samplings occur at 0, 7, 14, and 28 days after application. Determination of total nitrogen as sum of ammonium-ion and nitrate + nitrite concentrations.

Prior to application, for each soil the quantity of glucose to be added for optimum short-term respiration was determined (500g gluc./100g wet weight and 400 g gluc./100 wet weight to soil 1 and 2, respectively). Sampling occurs after 6 hours, 7, 14, 28 and 42 days (the last only for soil 1).

The determination of short-term respiration upon sampling by addition of glucose was done by means of the OxiTop system.

Findings:

The results for the two types of soil for the nitrogen turnover are presented below as percentage of inorganic nitrogen content (Nmin) deviating from the respective control values.

Table B.9.8.1 - Percentage deviation of Nmin from control.

Treatment group	Loamy sand (soil 1)				Silt (soil 2)			
	Deviation from control in % after							
	0 d	7 d	14 d	28 d	0 d	7 d	14 d	28 d
900 g as/ha (1-fold)	+ 33.7	- 12.39	- 26.75	- 19.01	- 2.94	-0.53	- 5.15	- 6.32
9000 g as/ha (10-fold)	+ 32.61	- 8.85	- 16.56	-14.44	- 2.39	- 0.53	- 1.06	- 4.50
dinoterb	+ 103.26	+ 38.94	- 17.20	- 35.21	- 3.13	+ 27.73	+ 46.57	+ 45.98

In the reference substance group, the nitrogen turnover was decreased to -35.21 % and stimulated to + 45.98% in comparison to the control for the loamy sand soil and the silt soil respectively.

This was a result of the mineralization of the mortified biomass. The study, therefore, fulfils the criterion of validity (deviation > 25%).

The results for the two types of soil for short-time respiration are presented below as percentage of deviation of the short time respiration rates from the respective control values.

Table B.9.8.2 - Percentage deviation of short time respiration rate from control.

Treatment group	Loamy sand (soil 1)					Silt (soil 2)				
	Deviation from control in % after									
	6 h	7 d	14 d	28 d	42 d	6 h	7 d	14 d	28 d	42 d
900 g as/ha (1-fold)	- 7.08	- 12.26	- 4.30	+ 2.60	+ 13.33	+ 10.5	+ 2.29	+ 84.06	- 7.31	-
9000 g as/ha (10-fold)	- 53.98	- 39.62	- 36.56	- 36.36	- 15.56	- 3.50	+ 2.29	+ 18.84	- 0.91	-
dinoterb	- 39.82	- 27.36	- 34.41	- 50.65	- 31.11	+ 28.50	+ 5.96	+ 26.09	- 26.94	-

The toxic standard inhibited short-term respiration by -31.1 % after 42 days for the loamy sand soil and -26.94% after 28 days for the silt soil. Therefore, the toxic standard fulfils the criterion of validity (deviation >25%).

Nitrogen turnover: since the deviation between both treated soils and the control soils for the nitrogen turnover was less than 25% after 28 days (loamy sand soil and silt soil, respectively), the influence from Dodine 400 SC on the nitrogen turnover is considered to be negligible up to 9000 g as/ha (10-fold).

Short-term respiration: At the end of the study, the deviation between the short-term respiration rate in both soils treated with the test substance Dodine 400 SC and the control soil was less than 25%. It is concluded that the influence from Dodine 400 SC on short-term respiration is considered to be negligible up to 9000 g as/ha.

B.9.8.2 Impact of the formulations on soil microbial activity (laboratory) (Annex IIIA 10.7.1)

No study conducted, not required, see point B.9.8.1.

B.9.8.3 Further laboratory, glasshouse of field testing to investigate impact on soil microbial activity (Annex IIIA 10.7.2)

No study conducted, not required, see point B.9.8.1.

B.9.8.4 Summary of effects on non-target micro-organisms - exposure and risk assessment for non-target micro-organisms

Dodine had no adverse effects on soil microflora at 12 mg/kg (equivalent to 9 kg/ha) in a 28-day study.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (Annex IIA 8.6; Annex IIIA 10.8)

Tier I: determination of the phytotoxic effects of dodine fungicide on vegetative vigor of non target plants.

Maggi, V. L. (1993a). California Agricultural Research USA, unpublished report # CAR 182-92 – J-2103.0

Guidelines and GLP:

The study was conducted according to US EPA § 122-1 (1982). Principles of GLP were complied with.

Test species and methods:

Dodine technical: batch 303/90, purity: 94.1 % was used to assess the phytotoxic effects of dodine. The test was performed in a greenhouse on ten crops (soybean, lettuce, radish, tomato, cucumber, cabbage, oat, ryegrass, corn and onion).

Seeds were planted in a sterilized sandy/loam soil and placed in the greenhouse (T° ranged from 13 to 35°C, humidity ranged from 20 to 75%). After emergence, young plants (at the 1-4 true leaf stage) were sprayed with a 1.74% solution of dodine (+ ethyl alcohol to increase solubility) at a dose rate equivalent to 2.9 kg as/ha. Evaluations of phytotoxicity were made 7, 14 and 21 days after treatment. Plant height and dry weight were assessed 21 days after treatment. Results were analysed statistically (ANOVA) and compared to an untreated control and a solvent control.

Findings:

The negative control treatment (ethyl alcohol solvent) caused no visible effect on the plants and plant height or plant weights were unaffected. The use of ethyl-alcohol as a diluent for dodine was therefore deemed adequate. At some occasions, treatments with dodine caused some significant effects and results are given in the following Table:

Table B.9.9.1 - Phytotoxicity and reduction of height or dry weight at 21 DAT.

Species	Untreated	Ethyl alcolhol	Dodine + Ethyl alcolhol		
	% phytotox	% phytotox	% phytotox	% reduction height	% reduction dry weight
Soybean	0	0	15	-	3
Lettuce	0	0	0	14	-
Radish	0	0	37.5	5	-
Tomato	0	0	10.0	1	-
Corn	0	0	0	-	-
Cucumber	0	0	40	4	15
Cabbage	0	0	31.3	12	21
Oat	0	0	0	2	-
Ryegrass	0	0	0	-	8
Onion	0	0	0	5	20

Radish, cucumber and cabbage yielded a treatment response to dodine which was greater than the trigger value of 25% when compared with those of the untreated control plants. The response seemed to be more of a systemic rather than contact effect. Aside from spotty or general chlorosis evident in these plants, there were apparent effects visible in new plant tissues in the way of necrotic spots, growth delays and/or mild stem and leaf distortion.

Soybeans exhibited only a slight phytotoxic response but it should be pointed out that the effect appeared strictly as a contact effect and not as a systemic one; the leaves which matured after the spray treatment was applied did not yield any signs of phytotoxicity.

The tomato plants yielded somewhat the same response as the soybeans in their ability to grow out of the effect of dodine treatment. Although the tomato plants did show a more general chlorotic

effect overall, the mean effect dropped from a 28.8% effect at the 14 DAT evaluation down to a 10% effect by the 21 DAT evaluation.

Although significant differences were detected for radish, cabbage and ryegrass, none of the ten species tested yielded evidence of a reduction of plant height any greater than 14% over those plants in the untreated control.

No significant differences were detected in dry weight. None of the ten species tested yielded evidence of a reduction in plant weight any greater than 21 %.

Dodine was shown to be phytotoxic on certain species. It appears that only radish, cucumber and cabbage yielded an effect greater than 25% in any of the test parameters evaluated in this study. These effects were evident in regards to plant phytotoxicity as evaluated on the 21 DAT evaluation only.

Tier I: determination of the phytotoxic effects of dodine fungicide on seedling emergence of non target plants.

Maggi, V. L. (1993b). California Agricultural Research USA, unpublished report # CAR 183-92 – J-2102.0

Guidelines and GLP:

The study was conducted according to US EPA § 122-1 (1982). Principles of GLP were complied with.

Test species and methods:

Dodine technical: batch 303/90, purity: 94.1 % was used to assess the phytotoxic effects of dodine. The test was performed in a greenhouse on ten crops (soybean, lettuce, radish, tomato, cucumber, cabbage, oat, ryegrass, corn and onion).

Ten seeds per species were planted in a sterilized sandy/loam soil and placed in the greenhouse (T° ranged from 4 to 35°C, humidity ranged from 20 to 75%). Immediately after planting and prior to first irrigation, the soil was sprayed with a 1.74% solution of dodine (+ ethyl alcohol to increase solubility) at a dose rate equivalent to 2.9 kg as/ha. Evaluations of phytotoxicity were made 7, 14 and 21 days after treatment. Percent emergence data was evaluated at 14 DAT. Plant height and dry weight were assessed 21 days after treatment. Results were analysed statistically (ANOVA) and compared to an untreated control and a solvent control.

Findings:

The negative control treatment (ethyl alcohol solvent) caused no visible effect on the plants and plant height or plant weights were unaffected. The use of ethyl-alcohol as a diluent for dodine was therefore deemed adequate.

No noticeable phytotoxicity was noted on any day of evaluation. Results of seedling emergence, plant height and dry weight are given in the following Table:

Table B.9.9.2 - Seedling emergence and reduction of height or dry weight.

Species	Untreated	Ethyl alcolhol	Dodine + Ethyl alcolhol		
	% emergence	% emergence	% emergence/ % reduction to untreated	% reduction height	% reduction dry weight
Soybean	95.0	90.0	97.5/0	7	11
Lettuce	87.5	100	85.0/2.5	17	14
Radish	85.0	80.0	87.5/0	1	9
Tomato	82.5	82.5	77.5/6	0	0
Corn	95.0	95.0	97.5/0	1	10
Cucumber	97.5	82.5	100/0	2	14
Cabbage	80.0	85.0	77.5/3	22	25
Oat	100	92.5	100/0	9	0
Ryegrass	80.0	85.0	90.0/0	5	0
Onion	85.0	87.5	70.0/15	0	11

None of the species tested yielded evidence of reduced emergence of more than 15%.

Cabbage yielded evidence of a reduction of plant height by 22% and of plant dry weight by 25%.

Dodine was shown to affect mainly cabbage which yielded evidence of a reduction of plant height by 22% and dry weight by 25%. When dodine-treated cabbage plants were compared with the ethyl alcohol-treated plants, reductions of only 6% and 9% were noted in the later case for the mean plant dry weight and mean plant height evaluations respectively.

Tier I: determination of the phytotoxic effects of dodine fungicide on seed germination of non target plants.

Maggi, V. L. (1993c). California Agricultural Research USA, unpublished report # CAR 184-92 – J-2102.0

Guidelines and GLP:

The study was conducted according to US EPA § 122-1 (1982). Principles of GLP were complied with.

Test species and methods:

Dodine technical: batch 303/90, purity: 94.1 % was used to assess the phytotoxic effects of dodine. The test was performed in a greenhouse on ten crops (soybean, lettuce, radish, tomato, cucumber, cabbage, oat, ryegrass, corn and onion).

For each species tested, 10 seeds were germinated in Petri dishes on treated blotter paper: Dodine at 7.8 ppm in 10 ml of ethyl alcohol was applied to the blotter sheets (dose rate equivalent to 2.9 kg as/ha). After treatment, dishes were left uncovered and allowed to dry. Once blotter papers were completely dry, ten seeds of each species were counted into each dish and irrigation water was added. The closed Petri dishes were left in an incubator at 25°C for 7 days to allow seed germination. Evaluations of germination were made on day 7 after treatment by measuring the seed

hypocotyls. Hypocotyls less than 5 mm in length were not considered germinated. Results were analysed statistically (ANOVA) and compared to an untreated control and a solvent control.

Findings:

Results are given in the following Table:

Table B.9.9.3 - Mean percent germination at 21 DAT.

Species	Untreated	Ethyl alcolhol	Dodine + Ethyl alcolhol	
	% germination	% germination	% germination	% reduction
Soybean	90.0	92.5	92.5	0
Lettuce	100	100	100	0
Radish	100	97.5	100	0
Tomato	95.0	97.5	87.5	7.5
Corn	95.0	97.5	95.0	0
Cucumber	100	100	100	0
Cabbage	95.0	97.5	97.5	0
Oat	80.0	90.0	85.0	0
Ryegrass	97.5	97.5	95.0	2.5
Onion	95.0	95.0	95.0	0

No statistically significant differences were observed between ay of the treatment means.

Only in two species, there was a notable reduction in germination, 2.5% for ryegrass and 7.5% for tomato without being statistically significant.

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

Activated sludge respiration inhibition test with Dodine technical (Contact time: 30 minutes)

Desmares-Koopmans, M. J. E. (2001). Notox / NL, unpublished report # 327138

Guidelines and GLP:

The study was conducted according to OECD Nº 209 (1984) and Dir. 87/302/EEC (part C, 1988). Principles of GLP were complied with.

Test species and methods:

Test substance: ISO common name: dodine; IUPAC name: 1-dodecylguanidinium acetate; echnical: batch S01106, purity 96.2%

Reference substance: toxic standard): defined solution containing 3,5-dichlorophenol (Aldrich, no. D 7,060-0; purity 97%).

Test system: activated sludge from municipal sewage plant ('s-Hertogenbosch, Netherlands) characterised as the amount of micro-organisms expressed as MLSS (Mixed Liquor Suspended Solids) per I of test medium.

Test design and procedures:

- preparation of the activated sludge for test: mix of synthetic sewage feed (16 ml) + test item/ref. item, made up to 300 ml with Milli-Q water followed by addition of 200 ml activated sludge; vessel: 1 L bottle with pipette inserted (aeration device);

- test design: 30 min contact time followed by 10 min of respiration measurement by means of an oxygen electrode in oxygen bottle (volume: 300 ml);

The test concentrations were: blank control (in duplicate); dodine: 18, 32, 56, 100, 180 and 320 mg/L; direct addition of dodine to the test vessels and 3,5-dichlorophenol: 3.2, 10, 32 mg/L.

Oxygen was measurement with electrode Tri Ox EO 200, WTW, Germany and pH was measured 10-min after measurement of respiration.

Findings:

The findings are summarised in the following table. The temperature of the test medium was at 21.4°C, the pH ranged from 6.6 to 7.0.

Table B.9.10 - Respiration rates in treated activated sludge.

Treatment (mg/l)	Concentration (mg/l)	Respiration rate (mg O_2 /l/h)	Inhibition (%)
Control 1	-	31	-
Control 2	-	27	-
Mean controls 1+2	-	29	-
Reference 1	3.2	23	21
Reference 2	10	15	48
Reference 3	32	5	83
Dodine 1	18	20	31
Dodine 2	32	16	45
Dodine 3	56	19	34
Dodine 4	100	10	66
Dodine 5	180	5	83
Dodine 6	320	2	93

The test can be regarded as valid and acceptable, since the mean respiration rate in all controls ranged from 27 to 31 mg O_2/L/hour with inter-control variation of 14% (limit: 15%) and the EC_{50} for the reference substance was at 10 mg/L which proves the system's susceptibility (acceptable range: 5-30 mg/L).

Under the conditions of this test, Dodine was toxic to waste water (activated sludge) bacteria at and above 18 mg/L, the lowest concentration tested. **The EC_{50} was found to be 52 mg as/L** with a 95% confidence interval ranging from 14 to 197 mg/L.

B.9.10.1 Summary of Effects on Other Non-Target Organisms

No significant differences were detected in dry weight. None of the ten species tested yielded evidence of a reduction in plant weight any greater than 21%

Dodine was shown to be phytotoxic on certain species. It appears that only radish, cucumber and cabbage yielded an effect greater than 25% in any of the test parameters evaluated in this study. These effects were evident in regards to plant phytotoxicity as evaluated on the 21 DAT evaluations only.

Dodine was shown to affect mainly cabbage which yielded evidence of a reduction of plant height by 22% and dry weight by 25%. When dodine-treated cabbage plants were compared with the ethyl alcohol-treated plants, reductions of only 6% and 9% were noted in the later case for the mean plant dry weight and mean plant height evaluations respectively.

Only in two of the ten species tested, there was a notable reduction in germination, 2.5% for ryegrass and 7.5% for tomato without being statistically significant.

Under the conditions of this test, Dodine was toxic to waste water (activated sludge) bacteria at and above 18 mg/L, the lowest concentration tested. **The EC_{50} was found to be 52 mg as/L** with a 95% confidence interval ranging from 14 to 197 mg/L.

B.9.11 References relied on

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection claimed Y/N	Owner
Annex IIA					
IIA, 8.1.1/01	Hakin B.	1990a	The acute oral toxicity (LD50) of dodine to the mallard duck; Huntingdon report # CMK 40/881487, GLP, Unpublished	Y	CAG
IIA, 8.1.1/02	Hakin B.	1990b	The acute oral toxicity (LD50) of dodine to the bobwhite quail; Huntingdon report # CMK 411881123, GLP, Unpublished	Y	CAG
IIA, 8.1.2/01	Hakin B.	1990a	The dietary toxicity (LC50) of dodine to the bobwhite quail; Huntingdon report # CMK 39/881199, GLP, Unpublished	Y	CAG
IIA, 8.1.2/02	Hakin B.	1990b	The dietary toxicity (LC50) of dodine to the mallard duck; Huntingdon report # CMK 38/881122, GLP, Unpublished	Y	CAG
IIA, 8.1.3/01	Pedersen C.A., Mumper J.A.	1993a	Dodecylguanidine acetate (Dodine) technical grade : six-week dietary toxicity and reproductior pilot study in bobwhite quail; Bio-Life Associates, Report # BLAL Nr 119-006-05, GLP, Unpublished	Y	CAG
IIA, 8.1.3/02	Pedersen C.A.	1994a	Dodecylguanidine acetate (dodine) technical grade : toxicity and reproduction study in bobwhite quail; Bio-Life Associates, Report #BLAL Nr 119-008-07, GLP, Unpublished	Y	CAG
IIA, 8.1.3/03	Pedersen C. A.	1999	Avian reproductive toxicity study with Dodecylguanidine acetate (dodine) technical in bobwhite quail; Bio-Life Associates, Report # BLAL Nr 108-029-07, GLP, Unpublished.	Y	CAG
IIA, 8.1.3/04	Pedersen C. A., Mumper J. A.	1993b	Dodecylguanidine acetate (Dodine) technical grade : six-week dietary toxicity and reproductior pilot study in mallard ducks; Bio-Life Associates, Report # BLAL Nr 119-007-06, GLP, Unpublished	Y	CAG

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection claimed Y/N	Owner
IIA, 8.1.3/05	Pedersen C. A.	1994b	Dodecylguanidine acetate (dodine) technical grade : toxicity and reproduction study in mallard ducks; Bio-Life Associates, Report # BLAL Nr 119-009-08, GLP, Unpublished	Y	CAG
IIA, 8.2.1/01	Caley, C. Y. Cameron, B. D. Chapleo, S. Knight, B.	1990a	Dodine : determination of acute toxicity (LC50) to rainbow trout (96h, semi-static) (EPA); Inveresk Research report # 7068, GLP, Unpublished	Y	CAG
IIA, 8.2.1/02	Caley, C. Y. Cameron, B. D. Chapleo, S. Knight, B.	1990b	Dodine : determination of acute toxicity (LC50) to bluegill sunfish (96h, semi-static) (EPA); Inveresk Research report # 7071, GLP, Unpublished.	Y	CAG
IIA, 8.2.1/03	Bettencourt M.J	1992	Dodine technical: Acute toxicity to sheepshead minnow (C*yprinodon variegates*) under flow-through conditions; Springborn Laboratories SLI report # 92-9-4416, GLP, Unpublished.	Y	CAG
IIA, 8.2.2/01	Sousa J.V.	1995	Dodine technical : the toxicity to fathead minnow (*Pimephales promelas*) during an early life stage exposure; Springborn Laboratories SLI report # 95-10-6126, GLP, Unpublished.	Y	CAG
IIA, 8.2.4.1/01	Putt A.E.	1992	Dodine technical: acute toxicity to daphnids (*Daphnia magna*) under flow-through conditions; Springborn Laboratories SLI report # 92-4-4245, GLP, Unpublished.	Y	CAG
IIA, 8.2.4.1/02	Caley C.Y.	1989	Dodine: determination of acute toxicity (LC50) to daphnia (48h, semi-static) (EPA); Inveresk Research, report # 7069, GLP Unpublished	Y	CAG
IIA, 8.2.4.1/03	Migchielsen M.H.J.	2002	Acute toxicity study in Daphnia magna with Dodine in a water-sediment system (Static); Notox. NL: report # 354825. GLP. Unpublished	Y	CAG
IIA, 8.2.4.1/04	Bettencourt M.J.	1992	Dodine technical : acute toxicity to mysid shrimp (*Mysidopsis bahia*) under flow-through conditions; Springborn Laboratories, SLI report # 92-9-4401, GLP, Unpublished	Y	CAG

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection claimed Y/N	Owner
IIA, 8.2.4.1/05	Dionne E.	1992	Dodine technical : acute toxicity to eastern oyster (*Crassostrea virginica*) under flow-through conditions; Springborn Laboratories, SLI report # 92-9-4404, GLP, Unpublished	Y	CAG
IIA, 8.2.5/01	Putt A.E.	1995	Dodine technical: the chronic toxicity to *Daphnia magna* under flow-through conditions; Springborn Laboratories, SLI report # 95-10-6162, GLP, Unpublished.	Y	CAG
IIA, 8.2.6/01	Hoberg J.R.	1993	Dodine : toxicity to the freshwater green alga, *Selenastrum capricornutum*; Springborn Laboratories, SLI report # 92-12-4550, GLP, Unpublished.	Y	CAG
IIA, 8.2.6/02	Hoberg J.R.	1995	Dodine - Toxicity to the freshwater green alga, *Selenastrum capricornutum* during a 15-day partial renewal test, Springborn Laboratories, SLI-report # 95-10-6147, GLP, Unpublished	Y	CAG
IIA, 8.2.7/01	Desmares-Koopmans M.J.E.	2002	Sediment-water chironomid toxicity test using water spiked with Dodine technical; Notox, report # 327127, GLP, Unpublished.	Y	CAG
IIA, 8.3.1.1/02	Servajean E.	2004	Determination of contact and oral acute toxicity of dodine to honey bees (*Apis mellifera*); Phytosafe, report # 04-99-037-ES, GLP, Unpublished.	Y	CAG
IIA, 8.3.2.1/01	Kühner C.	1997a	EXP 10343A: acute toxicity to the predatory mite, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in the laboratory; GAB/IFU, report # 97123/02-NLTp, GLP, Unpublished.	Y	CAG
IIA, 8.3.2.1/02	Jansen, J.-P.	2001a	Determination of LD50 of Syllit on protonymphs of the predatory mite *Typhlodromus pyri* Scheuten (Acari : Phytoseiidae) on detached bean leaves in the laboratory; Lab. d'Ecotox. Project test # TE.0212001, GLP, Unpublished	Y	CAG

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection claimed Y/N	Owner
IIA, 8.3.2.2/01	Kühner C.	1997b	EXP 10343A : acute toxicity to the aphid parasitoid, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) in the laboratory; GAB/IFU, report # 97123/02-NLAp, GLP, Unpublished.	Y	CAG
IIA, 8.3.2.2/02	Jansen, J.-P.	2001b	Side effects of Syllit on larvae of the green lacewing *Chrysoperla carnea* (Neuroptera; Chrysopidae) on detached bean leaves in the laboratory; Lab. d'Ecotox. Project test # CCE.01/2001, GLP, Unpublished	Y	CAG
IIA, 8.3.2.2/03	Jansen, J.-P.	2001c	Side effects of Syllit on larvae of the ladybird *Coccinella septempunctata* L. (Coleoptera; Coccinellidae) on detached bean leaves in the laboratory; Lab. d'Ecotox. Project test # CSE.01/2001, GLP, Unpublished	Y	CAG
IIA, 8.3.2.2/04	Jansen, J.-P.	2002	Side effects of Syllit on protonymph of the anthocorid bug *Orius insidiosus* (Heteroptera; Anthocoridae) on detached bean leaves in the laboratory; Lab. d'Ecotox. Project test # 0101/2001, GLP, Unpublished	Y	CAG
IIA, 8.4.1/01	Suteau, P.	1995	Dodine : Acute toxicity (14 day) to earthworms (*Eisenia foetida*). Artificial soil method; Rhone-Poulenc Agrochimie Report # SA 95248, GLP, Unpublished.	Y	CAG
IIA, 8.5/01	Kölzer, U	2002	Assessment of the side effects of Dodine 400 SC on the activity of the soil microflora; GAB/IFU report # 20011182/01-ABMF, GLP, Unpublished.	Y	CAG
IIA, 8.601	Maggi V.L.	1993a	Tier I : determination of the phytotoxic effects of dodine fungicide on vegetative vigor of non target plants; California Agricultural Research/ USA, Report # CAR 182-92, GLP, Unpublished	Y	CAG
IIA, 8.602	Maggi V.L.	1993b	Tier I : determination of the phytotoxic effects of dodine fungicide on seedling emergence of non target plants; Califomia Agricultural Researchl USA, Report # CAR 183-92, GLP, Unpublished	Y	CAG

Annex point / reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection claimed Y/N	Owner
IIA, 8.603	Maggi V.L.	1993c	Tier I : determination of the phytotoxic effects of dodine fungicide on seed germination of non target plants; California Agricultural Research/ USA, Report # CAR 184-92, GLP, Unpublished	Y	CAG
IIA, 8.7/01	Desmares-Koopmans M.J.E.	2001	Activated sludge respiration inhibition test with Dodine technical (Contact time : 30 minutes); Notox project 327138, GLP, Unpublished	Y	CAG
Annex IIIA					
IIIA, 10.1.0/01	Hirth, N.	2005	A study to evaluate residues of dodine 400 SC in *Tenebrio molitor* L. as feed source for wild birds and mammals following an application GAB Biotechnologie GmbH, Germany; report 20051278/01-NHTm GLP Unpublished	Y	CAG
IIIA, 10.1.0/02	Corman, C.	2006	Update risk assessment for long term exposure of birds to dodine Not applicable Unpublished	Y	CAG
IIIA, 10.2.1/01	Migchielsen M.	2004	Acute toxicity study in daphnia magna with Dodine 400 SC (Semi-static). Notox/NL; report # 413213 GLP, Unpublished,	Y	CAG
IIIA, 10.2.1/02	Migchielsen M.	2004	Fresh water algal growth inhibition test with Dodine 400 SC. Notox/NL; report # 413224, GLP Unpublished	Y	CAG
IIIA, 10.3/01	Klein, C., Schmitz, A. Hofer, M.	2006	91/414/EEC Review of dodine: refinement of the long-term mammalian risk assessment. SCC Scientific Consulting Company, Germany; project nº 169-003-02 Unpublished	Y	CAG
IIIA, 10.4.1/01	Servajen E.	2004	Determination of contact and oral acute toxicity of Syllit 400 SC to honey bees (*Apis mellifera*). Phytosafe / France; report # 04-99-038-ES, GLP Unpublished.	Y	CAG

PORTUGAL

MINISTÉRIO DA AGRICULTURA DO DESENVOLVIMENTO RURAL E DAS PESCAS
DIRECÇÃO-GERAL DE PROTECÇÃO DAS CULTURAS

Report prepared in the context of the application for first inclusion of dodine in Annex I of the Council Directive 91/414/EEC

DODINE

Volume 3-6

Annex B
Appendices

Summary, evaluation and assessments of the data.
List of tests and studies relied upon

2006

DODINE

ANNEX B

Appendix 1: Standard terms and abbreviations

2006

Appendix 1 Standard terms and abbreviations

Part 1 Technical terms

A	ampere
ACh	acetylcholine
AChE	acetylcholinesterase
ADI	acceptable daily intake
ADP	adenosine diphosphate
AE	acid equivalente
AFID	alkali flame-ionization detector or detection
A/G	albumin/globulin ratio
ai	active ingredient
ALD50	approximate median lethal dose, 50%
ALT	alanine aminotransferase (SGPT)
AOEL	acceptable operator exposure level
AMD	automatic multiple development
ANOVA	analysis of variance
AP	alkaline phosphatase
approx	approximate
ARC	anticipated residue contribution
ARfD	acute reference dose
as	active substance
AST	aspartate aminotransferase (SGOT)
ASV	air saturation value
ATP	adenosine triphosphate
BCF	bioconcentration factor
bfa	body fluid assay
BOD	biological oxygen demand
bp	boiling point
BSAF	biota-sediment accumulation factor
BSE	bovine spongiform encephalopathie
BSP	bromosulfophthalein
Bt	bacillus thuringiensis
Bti	bacillus thuringiensis israelensis
Btk	bacillus thuringiensis kurstaki
Btt	bacillus thuringiensis tenebrionis
BUN	blood urea nitrogen
bw	body weight
c	centi- (× 10-2)
ºC	degree Celsius (centigrade)
CA	controlled atmosphere
CAD	computer aided design
CADDY	computer aided dossier and data supply (an electronic dossier interchange and archiving format)
cd	candela
CDA	controlled drop(let) application
cDNA	complementary DNA
CEC	cation exchange capacity
cf	confer, compare to
CFU	colony forming units
ChE	cholinesterase
CI	confidence interval
CL	confidence limits
cm	centimetre
CNS	central nervous system
COD	chemical oxygen demand
CPK	creatinine phosphatase
cv	coefficient of varation

CXL	Codex Maximum Residue Limit (Codex MRL)
d	day
DES	diethylstilboestrol
DFR	dislodgeable foliar residue
DMSO	dimethylsulphoxide
DNA	deoxyribonucleic Acid
dna	designated national authority
DO	dissolved oxygen
DOC	dissolved organic carbon
dpi	days pot inoculation
DRES	dietary risk evaluation system
DT50	period required for 50 percent dissipation (define method estimation)
DT90	period required for 90 percent dissipation (define method estimation)
dw	dry weight
DWQG	drinking water quality guidelines
ξ	decadic molar extinction coefficient
EC50	median effective concentration
ECD	electron capture detector
ECU	European currency unit
ED50	median effective dose
EDI	estimated daily intake
ELISA	enzyme linked immonosorbent assay
e-mail	electronic mail
EMDI	estimated maximum daily intake
EPMA	electron probe micro analysis
ERC	environmentally relevant concentration
ERL	extraneous residue limit
F	field
F0	parental generation
F1	filial generation, first
F2	filial generation, second
FIA	fluorescence immuno assay
FID	flame ionization detector
FOB	functional observation battery
fp	freezig point
FPD	fame photometric detector
FPLC	fast protein liquid chromatography
g	gram
G	glasshouse
GAP	good agricultural practice
GC	gas chromatography
GC-EC	gas chromatography with electron capture detector
GC-FID	gas chromatography with flame ionization detector
GC-MS	gas chromatography-mass spectrometry
GEP	good experimental practice
GFP	good field practice
GGT	gamma glutamyl transferase
GI	gastro-intestinal
GIT	gastro.intestinal tract
GL	guideline level
GLC	gas liquid chromatography
GLP	good laboratory practice
GM	geometric mean
GMO	genetically modified organism
GMM	genetically modified micro-organism
GPC	gel-permeation chromatography

GPPP	good plant protection practice
GPS	global positioning system
GSH	glutathion
GV	granulosevirus
h	hour(s)
H	Henry´s Law constant (calculated as a unitless value) (see also K)
ha	hectare
Hb	haemoglobin
HCG	human chorionic gonadotropin
Hct	haematocrit
HDT	highest dose tested
hL	hectolitre
HEED	high energy electron diffraction
HID	helium ionization detector
HPAEC	high performance anion exchange chromatography
HPLC	high pressure liquid chromatography or high performance liquid chromatography
HPLC-MS	high pressure liquid chromatography – mass spectrometry
HPPLC	high pressure planar liquid chromatography
HPTLC	high performance thin layer chromatography
HS	Shannon-Weaver index
Ht	haemotocrit
I	indoor
I50	inhibitory dose, 50%
IC50	median immobilization concentration or median inhibitory concentration[1]
ICM	integrated crop management
ID	ionization detector
IEDI	internatinal estimated daily intake
IGR	insect growth regulator
im	intramuscular
inh	inhalation
ip	intraperitoneal
IPM	integrated pest management
IR	infrared
ISBN	international standard book number
ISSN	international standard serial number
iv	intravenous
IVF	in vitro fertilization
k	kilo
K	Kelvin or Henry´s Law constant (in atmospheres per cubic meter per mole) (see also H)[1]
Kads	adsorption constant
Kdes	apparent desorption coefficient
Koc	organic carbon adsorption coefficient
Kom	organic matter adsorption coefficient
kg	kilogram
L	litre
LAN	local area network
LASER	light amplification by stimulated emission of radiation
LBC	loosely bound capacity
LC	liquid chromatography
LC-MS	liquid chromatography – mass spectrometry
LC50	lethal concentration, median
LCA	life cycle analysis
LCLO	lethal concentration low

[1] The first time the abbreviation is used in a document, it should be defined (using a footnote to do so)

LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD50	lethal dose, median; dosis letalis media
LDLO	lethal dose low
LDH	lactate dehydrogenase
LOAEC	lowest obserbable adverse effect concentration
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOEC	lowest observable effect concentration
LOEL	lowest observable effect level
LOQ	limit of quantification (determination)
LPLC	low pressure liquid chromatography
LSC	liquid scintillation counting or counter
LSD	least squared denominator multiple range test
LSS	liquid scintilation spectometry
LT	lethal threshold
m	meter
M	molar
µm	micrometer (micron)
MC	moisture content
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MDL	method detection limit
MFO	mixed function oxidase
µg	microgram
mg	milligram
MHC	moisture holding capacity
min	minute(s)
mL	millilitre
MLT	median lethal time
MLD	minimum lethal dose
mm	millimetre
mo	month(s)
mol	Mole(s)
MOS	margin of safety
mp	melting point
MRE	maximum residue expected
MRL	maximum residue level or limit
mRNA	messenger ribonucleic acid
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
n	normal (defining isomeric configuration) or number of observations[1]
NAEL	no adverse effect level
nd	not detected
NEDI	national estimated daily intake
NEL	no effect level
NERL	no effect residue level
ng	nanogram
nm	nanometer
NMR	nuclear magnetic resonance
no	number
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration

[1] The first time the abbreviation is used in a document, it should be defined (using a footnote to do so)

NOED	no observed effect dose
NOEL	no observed effect level
NOIS	notice of intent to suspend
NPD	nitrogen-phosphorus detector or detection
NPV	nuclear polyhedrosis virus
NR	not reported
NTE	neurotoxic target esterase
OC	organic carbon content
OCR	optical character recognition
ODP	ozone-depleting potential
ODS	ozone-depleting substances
OM	organic matter content
op	organophosphorus pesticide
Pa	Pascal
PAD	pulsed amperometric detection
2-PAM	2-pralidixime
pc	paper chromatography
PC	personal computer
PCV	haematocrit (packed corpuscular volume)
PEC	predicted environmental concentration
PECA	predicted environmental concentration in air
PECS	predicted environmental concentration in soil
PECSW	predicted environmental concentration in surface water
PECGW	predicted environmental concentration in ground water
PED	plasma-emissions-detector
pH	pH-value
PHED	pesticide handler´s exposure data
PHI	pre-harvest interval
PIC	prior informed consent
pic	phage inhibitory capacity
PIXE	proton induced X-ray emission
pKa	negative logarithm (to the base 10) of the dissociation constant
PNEC	predicted no effect concentration
po	by mouth
POW	partition coefficient between n-octanol and water
POP	persistent organic pollutants
ppb	parts per billion (10 -9)
PPE	personal protective equipment
ppm	parts per million (10 -6)
ppp	plant protection product
ppq	parts per quadrillion (1 O
ppt	parts per trillion (10 -24)
PSP	phenolsulfophthalein
PrT	prothrombin time
PRL	practical residue limit
PT	prothrombin time
PTDI	provisional tolerable daily intake
PTT	partial thromboplastin time
QSAR	quantitative structure-activity relationship
r	correlation coefficient
r2	coefficient of determination
RBC	red blood cell
REI	restricted entry interval
Rf	retardation factor
RfD	reference dose
RH	relative humidity

RL50	median residual lifetime
RNA	ribonucleic acid
RP	reversed phase
rpm	rotations per minute
rRNA	ribosomal ribonucleic acid
RRT	relative retention time
RSD	relative standard deviation
s	second
SAC	strong adsorption capacity
SAP	serum alkaline phosphatase
SAR	structure/activity relationship
SBLC	shallow bed liquid chromatography
sc	subcutaneous
sce	sister chromatid exchange
SD	standard deviation
se	standard error
SEM	standard error of the mean
SEP	standard evaluation procedure
SF	safety factor
SFC	supercritical fluid chromatography
SFE	supercritical fluid extraction
SIMS	secondary ion mass spectroscopy
SOP	standard operating procedures
sp	species (only after a generic name)
SPE	solid phase extraction
SPF	specific pathogen free
spp	subspecies
sq	square
SSD	sulphur specific detector
SSMS	spark source mass spectrometry
STEL	short term exposure limit
STMR	supervised trials median residue
t	tonne (metric ton)
t1/2	half-life (define method of estimation)
T3	tri-iodothyroxine
T4	thyroxine
TADI	temporary acceptable daily intake
TBC	tightly bound capacity
TCD	thermal conductivity detector
TCLO	toxic concentration, low
TID	themionic detector, alkali flame detector
TDLO	toxic dose low
TDR	time domain reflectrometry
TER	toxicity exposure ration
TERI	toxicity exposure ration for initial exposure
TERST	toxicity exposure ration following repeated exposure
TERLT	toxicity exposure ration following chronic exposure
tert	tertiary (in a chemical name)
TEP	typical end-use product
TGGE	temperature gradient gel electrophoresis
TIFF	tag image file format
TLC	thin layer chromatography
TIm	median tolerance limit
TLV	threshold limit value
TMDI	theoretical maximum daily intake
TMRC	theoretical maximum residue contribution
TMRL	theoretical maximum residue limit
TOC	total organic carbon

Tremcard	Transport emergency card
tRNA	transfer ribonucleic acid
TSH	thyroid stimulating hormone (thyrotropin)
TWA	time weighted average
UDS	unscheduled DNA synthesis
UF	uncertainty factor (safety factor)
ULV	ultra low volume
UV	ultraviolet
v/v	volume ratio (volume per volume)
WBC	white blood cell
wk	week
wt	weight
w/v	weight per volume
ww	wet weight
w/w	weight per weight
XRFA	X-ray fluorescence analysis
yr	year
<	less than
≤	less than or equal to
>	greater than
≥	greater than or equal to

Part 2 Organisations and Publications

ACPA	American Crop Protection Association
ASTM	American Society for Testing and Materials
BA	Biological Abstracts (Philadelphia)
BART	Beneficial Arthropod Registration Testing Group
CA	Chemical Abstracts
CAB	Centre for Agriculture and Biosciences International
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCFAC	Codex Committee on Food Additives and Contaminants
CCGP	Codex Committee on General Principles
CCPR	Codex Committee on Pesticide Residues
CCRVDF	Codex Committee on Residues of Veterinary Drugs in Food
CE	Council of Europe
CIPAC	Collaborative International Pesticides Analytical Council Ltd
COREPER	Comite des Representants Permanents
EC	European Commission
ECB	European Chemical Bureau
ECCA	European Crop Care Association
ECDIN	Environmental Chemicals Data and Information Network of the European Communities
ECDIS	European Environmental Chemicals Data and Information System
ECE	Economic Commission for Europe
ECETOC	European Chemical Industry Ecology and Toxicology Centre
ECLO	Emergency Centre for Locust Operations
ECMWF	European Centre for Medium Range Weather Forecasting
ECPA	European Crop Protection Association
EDEXIM	European Database on Export and Import of Dangerous Chemicals
EHC (number)	Environmental Health Criteria (number)
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EMIC	Environmental Mutagens Information Centre
EPA	Environmental Protection Agency
EPO	European Patent Office
EPPO	European and Mediterranean Plant Protection Organization
ESCORT	European Standard Characteristics of Beneficials Regulatory Testing
EU	European Union
EUPHIDS	European Pesticide Hazard Information and Decision Support System
EUROPOEM	European Predictive Operator Exposure Model
FAO	Food and Agriculture Organization of the UN
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
FRAC	Fungicide Resistance Action Committee
GATT	General Agreement on Tariffs and Trade
GAW	Global Atmosphere Watch
GIFAP	Groupement International des Associations Nationales de Fabricants de Produits Agrochimiques (now known as GCPF)
GCOS	Global Climate Observing System
GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GEDD	Global Environmental Data Directory
GEMS	Global Environmental Monitoring System
GIEWS	Global Information and Early Warning System for Food and Agriculture
GRIN	Germplasm Resources Information Network
HRAC	Herbicide Resistance Action Committee

IARC	International Agency for Research on Cancer
IATS	International Academy of Toxicological Science
IBT	Industrial Bio-Test Laboratories
ICBB	International Commission of Bee Botany
ICBP	International Council for Bird Preservation
ICES	International Council for the Exploration of the Seas
ICPBR	International Commission for Plant-Bee Relationships
ILO	International Labour Organization
IMO	International Maritime Organisation
IOBC	International Organization for Biological Control of Noxious Animals and Plants
IPCS	International Programme on Chemical Safety
IRAC	Insecticide Resistance Action Committee
IRC	International Rice Commission
ISCO	International Soil Conservation Organization
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
JECFA	FAO/WHO Joint Expert Committee on Food Additives
JFCNT	Joint FAO/WHO Food and Animal Feed Contamination Monitoring Programme
JMP	Joint Meeting on Pesticides (WHO/FAO)
JMPR	Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
NATO	North Atlantic Treaty Organisation
NAFTA	North American Free Trade Agreement
NCI	National Cancer Institute (USA)
NCTR	National Centre for Toxicological Research (USA)
NGO	non-governmental organization
NTP	National Toxicology Programme (USA)
OECD	Organization for Economic Co-operation and Development
OLIS	On-line Information Service of OECD
PAN	Pesticide Action Network
RNN	Re-registration Notification Network
RTECS	Registry of Toxic Effects of Chemical Substances (USA)
SCPH	Standing Committee on Plant Health
SETAC	Society of Environmental Toxicology and Chemistry
SI	Systeme International d'Unites
SITC	Standard International Trade Classification
TOXLINE	Toxicology Information On-line
UN	United Nations
UNEP	United Nations Environment Programme
WCDP	World Climate Data Programme
WCP	World Climate Programme
WCRP	World Climate Research Programme
WFP	World Food Programme
WHO	World Health Organization
WTO	World Trade Organization
WWF	World Wildlife Fund

Part 3 International codes for technical & formulated pesticides*

Code	Term	Definition
AB	Grain bait	Special form of bait.
AE	Aerosol dispenser	A container-held formulation which is dispersed generally by a propellant as fine droplets or particles upon the actuation of a valve.
AL	Any other liquid	A liquid not yet designated by a specific code, to be applied undiluted.
AP	Any other powder	A powder not yet designated by a specific code, to be applied undiluted.
BB	Block bait	Special form of bait.
BR	Briquette	Solid block designed for controlled release of active ingredient into water.
CB	Bait concentrate	A solid or liquid intended for dilution before use as a bait.
CF	Capsule Suspension for Seed Treatment	A stable suspension of capsules in a fluid to be applied to the seed, either directly or after dilution.
CG	Encapsulated granule	A granule with a protective or granule release-controlling coating.
CL	Contact liquid or gel	Rodenticidal or insecticidal formulation in the form of a liquid/gel for direct application, or after dilution in the case of gels.
CP	Contact powder	Rodenticidal or insecticidal formulation in powder form for direct application. Formerly known as tracking powder (TP).
CS	Capsule suspension	A stable suspension of capsules in a fluid, normally intended for dilution with water before use.
DC	Dispersible concentrate	A liquid homogeneous formulation to be applied as a solid dispersion after dilution in water. (Note: there are some formulations which have characteristics intermediate between DC and EC).
DP	Dustable powder	A free-flowing powder suitable for dusting.
DS	Powder for dry seed treatment	A powder for application in the dry state directly to the seed.
DT	Tablet for direct application	Formulation in the form of tablets to be applied individually and directly in the field, and/or bodies of water, without preparation of a spraying solution or dispersion.
EC	Emulsifiable concentrate	A liquid, homogeneous formulation to be applied as an emulsion after dilution in water.
ED	Electrochargeable liquid	Special liquid formulation for electrostatic (electrodynamic) spraying.
EG	Emulsifiable Granule	A granular formulation to be applied as an oil-in-water emulsion of the active ingredient after disintegration in water, which may contain water insoluble formulants.
EO	Emulsion, water in oil	A fluid, heterogeneous formulation consisting of a solution of pesticide in water dispersed as fine globules in a continuous organic liquid phase.
ES	Emulsion for seed treatment	A stable emulsion for application to the seed either directly or after dilution.
EW	Emulsion, oil in water	A fluid, heterogeneous formulation consisting of a solution of pesticide in an organic liquid dispersed as fine globules in a continuous water phase.
FD	Smoke tin	Special form of smoke generator.
FG	Fine granule	A granule in the particle size range from 300 to 2500 µm.
FK	Smoke candle	Special form of smoke generator.
FP	Smoke cartridge	Special form of smoke generator.
FR	Smoke rodlet	Special form of smoke generator.
FS	Flowable concentrate for seed	A stable suspension for application to the seed either directly or after

* Note: based on *Catalogue of Pesticide Formulation Types and International Coding System*. Technical Monograph No.2, GIFAP, Brussels. Revised February 1989.

	treatment	dilution.
FT	Smoke tablet	Special form of smoke generator.
FU	Smoke generator	A combustible formulation, generally solid, which upon ignition releases the active ingredient(s) in the form of smoke.
	Special forms of smoke generators	
	Smoke candle	(FK)
	Smoke cartridge	(FP)
	Smoke pellet	(FW)
	Smoke rodlet	(FR)
	Smoke tablet	(FT)
	Smoke tin	(FD)
FW	Smoke pellet	Special form of smoke generator.
GA	Gas	A gas packed in pressure bottle or pressure tank.
GB	Granular bait	Special form of bait.
GE	Gas generating product	A formulation which generates a gas by chemical reaction.
GF	Gel for Seed Treatment	A homogeneous gelatinous formulation to be applied directly to the seed.
GG	Macrogranule	A granule in the particle size range from 2000 to 6000 µm.
GL	Emulsifiable gel	A gelatinized formulation to be applied as an emulsion in water.
GP	Flo-dust	Very fine dustable powder for pneumatic application in greenhouses.
GR	Granule	A free-flowing solid formulation of a defined granule size range ready for use.
	Special forms of granules:	
	Encapsulated granule (CG)	A granule with a protective or release-controlling coating.
	Fine granule (FG)	Particle size range from 300 to 2500 µm.
	Macrogranule (GG)	Particle size range from 2000 to 6000 µm.
	Microgranule (MG)	Particle size range from 100 to 600 µm.
GS	Grease	Very viscous formulation based on oil or fat.
GW	Water soluble gel	A gelatinized formulation to be applied as an aqueous solution.
HN	Hot fogging concentrate	A formulation suitable for application by hot fogging equipment, either directly or after dilution.
KK	Combi-pack solid/liquid	A solid and a liquid formulation, separately contained within one outer pack, intended for simultaneous application in a tank mix.
KL	Combi-pack liquid/liquid	Two liquid formulations, separately contained within one outer pack, intended for simultaneous application in a tank mix.
KN	Cold fogging concentrate	A formulation suitable for application by cold fogging equipment, either directly or after dilution.
KP	Combi-pack solid/solid	Two solid formulations, separately contained within one outer pack, intended for simultaneous application in a tank mix.
LA	Lacquer	Solvent-based, film-forming composition.
LS	Solution for seed treatment	A clear to opalescent liquid to be applied to the seed either directly or as a solution of the active ingredient after dilution in water. The liquid may contain water insoluble formulants.
ME	Micro-emulsion	A clear to opalescent, oil and water containing liquid, to be applied directly or after dilution in water, when it may form a diluted micro-emulsion or a conventional emulsion.
MG	Microgranule	A granule in the particle size range from 100 to 600 µm.
OF	Oil miscible flowable concentrate (oil miscible suspension)	A stable suspension of active ingredient(s) in a fluid intended for dilution in an organic liquid before use.
OL	Oil miscible liquid	A liquid, homogeneous formulation to be applied as a homogeneous liquid after dilution in an organic liquid.
OP	Oil dispersible powder	A powder formulation to be applied as a suspension after dispersion in an organic liquid.
PA	Paste	Water-based, film-forming composition.
PB	Plate bait	Special form of bait.
PC	Gel or paste concentrate	A solid formulation to be applied as a gel or paste after dilution with water.
PO	Pour-on	Solution for pouring on the skin of animals in a high volume (normally more than 100 ml per animal).
PR	Plant rodlet	A small rodlet, usually a few centimetres in length and a few

		millimetres in diameter, containing an active ingredient.
PS	Seed coated with a pesticide	Self defining.
RB	Bait (ready for use) Special forms of baits: Block bait (BB) Grain bait (AB) Granular bait (GB) Plate bait (PB) Scrap bait (SB)	A formulation designed to attract and be eaten by the target pests.
SA	Spot-on	Solution for spot application on the skin of animals in a low volume (normally less than 100 ml per animal).
SB	Scrap bait	Special form of bait.
SC	Suspension concentrate (= flowable concentrate)	A stable suspension of active ingredient(s) in a fluid, which may contain other dissolved active ingredient(s), intended for dilution with water before use.
SE	Suspo-emulsion	A fluid, heterogeneous formulation consisting of a stable dispersion of active ingredients in the form of solid particles and fine globules in a continuous water phase.
SG	Water soluble granule	A formulation consisting of granules to be applied as a true solution of the active ingredient after dissolution in water, but which may contain insoluble inert ingredients.
SL	Soluble concentrate	A clear to opalescent liquid to be applied as a solution of the active ingredient after dilution in water. The liquid may contain water insoluble formulants.
SO	Spreading oil	Formulation designed to form a surface layer on application to water.
SP	Water soluble powder	A powder formulation to be applied as a true solution of the active ingredient after dissolution in water, but which may contain insoluble inert ingredients.
SS	Water soluble powder for seed treatment	A powder to be dissolved in water before application to the seed.
ST	Water soluble tablet	Formulation in form of tablets to be used individually, to form a solution of the active ingredient after disintegration in water. The formulation may contain water insoluble formulants.
SU	Ultra-low volume (ULV) suspension	A suspension ready for use through ULV equipment.
TB	Tablet Special forms of tablets: DT - tablets for direct application ST - tablets for dissolution in water WT - tablets for dispersion in water	Pre-formed solids of uniform shape and dimensions, usually circular, with either flat or convex faces, the distance between faces being less than the diameter.
TC	Technical material	A material resulting from a manufacturing process comprising the active ingredient, together with associated impurities. This may contain small amounts of necessary additives.
TK	Technical concentrate	A material resulting from a manufacturing process comprising the active ingredient, together with associated impurities. This may contain small amounts of necessary additives and appropriate diluents. For use only in the preparation of formulations.
(TP)	(Tracking powder)	(Discontinued terms. Refer to CP)
UL	Ultra-low volume (ULV) liquid	A homogeneous liquid ready for use through ULV equipment.
VP	Vapour releasing product	A formulation containing one or more volatile active ingredients, the vapours of which are released into the air. Evaporation rate is normally controlled by using suitable formulations and/or dispensers.
WG	Water dispersible granules	A formulation consisting of granules to be applied after disintegration and dispersion in water.
WP	Wettable powder	A powder formulation to be applied as a suspension after dispersion in water.
WS	Water dispersible powder	A powder to be dispersed at high

	for slurry seed treatment	concentration in water before application as a slurry to the seed.
WT	Water dispersible tablet	Formulation in the form of tablets to be used individually, to form a dispersion of the active ingredient after disintegration in water.
XX	Others	Temporary categorization of all other formulations not listed above.

DODINE

ANNEX B

Appendix 2: Specific terms and abbreviations

2006

Appendix 2 Specific terms and abbreviations

Part 1 Technical terms

2AA	2-aminoanthracene
Abs	Absolute
A/G ratio	Albumin/globulin ratio
Alb	Albumin
AP	Alkaline phosphatase
c	Completely reversible
Ca^{++}	Calcium
CHO	Chinese hamster ovary
Cl^{-}	Chloride
CMC	Carboxymethyl cellulose
CP	Cyclophosphamide
d	Desquamation
DMN	Dimethylnitrosamine
DNCB	2,4-dinitrochlorobenzene
EMS	Ethyl methane sulphonate
excl.	Excluding
F	Female
FCA	Freund's complete adjuvant
σg	Standard geometric deviation
GGT	γ-glutamyl transpeptidase
GI	Gastro-intestinal
Glob	Globulin
HGPRT	Hypoxanthine-guanine phosphoribosyl transferase
incl.	Including
i.v.	Intravenous
K^{+}	Potassium
LD	Less dense background lawn of bacterial growth
Lymph	Lymphocytes
M	Male
M&K	Maximization test of Magnusson and Kligman
MMAD	Mass median aerodynamic diameter
MMC	Mitomycin C
MMS	Methyl methane sulphonate
MNC	Micronucleated cells
MPV	Mean platelet volume
n	Not reversible
N.	Necrosis
Na^{+}	Sodium
NaCl	Sodium chloride
nc	Not completely reversible
NCE	Normochromatic erythrocyte
Neut seg	Neutrophils segmented
NG	Normal background lawn of bacterial growth
4-NQO	4-nitroquinolina N-oxide

PCE	Polychromatic erythrocyte
Plt	Platelet count
p.o.	*Per os*
POEM	Predictive Operator Exposure Model
QA	Quality Assurance
RDW	Red cell distribution width
Rel	Relative
RMS	Rapporteur Member State
S9	Liver homogenate fraction
S9 Mix	Metabolic activation mixture
sd	Standard deviation
SE	Standard error
Soln.	solution
6TG	6-thioguanidine
Trp+	Tryptophan independent
↑	Increase
↓	Decrease

Part 2 Organisations and Publications

CAG	Chimac-Agriphar
DFG	Deutsche Forschungsgemeinschaft
DGPC	Direcção-Geral de Protecção das Culturas