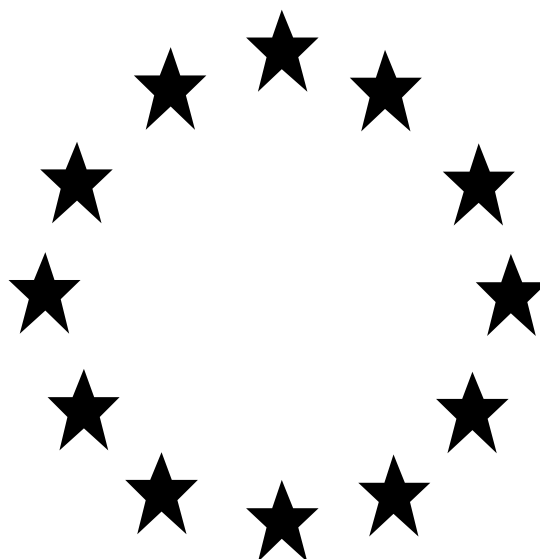




Additional Report to the DAR

- public version -

**Risk assessment provided by the rapporteur Member State
Italy for the existing active substance**

CHLOROPICRIN

**upon resubmission in the framework of the accelerated procedure in
accordance with Commission Regulation (EC) No 33/2008**

**Volume 3
Annex B9**

March 2010

Ministry of Health

Rome

Italy

**Addendum to Draft Assessment Report
regarding resubmission of Chloropicrin**



Chloropicrin

Volume 3

Annex B.9: Ecotoxicology

March 2010

B.9 ECOTOXICOLOGY

Background Information

Parent compound

The assessment has been performed on the active substance Chloropicrin based on a single application of 448 Kg/ha (equivalent to 272 l/ha) to soil. The application is made by pre-plant soil injection followed by the placement of a tarp covering for 8 days. A virtually impermeable film is employed, which reduces emissions.

Formulation

Chloropicrin is not formulated as a bait or granule and is not used in the treatment of seed. The active substance and preparation are of the same formulation, as the product contains 99% of Chloropicrin.

B.9.1 Effects on birds (IIA 8.1, IIA 10.1)

In response to requests made in the DAR2005 (supported by the EFSA and several MS), studies into the acute oral, dietary, long-term and inhalation toxicity to birds have been carried out.

B.9.1.1 Acute oral/dietary toxicity

Acute oral toxicity (IIA 8.1.1)

Hubbard, P.M., Beavers, J.B., 2009a Chloropicrin: An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential Testing Procedure. [REDACTED]
[REDACTED] Unpublished report No. 650-101. Owner European Chloropicrin Group. 2009a. (IIA 8.1.1/01)

GLP: Yes with the following exceptions:

1. Test substance characterisation and stability of the test substance under storage conditions at the test site were determined at the time of use however the determination was not carried out according to Good Laboratory Practice Standards.
2. Periodic analyses of feed and water for potential contaminants were not performed according to Good Laboratory Practice standards, but were performed using a certified laboratory and standard U.S. Environmental Protection Agency analytical methods.

The study was carried out in accordance with OECD guideline number 223.

Method

A three-stage test design was used in this experiment. Male northern bobwhites (26-28 weeks old, 181-230 g) were randomly assigned to the test pens. At the start of each stage, a single encapsulated dose of the test substance was orally inserted and manually manipulated directly into the crop of each bird. Each bird was individually weighed and dosed on the basis of milligrams of test substance per kilogram of body weight (mg/kg). The dosage of Chloropicrin at each stage is shown in Table B 9.1.1-1. For stages 2 and 3, the choice of test concentrations were decided upon the results of the LC50 from the previous stage.

Table B 9.1.1-1: Chloropicrin Treatment Groups

Test Stage	Target Dosage ¹ (mg/kg bw)	Achieved Dosage ² (mg/kg bw)	Pens per Group	Birds per Pen (Males)
1	35	34	1	1
1	130	131	1	1
1	480	480	1	1
1	1768	1768	1	1
2	23	23	1	1
2	29	29	1	1
2	37	37	1	1
2	47	46	1	1
2	59	60	1	1
2	75	75	1	1
2	95	94	1	1
2	121	120	1	1
2	154	153	1	1
2	195	196	1	1
3	42	42	2	1
3	53	53	2	1
3	67	67	2	1
3	85	85	2	1
3	108	108	2	1

¹ Target Dosages used for reporting purposes.

² Achieved Dosages used for calculating the LD₅₀ value and confidence intervals.

Birds were acclimatized to the test facility (11 weeks) and to the cage (3 weeks) and fasted at least 17 hours prior to dosing at each test stage.

All birds were observed at least once daily, and a record was maintained of all mortality, signs of toxicity and abnormal behaviour. Body weights were measured individually at the initiation of each stage and on Days 3, 7 and 14 of each stage and on Day 17 of Stage 2.

Average temperature was 23.4 °C , relative umidity of 60%, 8 h light photoperiod.

The LD50 was calculated using mortality data from all the 3 stages.

Results:

Generally there was a clear dose response relation; reversals in the mortality pattern occurred only at one instance, in the range of dosages from 53 to 85 mg/kg.

There were no mortalities at the 47 mg/kg dosage level or dosage levels below that level. There was 100 % mortality in the 95 mg/kg dosage level and all the dosage levels above that value. Reversals in the mortality pattern occurred in the range of dosages from 53 to 85 mg/kg. Achieved dosages were used for calculating the LD₅₀ value and confidence intervals.

During Stage 1 of the test, four birds, one at each dosage, were dosed with Chloropicrin at 35, 130, 480 and 1768 mg/kg. No regurgitation was noted for any of the dosed birds. Signs of toxicity were first noted in the birds at the 480 and 1768 mg/kg dosages approximately thirty minutes after dosing, when both birds were noted with a slight loss of coordination and a slight ruffled appearance. Signs of toxicity (ruffled appearance) were first noted at the 130 mg/kg dosage approximately 1h20' after dosing and at the 35 mg/kg level approximately 4h40' after dosing. Mortalities were observed approximately 1h50' after dosing in the 130 and 1768 mg/kg dosage, and 1h20' after dosing in the 480 mg/kg dosage.

During Stage 2 of the test, ten birds were dosed with Chloropicrin at 23, 29, 37, 47, 59, 75, 95, 121, 154 and 195 mg/kg. No regurgitation was noted for any of the dosed birds. At 23 and 29 mg/kg dosages, a ruffled appearance was observed within five hours of dosing; however both had recovered by the afternoon of Day 1. At the 37 and 47 mg/kg dosages, signs of toxicity were observed approximately three hours and thirty minutes after dosing. Based on feed consumption levels, both birds suffered with anorexia from Day 0 to Day 3. The bird at the 37 mg/kg dosage recovered by the afternoon of Day 4 and the bird at the 47 mg/kg dosage had recovered by the morning of Day 6. The bird at the 59 mg/kg dosage first exhibited signs of toxicity approximately three hours and thirty minutes after dosing, however the bird had recovered by the morning of Day 7. The bird at the 75 mg/kg dosage first showed signs of toxicity one hour and fifty minutes after dosing. The bird was found dead on the morning of Day 1. Birds dosed with 95, 121 and 195 mg/kg exhibited one or more signs of toxicity within two hours of dosing. All birds at these dosage levels were found dead within five hours of dosing.

During Stage 3 of the test, ten birds (two per level) were dosed with Chloropicrin at 42, 53, 67, 85 and 108 mg/kg. No regurgitation was noted for any of the dosed birds. At the 42 mg/kg dosage level, signs of toxicity were first observed approximately three hours and forty minutes after dosing. One bird had recovered by the afternoon of Day 2 while the other displayed signs of toxicity through to the afternoon of Day 4. Both birds were normal in appearance and behaviour from the morning of Day 5 until test termination.

At the 53 mg/kg dosage level, signs of toxicity were first observed approximately three hours and forty minutes after dosing when both birds were noted with ruffled appearance and a slight loss of coordination. On the morning of Day 1 one bird was found dead. The second bird improved from the afternoon of Day 2, and was normal in appearance and behaviour through to the end of the test. At the 67 mg/kg dosage, signs of toxicity, including ruffled appearance, loss of coordination and lower limb weakness were first noted approximately three hours and forty minutes after dosing. The first bird was found dead five hours and fifteen minutes after dosing and the second bird was found on the morning of Day 1.

At the 85 mg/kg dosage, signs of toxicity were first noted in one bird one hour and forty minutes after dosing. The remaining bird continued to display signs of toxicity, including anorexia, through the afternoon of Day 5. The second bird showed signs of toxicity, including ruffled appearance, loss of coordination, anorexia and prostrate posture approximately two

hours after dosing and died about five hours and ten minutes after dosing. The single surviving bird was normal in appearance and behaviour from the morning of Day 6 until the test ended.

At the 108 mg/kg dosage, multiple signs of toxicity were noted in both birds approximately 1h40' after dosing. The first mortality occurred approximately 2 hours after dosing. The second bird was found on the morning of Day 1. Signs of toxicity noted at this dosage level were ruffled appearance, loss of coordination and lower limb weakness.

A summary of the mortality and bodyweight data is given in Table 9.1.1-2

Table B 9.1.1-2: Chloropicrin Treatment Groups

Test Stage	Target Dosage ¹ (mg/kg)	Achieved Dosage ² (mg/kg)	Number dead / Number exposed (#)	Changes in bodyweight (%)
1	35	34	0/1	-1
1	130	131	1/1	n.a.
1	480	480	1/1	n.a.
1	1768	1768	1/1	n.a.
2	23	23	0/1	7.7
2	29	29	0/1	1
2	37	37	0/1	-3.5
2	47	46	0/1	-16
2	59	60	0/1	-27.3
2	75	75	1/1	n.a.
2	95	94	1/1	n.a.
2	121	120	1/1	n.a.
2	154	153	1/1	n.a.
2	195	196	1/1	n.a.
3	42	42	0/2	-2.4 / 0.5
3	53	53	1/2	1.6 / n.a
3	67	67	2/2	n.a.
3	85	85	1/2	-31 / n.a
3	108	108	2/2	n.a.

¹ Target Dosages used for reporting purposes.

² Achieved Dosages used for calculating the LD₅₀ value and confidence intervals.

n.a. not available due to mortality

Conclusions:

The acute oral LD₅₀ value for northern bobwhite exposed to Chloropicrin as a single oral dose was calculated to be 62 mg/kg/bw with a 95 % confidence interval of 43 mg/kg to 84 mg/kg. The slope of the dose-response line was 8.6 and the Chi-square value was 6.6.

Acceptability - The study is considered acceptable.

Acute Inhalation Toxicity (IIA 8.1.1/02)

Weinberg, J.T., 2008. A 5-day Whole-Body Inhalation Toxicity Study of Chloropicrin in Northern Bobwhite Quail. [REDACTED] Unpublished Report no.: [REDACTED]-697002. Owner European Chloropicrin Group (IIA 8.1.1/02).

GLP: Yes (Deviations: None)

Guideline: OECD number 412.

Deviations:

1. The test system used was the northern bobwhite quail.
2. The length of the study was only 5 days.
3. The length of each daily exposure was only 4 hours.
4. Clinical pathology evaluations were not conducted

Method

The study was performed to assess the inhalation toxicity of Chloropicrin administered via whole-body inhalation to the northern bobwhite (*Colinus virginianus*) for a period of four hours over 5 consecutive days. Birds were 23 weeks old at test initiation, males weighed 166 g – 222 g and females: 178 g – 232 g. Animals were acclimated for at least 29d before testing. Males and females were assigned into three treatment groups and one control and received the dosage of Chloropicrin as indicated in Table B 9.1.1-3. Chloropicrin concentration was analyzed approximately every 24 to 50 minutes using a GC with ECD detector. The achieved dosages are the overall means of the mean concentrations measured on all study days.

Table B 9.1.1-3: Chloropicrin Treatment Groups

Test roup	Target Dosage ¹	Achieved Dosage ²	Birds per Group	
	(ppb)	(ppb)	Male	Female
Control	0	0	15	15
Group 2	100	96	15	15
Group 3	300	303	15	15
Group 4	750	659	16	14

¹ Target Dosages used for reporting purposes.

² Achieved Dosages used for calculating the NOAEL value (mean value of all study days combined).

The initiation of the study was staggered over a 3-day period with study day 0 defined by the first animal exposure for the first replicate of animals. Exposure to the test substance or filtered air was conducted in four 1.0 m³ 1000-L stainless steel and glass whole-body inhalation exposure chambers (one chamber per group). During the exposure periods, food and water were withheld.

Actual environmental conditions were: Temperature 21.1-22.8 °C , humidity 38-50.7 %, minimum of 10 air changes/hour. Following the exposure period, the high concentration exposure atmosphere was evaluated for the presence of aerosols.

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily and physical examinations weekly. Individual body weights were recorded pre-test and prior to the first and fifth exposures.

Following five days exposure, ten quail per sex in the control group and treatment groups 2 and 3 were euthanized. In group 4, 11 males and 9 females were euthanized. The remaining five birds per group per sex were euthanized following a 14-day non-exposure recovery period. Complete necropsies were conducted on all animals and selected tissues were examined microscopically.

Results:

No mortalities occurred at any of the three treatment group concentrations. There were no test substance-related effects on survival, organ weight or macroscopic observations.

There were no test substance-related clinical observations in females. Gasping, increased activity and complete closure of the right eye were reported in the 659 ppb a.s. group male after the fourth and/or the fifth exposure.

Body weights (summarized in Table B 9.1.1-4) were significantly lower in the 659 ppb a.s. group male during the study day 0 to 4 interval (Dunnett's test). This was likely attributed to one male with a total body loss of 57g compared to an average group loss of 10g. This decrease in weight was not observed in females.

Table B.9.1.1-4 Mean body weights (g).

Experimental Group	Sex	2d Pre-test	S.D.	Day 0 Treatment	S.D.	Day 4 Treatment	S.D.
Control	Male	199	12.3	198	12.2	194	13.3
0 ppb a.s.	Female	199	14	200	14.1	195	14.3
96 ppb a.s.	Male	198	12.9	195	11.8	193	12.6
	Female	199	14.9	197	15.8	191	16.1
303 ppb a.s.	Male	198	12.0	197	10.3	190	10.5
	Female	199	15.8	197	13.4	193	12.1
659 ppb a.s.	Male	196	14.2	196	14.4	185	14.0
	Female	200	14.5	199	15.1	191	14.1
S.D = Standard Deviation							

On microscopic examination of male and female birds in the 303 and 659 ppb a.s., changes were observed in the respiratory tract. These birds suffered from parabronchial smooth muscle hyperplasia with degeneration of the epithelial lining in the lung, squamous metaplasia and subepithelial stromal hyperplasia of the respiratory epithelium lining the middle concha of the nasal cavity, and respiratory epithelial atrophy with deciliation of the trachea. Following a two week recovery period, there was a decreased incidence and severity of the lesions in the respiratory tract. In the 659 ppb a.s. treatment group, two males and one female had minimal to mild parabronchial smooth muscle hyperplasia. However, no birds

from the 303 ppb a.s. were affected, indicating there was almost complete recovery from the inhalation effects of chloropicrin.

Conclusions:

The test fulfils the OECD validity criteria.

Administration of chloropicrin to male and female northern bobwhite quail *via* whole-body inhalation at exposure levels of 96, 303 or 659 ppb a.s. for 4 hours period for five consecutive days was well tolerated with no mortality. The no-observed-effect level (NOEL) and the no-observed-adverse-effect level (NOAEL) was 96 ppb a.s., based on clinical observations of gasping in the 659 ppb a.s. group males and microscopic changes in the respiratory tract of the 303 and 659 ppb group males and females. However, following a 2-week recovery period, there were no test substance-related clinical findings, and microscopic findings were resolved or present at a lower incidence and/or severity.

Acceptability - This study is considered acceptable.

Short-term dietary toxicity (IIA 8.1.2)

In response to comments from EFSA a dietary study in the mallard and bobwhite quail was carried out.

Hubbard, P.M., Martin, K.H., and Beavers, J.B., 2009a. Chloropicrin: A Dietary LC₅₀ Study with the Mallard. [REDACTED] Unpublished Report No.: 650-106. Owner European Chloropicrin Group (IIA 8.1.2/01)

GLP: Yes, except for periodic analyses of feed and water for potential contaminants

Guideline: EPA guideline OPPTS Number 850.2200, OECD "Avian Dietary Toxicity Test" 205, ASTM E857-87

Deviation: during the test, the temperature and humidity were both slightly outside of the ranges recommended in OECD 205. These deviation are not considered to have affected the reliability of results.

Method:

Chloropicrin was administered to 7 groups (2pens per group) of 10 ducklings of Mallard duck (*Anas platyrhynchos*) at nominal concentrations of 0, 17.8, 31.6, 56.2, 100, 178, 316, 562 ppm Chloropicrin in the diet for 5 days. 30 mallard ducklings were assigned to the control groups. Birds were acclimated for 8 days. At test initiation, birds were 10d old and weighed 146-193 g.

Encapsulated Chloropicrin was prepared by mixing it with the basal ration. The resulting achieved test concentrations (corrected to 100 % active substance) were: 85, 83, 97, 86, 84, 98 and 84 %, of the nominal values, respectively. Homogeneity of the test substance in the diet was evaluated by collecting six samples from the 17.8 and 562 ppm test diets at preparation Day 0. Samples were collected from the feed troughs of the control group and each treatment group on Day 0 and 5 to assess the stability of the test substance under actual test conditions. Concentrations of Chloropicrin were determined by (GC) equipped with an electron capture detector (ECD).

Environmental conditions were: T=28.8 ± 1.4 °C (brooding pens), Room temperature: 23.6 ± 0.4°C; Humidity: 53 ± 9 %, Photoperiod: 16 hours of light, Air change: 15 room air volumes every hour

From test initiation until termination, all birds were observed at least twice daily for all signs of toxicity and abnormal behavior. Individual body weights were measured at the initiation of

the test (Day 0), at the end of the exposure period on Day 5 and at termination of the test on Day 8. Following the five-day exposure period all groups were given untreated basal diet for three days. Average feed consumption values were determined daily by pen for each treatment group and the control group during day 0-8.

Results:

No mortalities occurred in the test birds at any dose level. There were no overt signs of toxicity up to 316 ppm a.s. and all birds at these concentrations were normal in appearance and behaviour throughout the test. At the 562 ppm a.s. test concentration, signs of toxicity including lethargy and loss of coordination were first noted in all birds on the afternoon of Day 1 and continued to be displayed through to the morning of Day 7. All birds had recovered by the afternoon of Day 7 and were normal in appearance and behaviour for the remainder of the test.

When compared to the control group, there were no treatment-related effects on body weight or on feed consumption up to 316 ppm a.s. during the exposure period. There was a reduction in mean body weight gain in birds treated at the 562 ppm a.s. during the exposure period (Day 0 – 5) but this parameter was comparable to the control group during the post-exposure period (Days 6 – 8). There was a treatment related reduction in mean feed consumption at the 562 ppm a.s. from Day 0 to Day 1 but from Day 1 – 5 it was comparable to the control. During the post-exposure period, mean feed consumption at all test concentrations was comparable to or exceeded mean feed consumption by the control group.

The test is valid because no mortality occurred in the control and in the lowest concentration, the concentration of Chloropicrin was maintained between above 80% of the nominal values during the 5 days test period.

Conclusions:

Since no mortality occurred, the LC_{50} for mallard duck was determined to be greater than the highest dosage tested, i.e. $LC_{50} > 562$ ppm (243 mg a.s./kg bw/day). The NOEC for mortality was 562 ppm a.s. Based on a reduction in body weight gain and signs of toxicity in the 562 ppm treatment group, the NOEC was 316 ppm (140 mg a.s./kg bw/day).

Acceptability: The study is considered acceptable.

Hubbard, P. M., Martin, H. K., Beavers, B. J., 2009b. Chloropicrin: A Dietary LC_{50} Study With The Northern Bobwhite. [REDACTED] Unpublished report no. 650-105. Owner European Chloropicrin Group. (IIA 8.1.3/01).

GLP: Yes, except for periodic analyses of feed and water for potential contaminants,

Guidance: EPA guideline OPPTS Number 850.2200, U.S. EPA FIFRA Guidelines Subdivision E Section 71-2, OECD guideline number 205.

Deviation: during the test, the temperature and humidity were both slightly outside of the ranges recommended in OECD 205. These deviations are not considered to have affected the reliability of results.

Chloropicrin extract was administered to groups (2pens per group) of 10 juvenile northern bobwhite quail (*Colinus virginianus*) at concentrations of 0, 17.8, 31.6, 56.2, 100, 178, 316, 562, ppm a.s. in the diet for 5 days. 30 juvenile bobwhite chicks were assigned to the control group. Birds were acclimated to the caging and facilities for 10 days (from the day of hatch). At test initiation, birds were 10d old and weighed 19-26 g.

Encapsulated Chloropicrin was prepared by mixing it with the basal ration. The resulting achieved test concentrations (corrected to 100 % active substance) were: 85, 83, 97, 86, 84, 98 and 84 %, of the nominal values, respectively. Homogeneity of the test substance in the diet was evaluated by collecting six samples from the 17.8 and 562 ppm test diets at preparation Day 0. Samples were collected from the feed troughs of the control group and each treatment group on Day 0 and 5 to assess the stability of the test substance under actual test conditions. Concentrations of Chloropicrin were determined by (GC) equipped with an electron capture detector (ECD).

Environmental conditions were: T=38 ± 2°C 28.8 ± 1.4 °C (brooding pens), Humidity: 33 ± 13%, Photoperiod: 16 hours of light, Air change: 15 room air volumes /hour

From test initiation until termination, all birds were observed at least twice daily for all signs of toxicity and abnormal behavior. Individual body weights were measured at the initiation of the test (Day 0), at the end of the exposure period on Day 5 and at termination of the test on Day 8. Following the five-day exposure period all groups were given untreated basal diet for three days. Average feed consumption values were determined daily by pen for each treatment group and the control group during day 0-8.

LC50 was calculated by Probit analysis.

Results:

There were no mortalities in the control group and up to 178 ppm a.s. treatment. A single mortality occurred on Day 8 at the 100 ppm a.s. test concentration. The bird was observed to have injuries that were considered to have caused the mortality, but these were deemed to be incidental to treatment (the gross necropsy revealed some bruising at the base of the cranium). There was 10 % mortality at the 316 ppm a.s. test concentration and 70% mortality at the 562 ppm a.s. test concentration. These mortalities were considered to be treatment related.

Table B.9.1.1-5: Cumulative mortality

Nominal Concentration (ppm a.s.)	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
	Exposure Period						Post-Exposure Period		
Control: 0	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30	0/30
17.8	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
31.6	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
56.2	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
100	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	1/10
178	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
316	0/10	0/10	0/10	1/10	1/10	1/10	1/10	1/10	1/10
562	0/10	0/10	0/10	3/10	6/10	7/10	7/10	7/10	7/10

No overt signs of toxicity or abnormal appearance or behavior were noted among the treatment birds at the 17.8, 31.6, 56.2, 100 and 178 ppm a.s. test concentrations. On day 8 of the test, one bird in the 100 ppm a.s. test concentration was found dead. This bird had been normal in appearance and behaviour for all observations preceding its death.

There was no apparent effect upon body weight gain at the 17.8, 31.6, 56.2, 100 and 178 ppm a.s. test concentrations. However, there was a slight, but concentration responsive, reduction in body weight gain from Day 0 to Day 5 for the surviving birds in the 316 and 562 ppm a.s. treatment group when compared to the control group. There were no effects on feed

consumption up to 178 ppm a.s. during any feed consumption interval. However, there was a slight reduction in feed consumption at the 316 and 562 ppm a.s. test concentrations during Days 0, 1, and 2 of the exposure period that was considered to be related to treatment.

Table B.9.1.1-6: Mean daily dietary dose (mg a.i./Kg/d). Food consumption and body weight were averaged over day 0-5.

Test Concentration (ppm a.i.)	Mean Body Weight (g)	Mean Feed Consumption (g/bird/day)	Estimated Daily Dietary Dose* (mg a.i./kg/day)
0	29.7	6.0	6
17.8	29.0	6.6	4
31.6	27.0	6.7	8
56.2	28.4	6.3	
100	26.8	6.1	
78	28.5	6.9	43
316	27.6	5.8	67
562	27.5	6.0	122

* Calculated using unrounded data, calculations using data rounded to one decimal place may vary slightly.

$$\text{Daily Dietary Dose (mg a.i./kg body weight/day)} = \frac{\text{Test Concentration (mg a.i./kg)} \times \text{Daily Feed Consumption (g/bird/day)}}{\text{Body Weight (g/bird)}}$$

The test is valid because no mortality occurred in the control and in the lowest concentration, the concentration of Chloropicrin was maintained between above 80% of the nominal values during the 5 days test period.

Conclusions:

Under the conditions of this study, the LC₅₀ value for Chloropicrin on the Northern Bobwhite was determined to be 476 ppm a.s., with a 95 % confidence interval of 374 to 671 ppm a.s. Based on signs of toxicity and mortality at the 316 ppm a.s. treatment level, the 'No-observed effect concentration' was 178 ppm a.s. (43 mg a.i./Kg/d).

The RMS converted the LC₅₀=476 ppm in LC₅₀= 108 mg a.i./Kg/ (averaging data of mean food consumption and mean body weight from all the dose levels).

Acceptability- This study is considered acceptable.

Plant protection product

Acute oral toxicity (IIIA 10.1.1)

Not applicable. Since the active substance is identical to the plant protection product, separate studies on the plant protection product are not considered necessary.

B.9.1.2 Subchronic toxicity and reproduction (IIA 8.1.3)

Frey, L.T., Martin, K.H., Beavers, J.B. and Jaber, M. 2009. Chloropicrin: A Reproduction Study with the Northern Bobwhite. [REDACTED] Report no.: 650-104. Owner European Chloropicrin Group. (IIA 8.1.4/01)

GLP: Yes with the exceptions of periodic analyses of water and feed for potential contaminants that were performed using a certified laboratory and standard U.S. EPA analytical methods.

Guideline: draft OECD Test Guideline for the Testing of Chemicals. Proposal for a New Test Guideline. Avian Reproductive Toxicity Test in the Japanese Quail or Northern Bobwhite.

A study was performed to assess the reproductive toxicity of Chloropicrin during a relatively long (sub-chronic) exposure of the test substance administered orally to the northern bobwhite (*Colinus virginianus*).

Based on the effects observed in the pilot reproduction study, 100 ppm a.s. was selected as the highest test concentration for the definitive reproduction study. Northern bobwhite (80 males and 80 females) were randomly assigned into one control group and three treatment groups. Each treatment and control group contained 20 pairs of birds with one female and one male per pen. Only those pairs with proven ability to produce a fertile egg were used in the test. The birds were fed a nominal dose of either 10, 50, or 100 ppm a.s. of encapsulated Chloropicrin for a period of six weeks while the control group was fed the diet only. All offspring received the basal diet without the test substance but had an additional water-soluble vitamin and electrolyte mix in their water.

When available, eggs were collected daily based on indiscriminate selection, starting with the pre-treatment period and set weekly for incubation. From these eggs, a random selection was made and each week, these eggs were examined for egg shell thickness. The remaining eggs were candled prior to incubation to detect infertile eggs or embryo mortality. Following 21 days of incubation, the eggs were placed in a hatcher and allowed to hatch. On completion of hatching, the hatchlings were removed from the hatcher and the individual body weights of each hatchling were determined.

All birds were observed at least once daily and a record was maintained of all mortalities and clinical abnormalities. Prior to the initiation of the pre-treatment period, all eggs laid during the acclimation period were counted and selected eggs from each pen were incubated in order to identify successful breeding pairs of birds. Breeding pairs used in the test had produced at least one fertile egg during the acclimation period. In addition, all offspring were observed daily from hatching until day 14 and a record was maintained of all mortalities and clinical observations. Individual body weights were measured at the start of the pre-treatment period, at the start of the treatment period and at adult termination. Feed consumption for each pen was measured weekly throughout the test.

Diet samples were collected from the 10.0, 50.0 and 100 ppm a.s. test concentrations, and were analyzed by gas chromatography to evaluate the homogeneity of the test substance in the diet. Samples collected during the test to verify test substance concentrations for the 10.0, 50.0 and 100 ppm a.s. diets had means of 8.08 ppm a.s., 43.4 ppm a.s. and 103 ppm a.s. respectively.

Results:

Range-finding:

In the range-finding study, when birds were administered with a test concentration of 200 ppm a.s., a single mortality was observed. However, this mortality was not considered to be treatment related. When birds were administered a dose of 300 ppm a.s., four mortalities occurred. Of these four mortalities, three mortalities were considered to be treatment related.

Definitive Test:

In the definitive test, eight incidental mortalities occurred during the course of the treatment phase, four in the control group, three in the 50 ppm a.s. treatment group and one in the 100 ppm a.s. treatment group. None of the treatment phase mortalities that occurred were considered to be related to the treatment.

No overt signs of toxicity were observed at any of the concentrations tested. Incidental clinical observations noted during the test included those that are normally associated with injuries and penwear. There were four mortalities in the control, one male who was euthanized and three females, two of which were found dead and one of which was euthanized. The euthanized birds were found to be in a debilitating condition. At necropsy, these birds were all found to be thin or emaciated. Prior to death, these birds all suffered from lesions on the legs, feet and head. In addition to this, some birds also suffered feather loss. Internally, it was noted that there was a loss of muscle mass as well as distension of the intestines and ceca and damage to the spleen and kidneys.

Three mortalities (two male birds and one female bird) were observed in the 50 ppm a.s. treatment group. One of the male birds was euthanized on Day 7 of Week 5 due to a debilitated condition. The two other mortalities were both found dead, with the male found dead on Day 5 of Week 7 and the female found dead on Day 2 of Week 8. At necropsy, the male bird that was euthanized was found to be emaciated with a body weight of 114g, as was the female bird found dead which had a body weight of 114g. However, the male that was found dead was not found to have lost a significant amount of weight, with a body weight of 164 g. External signs of toxicity include lesions on the legs and feet, dried blood on abdominal feathers and a pasty vent. In addition, internal signs of toxicity included a loss of muscle mass, no fat in the coronary groove, distension of the intestines and ceca and discoloration of the kidneys, spleen and cecal contents. The ovary of the female bird was regressed while in one of the male birds, the testes were small, approximately 1.2 cm in length.

The only mortality in the 100 ppm a.s. treatment group was a female bird that was found dead on Day 1 of Week 8. At necropsy, the bird was reported to be emaciated, with a body weight of 102 g. Prior to death, it was noted the bird had lesions on its feet, lameness, laboured breathing and ruffled appearance. External signs of toxicity included lesions on the left foot, a pasty vent and feather loss on the neck. Internal signs of toxicity included a loss of muscle mass with the keel prominent, no fat in the coronary groove, old lesions of egg yolk peritonitis in the abdominal cavity, distension of the large intestines and discoloration of the kidneys. The liver was friable with a fluid-filled cyst on the ventral surface of the right lobe and the ovary was regressed. On examination of the birds at necropsy, the nature of the lesions observed at necropsy indicated that none of the treatment phase mortalities that occurred were considered to be treatment related.

There were no apparent treatment-related effects upon feed consumption at the 10, 50 and 100 ppm a.s. test concentrations. A slight decrease in food consumption was observed in the 10 and 50 ppm a.s. treatment groups during Week 2 of the pre-treatment phase that were significantly different from the control value at $p < 0.05$. There were no statistically significant differences between the control group and the 10 or 50 ppm a.s. treatment groups at any of the

treatment phase feed consumption intervals. In the 100 ppm a.s. treatment group, during weeks 3 and 4, there were slight decreases (<10%) in feed consumption that were significantly different from the control values at $p < 0.05$ and $p < 0.01$, respectively.

There were no treatment related effects on reproductive performance at any of the concentrations tested. When compared to the control group, there were no statistically significant differences in any of the reproductive parameters measured during the treatment phase in the 10, 50 or 100 ppm a.s. treatment groups. In addition, there were no treatment-related effects on egg shell thickness at the 10, 50 or 100 ppm a.s. test concentrations. There were no statistically significant differences between the control group and the 10 or 100 ppm a.s. treatment groups; however, there is a statistically significant decrease in shell thickness in the 50 ppm a.s. test group compared to the control value at $p < 0.05$. However, as the difference is very small (<10%) and it is not concentration responsive, the difference was not considered to be treatment related.

Body weights of birds were measured individually at the initiation of the pre-treatment period, at the start of the treatment period and at adult termination. There were no apparent treatment-related effects upon adult body weight at any of the concentrations tested and no statistically significant differences between the control group and the three treatment groups at any of the body weight intervals.

Conclusions:

There were no treatment-related mortalities, overt signs of toxicity or treatment-related effects upon body weight or feed consumption at any of the concentrations tested. Additionally, there were no treatment-related effects upon any of the reproductive parameters measured at 10, 50 or 100 ppm a.s. test concentrations. Based on the results of this study, the no-observed-effect concentration for Northern bobwhite exposed to chloropicrin in the diet was 100 ppm a.s., the highest concentration tested. The results of the range-finder test at the high dose concentration support the methodology and validity of the 100 ppm a.s. end-point.

This study is considered to be acceptable

B.9.1.3 Supervised cage or field trials (IIIA 10.1.2)

Please refer to comments made in the DAR 2005.

B.9.1.4 Acceptance of bait, granules or treated seeds by birds (IIIA 10.1.3)

Please refer to comments made in the DAR 2005.

B.9.1.5 Effects of secondary poisoning (IIIA 10.1.4)

Please refer to comments made in the DAR 2005.

B.9.1.6 Risk assessment

A summary of the acute toxicity of Chloropicrin to birds is provided in Table B.9.1.6-1

Table B.9.1.6-1- Acute, short-term and long term avian toxicity data for Chloropicrin

Endpoint	Species	Result	End point	Reference
Acute oral LD₅₀	Bobwhite quail/ Mallard	62 mg/kg	LD ₅₀	Hubbard, P.M. and Beavers, J.B. 2009
Short term (dietary)	Bobwhite quail/ Mallard	108 mg/kg bw/d (476 ppm)*	LC ₅₀	Hubbard, P.M. <i>et al</i> , J.B. 2009
Reproductive Toxicity	Bobwhite quail	24.2 mg/kg bw/d (100 ppm)	Reproductive NOEC	Frey, L.T. <i>et.al.</i> 2009

* calculated by the RMS by conversion from CC50 expressed as ppm (The LC50 from the dietary study with bobwhite quail calculated by the Notifier was slightly higher: 115 mg/kg b.w./day).

In the original DAR 2005 the RMS pointed that for birds the dietary route of exposure could not be negligible. Since the treated field remains covered by tarp till 8 day after application of Chloropicrin, terrestrial vertebrates will not gain access to the treated area during this lapse of time. Therefore birds will not be exposed to in-field food items contaminated at initial concentration, but concern remains for off-field and in-field (after tarp removal) exposure of birds to contaminated food that might still contain significant residues. Therefore data on residues on food items and a risk assessment for mammals was considered necessary.

In the updated Dossier presented in April 2006, the Notifier submitted a literature study on plant metabolism (Lawrence, 1990). This study showed that the radiocarbon present in plants grown in soil treated with [¹⁴C]Chloropicrin consisted of natural plant biochemical components which had incorporated the radiolabelled carbon atom. Furthermore, the major concentrations of radiocarbon in each plant tissue were generally associated with the natural plant products present in the tissue. This would indicate that birds and mammals feeding on vegetation grown in areas treated with Chloropicrin not likely to be exposed to significant levels of residues in plants tissue. The results of this study confirm that Chloropicrin is not a systemic substance.

Within the dossier submitted for the present resubmission, the Notifier included a study on residues on several food items (Bartolomè, 2009, see Other/Special Studies, IIA 8.16.1) and presented a Tier 1 and Tier 2 risk assessment for birds.

Tier 1

A Tier 1 assessment was made with reference to the 'leafy crop' scenario which the Notifier states to cover the application of Chloropicrin to Tomatoes, considering a small insectivorous bird (10 g) and medium-sized herbivorous bird (300 g). For both indicator birds, the TER acute, short-term, long term were well below the triggers (see Table B.9.1.6-2), indicating high risk.

Table B.9.1.6-2: Tier 1: Acute, Short-Term and Long-Term Risk Assessment for small insectivorous birds (yellow wagtail) in tomatoes

	FIR/ bw	Application rate (kg/ha)	RUD	ETE	Endpoint (mg/kg)	TER	Trigger
Acute	1.04	448	52	24,228	62	0.0026	10
Short-term	1.04	448	29	13,512	115.2	0.0085	10
Long-term	1.04	448	29	13,512	24.2	0.0018	5

Figures highlighted in **bold** are below the trigger values set by Annex VI of Directive 91/414 EEC.

Table B.9.1.6-3: Tier 1: Acute, Short-Term and Long-Term Risk Assessment for medium sized herbivorous birds in tomatoes.

	FIR/ bw	Application rate (kg/ha)	RUD	ETE	Endpoint (mg/kg)	TER	Trigger
Acute	0.76	448	87	29,622	62	0.0021	10
Short-term	0.76	448	40	13,619	115.2*	0.0085	10
Long-term	0.76	448	40	13,619	24.2	0.0004	5

The LC50 from the dietary study with bobwhite quail used by the Notifier is slightly higher than that calculated by the RMS (108 mg/kg b.w./day) .

Comments of the RMS: Chloropicrin is proposed to be applied to bare soil, therefore the reference to 'leafy crop' is not appropriate. A tomatoes crop will not be established when Chloropicrin is applied.

Tier 2- Refined Risk Assessment for Birds

For the sake of clarity and to keep the present Addendum as concise as possible, the original Tier 2 risk assessment submitted by the Notifier is included in Annex I. Briefly, the risk assessment was carried out for a relevant small insectivorous pan-European bird (Yellow wagtail, *Motacilla flava*, weighing 17.6 g), a small vermivorous bird (Passeriformes) and a medium sized herbivorous focal species (Woodpigeon, *Columba palumbus*, weighing 490g), feeding on non-grass herbs and on seeds. Reference was made to SANCO Guidance Document on Risk Assessments for Birds and Mammals (Anonymous, 2002) and to "Revision of Guidance Document under Council Directive 91/414/EEC, SANCO/4145/2000 – final of 25 September 2002 (Scientific Opinion of the Panel on Plant protection products and their Residues (PPR) on the Science behind the Guidance Document on Risk Assessment for birds and mammals, June 2008"

The measured residues of Chloropicrin in insects, plant matter and earthworms and its decline in the various food items were retrieved from the study by Bartolomé (2009) and were used to establish the initial concentration of Chloropicrin in the diet and to refine the ftwa (see

tables below). As chloropicrin volatilizes rapidly the Notifier considered justified to apply also 1-day and 5-day TWA values for the acute and short-term risk assessment, respectively.

Table B.9.1.6-4: Residue decline in arthropods from Bartolome (2009)

Species	Type of test	DT ₅₀ (mean) (days)
<i>Carabid Beetle</i>	Field	0.6
<i>Crickets</i>	Field	2.2
<i>Mealworms</i>	Field	2.2
<i>Blowfly pupae</i>	Field	1.4
Overall mean (arthropods) = 1.667		
<i>Earthworms</i>	Field	0.7

Table B.9.1.6-5 Residues measured decline in food items (Bartolome,2009)

Species	Chloropicrin residues (mg/kg) detected at day		
Day	7	8 *	14
<i>Earthworms</i>	3.67	2.67*	0.4

Species	Chloropicrin residues (mg/kg) detected at day		
Day	7	8 *	14
<i>Blow Fly pupae</i>	204.67	131.0	9
<i>Carabid beetles</i>	357.67	213.5	9.67
<i>Meal worms</i>	598.33	396.1	33.33
<i>Crickets</i>	52.5	42.51	12.0
Overall Insects	303.3	195.8*	16.0

Species	Chloropicrin residues (mg/kg) detected at day		
Day	7	8 *	14
<i>Pea seeds</i>	1.3	1.257	1.03
<i>Barley seeds</i>	22.5	18.27	5.23
Overall Seeds	11.9	9.76*	3.13

* interpolated, assuming first-order degradation

Further refinements were also made for:

- proportion of food type in the diet (PD).
- proportion of diet obtained from the treated area (PT). It was considered that
- avoidance (AV).

The results of the risk assessment proposed by the Notifier are reported in tables Table B9.1.6-6- Table B9.1.6-9. The comments of the RMS are provided at the end of the chapter.

Table B.9.1.6-6: Tier 2 TER Calculations for Chloropicrin: Acute, Short-Term and Long-Term Risk Assessment for small insectivorous birds (yellow wagtail) in tomatoes

	FIR/bw	Concentration in diet (mg/kg)	PD	PT	AV	f _{twa}	ETE	Endpoint (mg/kg b.w./day)	TER	Trigger
Acute	0.875	195.8	0.25	0.33	0.5	0.8182 [^]	5.78	62	10.73	10
Short-term	0.875	195.8	0.25	0.33	0.5	0.4208 [^]	2.97	115.2**	38.79	10
Long-term	0.875	195.8	0.25	0.33	-	0.1145	1.62	24.2	14.94	5

[^] As chloropicrin volatilizes rapidly the Notifier considered justified to apply also 1-day and 5-day TWA values for the acute and short-term risk assessment, respectively (see comment of the RMS, below).

** The LC50 from the dietary study with bobwhite quail recalculated by the RMS is slightly lower (108 mg/kg b.w./day) .

Table B.9.1.6-7: Tier 2 TER Calculations for Chloropicrin: Acute, Short-Term and Long-Term Risk Assessment for Small vermivorous Birds (Passeriformes) in Tomatoes.

	FIR/bw	Concentration in diet (mg/kg)	PD	PT	AV	f _{twa}	ETE	Endpoint (mg/kg b.w./day)	TER	Trigger
Acute	1.90	2.67	0.25	0.33	0.5	0.6347 [^]	0.133	62	466	10
Short-term	1.90	2.67	0.25	0.33	0.5	0.2477 [^]	0.052	115.2**	2215	10
Long-term	1.90	2.67	0.25	0.33	-	0.048	0.020	24.2	1210	5

[^] As chloropicrin volatilizes rapidly the Notifier considered justified to apply also 1-day and 5-day TWA values for the acute and short-term risk assessment, respectively (see comment of the RMS, below).

** The LC50 from the dietary study with bobwhite quail recalculated by the RMS is slightly lower (108 mg/kg b.w./day) .

Table B.9.1.6-8: Tier 2 TER Calculations for Chloropicrin: Acute, Short-Term and Long-Term Risk Assessment for medium herbivorous birds (Woodpigeon feeding on non-grass herbs) in Tomatoes

	FIR/bw	Concentration in diet (mg/kg)	PD	PT	AV	f _{twa}	ETE	Endpoint (mg/kg b.w./day)	TER	Trigger
Acute	0.69	9.76	0.325	0.33	0.5	0.5929	0.214 [^]	62	289.7	10
Short-term	0.69	9.76	0.325	0.33	0.5	0.2143	0.077 [^]	115.2**	1496	10
Long-term	0.69	9.76	0.325	0.33	-	0.041	0.030	24.2	807	5

[^] As chloropicrin volatilizes rapidly the Notifier considered justified to apply also 1-day and 5-day TWA values for the acute and short-term risk assessment, respectively (see comment of the RMS, below)

** The LC50 from the dietary study with bobwhite quail recalculated by the RMS is slightly lower (108 mg/kg b.w./day) .

Table B.9.1.6-9: Tier 2 TER Calculations for Chloropicrin: Acute, Short-Term and Long-Term Risk Assessment for medium herbivorous birds (Woodpigeon feeding on seeds) in Tomatoes

	FIR/bw	Concentration in diet (mg/kg)	PD	PT	AV	f _{twa}	ETE	Endpoint (mg/kg b.w./day)	TER	Trigger
Acute	0.076	9.76	0.51	0.33	0.5	0.5929-	0.037^	62	1676	10
Short-term	0.076	9.76	0.51	0.33	0.5	0.2143	0.013^	115.2**	8861	10
Long-term	0.076	9.76	0.51	0.33	-	0.041	0.005	24.2	4840	5

^ As chloropicrin volatilizes rapidly the Notifier considered justified to apply also 1-day and 5-day TWA values for the acute and short-term risk assessment, respectively (see comment of the RMS, below).

** The LC50 from the dietary study with bobwhite quail recalculated by the RMS is slightly lower (108 mg/kg b.w./day).

Comments :

General:

The Notifier states that the “Revision of Guidance Document under Council Directive 91/414/EEC, SANCO/4145/2000 – final of 25 September 2002 (Scientific Opinion of the Panel on Plant protection products and their Residues (PPR) on the Science behind the Guidance Document on Risk Assessment for birds and mammals, June 2008” has been consulted in order to implement its recommendations in the use of Tier 1 endpoints and in the choice of Tier 2 refinements, where required. Nevertheless, the risk assessment has been conducted in relation to “leafy crops” (tomatoes), using pigeon as representative species of a medium herbivorous birds. It should be noted that for chemicals applied on bare soil like Chloropicrin, the above cited guideline recommends the following generic focal species: granivorous bird (finch), omnivorous bird (lark) and insectivorous birds (wagtail).

As chloropicrin volatilizes rapidly the Notifier considered justified to apply also 1-day and 5-day TWA values for the acute and short-term risk assessment, respectively. The RMS does not consider this approach acceptable for the acute exposure (mean insect DT50=1.7) while for the short-term exposure it can be reasonable, considering that after tarp removal the degradation of chloropicrin will be faster than that calculated under the tarp.

Small insectivorous birds (yellow wagtail)

Based on the risk assessment presented by the Notifier and reported in Table B9.1.6-6, the TER values for acute is very close to the trigger of 10, while the TER for short-term and long-term are above the triggers. The RMS stresses that in the ETE calculation, all the input parameters have been refined and therefore the robustness of the assumptions should be clearly demonstrated. Some comments are made hereafter.

Twa - the use of the twa factor for the calculation of the acute ETE is not considered appropriate (note that the mean insect DT50=1.7). The removal of this factor would be sufficient to lower the acute TER below the trigger (TER=8.8).

PD - It is agreed with the Notifier that, considering that Chloropicrin is applied pre-planting by direct soil incorporation in fields with no vegetation, only be the proportion of ground dwelling insects in the wagtails diet is relevant. Nevertheless no scientific support is given for the setting of relative proportions of ground-dwelling, leaf-dwelling and airborne invertebrates to 0.25 and 0.4 and 0.35 respectively. The RMS comments that the proportion of arthropods type will depend of the landscape (presence of crops, bare soil, grassland) and

notes that would be enough to consider a PD =0.3 for calculating a TER<10 (keeping all the other refinements).

PT – The Notifier reports that wagtails usually search for prey on sites with short vegetation (grassland and marginal areas) and some species are particularly known also to feed on bare soil. Their diet varies more over time than between different species of the genus and consists mainly of arthropods. As such the proportion of foraging time spent by a wagtail in a Chloropicrin treated field is set to 33% to give a PT value of **0.33**. Contamination of marginal areas and adjacent grasslands has been shown to be negligible and so only time spent in the treated field is considered relevant.

The RMS agrees that in the late spring or summer (when Chloropicrin is typically applied) it would be unlikely that yellow wagtails (*Motacilla flava*) would spend all their time foraging in a field treated with Chloropicrin, because of limited extension of treated area, the absence of vegetation and therefore the limited insect life. Nevertheless quantitative field data to support the proposed PT are not provided.

Small vermivorous bird (Passeriformes)

Based on the risk assessment presented by the Notifier and reported in Table B9.1.6-7, the TER values for acute, short-term and long-term risk are well above the triggers. The RMS recalculated the TER without the refinement for PD, PT and AV (keeping them=1, as worst case) and excluding the use of fwa for the acute risk assessment. This exercise provided TER_{acute}= 12, TER short-term= 86, TER long-term= 100. It is concluded that a low risk is predicted for bird feeding on worms containing Chloropicrin residues.

*Medium sized herbivorous focal species (Woodpigeon, *Columba palumbus*, weighing 490g), feeding on non-grass herbs and on seeds.*

For the non-grass herbs, the Notifier used the same residue as in seeds. From the field residue study (Bartolomè, 2009), the residues on foliage were lower than those measured on seed. Based on the risk assessment presented by the Notifier and reported in Table B9.1.6-8, the TER values for acute, short-term and long-term risk are well above the triggers. Even without the refinement for PD, PT, AV and excluding the use of fwa for the acute risk assessment), the TER would still indicate an acceptable for bird feeding on non-grass herbs and seeds containing Chloropicrin residues.

Conclusion

The acute, short-term and long-term risk assessment for medium herbivorous and small vermivorous birds is acceptable. Anyhow, since Chloropicrin is applied to bare soil, a small granivorous bird (finch) is considered more appropriate than a pigeon (feeding on seeds) as representative of granivorous birds. A risk assessment for this bird should be used for the purpose of the present risk assessment.

For insectivorous birds, some of the refinements proposed are not sufficiently supported/documented and therefore it is concluded that an acceptable risk has not been demonstrated.

Risk assessment from inhalation exposures

In order to determine the risk of off-site inhalation of Chloropicrin to birds a 5 day inhalation toxicity study was carried out. This study was designed to establish the risk of Chloropicrin vapors to birds in areas adjacent to treated fields where the Chloropicrin vapor is unavoidable. This would be relevant to birds that are not capable of moving away from treated zones such as incubating mothers or nestlings. The results of this study are summarized below.

Table B9.1.6-10: Summary of Effects of Inhaled Chloropicrin to Birds

Test species	Test system	Duration of exposure	Results	Reference
Northern Bobwhite Quail	Inhalation exposure	5 days	LC50 >659 ppb NOAEL 96 ppb 0.647 mg/m ³	Weinberg, J. (2009)

The relevant exposure level to compare to this endpoint comes from the field emission study presented in IIIA 7.3.3/01. This study determined the field exposure levels at various distances from the treated sites (1, 20, 50m). From data measured at 1 and 20m (see tables below) it can be noted that the weighed mean concentration at 20 m are very similar to the ones measured at 1 m distance and on some occasion even higher. Therefore it can be considered adequate to use data relative to 20 m distance for the purpose of this risk assessment.

Table B.9.1.6-11 - chloropicrin concentrations at the poles 1 m away from the edge of the treated plot.

POLES 1 m far away the edge of the treated plot										
Day	Sample n°	North		East		South		West		Weighted mean
		Conc. in air mg/m ³	Sampling time minutes	Conc. in air mg/m ³	Sampling time minutes	Conc. in air mg/m ³	Sampling time minutes	Conc. in air mg/m ³	Sampling time minutes	
1	Sample 1	0.0236	440	0.0000	416	0.0031	392	0.0110	539	0.0098
	Sample 2	0.0857	498	0.0116	545	0.0450	454	0.0946	554	0.0594
	Sample 3	0.0354	509	0.0191	503	0.0000	501	0.0095	503	0.0161
	D W Mean	0.0491	1447	0.0109	1464	0.0161	1347	0.0395	1596	0.0293
2	Sample 4	0.0135	466	0.0150	470	0.0000	468	0.0060	470	0.0086
	Sample 5	0.0343	579	0.0425	579	0.0207	582	0.0102	579	0.0269
	Sample 6	0.0012	490	0.0071	489	0.0095	491	0.0000	490	0.0045
	D W Mean	0.0174	1535	0.0228	1538	0.0108	1541	0.0057	1539	0.0142
3	Sample 7	0.0134	780	0.0042	780	0.0082	783	0.0032	788	0.0072
	Sample 8	0.0008	633	0.0009	637	0.0043	633	0.0006	635	0.0017
	D W Mean	0.0078	1413	0.0027	1417	0.0065	1416	0.0021	1423	0.0047
	Sample 9	0.3077	797	0.0603	735	0.0289	801	0.0305	799	1.076
4	Sample 10	0.0008	647	0.0017	643	0.0010	649	0.0041	635	0.0019
	D W Mean	0.1702	1444	0.0329	1378	0.0164	1450	0.0188	1434	0.0599
5	Sample 11	0.0069	789	0.0017	782	0.0006	786	0.0010	799	0.0025
	Sample 12	0.0008	649	0.0000	658	0.0000	653	0.0000	646	0.0000
	D W Mean	0.0058	1438	0.0009	1440	0.0003	1439	0.0006	1445	0.0014
	Sample 13	0.0331	781	0.0314	775	0.0138	824	0.0199	785	0.0244
6	Sample 14	0.0000	665	0.0090	585	0.0019	667	0.0259	650	0.0091
	D W Mean	0.0179	1446	0.0218	1360	0.0085	1491	0.0226	1435	0.0175
7	Sample 15	0.0175	767	0.0016	766	0.0050	770	0.0101	764	0.0086
	Sample 16	0.0040	666	0.0000	669	0.0180	669	0.0983	663	0.0299
	D W Mean	0.0112	1433	0.0009	1435	0.0110	1439	0.0511	1427	0.0185
	Sample 17	0.0056	759	0.0658	754	0.0348	763	0.0570	725	0.0406
8	Sample 18	0.0008	633	0.0000	615	0.0014	631	0.0064	632	0.0022
	D W Mean	0.0034	1392	0.0363	1369	0.0197	1394	0.0334	1387	0.0231
9	Sample 19	0.0119	817	0.0016	817	0.0032	822	0.0008	819	0.0044
	Sample 20	0.0007	663	0.0010	443	0.0000	663	0.0006	643	0.0005
	D W Mean	0.0069	1480	0.0014	1260	0.0018	1485	0.0007	1462	0.0027
10	Sample 21	0.0190	753	0.0173	756	0.0000	750	0.0008	750	0.0093
	Sample 22	0.0000	667	0.0000	663	0.0000	673	0.0000	673	0.0000
	D W Mean	0.0101	1420	0.0092	1419	0.0000	1423	0.0004	1423	0.0049
	Sample 23	0.0130	772	0.0076	775	0.0080	769	0.0158	770	0.0111
11	Sample 24	0.0000	653	0.0000	659	0.0000	662	0.0000	652	0.0000
	D W Mean	0.0071	1425	0.0041	1434	0.0043	1431	0.0086	1422	0.0060
12	Sample 25	0.0000	826	0.0000	828	0.0000	828	0.0000	829	0.0000
	Sample 26	0.0000	591	0.0000	590	0.0000	593	0.0000	592	0.0000
	D W Mean	0.0000	1417	0.0000	1418	0.0000	1421	0.0000	1421	0.0000
	Sample 27	0.0000	834	0.0000	836	0.0000	838	0.0000	834	0.0000
13	Sample 28	0.0000	645	0.0000	645	0.0000	638	0.0000	649	0.0000
	D W Mean	0.0000	1479	0.0000	1481	0.0000	1476	0.0000	1483	0.0000
14	Sample 29	0.0000	820	0.0000	819	0.0000	829	0.0000	818	0.0000
	Sample 30	0.0000	598	0.0000	598	0.0000	597	0.0000	596	0.0000
	D W Mean	0.0000	1418	0.0000	1417	0.0000	1426	0.0000	1414	0.0000

Table B.9.1.6-12 - chloropicrin concentrations at the poles 20m away from the edge of the treated plot.

Day	Sample n°	POLES 20 m away from the edge of the treated plot										Weighted Mean
		North		East		South		West		Mean		
		Conc. in air mg/m3	Sampling time minutes	Conc. in air mg/m3	Sampling time minutes	Conc. in air mg/m3	Sampling time minutes	Conc. in air mg/m3	Sampling time minutes			
1	Sample 1	0.0250	435	0.0031	421	0.0016	390	0.0100	535		0.0102	
	Sample 2	0.0763	534	0.0092	534	0.0748	535	0.1005	506		0.0657	
	Sample 3	0.1613	500	0.0066	513	0.0000	502	0.0014	513		0.0418	
	D W Mean	0.0900	1469	0.0066	1468	0.0285	1427	0.0390	1614		0.0411	
2	Sample 4	0.0822	456	0.0024	559	0.0000	451	0.0000	458		0.0202	
	Sample 5	0.0190	586	0.0378	585	0.0104	586	0.0058	588		0.0182	
	Sample 6	0.0000	482	0.0020	489	0.0000	483	0.0000	482		0.0005	
	D W Mean	0.0319	1524	0.0149	1633	0.0040	1520	0.0022	1528		0.0133	
3	Sample 7	0.0504	782	0.0063	790	0.0069	783	0.0080	798		0.0178	
	Sample 8	0.0007	637	0.0000	633	0.0006	635	0.0000	636		0.0005	
	D W Mean	0.0281	1419	0.0035	1423	0.0041	1418	0.0047	1434		0.0101	
	4	Sample 9	0.2960	795	0.0559	804	0.0066	804	0.0000	802		0.0891
Sample 10		0.0020	651	0.0014	295	0.0000	647	0.0007	648		0.0010	
D W Mean		0.1636	1446	0.0413	1099	0.0036	1451	0.0003	1450		0.0528	
5		Sample 11	0.0064	793	0.0016	750	0.0005	789	0.0014	614		0.0026
	Sample 12	0.0000	651	0.0000	485	0.0000	655	0.0000	651		0.0000	
	D W Mean	0.0035	1444	0.0010	1235	0.0003	1444	0.0007	1265		0.0014	
	6	Sample 13	0.0362	765	0.0083	796	0.0106	791	0.0115	798		0.0165
Sample 14		0.0006	668	0.0008	562	0.0015	668	0.0000	667		0.0007	
D W Mean		0.0196	1433	0.0052	1358	0.0064	1459	0.0063	1465		0.0094	
7		Sample 15	0.0195	769	0.0043	769	0.0058	774	0.0038	766		0.0084
	Sample 16	0.0008	669	0.0033	580	0.0093	666	0.0379	666		0.0131	
	D W Mean	0.0108	1438	0.0039	1349	0.0074	1437	0.0197	1432		0.0105	
	8	Sample 17	0.2175	761	0.0671	756	0.0446	763	0.0249	762		0.0885
Sample 18		0.0000	610	0.0010	615	0.0008	630	0.0026	632		0.0011	
D W Mean		0.1207	1371	0.0374	1371	0.0248	1393	0.0148	1394		0.0492	
9		Sample 19	0.0118	819	0.0010	828	0.0025	823	0.0005	818		0.0039
	Sample 20	0.0000	665	0.0000	582	0.0000	663	0.0000	663		0.0000	
	D W Mean	0.0065	1484	0.0006	1410	0.0014	1486	0.0003	1481		0.0022	
	10	Sample 21	0.0162	757	0.0031	760	0.0000	754	0.0043	751		0.0059
Sample 22		0.0000	667	0.0000	662	0.0000	672	0.0000	673		0.0000	
D W Mean		0.0086	1424	0.0017	1422	0.0000	1426	0.0023	1424		0.0031	
11		Sample 23	0.0089	770	0.0036	778	0.0059	771	0.0099	772		0.0071
	Sample 24	0.0000	654	0.0000	652	0.0000	662	0.0000	659		0.0000	
	D W Mean	0.0048	1424	0.0020	1430	0.0032	1433	0.0053	1431		0.0038	
	12	Sample 25	0.0000	827	0.0000	827	0.0000	827	0.0000	827		0.0000
Sample 26		0.0000	593	0.0000	593	0.0000	593	0.0000	593		0.0000	
D W Mean		0.0000	1420	0.0000	1419	0.0000	1420	0.0000	1420		0.0000	
13		Sample 27	0.0000	836	0.0000	836	0.0000	834	0.0000	836		0.0000
	Sample 28	0.0000	648	0.0000	645	0.0000	638	0.0000	648		0.0000	
	D W Mean	0.0000	1484	0.0000	1481	0.0000	1472	0.0000	1484		0.0000	
	14	Sample 29	0.0000	821	0.0000	821	0.0000	829	0.0000	820		0.0000
Sample 30		0.0000	599	0.0000	598	0.0000	536	0.0000	597		0.0000	
D W Mean		0.0000	1420	0.0000	1419	0.0000	1365	0.0000	1417		0.0000	

There is no relevant trigger value set by Annex VI of Directive 91/414 EEC for this type of exposure scenario, however a very conservative approach would be to compare the inhalation NOAEL to the relevant exposure levels and apply a trigger value of 10 to the calculated TER.

In the first tier the maximum mean concentration measured for each of the 4 wind directions is considered. This is 0.1636 mg/m³, measured at North on day 4. The corresponding TER is 4, which is below the trigger and indicates an unacceptable risk.

The Notifier proposed to use the average airborne concentration in air of 0.0237 mg/m³ equivalent to 3.5 ppb, calculated from the weighed mean for the 4 wind directions over the 5 day period (days 1 to 5) after application. The RMS does not agree with this proposal and consider more adequate to average the mean daily concentrations measured at North over days 1-5, to take into account the worst case wind direction exposure. The calculated concentration is 0.0634 mg/m³. Using this figure, the TER would be just above the trigger of 10, indicating acceptable risk. It is pointed out that in this assessment the NOAEC of 96 ppb has been used instead of the LC50 >659 ppb, and therefore the assessment addresses also the sublethal effects.

Table B.9.1.6-13 : Toxicity Exposure Ratio for Inhalation Exposure to Birds

Test species	Exposure Levels	Duration of exposure	End Point	TER	Trigger
Northern Bobwhite Quail	0.1636 mg/m ³ (mean day 4, north)	5 days	5 day NOAEL 96 ppb 0.647 mg/m ³	4	10
	0.0634 mg/m ³ (average over day 1-5, north)			10.2	

Conclusion: The calculated TER in the above table indicates that there are no adverse effects expected due to Chloropicrin exposure to birds in areas adjacent to treated fields.

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

Please refer to comments made in the DAR 2005.

B.9.2.1 Acute toxicity

The acute toxicity of Chloropicrin has been established in studies on Rainbow trout (*Oncorhynchus mykiss*), Bluegill Sunfish (*Lepomis macrochirus*), *Daphnia magna* and green alga (*Selenastrum capricornutum*), which have been summarized in the original DAR 2005. Since chloropicrin has an herbicidal action (such as effects on seed germination, see section B3), the need of a test with a second species of algae and with macrophyte has been pointed out by EFSA and R SM (Reporting table, november 20069). In response to these requests, a test with these organisms have been submitted by the Notifier in the resubmission dossier and are summarized in the relevant chapter below (IIA 8.4/02 and IIA 8.6/01)

Active substance (Chloropicrin)

Acute toxicity to fish (IIA 8.2.1)

Please refer to DAR 2005.

Acute toxicity to aquatic invertebrates (IIA 8.2.4)

Please refer to DAR 2005.

Effects on algal growth (IIA 8.2.6)

For the study on *Selenastrum capricornutum* (IIA 8.4/01, Flatman, 2004), please refer to DAR 2005.

An additional test was conducted to assess the inhibitory effect of Chloropicrin on the growth of the freshwater diatom *Navicula pelliculosa*, summarized hereunder.

Wilby, J.J., 2009a, Chloropicrin Algal Growth Inhibition Assay. Huntingdon Life Science Ltd., UK. Unpublished report no.: SXC0022. Owner European Chloropicrin Group. (IIA 8.4/02)

GLP: Yes.

Guideline: OECD 201 and EC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 1992) Part C, Method 3 "Algal Inhibition Test"

Method:

The study aimed at assessing the inhibitory effect of Chloropicrin on the growth of the freshwater diatom *Navicula pelliculosa*, Strain No. SAG 1050-3. The study was conducted under sealed conditions due to the volatility of the test substance (glass Wheaton vials ca. 35 ml each completely filled).

Algal cultures of 1×10^4 /mL were exposed to Chloropicrin for 120 hours at nominal test concentrations of 9.39, 20.7, 45.5, 100, 220 µg/l. Test media were prepared in OECD medium by the direct addition of the test substance to the dilution medium. Thirty flasks were established for the control group and fifteen flasks for each test group, plus an additional flask at nominal concentrations of 9.39 and 220 µg/l which contained test medium but no algal cells to obtain information on absorption/adsorption of chloropicrin by algal cells. The cultures were incubated under continuous illumination at temperatures in the range of 22.2 to 23.4°C.

Cell numbers were counted daily using a haemocytometer. The estimate of cell numbers in each sample was based on the mean of eight consecutive counts depending on the cell density of the cultures. At the end of the test, aliquots were taken from each test flask at the three highest concentrations (45.5, 100 and 220 µg/l) where growth had been severely inhibited, and from each control flask and exposed in fresh culture medium for up to 10 days to check if the inhibition was algistatic or algicidal. Chloropicrin concentrations were analyzed by GC-ECD at the start and end of test.

Results:

The measured levels of chloropicrin in samples of freshly prepared test cultures ranged between 47 and 79% of their nominal values. After 120h, the measured levels had decreased, ranging from the limit of detection of the analytical method (0.125 µg/l) to 25% of nominal.

Test results are expressed in terms of the area under the growth curve and growth rate using the geometric mean of measured concentrations (see Table below).

Table B.9.2.-1: Cell densities

Exposure concentrations (µg chloropicrin/L)		Replicate number	Cell densities (cells/mL)				
Nominal	Mean Measured		24 hours	48 hours	72 hours	96 hours	120 hours
Control	nd	R ₁	16250	78750	245000	493750	761250
		R ₂	8750	83750	245000	525000	728750
		R ₃	15000	82500	261250	551250	763750
		R ₄	15000	83750	277500	542500	755000
		R ₅	13750	86250	242500	517500	740000
		R ₆	13750	78750	256250	505000	741250
		Mean	13750	82292	254583	522500	742917
9.39	0.528	R ₁	12500	83750	250000	527500	741250
		R ₂	13750	78750	260000	488750	765000
		R ₃	18750	86250	237500	581250	763750
		Mean	15000	82917	249167	532500	756667
20.7	0.840	R ₁	16250	83750	175000	431250	622500
		R ₂	15000	81250	192500	456250	595000
		R ₃	17500	78750	182500	487500	642500
		Mean	16250	81250	183333	458333	620000
45.5	2.90	R ₁	10000	31250	35000	35000	108750
		R ₂	8750	32500	31250	48750	95000
		R ₃	11250	25000	26250	43750	108750
		Mean	10000	29583	27500	42500	104167
100	2.98	R ₁	3750	8750	16250	25000	43750
		R ₂	5000	7500	12500	21250	53750
		R ₃	3750	8750	11250	23750	51250
		Mean	4167	8333	13333	23333	49583
220	98.2	R ₁	2500	5000	7500	13750	10000
		R ₂	1250	5000	8750	12500	12500
		R ₃	2500	5000	6250	10000	11250
		Mean	2083	5000	7500	12083	11250

nd : none detected (less than the limit of detection of 0.125 µg/L).

R₁-R₆ : replicate number.

Note : the initial cell density was estimated as 1.13×10^4 cells/mL.

Calculated values for the levels of inhibition of growth rate and biomass are given in Table B 9.2.-2.

Table B.9.2.-2: Inhibition of growth of algae exposed for 120 hours to Chloropicrin

Nominal concentration (µg/l)	Mean Measured Chloropicrin Concentration (µg/l)	Area under curve at 120 hours *	% Inhibition *	Growth rate (0-120 hours)	% Inhibition*
Control	Nd	28.8	0.0	0.045	0.0
9.39	0.528	29.1	-1.1	0.045	-0.4
20.7	0.840	24.1	16.3	0.040	4.2
45.5	2.90	2.80	90.3	0.014	45.6
100	2.98	0.695	97.6	0.004	62.9
220	9.82	-0.305	101.1	-0.004	97.4

* Percentage inhibition values calculated using non-rounded data

The median effective concentrations for inhibition of growth based on biomass and average specific growth rates (E_bC_{50} and E_rC_{50} , respectively), the corresponding EC10 values and NOEC were:

Time (hours)	EC_{50} (µg/l)	95% confidence limits (µg/l)
E_bC_{50} (120 hours)	1.34	1.27 – 1.41
E_bC_{10} (120 hours)	0.731	0.687- 0.781
E_rC_{50} (0 - 120 hours)	2.90	2.89– 2.91
E_rC_{10} (0 - 120 hours)	2.42	2.40-2.43

¹ based on mean measured concentrations

In the recovery phase it was found that the effects were reversible up to and including 2.98 µg/L (measured) (100 µg/L nominally), i.e. the cultures resumed growth. At 9.82 µg/L (220 µg/L nominal) recovery was not evident until day 10. The test demonstrated that at these concentrations Chloropicrin was algistatic.

Further endpoints for both growth and biomass (based on mean measured concentrations) are: NOEC: 0.528 µg/l, LOEC: 0.840 µg/l, NOAEC: 9.82 µg/l.

Validity of the test

The test was considered valid because:

- The mean increase in cell number in control cultures was 18.5 in 72h.
- the mean coefficient of variation for daily growth rates in control cultures ranged between 5.6% and 73.8% (mean value 22.3%); (Note: the 73% was determined during the initial 24h, and decreased to between 5.6 and 13.1% during the remaining 96h of the study. This variance was attributed to the sealed exposure system).

- the coefficient of variation of average specific growth rates was 0.65%.

Conclusions:

Under the conditions of this test, Chloropicrin inhibited the growth of *Navicula pelliculosa*, Strain No. SAG 1050-3 at concentrations tested in excess of 0.528 µg/l. Based on mean measured concentrations, the E_bC_{50} was 1.34 µg/L (1.27 – 1.41). The results indicate that chloropicrin was algistatic to *Navicula pelliculosa* at concentrations up to and including 9.82 µg/L.

Acceptability: Not acceptable because exponential growth in control is not demonstrated throughout the 120h test period.

Comments

The RMS calculated that the increase in cell number in the first 72h was 22.5, (initial concentration = 1.12×10^4 cell/ml, mean cell number at 72h = 254583). This fulfills the OECD relevant criterion for test validity. Nevertheless, the RMS notes that the test is prolonged up to 120h and a plot with growth curves is not included in the study report. Based on values in table 9.2 -1, an exponentially growth in controls seems not demonstrated. This conclusion is supported also by the mean growth rate reported in the study for each 24h period: 0.012, 0.075, 0.047, 0.030, 0.015.

Effects on aquatic plants (IIA 8.2.8)

In response to comments from EFSA requesting a study on aquatic macrophytes the effect of chloropicrin on the growth of the aquatic higher plant *Lemna minor* was performed

Wilby, J.J, 2009b, Chloropicrin Higher Plant (*Lemna*) Growth Inhibition Test. Huntingdon Life Science Ltd., UK. Unpublished report no.: SXC0023. Owner European Chloropicrin Group. (IIA 8.6/01)

GLP; Yes

Methods:

The test was run under non-axenic conditions. The seven day study was conducted under semi-static conditions. The media were renewed daily due to the volatility of the test substance.

Lemna plants were exposed to chloropicrin dispersed in nutrient medium at nominal concentrations of 1.49, 4.77, 15.3, 48.8, 156 and 500 µg/L. The plants were maintained in a temperature controlled incubator at 22.5 to 25.7°C under continuous illumination ranging between 7353 and 8390 lux (mean values). The test media were prepared by dilution of a aqueous stock solution in which the test substance was added directly to culture medium (SIS). Six replicate vessels were prepared for the control group and three replicate vessels for each of the test groups. Twelve fronds of *Lemna* (four plants of three fronds) were added to each flask. An additional vessel was established on Days 0, 3 and 6 of the test at nominal concentration of 0.0488 mg/L without *Lemna* fronds, for analysis after 24 hours.

Measured concentrations of chloropicrin ranged between 60% and 118% of nominal in freshly prepared media, except on one occasion at the lowest level, which was 41% of nominal. Measured concentrations in expired (24-hour-old) media had decreased, ranging

from less than the limit of detection of the analytical method (0.125 µg/L) to 12% of nominal. These results were not unexpected given the volatile nature of the test substance.

The geometric mean was used to determine the overall measured levels of chloropicrin during the test. The resulting mean measured concentrations were 0.168, 0.309, 1.31, 3.34, 17.9 and 140 µg/L.

The area under the curve was divided by initial count and total time to give AUCP, where a value of 1 represents no growth and a value of 0 represents complete toxicity (all *Lemna* killed). In order to estimate the concentration at which 5%, 50% and 90% inhibition of growth occurred (EC₅, EC₅₀ and EC₉₀), sigmoidal curves were fitted to AUCP and growth rate. For both variables, 0% inhibition was defined as the control mean and 100% inhibition was defined as no growth. The minimum of the curve (for infinite concentration) was bounded between 0 and 1 for AUCP and between -1000 and 0 for growth rate.

Multiple comparison tests were applied. The F1 test for monotonicity (Healy 1999) was first applied. If this was not significant at the 5% level, then each of the test substance treated groups was compared with the control using a two-sided Williams test for monotonic trend (Williams 1971, 1972). If the monotonicity test was significant at the 5% level, indicating a non-monotone dose-response, then a two-sided Dunnett's test (Dunnett 1955, 1964) was applied instead.

Results:

Growth was monitored daily by counting the number and observing the condition of fronds in each vessel. After 7 days of exposure, chlorotic or pale fronds were observed in all cultures exposed to chloropicrin at nominal concentrations of 48.8 to 500 µg/L. Shortened roots were observed in all cultures exposed at nominal concentrations of 15.3 to 500 µg/L; white and/or detached roots were also present at 156 and 500 µg/L. Gibbosity (humped or swollen fronds) was observed at nominal concentrations of 15.3 and 48.8 µg/L which was accompanied by colony break up at nominal concentrations of 156 and 500 µg/L.

Conclusions:

After 7 days of exposure of *Lemna minor* to chloropicrin, the E_bC₅₀, E_rC₅₀ and E_yC₅₀ for inhibition of growth (as area under the curve, specific growth rate and yield) were 14.0, 37.9 and 6.80 µg/L respectively, based on mean measured concentrations.

The "no observed effect concentration" (NOEC) of chloropicrin for area under the growth curve and yield (increase in dry weight) was 0.309 µg/L and for growth rate it was 3.34 µg/L.

The following values were derived from the data, based on mean measured concentrations:

Table B.9.9-1: Summary of the results of the study

Day 7	Mean measured chloropicrin concentration (µg/L).
Area under the growth curve	
E _b C ₅₀	14.0 (11.4, 17.2)
No-observed effect concentration	0.309
Average specific growth rate	
E _r C ₅₀	37.9 (32.2, 50.1)
No observed effect concentration	3.34
Yield (Dry weight)	
E _v C ₅₀	6.80 (4.67, 10.1)
No observed effect concentration	0.309

() : 95% confidence limits

The test fulfils the validity criterion of the doubling time of frond number in the control cultures that must be less than 2.5 days (60 hours), corresponding to approximately eight-fold increase in seven days. The doubling time of fronds in the control vessels during the test was calculated to be 1.98 days, which corresponds to an appropriate twelve-fold increase in frond number indicating the validity of the test.

Acceptability: This study is considered to be acceptable.

Plant protection products

a) Fish (IIIA 10.2.1)

Please refer to comments made in the DAR 2005.

b) Aquatic Invertebrates (IIIA 10.2.1)

Please refer to comments made in the DAR 2005.

c) Effects on Algal Growth (IIIA 10.2.1)

Please refer to comments made in the DAR 2005.

In summary, Chloropicrin is very acutely toxic to fish, daphnia and algae, as indicated by 96-hour LC₅₀ values of 4.8 µg/l for Rainbow trout (*Oncorhynchus mykiss*), 4.8 µg/l for Bluegill Sunfish (*Lepomis macrochirus*), 48-hour EC₅₀ (immobilisation) for *Daphnia Magna* of 0.15 mg/l, E_bC₅₀ for *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) of 0.01 µg/l and the E_vC₅₀ for *Lemna gibba* was 6.8 µg/l). Since the active substance is equivalent to the formulated product, Chloropicrin 99, the same values apply to both.

Hazard Classification/Labelling

Classification of the active substance for environmental effects according to 67/548/EEC Hazard

Please refer to comments made in the DAR 2005.

Classification/Labelling of plant protection products

Please refer to comments made in the DAR 2005.

B.9.2.2 Chronic toxicity

Active substance

Chronic toxicity to fish (IIA 8.2.2)

Lee M.R. 2009. Chloropicrin - Early Life-Stage Toxicity Test with Rainbow Trout (*Oncorhynchus mykiss*) Following OECD Guideline #210 and OPPTS Draft Guideline 850.1400. [REDACTED]

[REDACTED] Unpublished report No. 13939.6100 Owner European Chloropicrin Group. (IIA 8.2.4/01)

GLP: Yes

Guidance: OECD guideline number 210 and OPPTS Draft guideline 850.1400.

The objective of this study was to determine the No-Observed-Effect Concentration (NOEC), the Lowest-Observed-Effect Concentration (LOEC) and the Maximum Acceptable-Toxicant Concentration (MATC) of chloropicrin for rainbow trout (*Oncorhynchus mykiss*) embryos and larvae under flow-through conditions.

Method

Following a preliminary range finding test, nominal chloropicrin concentrations of 0.38, 0.75, 1.5, 3.0, 6.0 and 12 µg a.s./L were selected for the definitive test. A 0.70 mg a.s./mL diluter stock solution was prepared prior to test initiation and as needed during the study. Newly fertilized eggs of rainbow trout, 0.5h old at test initiation, were used. Eggs were impartially distributed in the 28 egg incubation cups until each cup contained 50 eggs. Two cups per replicate vessel were placed on the rocker arm apparatus in the respective exposure aquarium (200 eggs per treatment or control).

Test aquaria (exposure solution volume of 15 L) were positioned in a water bath at 12 ± 2 °C. During the incubation phase and prior to the larval development to the swim-up stage, the test area was maintained in continuous darkness. A 16-hour light photoperiod (760 to 1000 lux) was established once all larvae (with the exception of three larvae in the highest nominal concentration tested, 12 µg a.s./L) had reached the swim-up stage. Well water was used.

Measures during the study gave the following ranges: pH: 6.3 to 7.7, Specific conductivity: 210 to 290 µmhos/cm and Total alkalinity as CaCO₃: 18 to 24 mg/L, total hardness as CaCO₃: 36 to 52 mg/mL.

Due to the high volatility of the test substance in water, the diluter was calibrated to deliver approximately 10 aquarium volumes per 24-hour period.

Larvae were fed live brine shrimp nauplii (*Artemia salina*) three times daily beginning on day 9 post-hatch, when the rainbow trout began to exhibit swim-up behavior. Larvae were not fed during the final 48 hours of the test prior to determining weight measurements at test termination. The test lasted 90 day (60 days post-hatch).

Due to the high volatility of the test substance, potentially resulting in variable recoveries, the exposure system was sampled twice weekly, with the exception of the final week of the exposure period, during which the system was sampled once. Three quality control (QC) samples were prepared at each sampling interval and the results of their analyses were used to judge the precision and quality control maintained during the analysis of exposure solution samples.

Results:

Analytical results

Analytical results on days 63, 68 and 70 yielded variable recoveries between replicates of the 12 µg a.s./L nominal treatment level. Following a complete diluter inspection, it was discovered that test solution delivery to the sampled vessel (replicate A) was being impeded by a cleaning brush fiber. Following removal of the obstruction, solution delivery calibration was checked and determined to be within acceptable ranges. Analytical measurements performed on days 75 and 77 showed declining test concentrations. Due to these reduced exposure levels, stock solution delivery was again increased by 20%. Analysis of test solutions on day 81 yielded recoveries that were more consistent with previous intervals and expectations.

The mean exposure concentrations ranged from 67 to 95% of the nominal levels and defined the treatment levels as 0.29, 0.71, 1.1, 2.5, 4.6 and 8.0 µg a.s./L. Details see Table B.9.2.1-1

Table B.9.2.1-1

Nominal Concentration (µg a.i./L)	Mean Measured^a Concentration (SD)^b (µg a.i./L)	RSD^c (%)	N	Mean Percent of Nominal^a (%)
0.38	0.29 (0.071)	24	27	77
0.75	0.71 (0.19)	26	27	95
1.5	1.1 (0.26)	23	27	75
3.0	2.5 (0.60)	24	27	84
6.0	4.6 (1.1)	25	27	76
12	8.0 (2.2)	28	27	67

^a Mean measured concentrations and percent of nominal were calculated using the actual analytical (unrounded) results and not the rounded values..

^b SD = Standard Deviation.

^c RSD = Relative Standard Deviation.

Biological results

Mean embryo viability determined on test day 18 at all treatment levels ranged from 95-100%. Day 0 post-hatch was determined to be day 30 when no more than 10% unhatched viable embryos remained in any embryo incubation cup. At the completion of the hatching period (day 30), 100% survival was observed among embryos in the control and all treatment levels tested. Since embryo hatching success was 100% in all treatment levels tested and no deformed larvae were observed at hatch, these endpoints were not statistically analyzed. At test termination (60 days post-hatch), larval survival in the control averaged 98%.

Based on the data obtained during this study, growth (total body length) was the most sensitive indicator of the toxicity of chloropicrin to rainbow trout. The No-Observed-Effect Concentration (NOEC) was determined to be 2.5 µg a.s./L. The Lowest-Observed-Effect Concentration (LOEC) was determined to be 4.6 µg a.s./L. The Maximum-Allowable-Toxicant Concentration (MATC) for chloropicrin to rainbow trout embryos and larvae was estimated to be 3.4 µg a.s./L. Details see Table B.9.2.1-2

Table B.9.2.1-2

Nominal Concentration (µg a.i./L)	Mean Measured ^a Concentration (µg a.i./L)	Larval survival (%)	Mean total length (mm)	Mean dry weight (g)
control	<LOD	98	55.2	0.2819
0.38	0.29	98	55.4	0.2911
0.75	0.71	100	54.4	0.2713
1.5	1.1	98	54.6	0.2744
3.0	2.5	100	54.8	0.2753
6.0	4.6	93	53.6*	0.2655*
12	8.0	49*	53.1 [#]	0.2608 [#]

* Significantly reduced compared to the control, based on Williams' Test.

[#] This treatment level was excluded from further statistical analyses due to survival effect observed.

a Values presented represent percent viability of exposed eggs determined on test day 18.

b Values presented represent hatching success at the completion of hatch (test day 30).

c Standard deviation for each replicate is presented in parentheses.

d Significantly reduced compared to the control, based on Williams' Test.

e This treatment level was excluded from further statistical analyses due to survival effect observed.

The test fulfilled the OECD 210 validity criteria, because the DO was well above 60% of air saturation throughout the test, Temperature during the entire test was 11-12°C (except at concentration 0.75 where a range 10-12°C was recorded), mean embryo viability determined on test day 18 in all treatments ranged from 95 to 100%.

Conclusions:

The most sensitive endpoints were the growth parameters, i.e. total length and dry weight. Based on mean measured concentrations, the NOEC was determined to be 2.5 µg a.s./L and the LOEC was 4.6 µg a.s./L.

Acceptability: This study is considered to be acceptable.

Bioconcentration in fish (IIA 8.2.3)

Please refer to comments made in the DAR 2005.

Chronic toxicity to aquatic invertebrates (IIA 8.2.5)

In response to comments from MS to the original DAR 2005, further considerations is given to aquatic chronic risk to invertebrates following exposure to Chloropicrin. To this purpose the Notifier submitted a reproduction test with *Daphnia magna*, which is summarized below.

Jenkins, C.A. 2009. Chloropicrin Prolonged Toxicity to *Daphnia magna*,. Huntingdon Life Sciences Ltd., UK. Unpublished report No. SXC0011. Owner European Chloropicrin Group. (IIA 8.3.2.1/01)

GLP: Yes

Guidelines: OECD Guideline for Testing of Chemicals, No. 211 "*Daphnia magna*, reproduction test" (adopted 21 September 1998).

Method:

The effect of chloropicrin on the reproduction of *Daphnia magna* was assessed under semi-static exposure conditions over a period of 21 days. The study comprised two formulation trials, a range finding test and a definitive test.

Groups of ten, individually-housed *Daphnia* (<24h old) were exposed for 21 days to the test substance prepared at nominal concentrations of 0.500, 0.990, 3.00, 8.90, 27.0 and 80.0 µg/L. Twenty animals were exposed in the water control,

The test media were prepared from a concentrated aqueous stock solution made in Elendt M4 medium. The study was conducted in completely filled and sealed vessels to minimise losses of the test substance by volatilisation and the aqueous stock solution and the test media were renewed each day.

Daily records were taken of the numbers of floating, immobile, dead and gravid (animals with eggs in the brood pouch) parental *Daphnia*, general appearance, live, dead or floating neonates and the presence of aborted eggs and moulted carapaces.

Animals were daily fed with *Pseudokirchneriella subcapitata*.

At the end of the definitive test, after observations of the *Daphnia* was made, the body length of each surviving adult was measured using a microscope and stage mounted graticule.

The temperature ranged from 17.1 to 21.1°C, the hardness from 240 to 270 mgCaCO₃/l, pH from 7.1 to 8.08 and DO from 82 to 106%.

Results:

Measured concentrations of chloropicrin ranged between 67 and 118% of their nominal values in freshly prepared media, except at the start of the test at the lowest level, which was 816% of nominal (these value were attributed to contaminated preparation flask, that was replaced on day 5, and were not included in the calculation of the mean measured concentration). Measured concentrations in expired (24-hour-old) media had decreased, ranging from below the limit of detection of the analytical method (0.125 µg/L) to 30% of nominal except on Day 1 at the lowest test level, which was 74% of nominal. The losses observed during the study were not unexpected given the volatile nature of the test substance. Based on a geometric mean, the overall measured levels of chloropicrin during the test were 0.160, 0.241, 0.579, 1.10, 4.27 and 28.3 µg/L (see Table below).

Measured Chloropicrin concentrations

Nominal Conc. (µg/L)	Day 0 fresh	% N	Day 1 expired	% N	% T0	Day 7 fresh	% N	Day 8 expired	% N	% T7	
Control	nd	-	nd	-	-	nd	-	nd	-	-	
0.5	4.08*	816	0.372*	74	9	0.337	67	nd	-	-	
0.99	1.17	118	nd	-	-	0.738	75	nd	-	-	
3.0	2.23	74	<loq	-	-	2.28	76	0.461	15	20	
8.9	7.26	82	0.744	8	10	8.45	95	nd	-	-	
27	21.5	80	3.59	13	17	23.5	87	0.320	1	-	
80	70.5	88	24.3	30	34	76.1	95	10.7	13	14	
Nominal Conc. (µg/L)	Day 14 fresh	% N	Day 15 expired	% N	% T14	Day 20 fresh	% N	Day 21 expired	% N	% T20	Overall geometric mean [#] , µg/L
Control	nd	-	nd	-	-	<loq	-	nd	-	-	-
0.5	0.452	90	nd	-	-	0.447	89	nd	-	-	0.160
0.99	0.853	86	nd	-	-	0.991	100	nd	-	-	0.241
3.0	2.33	78	nd	-	-	2.33	78	nd	-	-	0.579
8.9	7.46	84	nd	-	-	7.07	79	nd	-	-	1.10
27	23.3	86	0.399	1	2	20.6	76	0.3	1	1	4.27
80	70.4	88	14.5	18	21	69.1	86	1.88	2	3	28.3

nd : none detected (< 0.125 µg/L; limit of detection, Appendix 3)

loq : limit of quantification (<0.25 µg/L)

%N : measured concentration expressed as a percentage of the nominal concentration.

% Tn : measured concentration ca. 24 hours after preparation expressed as a percentage of the concentration at the time of preparation.

: where measured concentrations were below the limit of detection, a value of 0.0625 µg/L was used in test calculations and where levels were below the limit of quantification, the limit of detection was used in test calculations.

The numbers of dead parental *Daphnia* observed during the definitive test are given in Table B 9.5-1.

Table: B.9.5-1: Cumulative number of dead parental *Daphnia*

Observation time (day)	Percentage mortality / measured Chloropicrin concentration (µg/L)						
	Control	0.160	0.241	0.579	1.10	4.27	28.3
2	0	0	0	0	0	0	0
4	0	10	0	0	0	0	0
7	0	20	0	0	0	10	40
14	5	20	0	0	0	10	50
21	15	20	0	11	0	10	60

There was a clear dose-response relationship for mortality of parent organisms except for an increased mortality at the lowest treatment level, 0.16 µg/L (mean measured). However, in this dose level initial measured concentrations were 816% of nominal. The observed mortality of 20% is thus likely to be result of a dosing error. In later time intervals no such irregularity was observed.

Based on parental mortality, the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were 4.27 µg/L and 28.3 µg/L, respectively. The 21-day EC₅₀ value for the parental generation was 25.9 µg/L.

Statistical analysis of the lengths of surviving adults after 21 days of exposure to chloropicrin identified no significant effect when compared to the control group, giving a LOEC of >28.3 µg/L and a NOEC of 28.3 µg/L.

Statistical analysis of the total number of live neonates produced by each surviving adult in the test groups compared to the control group indicated that reproduction was significantly reduced at a chloropicrin concentration of 28.3 µg/L (50.6%; p<0.001), giving a NOEC of 4.27 µg/L and a LOEC of 28.3 µg/L. The 21d EC₅₀ for reproduction was 28.2 µg/L. The numbers of live neonates produced during the test are given in Table B 9.5-2:

Table B.9.5-2: The number of live neonates during the test.

Chloropicrin (µg/L)	Cumulative number of neonates/adult (all live neonates)	
	Mean	sd
Control	88.06	17.31
0.160	86.88	19.69
0.241	90.50	18.75
0.579	80.13	18.35
1.10	89.90	16.86
4.27	87.33	13.17
28.3	43.50	12.26

The time taken to produce the first brood of neonates was not significantly reduced at any chloropicrin level employed in the test, consequently, the NOEC for this parameter was 28.3 µg/L and the LOEC was >28.3 µg/L.

At 4.27 and 28.3 µg/L, some of the parental animals appeared pale at intervals during the test. Pale animals were also observed on occasions in the control group and at the other test levels but the numbers affected were low and observations were infrequent.

The test fulfills the OECD validity requirements for parent mortality (<20%) and number of live offspring per surviving parent animal (>60).

Conclusions:

Based on parental mortality, size of parents, reproduction and time to brood, the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were 4.27 µg/L and 28.3 µg/L, respectively. The 21d EC₅₀ mortality for the parental generation was 25.9 µg/L. The 21d EC₅₀ reproduction was 28.2 µg/L. All endpoints are based on mean measured concentrations.

Acceptability: This study is considered to be acceptable.

Comments: The analytical measures of Chloropicrin in the test solutions show that the concentration was not maintained in the 24h renewal period. This indicates that the test system was not suitable for a volatile chemical such Chloropicrin. Anyway considering that mean measured concentrations were used and that test solutions were renewed daily, the results have been considered sufficiently adequate to be used in the risk assessment. In addition D.magna was the less sensitive organisms in the acute tests.

Effects on sediment dwelling organisms (IIA 8.2.7)

Please refer to comments made in the DAR 2005.

Plant protection products (IIIA 10.2.4)

Please refer to comments made in the DAR 2005.

B.9.2.3 Risk assessment

Chloropicrin is intended as a soil fumigant, and not for direct application to water. In soil fumigation, Chloropicrin under ambient temperature and pressure quickly volatilises and will vent upwards into the atmosphere. The fate and behaviour of Chloropicrin indicates that the main route of exposure for aquatic organisms is drainage from treated fields.

Since the preparation does not contain any co-formulants and consists entirely of Chloropicrin, studies on the effect of only Chloropicrin technical to aquatic organisms are made available.

The maximum application rate of 448 kg/ha (272 l/ha) is considered in the risk assessment. Drainage is the chosen entry route for which the aquatic risk assessment is conducted.

Acute risk

The results of these studies are given in Table 9.2.3-1.

Table B.9.2.3-1: Summary of effects of Chloropicrin to aquatic species

Test species	Test system	Duration of exposure	Results	References
<i>Oncorhynchus mykiss</i>	Acute	96 h	LC ₅₀ : 4.8 µg/l.	Flatman, D. (2004)
<i>Lepomis macrochirus</i>	Acute	96 h	LC ₅₀ : 48 µg/l.	Flatman, D. (2004)
<i>Daphnia magna</i> (Straus)	Acute	48 h	EC ₅₀ : 150 µg/l.	Flatman, D. (2004)
<i>Pseudokirchneriella subcapitata</i> (formerly <i>Selenastrum capricornutum</i>)	72 h	72 h	E _b C ₅₀ (72 hours): 0.11* µg/l E _r C ₅₀ (72 hours): 0.16 µg/l	Flatman, D. (2004)
<i>Navicula pelliculosa</i>	Not accepted			
<i>Lemna minor</i>		7 days	E _b C ₅₀ , (7 days) 14.0 µg/L E _r C ₅₀ (7 days) 37.9 µg/L E _y C ₅₀ (72 hours): 6.80 µg/l	Wilby, J.J, 2009b,

* nominal value (below the analytical detection limit)

The risk assessment for acute exposure to aquatic organisms has been performed by comparing the acute LC₅₀/EC₅₀ values for the representative species with the predicted concentrations in surface waters (PEC_{sw}). The PEC_{sw} calculation was revised based on comments from member states and EFSA for drainage, which is considered to be the major exposure route of entry of Chloropicrin into water bodies (see Section B8, Table 8.5.1). The maximum PEC for chloropicrin was estimated to be 6.573 µg as/l (D6 ditch). For the other 3 scenarios (R2,R3,R4) the PEC values were 0 µg as/l. The following table (Table 9.2.3-2) presents 'toxicity exposure ratios' (TER_{st}) calculated accordingly.

Table B.9.2.3-2: Acute Toxicity/exposure ratios for chloropicrin to aquatic organisms
(application of Chloropicrin to soil at the maximum rate of 448 kg/ha).

Method of Application	Test System	Test species	Effect Concentration (µg as/l)	PEC _{sw} # (µg as/l)	TER	Trigger
Soil Injection	Acute	Fish <i>Oncorhynchus mykiss</i>)	4.8	6.573	0.7	< 100
	Acute	Invertebrate	150	6.573	22.8	< 100
	72 hours	Algae 1	0.11	6.573	0.017	< 10
	7 days	Aquatic plants	6.80	6.573	1.0	< 10

based on FOCUS Step 3, the worst of 4 relevant scenarios (D6, ditch)

Values in **bold** are below the relevant Annex VI Trigger

Comment: The acute TER values calculated for the most susceptible species using the worst case PEC_{sw} (D6 scenario) calculated after tarp removal on day 8 and subsequent transference via drains to representative water bodies ranged from 0.017 to 22.8 and were below the relevant trigger values of Annex VI (10 or 100). A high risk for the D6 ditch scenario is predicted. For the other 3 scenarios (R2,R3,R4) the PEC values are calculated equal to 0 µg as/l, therefore since exposure is unlikely no acute risk is expected.

NOTE: The Notifier does not discuss this outcome because he used PEC_{initial} values much higher, which gave a conclusion of no risk. The use of PEC_{twa} is a refinement made by the RMS.

Chronic risk

The TER values calculated using initial PEC_{initial} and PEC_{twa} values for the D6 scenario are reported in the table below. For the other 3 scenarios (R2,R3,R4) the PEC values are calculated equal to 0 µg as/l,

Table B.9.2.3-3: Chronic Toxicity/exposure ratios for chloropicrin to aquatic organisms
(application of Chloropicrin to soil at the maximum rate of 448 kg/ha).

Method of Application	Test System	Test species	Effect Concentration (µg as/l)	PEC _{sw} # (µg as/l)	TER	Trigger
Soil Injection						
	Flow-through (F-ELS test)	Fish <i>Oncorhynchus mykiss</i>	NOEC: 2.5	6.573 (PEC _i) 0.403 (50d PEC _{twa})	0.4 5	< 10
	semistatic (reproduction test)	Invertebrate (<i>D. magna</i>)	NOEC: 4.27*	6.573 (PEC _i) 0.859 (24d PEC _{twa})	0.6 5	< 10

based on FOCUS Step 3, the worst of 4 relevant scenarios (D6, ditch)

Values in **bold** are below the relevant Annex VI Trigger

based on FOCUS Step 3, the worst of 4 relevant scenarios (D6, ditch)

Values in **bold** are below the relevant Annex VI Trigger

The TER values for the chronic toxicity calculated based on PEC_{initial} and PEC_{twa} are below trigger value of 10. Consequently the chronic risk from the proposed use to aquatic species is considered to be unacceptable for the D6 scenario. For the other 3 scenarios (R2,R3,R4) the PEC values are calculated equal to 0 µg as/l, therefore since exposure is unlikely no chronic risk is expected.

NOTE: The PEC initial values used by the Notifier in his risk assessment were much higher than those calculated in section B8 of the present Addendum. As a consequence, no risk was identified. The use of PEC_{twa} is a refinement made by the RMS.

Risk from metabolites

PEC estimates have been generated for the soil metabolite dichloronitromethane, DCNM. Surface water concentrations were estimated based on worst-case assumptions (see Section B8). The maximum PEC for DCNM was estimated to be 4.350 µg/L (D6 Thiva, ditch). Assuming ten-fold toxicity of the metabolite compared to the parent, toxicity exposure ratios' (TER) were calculated for the metabolite DCNM; see Table 9.2.3-3.

Table B.9.2.3-3: Toxicity/exposure ratios for DCNM to aquatic organisms

Method of Application	Test System	Test species	Effect Concentration* (µg as/l)	PEC _{SW} # (µg as/l)	TER	Trigger
Soil Injection	Acute	Fish	0.48	4.350(PECi)	0.1	< 100
	Long Term (F-ELS)	Fish	NOEC: 0.25	4.350(PECi) 0.306 (50dPEC _{twa})	0.06 0.8	< 10
	Acute	Invertebrate	15	4.350(PECi)	3.4	< 100
	Long term (reproduction)	Invertebrate	NOEC: 0.43	4.350(PECi) 0.601 (21d PEC _{twa})	0.1 0.7	< 10
	72 hours	Algae 1	0.011	4.350(PECi)	0.003	< 10
	7 days	Aquatic plants	0.68	4.350(PECi)	0.15	< 10

* assuming ten-fold toxicity of the metabolite DCNM compared to the parent, chloropicrin

based on FOCUS Step 3, the worst of 4 relevant scenarios (D6 Thiva, ditch)

Values in **bold** are below the relevant Annex VI Trigger

All the acute and chronic TER estimates were below the relevant trigger values of Annex VI (100 or 10). For algae, the TER was as low as 0.003. Even if the metabolite is assumed to be as toxic to aquatic organisms as chloropicrin, the resulting TER estimates would still be below the triggers.

Conclusion: The risk from the metabolite following the proposed use of chloropicrin to aquatic species is considered to be not acceptable for the scenario D6. For the other 3 scenarios (R2,R3,R4) the PEC values are calculated equal to 0 µg as/l, therefore since exposure is unlikely no acute or chronic risk is expected.

Sediment organism

Please refer to comments made in the DAR 2005.

Conclusion

According to the proposed GAP, four exposure scenarios have been considered in the section "Fate and behaviour" : D6,R2,R3,R4. For all the R scenarios, the PEC_{initial} values are calculated equal to 0 µg as/l, hence no exposure is predicted and no risk to aquatic organisms is expected. For the D6 scenario an unacceptable acute and chronic risk for both Chloropicrin and its metabolite DCNM has been calculated.

In conclusion some safe scenarios have been identified. The risk for the D6 scenario should be addressed at MS level for the authorization of the product at national level.

Bioaccumulation

Please refer to comments made in the DAR 2006.

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

B.9.3.1 Toxicity

Please refer to comments made in the DAR 2005 and to Section 3 of the present addendum..

B.9.3.2 Risk assessment

Dietary exposure

In the original DAR 2005 the RMS pointed that for mammals the dietary route of exposure could not be negligible. The RMS recognized that mammals will not be exposed to in-field insects contaminated at initial concentration, but pointed out that off-field and in-field (after tarp removal) exposure of mammals to contaminated food cannot be excluded and might still be significant. Therefore data on residues on food items and a risk assessment for mammals was considered necessary.

In the updated Dossier presented in April 2006, the Notifier submitted a literature study on plant metabolism (Lawrence, 1990). This study showed that the radiocarbon present in plants grown in soil treated with [¹⁴C]Chloropicrin consisted of natural plant biochemical components which had incorporated the radiolabelled carbon atom. Furthermore, the major concentrations of radiocarbon in each plant tissue were generally associated with the natural plant products present in the tissue. This would indicate that birds and mammals feeding on vegetation grown in areas treated with Chloropicrin not likely to be exposed to significant levels of residues in plants tissue. The results of this study confirm that Chloropicrin is not a systemic substance.

Within the dossier submitted for the present resubmission, the Notifier included a study on residues on several food items (Bartolomè, 2009, see Other/Special Studies, IIA 8.16.1) and presented a Tier 2 risk assessment for mammals.

The risk assessment was based on brown hare (*Lepus europaeus*), chosen as focal species representative of medium sized herbivorous mammals (3.5 Kg). For the sake of clarity and to keep the present Addendum as concise as possible, the original risk assessment submitted by the Notifier is included in Annex I. Briefly, the concentration of the test substance in diet has been refined based on the same data of measured residues and calculated DT50 used in the risk assessment for birds (Bartolomè, 2009). Further refinements were also made for PD, PT and ftwa. The Notifier concludes that the risk posed by Chloropicrin to herbivorous mammals foraging in treated field of tomatoes is acceptable. The RMS objects on the choice of the hare as "focal" species. Since Chloropicrin is applied to bare soil, a small omnivorous mammal is thought to be a more representative species than hare, that is a strictly herbivorous species. In case of pesticides applied to bare soil, the recently adopted revision of the Guidance Document SANCO/4145/2000 (EFSA 2008¹) recommends to consider as generic focal species a small omnivorous mammal (mouse).

Inhalation exposure-

The comparison of the highest off field emission of Chloropicrin (Trevisan, 2009) with the inhalation toxicity value for mammals indicates a low risk for wild mammals exposed to Chloropicrin in air.

Conclusion - the presented dietary risk assessment is not judged acceptable and the point is considered still open.

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

B.9.4.1 Acute toxicity to bees

In the DAR 2005, concern was expressed for effects on bees (flying and foraging in the areas adjacent to the treated field and on bees present in the hives possibly located there), following inhalation exposure and contact exposure to the deposits on off-field vegetation. In response to this concern, the Notifier presented a study aimed at assessing the potential effect of chloropicrin vapours on honey bees, which is summarized below.

Active substance (IIA 8.3.1.1)

Porch, J *et al.* 2009- Chloropicrin: An acute vapour exposure toxicity study with the honey bee. Wildlife International, USA. Unpublished report no.: 650-107. Owner European Chloropicrin Group. (IIA 8.7/01)

GLP: Yes (except for the analysis of well water for potential contaminants).

Guideline: U.S. Environmental Protection Agency Series 850 – Ecological Effects Test
Guidelines OPPTS Number 850.3020, EPPO guideline 170 and OECD 214

Methods:

In an acute contact vapour toxicity study with the honey bee (*Apis mellifera*), Young adult worker honey bees were exposed to five doses of chloropicrin ranging from 0.18 to 12 ppm (measured by GC with ECD). Bees were held in stainless steel cylinders (9x9cm) with perforations for ventilation covered with a petri dish, which were inserted in a sealed Tedlar® bags (where the exposure atmosphere was reached) for 60 minutes. The exposure duration of 60 minutes was justified as the longest period for which it appeared untreated bees would satisfactorily tolerate static conditions. A negative control group, exposed to untreated air, was also maintained. Each control and treatment group contained three replicates of 20 bees each. Feed bottles were removed during exposure. Bees were maintained in continuous darkness except during periods of dosing and observations. Additional groups of bees were treated with the positive control substance dimethoate (0.05, 0.10 and 0.30 µg a.s./bee, plus an acetone solvent control group). Positive control groups were dosed by administering a 2 µl droplet to the abdomen and/or thorax of each bee. Observations of mortality and other signs of toxicity were made twice on Day 0 following the completion of dosing and then at approximately 24 and 48 hours.

Results:

The measured Chloropicrin concentrations are reported in the following table.

Nominal and Actual Exposure Bag Concentrations

Test Substance	Sample Number	Exposure Bags Concentration (ppb)			
		Nominal		Measured ¹	% of Nominal
		Exposure (ppm)	Atmosphere (ppb)		
Chloropicrin		0	0	< LOD	-
Analysis Date	2	0.1	177	176	99
5/6/09	3	0.5	531	341	64
	4	1.0	1239	680	55
	5	5.0	5568	5805	104
	6	10.0	12347	12228	99

¹Limit of Detection (LOD) is the lowest analytical standard (62 ppb)

Treatment group mean mortality at the end of the test ranged from 3 to 12%, and did not occur in a dose responsive pattern. Mean mortality in the negative control group was 7%. Based on these results, the 48-hour LC₅₀ for honey bees exposed to chloropicrin for sixty minutes was determined to be greater than 12 ppm. The no-observed effect concentration (NOEC) was 12 ppm, the highest concentration tested.

Conclusions:

As there were no treatment related mortality at any dose concentration the 1 hour inhalation NOEC was 12.2 ppm (measured).

The inhalation 48-hour LC₅₀ value for honey bees exposed to chloropicrin vapours for sixty minutes was determined to be greater than 12 ppm. The corresponding 48 h NOEC 48 hour was =12 ppm, the highest concentration tested.

Acceptability - This study is considered to be acceptable.

Comments:

Ideally the positive control should have been administered using the same exposure system as for the test substance, i.e. gas chamber. Anyhow, it should be considered that the study is a non-standard study, available guidelines do not address the inhalation exposure and OECD 214 does not require a positive control to be tested.

B.9.4.2 Risk assessment

In order to determine the risk of off-site inhalation of Chloropicrin to bees a 1 hour inhalation toxicity study was carried out (IIA 8.7/01). This study was designed to establish the risk of Chloropicrin vapours to bees in areas adjacent to treated fields where the Chloropicrin vapour is unavoidable. This would be relevant to beehives as well as to bees foraging in the vicinity. The results of this study are summarized below.

Table B.9.4.2.1: Summary of effects of Chloropicrin to Bees

Test species	Test system	Duration of exposure	Results	Reference
Honeybees	Inhalation exposure	1 hour	48h LC50 > 12.2 ppm NOEC 12.2ppm (82.2 mg/m ³)	(Porch, J. et al. 2009)

The relevant exposure level to compare to the above inhalation toxicity endpoint comes from a field emission study (see Section 3, Trevisan, 2009, Annex IIIA, 7.3.3.01 (EU-7.2.1.2), "Cholopicrin: concentration in air and operator exposure assessment during and after soil application"). This study determined the field exposure levels at various distances from a fumigated field. The maximum measured air concentration (single measure) in this study was used for the risk assessment to bees, i.e. 0.3077 mg/m³ equivalent to 45.9 ppb, measured at 1 metre from plot margin on day 4 (the weighed mean for day 4 was 0.0599 mg/m³). This concentration is considered to be relevant for bees foraging on adjacent areas but it is also used as worst case for the purposes of assessing the potential risk to bee hives present in the areas adjacent the treated field,.

Table B.9.4.2.2 Chloropicrin concentration in air at the poles 1m away from the edge of the treated plot.

Day	POLES 1 m far away the edge of the treated plot										Weighted mean	
	North			East			South			West		
	Sample n°	Conc. in air mg/m ³	Sampling time minutes	Conc. in air mg/m ³	Sampling time minutes	Conc. in air mg/m ³	Sampling time minutes	Conc. in air mg/m ³	Sampling time minutes	Conc. in air mg/m ³		Sampling time minutes
1	Sample 1	0.0236	440	0.0000	416	0.0031	392	0.0110	539	0.0098		
	Sample 2	0.0857	498	0.0116	545	0.0450	454	0.0946	554	0.0594		
	Sample 3	0.0354	509	0.0191	503	0.0000	501	0.0095	503	0.0161		
	D W Mean	0.0491	1447	0.0109	1464	0.0161	1347	0.0395	1596	0.0293		
2	Sample 4	0.0135	466	0.0150	470	0.0000	468	0.0060	470	0.0086		
	Sample 5	0.0343	579	0.0425	579	0.0207	582	0.0102	579	0.0269		
	Sample 6	0.0012	490	0.0071	489	0.0095	491	0.0000	490	0.0045		
	D W Mean	0.0174	1535	0.0228	1538	0.0108	1541	0.0057	1539	0.0142		
3	Sample 7	0.0134	780	0.0042	780	0.0082	783	0.0032	788	0.0072		
	Sample 8	0.0008	633	0.0009	637	0.0043	633	0.0006	635	0.0017		
	D W Mean	0.0078	1413	0.0027	1417	0.0065	1416	0.0021	1423	0.0047		
	Sample 9	0.3077	797	0.0603	735	0.0289	801	0.0305	799	0.1076		
4	Sample 10	0.0008	647	0.0017	643	0.0010	649	0.0041	635	0.0019		
	D W Mean	0.1702	1444	0.0329	1378	0.0164	1450	0.0188	1434	0.0599		
	Sample 11	0.0069	789	0.0011	782	0.0006	786	0.0010	799	0.0025		
	Sample 12	0.0000	649	0.0000	658	0.0000	653	0.0000	646	0.0000		
5	D W Mean	0.0038	1438	0.0009	1440	0.0003	1439	0.0006	1445	0.0014		
	Sample 13	0.0331	781	0.0314	775	0.0138	824	0.0199	785	0.0244		
	Sample 14	0.0000	665	0.0090	585	0.0019	667	0.0259	650	0.0091		
	D W Mean	0.0179	1446	0.0218	1360	0.0085	1491	0.0226	1435	0.0175		
6	Sample 15	0.0175	767	0.0016	766	0.0050	770	0.0101	764	0.0086		
	Sample 16	0.0040	666	0.0000	669	0.0180	669	0.0983	663	0.0299		
	D W Mean	0.0112	1433	0.0009	1435	0.0110	1439	0.0511	1427	0.0185		
	Sample 17	0.0056	759	0.0058	754	0.0348	763	0.0570	725	0.0406		
7	Sample 18	0.0008	633	0.0000	615	0.0014	631	0.0064	632	0.0022		
	D W Mean	0.0034	1392	0.0063	1369	0.0197	1394	0.0334	1357	0.0231		
	Sample 19	0.0119	817	0.0016	817	0.0032	822	0.0008	819	0.0044		
	Sample 20	0.0007	663	0.0010	443	0.0000	663	0.0006	643	0.0005		
8	D W Mean	0.0069	1480	0.0014	1260	0.0018	1485	0.0007	1462	0.0027		
	Sample 21	0.0190	753	0.0173	756	0.0000	750	0.0008	750	0.0093		
	Sample 22	0.0000	667	0.0000	663	0.0000	673	0.0000	673	0.0000		
	D W Mean	0.0101	1420	0.0092	1419	0.0000	1423	0.0004	1423	0.0049		
9	Sample 23	0.0130	772	0.0076	775	0.0080	769	0.0158	770	0.0111		
	Sample 24	0.0000	653	0.0000	659	0.0000	662	0.0000	652	0.0000		
	D W Mean	0.0071	1425	0.0041	1434	0.0043	1431	0.0086	1422	0.0060		
	Sample 25	0.0000	826	0.0000	828	0.0000	828	0.0000	829	0.0000		
10	Sample 26	0.0000	591	0.0000	590	0.0000	593	0.0000	592	0.0000		
	D W Mean	0.0000	1417	0.0000	1418	0.0000	1421	0.0000	1421	0.0000		
	Sample 27	0.0000	834	0.0000	836	0.0000	838	0.0000	834	0.0000		
	Sample 28	0.0000	645	0.0000	645	0.0000	638	0.0000	649	0.0000		
11	D W Mean	0.0000	1479	0.0000	1481	0.0000	1476	0.0000	1483	0.0000		
	Sample 29	0.0000	820	0.0000	819	0.0000	829	0.0000	818	0.0000		
	Sample 30	0.0000	598	0.0000	598	0.0000	597	0.0000	596	0.0000		
	D W Mean	0.0000	1418	0.0000	1417	0.0000	1426	0.0000	1414	0.0000		

As the standard HQ assessment is not applicable to concentrations in air and there is no relevant trigger value set by Annex VI of Directive 91/414 EEC for inhalation exposure

scenario, a proposed approach would be to compare the inhalation NOEC to the relevant exposure levels and apply a trigger value of 10 to the calculated TER.

Table B.9.4.2.3 Toxicity Exposure Ratio for Inhalation Exposure to Bees

Test species	End Point	Distance from field	Exposure Levels	TER	Trigger
Honeybee (<i>Apis mellifera</i>)	LC50 > 12ppm (12,200 ppb), 82.2 mg/m ³	1 m	45.9 ppb 0.3077 mg/m ³	> 266	10

The calculated TER values is well above the trigger and, in addition, it is an unbounded value (greater than) and includes worst case assumptions. This indicates that there are no adverse effects expected to bees due to exposure via inhalation of Chloropicrin in areas adjacent to treated fields.

The other envisaged possible route of exposure, i.e. contact exposure to deposits on off-field vegetation, is not specifically addressed by the Notifier. Considering the results of the above risk assessment, the relevance of this exposure is expected to be negligible and the corresponding risk to bees low.

B.9.5 Effects on other arthropod species (IIA 8.3.2, IIIA 10.5.1)

B.9.5.1 Toxicity

Please refer to comments made in the DAR 2005 and Reporting table (17.11.2006).

Active substance (IIA 8.3.2)

Please refer to comments made in the DAR 2005 and Reporting table (17.11.2006).

B.9.5.1.a - Using artificial substrates. (*Pardosa Spp.*)

Please refer to comments made in the DAR 2005 and Reporting table (17.11.2006).

B.9.5.1.b - Extended laboratory study (*Pardosa spp.*) (IIA 10.5.1 and 10.5.2)

Please refer to comments made in the DAR 2005 and Reporting table (17.11.2006).

B.9.5.1.c - Using artificial substrate (*Poecilus cupreus L.*)

Please refer to comments made in the DAR 2005 and Reporting table (17.11.2006).

B.9.5.1.d - Extended laboratory study (*Poecilus cupreus L.*)

Please refer to comments made in the DAR 2005 and Reporting table (17.11.2006).

B. 9.5.1-e Using artificial soil (*Folsomia candida*)

Please refer to comments made in the DAR 2005 and Reporting table (17.11.2006).

B.9.5.1-f - Extended laboratory study (*Folsomia candida*)

Please refer to comments made in the DAR 2005 and Reporting table (17.11.2006).

Plant protection product (IIIA 10.5.1)

Please refer to comments made in the DAR 2005.

B.9.5.2 Risk assessment

Please refer to comments made in the DAR 2006.

B.9.5.2.1 Tier I risk assessment for non-target arthropods

In principle, the value of 2 for the HQ is only applicable for the first tier LR50 tests with the species *Aphidius rophalosiphi* and *Typhlodromus pyri*. Anyhow the standard risk assessment for Non-target Arthropods (Terrestrial Ecotoxicology guidance document (SANCO/10329/2002 rev 2 final, 17 October 2002) has been developed for spray applications that result in a risk due to spray deposition and spray drift; hence for the application of Chloropicrin the calculation of the hazard quotients (HQs) is not appropriate. In any case pronounced effects on soil-dwelling arthropods can be expected in the field (bare soil under tarp).

Tests with *Pardosa* spp. and *Poecilus cupreus* on artificial substrates resulted in effects on mortality of up to 100% in both species. The effects recorded in each study are clearly above the trigger value of 30%, indicating the requirement for higher tier testing.

B.9.5.2.2 Higher Tier risk assessment

The off-field risk is considered to be low, due to the method of tarp application that prevents lateral movement of Chloropicrin in soil outside the tarp edge during the first 8d after application. The inhalation exposure route to non-target organisms off-site is possible. Data on concentrations in air are available (see IIA 7.3.3/01) which never exceeded 0.31 mg/m³ air at 1 metre distance of the treated field. The results of a bee inhalation study (IIA 8.7/01) provided a NOEC of ≥ 82.2 mg/m³ thus indicating that the off-field risk to non-target arthropods can be considered as very low.

Conclusion: Chloropicrin applied at field rate of 272 L/ha, had severe effect after initial exposure (4 days after treatment), but no effect on survival, prey consumption or reproduction were observed on the four non-target arthropods species tested upon exposure to 14 days aged residues (18d after treatment). Overall, in-field data indicate that the risk to ground dwelling arthropods could be acceptable based on the potential for recolonisation within an acceptable period.

B.9.6 Effects on earthworms (IIA 8.4.1, IIIA 10.6.1)

B.9.6.1 Acute toxicity

Active substance (IIA 8.4.1)

The acute risk assessment for the soil injection application is reported below (see DAR 2006)

Table 9.6.1 – 1: Acute Toxicity Exposure Ratio (TER) for earthworms

Method of application	Test species	LC _{50corr} (mg as/kg dw soil)	PEC _s (mg as/kg)	TER _A	Trigger
Soil Injection (20 cm depth)	Earthworm	37.75	149.33	0.253	< 10

Values in **bold** are below the relevant Annex VI Trigger

The TER value calculated for Earthworms is below the relevant Annex VI trigger value of 10 for application of Chloropicrin, indicating unacceptable risk.

Plant protection product (IIIA 10.6.1.1)

Please refer to comments made in the DAR 2006

B.9.6.2 Sub-lethal effects

The need of long-term risk assessment was identified in the original DAR. A request was made by the RMS and several MS for sublethal/chronic data to enable the assessment of the potential for earthworm to colonize the treated area. In response, the Notifier submitted a study to assess the effects of aged field soil residues on reproduction and growth of *Eisenia fetida* (see below). In addition, the Notifier commissioned a laboratory based artificial soil reproduction study using *Eisenia fetida*. However the Notifier informed that, due to the technical difficulty of dosing the artificial soil with the very small amounts of Chloropicrin required and the experimental issues associated with the relevant analytical methods for detection in the soil medium, this study faced significant delays. These delays meant that the results of this study are not within the timescale of this re-submission dossier. As such, no earthworm reproduction NOEC can be established at this stage.

Rogers, M. H., 2009b, Chloropicrin To Determine the Effects of Aged Soil Residues on Reproduction and Growth of the Earthworm, *Eisenia fetida*. Huntingdon Life Sciences Ltd., UK. Unpublished report SXC0029. Owner European Chloropicrin Group. (IIA 8.9.2/02)

GLP: Yes
Guideline: OECD 222 (2004)

A study was performed to determine the effects of aged soil residues of Chloropicrin to the reproduction and growth earthworm, *Eisenia fetida*, under laboratory conditions. Soil samples from two plots of field sites (England) that had previously been treated at an application rate of 271 L/ha were used. The soil was sandy-loam. Applications had been made approximately 28 and 56 days earlier for soil A and soil B, respectively. An untreated field soil was also used as a control group (10 replicates). Bulk samples (20x20x20cm) were taken along the midline of each plot.

Each replicate contained 10 adult worms with 6 replicates per treatment level for the treated field soils and 4 replicates for the artificial soil (OECD soil) group. Adult worms were individually rinsed with distilled water and weighed before being placed on the surface of the soil. They were fed cow faeces. Following 28 days exposure the mortality of the adult worms was assessed, adult worms were removed from the soil. Weights of live adult worms were recorded in treatment replicates at Time 0 and on Day 28. After 56 days the number of surviving juvenile worms per container was determined. The number of juvenile worms and weights of adult worms in soil A, soil B and laboratory soil control were analyzed by Dunnett's test and compared with the field soil control.

Field observations

At the time of collection of the soils for the in-life phase of the study, an evaluation of earthworm populations adjacent to the treated sites, and from the control soil site, was undertaken. Triplicate 0.5 metre square quadrates were placed at random at distances of 1, 5 and 10 metres (or 8m) from the edge of the treated sites. Triplicate 0.5 metre square quadrats were placed at distances of 1, 5 and 10 metres from the edge of the treated sites (8 m at one of the treated sites due to space limitations). Five litres of a 0.08% solution of formaldehyde was slowly watered onto each quadrat. Earthworms emerging were collected and retained for counting and identification. The top 20 cm of soil within each quadrat was subsequently removed and searched for earthworms.

Results:

The soil analytical results indicate a mean measured concentration of 0.200 mg/kg in the one month old treated soils and 0.275 mg/kg in the two month treated soils. After 56d, the mean measured concentration was 0.080 mg/kg and 0.132 mg/kg, in the one month and two months aged soils, respectively.

There were no treatment related mortalities in the adult worms and no abnormalities recorded at Day 28. A single mortality was recorded in Control group 2 (field soil).

Food consumption was reduced in all three groups using field soil compared to the control in artificial soil.

Bodyweights increased in all groups although the artificial soil control group showed the highest increase. The analysis shows that the artificial soil group and test soil B had significantly higher body weight compared to the field soil control group, while group A was not statistically different.

Table 9.6.2-01: Group mean bodyweights (mg): Adult worms

Group	Treatment	Characterisation	Day of study		Change
			Day 0	Day 28	
Group 1	Control 1	Artificial soil	377	618	+ 64*
Group 2	Control 2	Field soil	378	435	+ 15
Group 3	Test Soil A	Field soil 1 month post application	359	439	+ 22
Group 4	Test Soil B	Field soil 2 months post application	354	503	+ 42*

*- significantly different from field soil control.

On day 56 of the study the numbers of juveniles in the four groups were assessed. Juvenile counts in the two test soils were compared to the control field soil to determine if residual Chloropicrin had any effect on the relative control levels. In the control soil the mean number of juveniles was found to be 1.6 (no juveniles in 4 of ten replicates), in the one month aged sample the mean number of juveniles was found to be 0.5. There were no juveniles recovered in the 2 month treated soil. In the artificial soil control, 182 juveniles were counted. Statistical analysis was not carried out to compare treated soils with the field control.

The results of this study demonstrate that while the levels of reproduction in the field soils were extremely low that reproduction is possible in soils treated with Chloropicrin one month after treatment.

Evaluation of field populations of earthworms adjacent to the treated sites

In order to investigate the potential for recovery, the presence and abundance of earthworms in the soil adjacent to the treated area was established. There was no consistent difference between counts obtained in association with the plot aged one month (Group 3, A) as compared with the plot aged two months (Group 4, B), nor was there any consistent trend associated with distance from the treated plot in either case. Overall, the counts for groups 3 and 4 were comparable with, or slightly higher than the counts for the control field soil plot (Group 2).

SXC0029

Appendix 3 Field earthworm population assessment 20 May 2009 (Day 0)

Group/Treatment	Distance from treated plot	Replicate	Number of worms recovered		
			Count 1	Count 2	Total
Group 2 Control Field soil	N/A (within- plot)	1	2	1	3
		2	2	3	5
		3	0	3	3
		4	0	2	2
		5	2	4	6
		6	0	4	4
		7	1	3	4
		8	1	2	3
		9	3	3	6
		Mean	1.2	2.8	4.0
		sd			1.4
Group 3 Test Soil A (aged 1 month)	1 m	1	8	7	15
		2	2	3	5
		3	4	12	16
		Mean	4.7	7.3	12.0
		sd			6.1
	5 m	1	1	4	5
		2	0	5	5
		3	3	2	5
		Mean	1.3	3.7	5.0
		sd			0
	8 m	1	0	3	3
		2	1	5	6
		3	13	18	31
		Mean	4.7	8.7	13.3
		sd			15.4
Group 4 Test Soil B (aged 2 months)	1 m	1	8	1	9
		2	9	6	15
		3	0	1	1
		Mean	5.7	2.7	8.3
		sd			7.0
	5 m	1	5	4	9
		2	6	7	13
		3	6	7	13
		Mean	5.7	6.0	11.7
		sd			2.3
	10 m	1	1	2	3
		2	3	5	8
		3	3	4	7
		Mean	2.3	3.7	6.0
		sd			2.6
Count 1	Worms collected from soil surface following formalin treatment				
Count 2	Worms collected from soil removed/examined to 20 cm depth				
sd	standard deviation				

Acceptability –

Soil samples were taken from treated plots, but no mention is made in the report about the method of treatment (injection?), the tarping and its duration. This needs to be clarified. Pending on confirmation that the application procedure is relevant to the proposed GAP, the results of the in-phase study are acceptable only for the 28d survival and growth results. The results of the field populations provide supportive information about the potential for recovery.

Comments of the RMS

At the start of the test, the concentration in soil B (0.275 mg/kg) is higher than the concentration in soil A (one month aged, 0.200 mg/kg). This difference is maintained at the end of the study (day 56), with the concentration decreasing in both soils. No explanation is given in the report for this finding.

No adverse effects on adult earthworms (survival and growth) were recorded in worms exposed at aged residue up to 0.275 mg/kg (initial concentration).

The effects of chloropicrin treatment on reproduction measured in the laboratory are difficult to determine due to the extremely low reproduction in the field control and no statistical analysis was carried out. This indicates that the field control soil was not adequate for worm reproduction. The fact that in soil A a few juveniles were found while none was recorded in soil B might be explained by the fact that in soil A, although having a shorter aging time (one month), the concentration of chloropicrin was lower than that measured in soil B (two months aged). Therefore, due to the variability in chloropicrin concentrations, it is not correct to conclude that after one month aging, reproduction is demonstrated. The results of the in-house test are considered inconclusive. No NOEC can be derived.

The results of the field monitoring suffer from the same limitation due to the low number of juveniles found in the control field sample. The in-field worm population was not monitored after treatment. The concentrations of the test compound at various distances from the treated area are not reported and therefore the lack of a trend associated with distance cannot be discussed in terms of concentration.

In conclusion, the study provides evidence about the survival of worms 1 month after treatment both in-field and off-field, but not evidence about adequate reproduction.

B.9.6.3 Risk assessment

In the study on aged residues (Rogers, M. H., 2009b, see above), Chloropicrin concentration measured 2 months after treatment (0.275 mg/kg) was about 100 times lower than the NOEC mortality of the acute test (14d NOEC=21 mg/Kg). The results of this study confirm the lack of effects on survival and growth expected at these concentrations but from this study a NOEC for reproduction could not be retrieved. The finding of worms in areas adjacent to the treated area one month after treatment indicates the presence of a source of living worms with a potential to recolonize the treated area, but the study does not provide conclusive data on the time necessary for a successful reproduction and full recovery of the population.

Conclusion

The RMS considers the point still open and proposes to wait for the results of the ongoing reproduction test for a final risk assessment.

B.9.7 Effects on other soil non-target macro-organisms (10.6.2)

Please refer to comments made in the DAR 2006.

B.9.8 Effects on soil non-target micro-organisms (IIA 8.5, IIIA 10.7.1)

B.9.8.1 Toxicity

In the study by Carter (2003) summarized in the DAR 2005, the soil function resulted compromised at 98 days. The recovery of the observed „representative“ microbial populations (i.e., aerobic bacteria, aerobic bacterial spores, *Pseudomonas* species, Actinomycetes and fungi) within the 98 days is not sufficient *per se* to demonstrate recovery of the soil function within an acceptable period. In fact, in the study the microbial populations have not been

sorted by functional/taxonomic groups, so that it is difficult to relate results to nitrogen transformation micro-organisms. In addition, in the study the soil samples were taken from soil covered for 7 days with VIF, instead of the 8 days proposed in the updated GAP. To evaluate the impact of Chloropicrin on soil non-target micro-organisms and demonstrate recovery of the soil functions (nitrogen transformation and carbon transformation activity), two additional studies were submitted (one laboratory and one field study), which are summarized below..

Carter, J.N., 2008, Chloropicrin: Effects on Soil Non-Target Micro-Organisms: Nitrogen Transformation, unpublished report No.: SXC0019 (IIA 8.10.1/02)

Guideline : OECD Guideline for the Testing of Chemicals No. 216 "Soil Microorganisms: Nitrogen Transformation Test, (January 2000) and SETAC – Europe (March 1995) "Procedures for assessing the environmental fate and ecotoxicity of pesticides: 4 – Soil Microorganisms".

Deviation: test duration was 142d (instead of a maximum of 100d).

GLP : Yes

The test soil was a sandy loam soil obtained from a site in England that had received no pesticides or fertilisers for at least 2 years prior to sampling. The microbial biomass of the test soil was 3.12% of the total organic carbon (TOC = 1.2%). Nitrogen transformation was determined in soil covered for 7 days with Virtually Impermeable Film (Group 1 - untreated control soil, Group 2 - 1 x MAR chloropicrin, Group 3 - 5 x MAR chloropicrin), and in uncovered soil (Group 4 - untreated control soil, Group 5 - 1 x MAR chloropicrin). Chloropicrin was administered by soil injection at a soil depth of 20 cm, where MAR is Maximum Application Rate per year. Each group replicate was amended with lucerne meal (0.5%, dw soil) as a nitrogen source and incubated in the dark at 20 ± 2 °C. Replicates were sampled and soil extracts analysed at 0, 7, 14, 28, 56, 100 and 142 days for ammonium, nitrite and nitrate ion concentrations. Since the nitrite concentrations were negligible (less than 0.5 µg N/g soil dry weight) the values for total oxidised nitrogen were considered to represent the concentration of nitrate (µg N/g soil dry weight) in each soil sample. Assessments of the viability of representative microbial populations in the control and treated soils (aerobic bacteria, aerobic bacterial spores, *Pseudomonas* species, *Actinomycetes* and fungi) were made over the study period using standard agar pour plate counting procedure. This assessment indicated that Chloropicrin had no significant long term effect on populations of aerobic bacteria, aerobic bacterial spores or fungi but was shown to exert a long term influence on *Pseudomonas* bacteria and *Actinomycetes*, reducing the populations of these soils micro-organisms. The results for nitrogen transformation demonstrated that treatment of soil with Chloropicrin (1 x MAR – 272 litres/ha and 5 x MAR – 1360 litres/ha) and covering with virtually impermeable film for 7 days resulted in long term influence of nitrogen transformation activities in soil, evident from the results at the end of the study on Day 142 where the deviations in nitrogen transformation activity compared to non-treated control soil were greater than 25%. However, where virtually impermeable film covering was not employed it was noted that significant recovery of nitrogen transformation activity occurred in soil treated with Chloropicrin (1 x MAR) by the end of the study period.

Table 1 Analysis of ammonium levels in soil - group mean values ($\mu\text{g/g}$ dry weight soil)

Treatment group	Group	N	Day	Mean	Top Soil Sd	sed	Mean	Bottom Soil sd	sed	Soils without film comparison Mean sd sed
Control with film	1	3	0	8.79	0.449	0.403	8.46	0.837	0.450	
1 x MAR with film	2	3	0	4.00***	0.312		4.83***	0.405		
5 x MAR with film	3	3	0	3.45***	0.657		4.38***	0.220		
Control without film	4	3	0							14.31 1.039 0.746
1 x MAR without film	5	3	0							15.97 0.771
Control with film	1	3	7	23.95	1.250	0.711	24.88	1.220	0.678	
1 x MAR with film	2	3	7	15.54***	0.829		13.98***	0.518		
5 x MAR with film	3	3	7	11.81***	0.161		11.26***	0.558		
Control without film	4	3	7							4.42 0.492 0.618
1 x MAR without film	5	3	7							5.97 0.951
Control with film	1	3	14	1.24	0.507	0.619	0.00	0.000	0.522	
1 x MAR with film	2	3	14	11.53***	1.197		12.72***	1.101		
5 x MAR with film	3	3	14	9.80***	0.183		12.28***	0.127		
Control without film	4	3	14							0.00 0.00 1.936
1 x MAR without film	5	3	14							11.36++ 3.354
Control with film	1	3	28	1.22	0.122	2.119	0.00	0.000	1.859	
1 x MAR with film	2	3	28	88.07***	3.662		73.10***	0.783		
5 x MAR with film	3	3	28	59.66***	2.603		65.75***	3.866		
Control without film	4	3	28							5.54 0.196 1.347
1 x MAR without film	5	3	28							4.91+++ 2.324

Treatment group	Group	N	Day	Mean	Top Soil Sd	sed	Mean	Bottom Soil sd	sed	Soils without film comparison Mean sd sed
Control with film	1	3	56	1.38	0.518	7.653	0.95	1.021	4.181	
1 x MAR with film	2	3	56	94.36***	10.861		79.06***	7.320		
5 x MAR with film	3	3	56	99.48***	12.056		76.06***	4.903		
Control without film	4	3	56							1.95 0.220 1.663
1 x MAR without film	5	3	56							91.36+++ 2.872
Control with film	1	3	100	0.20	0.214	6.730	1.54	0.186	4.322	
1 x MAR with film	2	3	100	129.47***	8.444		86.53***	9.028		
5 x MAR with film	3	3	100	101.63***	11.508		67.62***	1.584		
Control without film	4	3	100							2.80 0.370 4.66
1 x MAR without film	5	3	100							150.33+++ 8.067
Control with film	1	3	142	2.82	0.457	2.142	0.28	0.176	5.536	
1 x MAR with film	2	3	142	113.64***	1.427		94.08***	5.947		
5 x MAR with film	3	3	142	126.06***	4.290		84.01***	10.126		
Control without film	4	3	142							2.11 0.625 0.854
1 x MAR without film	5	3	142							72.00+++ 1.341

n Number of replicates
sd Standard deviation
sed Standard error of the difference between groups
*** $p < 0.001$ (Dunnett's test)
++ $p < 0.01$ (t-test)
+++ $p < 0.001$ (t-test)

Table 2 Analysis of nitrate levels in soil - group mean values ($\mu\text{g/g}$ dry weight soil)

Treatment group	Group	N	Day	Mean	Top Soil Sd	sed	Mean	Bottom Soil sd	sed	Soils without film comparison Mean sd sed
Control with film	1	3	0	5.62	0.427	0.115	5.20	0.153	2.090	
1 x MAR with film	2	3	0	7.93***	0.486		7.16***	0.093		
5 x MAR with film	3	3	0	9.03***	0.993		8.52***	0.122		
Control without film	4	3	0							5.52 0.335 0.440
1 x MAR without film	5	3	0							5.64 0.684
Control with film	1	3	7	22.55	0.387	0.231	18.89	1.522	0.723	
1 x MAR with film	2	3	7	6.88***	0.122		7.14***	0.093		
5 x MAR with film	3	3	7	11.00***	0.275		10.11***	0.161		
Control without film	4	3	7							23.18 1.072 0.720
1 x MAR without film	5	3	7							5.72+++ 0.636
Control with film	1	3	14	54.81	1.152	0.625	49.19	3.073	1.480	
1 x MAR with film	2	3	14	11.02***	0.581		9.62***	0.426		
5 x MAR with film	3	3	14	13.13***	0.306		12.05***	0.483		
Control without film	4	3	14							47.77 1.519 0.966
1 x MAR without film	5	3	14							8.67+++ 0.703
Control with film	1	3	28	65.14	2.634	1.284	55.48	0.549	0.348	
1 x MAR with film	2	3	28	10.17***	0.641		7.85***	0.322		
5 x MAR with film	3	3	28	8.75***	0.253		7.47***	0.372		
Control without film	4	3	28							62.95 1.914 1.160
1 x MAR without film	5	3	28							7.83+++ 0.610

Treatment group	Group	N	Day	Top Soil			Bottom Soil			Soils without film comparison		
				Mean	Sd	sed	Mean	sd	sed	Mean	sd	sed
Control with film	1	3	56	85.31	10.723	5.350	52.40	27.082	12.774			
1 x MAR with film	2	3	56	3.21***	1.233		2.86*	0.316				
5 x MAR with film	3	3	56	9.23***	3.505		8.64*	0.878				
Control without film	4	3	56							39.25	12.710	7.347
1 x MAR without film	5	3	56							5.36++	0.618	
Control with film	1	3	100	91.64	5.122	2.438	96.35	27.303	12.873			
1 x MAR with film	2	3	100	3.17***	0.161		3.61***	0.405				
5 x MAR with film	3	3	100	11.16***	0.706		11.30***	0.246				
Control without film	4	3	100							78.01	3.882	2.328
1 x MAR without film	5	3	100							16.60+++	1.090	
Control with film	1	3	142	122.08	9.564	4.522	88.24	6.090	2.884			
1 x MAR with film	2	3	142	3.90***	0.581		2.56***	0.426				
5 x MAR with film	3	3	142	8.26***	0.461		9.94***	0.405				
Control without film	4	3	142							86.69	5.569	5.267
1 x MAR without film	5	3	142							45.48++	7.325	

n Number of replicates

sd Standard deviation

sed Standard error of the difference between groups

* $p < 0.05$ (Dunnett's test)

*** $p < 0.001$ (Dunnett's test)

++ $p < 0.01$ (t-test)

+++ $p < 0.001$ (t-test)

Acceptability: acceptable.

The variation between the replicate control samples in some of the controls exceeded 15 % in the longer time frame (56 – 100 days). This represents a deviation from the validity OECD criterion, but considering that in any case the results indicate a remarkable deviation of the treated groups from the control, this deviation does not affect the conclusion of the test.

Carter, J.N., 2009, Chloropicrin: Evaluation of Effects on Soil Non-Target Micro-Organisms following Commercial Application of Chloropicrin to Soil in the UK in 2009. Huntingdon Life Sciences Ltd., UK. Unpublished report No.: SXC0028. Owner European Chloropicrin Group. (IIA 8.10.1/03)

GLP: Yes for the in-phase study, except for the carbon-nitrogen content of lucerne meal and soil analysis. GLP compliance is claimed also for the field activities, although some of these were conducted by a non GLP contractor under the supervision of the GLP laboratory.

Guidelines: There were no guidelines applicable to the conduct of the field phase of this study. The test procedures used in the laboratory analytical phase of this study were conducted according to OECD Guideline for the Testing of Chemicals No. 216 "Soil Microorganisms: Nitrogen Transformation Test", OECD Guideline for the Testing of Chemicals No. 217 "Soil Microorganisms: Carbon Transformation Test", and SETAC – Europe (March 1995) "Procedures for assessing the environmental fate and ecotoxicity of pesticides: 4 – Soil Microorganisms".

Methods:

The test soil was a sandy loam soil obtained from a site in England (pH= 5.82). The microbial biomass of the test soil was 3.12% of the total organic carbon (TOC =0.7%).

Chloropicrin was tested at two treatment concentrations, the lower concentration was applied at a maximum application rate (MAR) of 272 litres Chloropicrin/hectare (achieved 283 l/ha) and the higher rate was at 1.5 x MAR (400 l/ha) (achieved 389 l/ha), the maximum achievable application rate, based on administration of the test material by soil injection at a depth of 20 cm. The two treated plot measured 3.5 m wide and 50m long.

Nitrogen and Carbon transformation were determined in soil, treated and untreated, covered for 8 days with Virtually Impermeable Film (VIF). Three replicate soil samples were taken from each plot on day 9,16, and 29 (28d following treatment) An assessment of the viability of representative microbial populations in the control and Chloropicrin treated soils in this study was also made. Portions of each soil group replicate were sampled on Day 0, Day 7, Day 14 and Day 28 of the laboratory phase. On Day 0 of the laboratory phase the soil samples were amended with lucerne meal as a nitrogen source (carbon: nitrogen ratio of 13.9:1), added at a

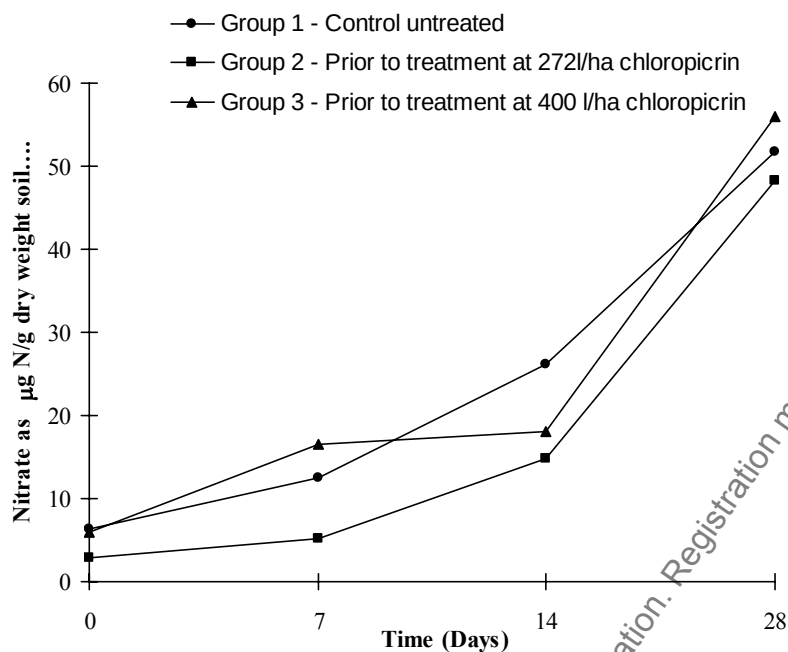
maximum of 0.5% of the soil dry weight. In addition, on Day 0, within 6 hours of amendment with lucerne meal and adjustment of moisture content, a 20 g soil sample was withdrawn from each of the 3 replicate soil samples per group. The soil samples were either stored in a deep freeze (-20 °C) and then thawed prior to extraction or extracted on the same day as sampling, and the supernatant solutions were analysed on the same day or transferred to a deep-freeze at approximately -20 °C to await analysis. Sampling in this manner was also performed on Days 7, 14 and 28 of the laboratory phase. For assessment of nitrogen transformation, soil sample extracts were analysed for nitrate and nitrite ion concentrations using continuous flow colorimetric autoanalyser. For assessment of carbon transformation, rates of carbon respiration were determined using an ADC 2250 Infrared Gas Analyser. Assessments of the viability of representative microbial populations in the control and treated soils (aerobic bacteria, aerobic bacterial spores, *Pseudomonas* species, *Actinomyces* and fungi) were made over the study period using standard agar pour-plate counting procedure.

Results:

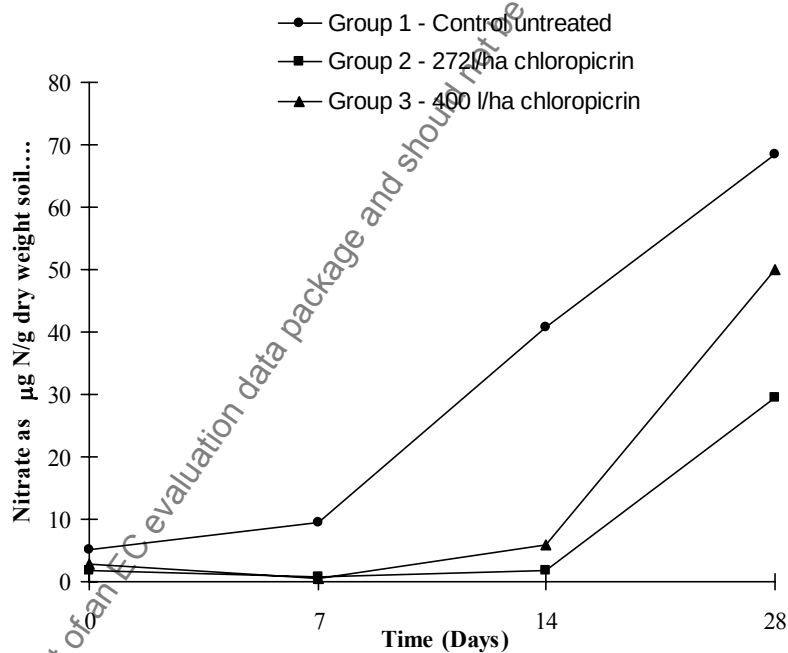
Nitrogen transformation in all soils sampled from the field on day 0 prior to application was evident from the increase in nitrate concentrations over the study period. Nitrate concentration in all groups differed by less than 25% at the end of the 28 d lab phase indicating that cumulative nitrate transformation activity was similar across the test site at the beginning of the study.

When the nitrate formation rate was calculated on a sequential basis, the differences between the treated groups and the control were larger than 25% already in the samples taken one day prior to application again reflecting the in-field variability. However, the deviations were not consistent, but varied considerably between the different measurement intervals. In contrast, the overall formation rates showed a much less variable pattern. Therefore in order to account for this background variation in natural field soils and to capture the treatment related overall pattern, the soil nitrate formation rate was also calculated on a cumulative basis, i.e. for the whole 0 - 28 day laboratory evaluation period for each field sampling day. This overall evaluation levels out short-term fluctuations in natural field soils and allowed detection of the underlying pattern of chloropicrin effects on the nitrate formation rate of the soil microflora. In the samples obtained prior to chloropicrin application, the overall nitrate formation rates deviated from the controls by -0.61% and 9.82% in the low and high dose groups, respectively. Following chloropicrin application, the deviations in the overall nitrate formation rates for treated soils sampled on study Days 9 and 16 ranged between -25.22 % and -72.63%, indicating clear short term effects of chloropicrin on the nitrate formation rate at both treatment rates. However, these effects diminished within one month following chloropicrin application. In the samples taken from the field on Day 29 the overall nitrate formation rates deviated from the controls by 8.13% and 20.84% in the low and high treatment groups, respectively. These deviations were less than the trigger value of 75% set in the OECD guideline 216.

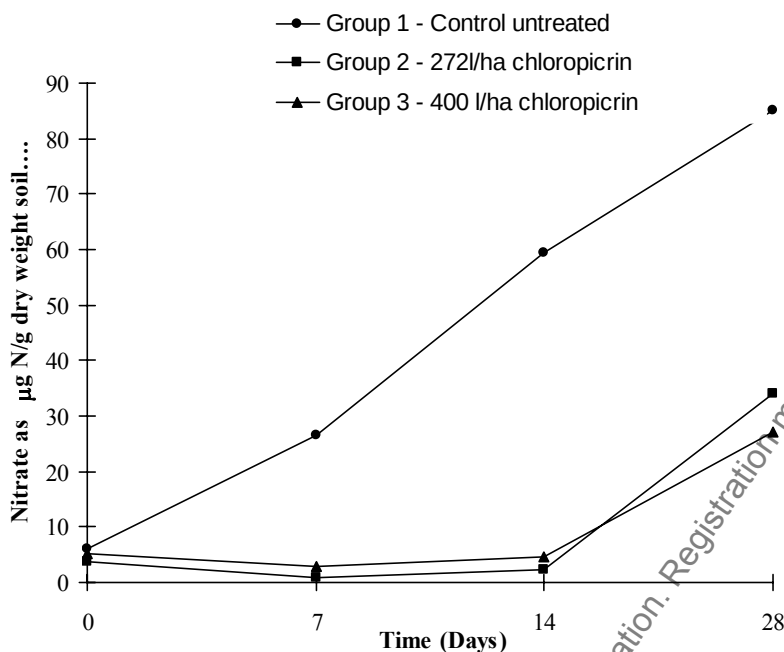
Nitrate concentrations, Day 1 prior to treatment



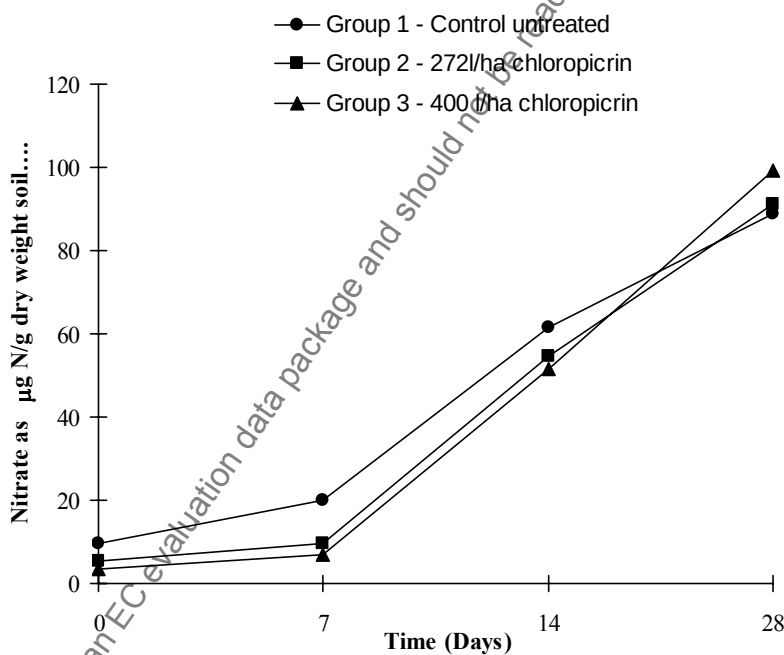
Nitrate concentrations, Day 9 following treatment



Nitrate concentrations, Day 16 following treatment



Nitrate concentrations, Day 29 following treatment

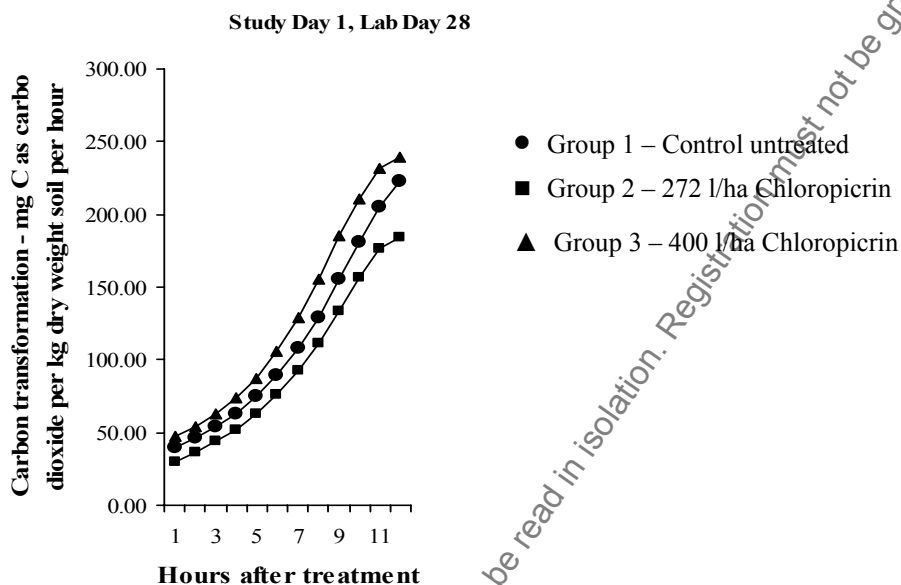


Carbon

Differences in carbon transformation rates in soil samples taken on Day 1 prior to chloropicrin application were -14.48% and 18.57% for the low and high treatment rates compared to the non- treated control. These were within the trigger value of 25% set by OECD guideline 217 at the end of the 28 day laboratory phase. After chloropicrin application, the overall carbon transformation rates in chloropicrin treated groups deviated from the non-treated control by Days 9 and 16). However, in the field samples taken on Day 29, at the end

of the 28 days laboratory phase the differences in the overall carbon transformation rates in the low and high chloropicrin treatment groups compared to the non-treated control were within the trigger value of 25% set by OECD guideline 217 (-9.88 % and 0.85% respectively) indicating that treatment related effects on carbon transformation observed for Day 16 samples were only transient.

Carbon transformation rates, Day 1 prior to treatment



Carbon transformation rates, Day 9 following treatment

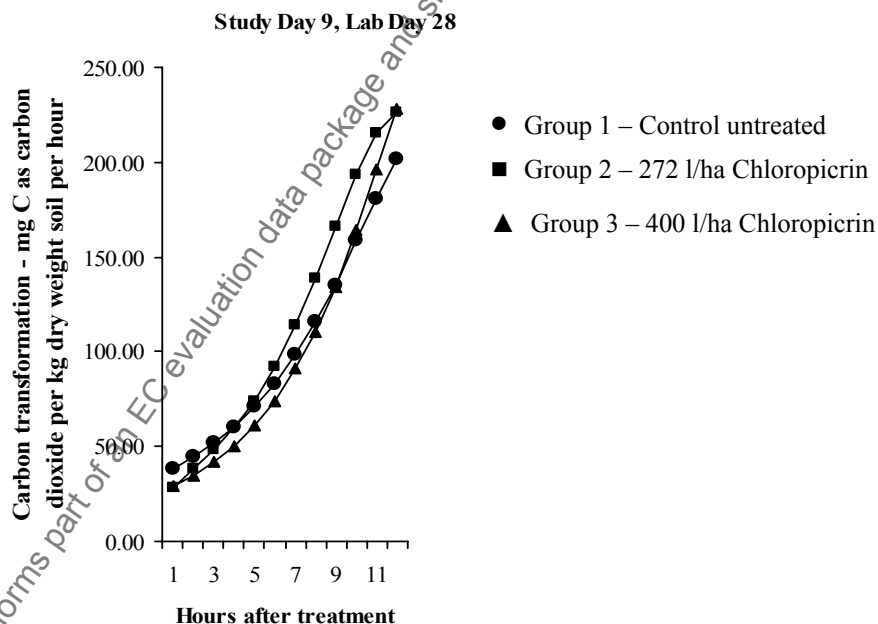
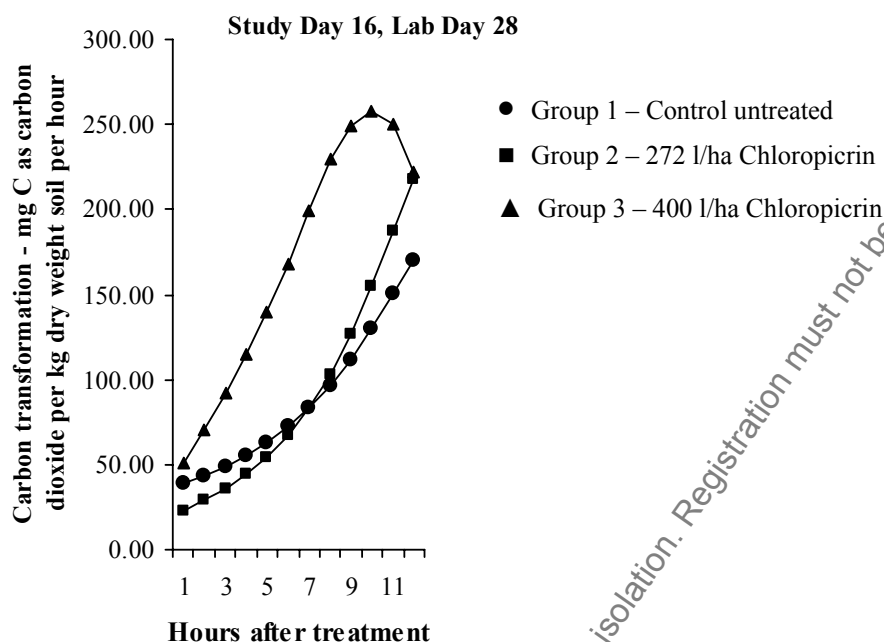
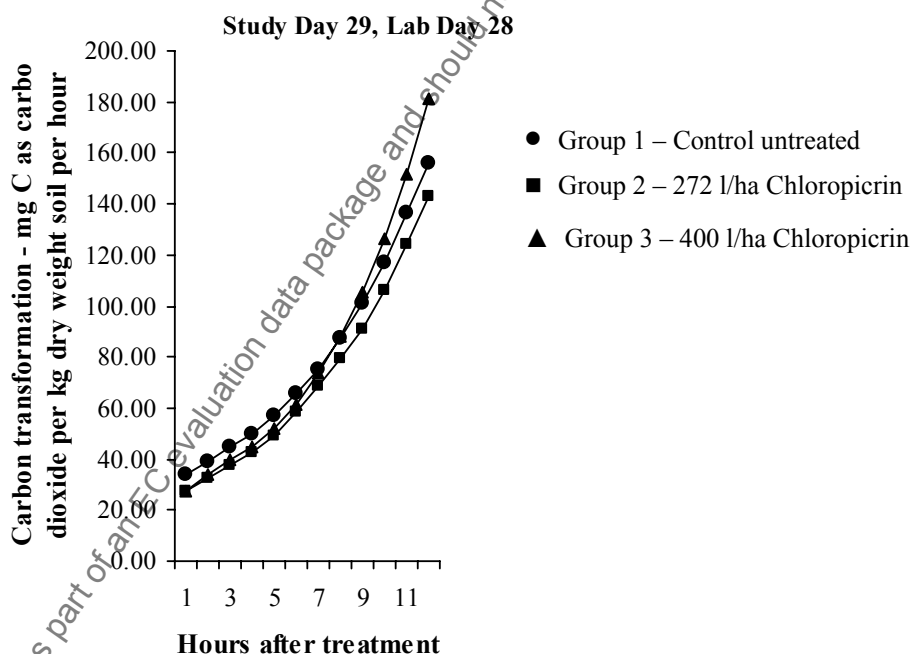


Figure IIA 8.10.1-7: Carbon transformation rates, Day 16 following treatment



Carbon transformation rates, Day 29 following treatment



Microbial populations

The assessment of the viability of representative microbial populations in the control and Chloropicrin treated soils indicated that Chloropicrin treatment exerted a transient inhibitory

effect on populations of aerobic bacteria, *Pseudomonas* species, Actinomycetes and in particular on fungi. By the end of the study period each of these microbial populations showed a recovery in viable numbers to levels comparable with those at the start of the study prior to treatment.

Conclusions:

It was concluded that the application of chloropicrin at field rates of 272 and 400l/ha resulted in consistent transient effects on soil microflora in terms of both carbon and nitrogen transformation rate. Relevant effects were observed until day 16 following treatment. At the end of the study (29 days following treatment), deviations in overall formation rates between the non-treated control and chloropicrin treated groups were below the regulatory trigger of $\pm 25\%$, indicating no long term effects on soil microbial populations and soil functions.

Acceptability: acceptable.

B.9.8.2 Risk assessment

In the laboratory study (Point IIA 8.10.1/02), treatment of soil with Chloropicrin (1 x MAR – 272 litres/ha and 5 x MAR – 1360 litres/ha) and covering with VIF for 7 days resulted in long term influence of nitrogen transformation activities in soil up to Day 142 (end of the study). Deviations in nitrogen transformation activity compared to non-treated control soil were greater than 25%, indicating unacceptable effects. A long-term reduction of population of *Pseudomonas* bacteria and *Actinomycetes* was also recorded. Although the study is not fully reliable, it provides evidence of long term effects of under laboratory conditions.

In the field study (Point IIA 8.10.1/03), the application of chloropicrin at field rates of 272 and 400l/ha (with 8d tarping) resulted in consistent transient effects on soil microflora in terms of both carbon and nitrogen transformation rate. Relevant effects were observed until day 16 following treatment. At the end of the study (29 days following treatment), deviations in overall formation rates between the non-treated control and chloropicrin treated groups were below the regulatory trigger of $\pm 25\%$, indicating no long term effects on soil microbial populations and soil functions. By the end of the study period, microbial populations showed a full recovery in viable numbers.

From the field study, it can be concluded that field applications of Chloropicrin are expected to have no long term influence on soil micro-flora under field conditions. Transient effects are observed, which recovered within one month. Hence, an acceptable risk is posed to soil microorganism.

B.9.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (IIA 8.6, IIA 10.8)

Active substance

Effects on Terrestrial Vascular Plants (IIA 8.12)

Weinberg, J.T. 2009. Toxicity of Chloropicrin to six plant species: corn, soybean, ryegrass, oilseed rape, cucumber and sunflower. WIL Research Laboratories, USA. Unpublished report WIL- 697004. Owner European Chloropicrin Group. (IIA 8.12/01),

GLP: Yes

There are no standardized guidelines for conducting vapor-phase plant studies. This study has been designed based on the following OECD methods, with modifications for gaseous exposure.

Organisation for Economic Cooperation and Development (OECD) Guideline for The Testing of Chemicals 208, Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test, Draft Guideline, September, 2003.

Organisation for Economic Cooperation and Development (OECD) Guideline for The Testing of Chemicals 227, Terrestrial Plant Test: Vegetative Vigor Test, July, 2006.

The objective of this study was to evaluate potential adverse effects of Chloropicrin on seed germination and vegetative vigor (young plant growth) when administered as a vapor for 2 consecutive daily 6-hour exposure periods. Effects were established by identifying endpoints based on analyzed air concentrations. During the course of the study vegetative vigor was identified as being the most sensitive endpoint while seedling emergence was largely unaffected, except in the ryegrass where some effects were observed at the highest test concentrations.

For the seedling emergence portion of the study, 3 sunflower, corn, soybean and cucumber seeds, 10 ryegrass seeds and 5 oilseed rape seeds were planted in each of 10 pots for a total of 30, 30, 30, 30, 100 and 50 seeds for each test concentration.

For the vegetative vigour of the study, 2 sunflower, corn, soybean and cucumber seeds, 10 ryegrass seeds and 5 oilseed rape seeds were planted in each of 10 pots for a total of 20, 20, 20, 20, 100 and 50 seeds for each test concentration.

Table B.9.9-1: Target vapour exposure levels in seedling emergence study:

PARAMETER Group (Group 1 = controls)	Mean Chloropicrin Test Concentration (% of target)		
	Group 2 (200 ppb)	Group 3 (1,000 ppb)	Group 4 (10,000 ppb)
Nominal Concentrations	251 (126)	1082 (108)	9771 (98)
Analyzed Concentrations	199 ppb (100)	1018 ppb (102)	10082 (101)

Table B.9.9-2: Target vapour exposure levels in vegetative vigour study:

PARAMETER	Mean Chloropicrin Test Concentration (% of target)		
	Group 2 (200 ppb)	Group 3 (1,000 ppb)	Group 4 (10,000 ppb)
Nominal Concentrations	226 (113)	1057 (106)	9940 (99)
Analyzed Concentrations	204 ppb (102)	1046 ppb (105)	9880 ppb (99)

Based on the results from range-finding exposures, nominal exposure levels for the definitive study were 0, 0.2, 1.0 and 10 ppm (0, 200, 1000 and 10000 ppb). The measured concentrations varied between 100 and 102 % for the seedling emergence and between 99 and 105% for the vegetative vigour part.

On the day following the 14-day observation period, number and percent (%) emergence by replicate pot was calculated for each exposure concentration. Surviving plants were harvested (clipped) at the soil surface and weighed by replicate pot to determine wet biomass. Plant material was then dried at approximately 60 °C to a constant weight. The dried biomass weight was recorded for each replicate pot.

On the day following the 21-day observation period, surviving plants were harvested (clipped) at the soil surface and weighed by replicate pot to determine wet biomass. Plant material was then dried at approximately 60 °C to a constant weight. The dried biomass weight was recorded for each replicate pot.

Results:

There were no treatment related effects noted on seedling emergence at any of the concentration levels in any of the tested species except in Ryegrass which demonstrated some minor effects at the highest dose in wet biomass. This was attributed to the fact that the Ryegrass seeds were planted at a much shallower soil depth than other species.

Vegetative vigour was demonstrated to be the most sensitive endpoint with significant effects being noted at the highest concentration in all species.

Table B.9.9-3: Vegetative vigour study:

Parameter Test Group Species	Wet weight [g]				EC ₅₀ estimate [ppm]
	Group 1 (Control)	Group 2 (0.2 ppm)	Group 3 (1 ppm)	Group 4 (10 ppm)	
Sunflower	129.9 (100%)	97.1*+ (75%)	113 (87%)	12.1* (9%)	5.3
Oilseed rape	68.6 (100%)	55.7 (81%)	37.3*# (54%)#	6.7* (10%)	n.a.#
Soybean	19.4 (100%)	17.1 (88%)	14.1 (73%)	10.9* (56%)	>10
Corn	15.8 (100%)	22.7 (144%)	21.9 (139%)	25.8 (163%)	>10
Cucumber	150.2 (100%)	138.5 (92%)	155.3 (103%)	0* (0%)	5.6
Ryegrass	12.1 (100%)	11.4 (94%)	12.5 (103%)	9* (74%)	>10

* Statistically significantly different from the control

+ no dose-response relation, thus not considered to be treatment related

Result of physical damage due to handling, not considered to be treatment related

Table B.9.9-4 Vegetative vigour study:

Parameter Test Species	Dry weight [g]				EC ₅₀ estimate [ppm]
	Group 1 (Control)	Group 2 (0.2 ppm)	Group 3 (1 ppm)	Group 4 (10 ppm)	
Sunflower	10.8 (100%)	8.7 (81%)	9.2 (85%)	1.1* (10%)	5.2
Oilseed rape	6.3 (100%)	4.7 (75%)	3.2*# (51%)#	0.5* (8%)	n.a.#
Soybean	3.4 (100%)	3 (88%)	2.4 (71%)	1.8* (53%)	>10
Corn	1.2 (100%)	1.7 (142%)	1.7 (142%)	2 (167%)	>10
Cucumber	10.6 (100%)	10.6 (100%)	11.2 (106%)	0* (0%)	5.7
Ryegrass	1.4 (100%)	1.4 (100%)	1.4 (100%)	1.0* (71%)	>10

* Statistically significantly different from the control

Result of physical damage due to handling, not considered to be treatment related

A test substance-related decrease in wet and dry biomass was evident at the 10,000 ppb exposure level for sunflower, oilseed rape, soybean, cucumber, and annual ryegrass compared to the control groups. Cucumber plants did not survive after exposure to 10,000 ppb (10 ppm); therefore, there were no biomass weight data available for analysis. Lower wet and dry biomass was observed at the 1000 ppb exposure level for oilseed rape. Although the lower weight was statistically significant, plant tissue loss due to mechanical damage to the specimens introduced a confounding effect on both wet and dry biomass with this plant species. The damage occurred during daily observations which required moving of the plants and trays during observation sessions. Since the lower biomass was the result of physical damage, it was not considered to be test substance-related. A statistically-significant decrease in wet biomass at the 200 ppb (0.2 ppm) exposure level for sunflower was also observed; this observation did not follow a dose-response relationship and there were no differences in dry biomass compared to the control group. Therefore, the lower wet biomass in the 200 ppb (0.2 ppm) sunflower group was not considered to be a test substance-related effect.

Conclusions:

The no-observed-effect concentration (NOEC) for Chloropicrin vapour exposure of six plant species for 2 consecutive days was 1 ppm (1000 ppb) equivalent to 6.7 mg/m³, and the overall EC₅₀, taking into account the effects noted on all six species was determined to be 5.2 ppm (5200 ppb) equivalent to 35 mg/m³.

This study is considered to be acceptable.

Risk assessment

Table B.9.9-5.1 Summary of Effects of Vapor Exposure of Chloropicrin to Plants

Test species	Test system	Duration of exposure	Results	Reference
Monocots (Corn, Ryegrass) Dicots (Rape, Sunflower, Cucumber, Soybean)	Vapor exposure	2 periods of 6 hours exposure.	NOEC: 1 ppm (1000 ppb) equivalent to 6.7 mg/m ³ EC ₅₀ : 5.2 ppm (5200 ppb) equivalent to 35 mg/m ³	Weinberg, J.T. 2009

The risk assessment, as worst case, has been carried out taking into account the NOEC= 1000ppb level from the above study and the exposure levels as derived from the field emission study (Trevisan 2009, IIIA 7.3.3/01) the TER values in Tables B.9.9-6 are calculated. This study determined the field exposure levels at various distances from the treated sites. For the purposes of this risk assessment the most relevant end point is the maximum measured air concentration of 0.3077 mg/m³ equivalent to 45.9 ppb (1 metre from plot margin), which is considered to be relevant for non-target plants growing on adjacent areas. There is no relevant trigger value set by Annex VI of Directive 91/414 EEC for this type of exposure scenario, however a conservative approach would be to compare the vapor exposure EC₅₀ to the relevant exposure levels and apply the trigger value of 5 to the calculated TER (according to the Terrestrial Guidance Document).

Table B.9.9-5.2 Toxicity Exposure Ratio for Vapor Exposure of Chloropicrin to Plants

Test species	Exposure Levels	Duration of exposure	End Point	TER	Trigger
Monocots (Ryegrass, Corn) Dicots (Rape, Sunflower, Cucumber, Soy)	45.9 ppb 0.3077 mg/m ³	4 periods of 6 hours exposure.	NOEC 1ppm (1000 ppb) 6.7 mg/m ³	21.8	5

The calculated worst case TER in the above table indicates that there are no adverse effects expected due to Chloropicrin exposure to any non target plants in areas adjacent to treated fields.

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

In the DAR 2005 it was commented that, pending on reliable exposure data, a study might be necessary to assess the effect of chloropicrin to microorganisms in STP. EFSA supported this conclusion. In the resubmission dossier, the Notifier presented a study, which is summarized below.

Dickinson, R. 2009. Chloropicrin Activated Sludge: Respiration Inhibition Test. Huntingdon Life Science Ltd., UK. Unpublished report number SXC0014. Owner European Chloropicrin Group. (IIA 8.15/01)

GLP: Yes

Guideline: EC Directive 88/302, Method C.11, 'Biodegradation - Activated Sludge Respiration Inhibition Test' and OECD Test Guideline 209, 'Activated Sludge, Respiration Inhibition Test'. The test procedures were also based on methods outlined in United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances (OPPTS) Method 850.6800, 'Modified Activated Sludge, Respiration Inhibition Test for Sparingly Soluble Chemicals', Public Draft, April 1996.

Method

A sample of activated sludge was obtained from a domestic plant. In the laboratory, the sample was maintained under aerobic conditions until required. The concentration of suspended solids in a homogenised sample was determined on the day of collection and immediately before the start of the test. On the day of collection, the mixed liquor suspended solids (MLSS) content of the activated sludge was then calculated. Synthetic sewage (50 mL/L) was added to the stock of activated sludge and aerated overnight.

On the day of the test, the MLSS content of the sludge was determined (in triplicate) and adjusted to 4 g/L by settling the sludge and removing a portion of supernatant. Aliquots (200 mL) were then added to each mixture to give a final MLSS concentration of 1.6 g/L.

The test substance was added to 500 mL of reverse osmosis water at nominal concentrations of 10, 30, 100, 300 and 1000 mg/L. These preparations were incubated under the same conditions as the inoculated test mixtures, sampled and the achieved test substance concentrations verified using a Gas Chromatography method of analysis. The levels measured in these samples, after thirty minutes incubation, were considered representative of the levels achieved in inoculated test mixtures.

Samples of activated sludge (suspended solids 1.6 g/L), fed with synthetic sewage, were exposed for thirty minutes to the test substance at measured concentrations of 7.82, 18.5, 46.5, 93.35 and (triplicate) 287 mg/L. Their rates of oxygen consumption were determined and compared with controls, containing activated sludge and synthetic sewage alone, which were established at the beginning and end of the test.

Results:

The specific respiration rates of control cultures established at the end of the test (24.5 mgO₂/g/h) was 96% of the rate of that established at the start (25.4 mgO₂/g/h). Sludge respiration rates were progressively reduced in the presence of increasing concentrations of 3,5-DCP. The thirty minute 50% effect concentration (EC₅₀) for 3,5-DCP was calculated to be 7.2 mg/L (95% confidence limits, 6.3 - 8.1 mg/L). These results show that the sample of activated sludge employed was sensitive to inhibition.

Concentration-related inhibition of respiration rates was observed at concentrations between 7.82 and 46.5 mg/L with the highest concentration (triplicate, 287 mg/L) showing 96 to 100% inhibition.

The thirty minute EC₅₀ of the test substance was calculated to be 6.4 mg/L (95% confidence limits, 5.3 - 7.4 mg/L). The thirty minute EC₂₀ and EC₈₀ values were calculated to be 1.9 and 21.6 mg/L (95% confidence limits, 1.3 - 2.6 and 18.7 - 25.2 mg/L) respectively.

Concentration-related inhibition of respiration rates was observed at all the tested concentrations, (7,8-287 mg/L) with the highest concentration (287) showing 96 to 100% inhibition.

The EC₅₀ of the test substance was calculated to be 6.4 mg/L (95% confidence limits, 5.3 - 7.4 mg/L). The EC₂₀ and EC₈₀ values were calculated to be 1.9 and 21.6 mg/L, (95% confidence limits, 1.3 – 2.6 and 18.7 – 25.2 mg/L) respectively.

Conclusions:

The 30' EC₅₀ for microbial population in STP is 6.4 mg/L.

Test mixture	Temperature (°C)		pH		Specific respiration rate mgO ₂ /g/h	% Inhibition
	Initial	Final	Initial	Final		
Control (1)	21.2	21.8	7.4	7.4	25.4	-
Measured chloropicrin Concentration (mg/L)						
7.82	22.5	22.2	7.4	7.4	11.2	55
18.50	21.8	21.9	7.4	7.4	5.6	78
46.50	22.0	22.0	7.4	7.4	2.2	91
93.35	22.5	22.1	7.4	7.6	1.3	95
287.0	22.2	22.0	7.4	7.4	1.1	96
287.0	21.0	21.3	7.4	7.5	0	100
287.0	20.8	21.2	7.3	7.4	0.5	98
3,5-DCP (mg/L)						
3	21.3	21.7	7.5	7.4	17.6	29
10	21.2	21.7	7.4	7.5	10.4	58
32	21.2	21.7	7.4	7.5	4.5	82
Control (2)	21.3	21.8	7.4	7.3	24.5	-

The three-hour EC₅₀ for 3,5-DCP (7.2 mg/L) fulfilled the validity criterion relating to sensitivity to inhibition (acceptable EC₅₀ range 5 to 30 mg/L), and that relating to the respiration rates in the control (variation not greater than 15%) was also satisfied.

Acceptability: Acceptable.

The derived EC₅₀ is below the lowest test concentration, hence theoretically not fully reliable. Nevertheless, due to the nature of the endpoint, which according to OECD 209 merely represents a guide to the likely toxicity of the test substance, it is concluded that the information can be used for the risk assessment.

Risk assessment

As Chloropicrin is applied directly to soil by direct injection direct contamination of the STP would not be possible. Furthermore the maximum PEC in surface water was determined to be 6.6 ug/l, which is three orders of magnitude less than the EC₅₀ for STP. Therefore a conclusion of low risk can be drawn.

B.9.11 Metabolites

Toxicity of this metabolite has not been specifically addressed by the Notifier.

The major metabolite of Chloropicrin is Dichloronitromethane. Dichloronitromethane appears to be formed as a breakdown product of Chloropicrin in several metabolic systems. Formation of Dichloronitromethane has been shown to be conserved in animal, soil and aquatic systems. Thus, Chloropicrin ecotoxicology studies in animals and ecotoxicology trials in soil systems will also have inherently investigated the ecotoxicological effects of its main metabolite, Dichloronitromethane, in concentrations much higher than those that would be found in the field. Dichloronitromethane has been shown to be only transiently formed in these systems and, as Chloropicrin is not applied repeatedly, will not pose a chronic or long term risk to organisms. Considering the transient nature of Dichloronitromethane, its simplicity and similarity to its parent compound, its toxicological nature and the fact that it occurs as a 20% dechlorination breakdown product of Chloropicrin, it would be highly unlikely that this metabolite could be of any more concern than its parent compound.

Therefore it is concluded that sufficient evidence is available to demonstrate the low concern of Dichloronitromethane when compared to Chloropicrin. As such testing is not deemed necessary to prove its environmental safety.

Other/Special Studies (IIA 8.16.1)

Hubbard P.M., Beavers, J.B., 2009b. A Comparison of Technical Chloropicrin vs Microencapsulated Chloropicrin in an Acute Oral Toxicity Study with the Northern Bobwhite. [REDACTED] Unpublished report No.: 650-102. Owner European Chloropicrin Group. (IIA 8.16.1/01)

GLP: Yes, with the following exceptions:

1. Test substance characterisation and stability of the test substance under storage conditions at the test site were not determined prior to their use in the study according to GLP standards.
2. Periodic analyses of feed and water for potential contaminants were not performed according to GLP standards, but were performed using a certified laboratory and standard U.S Environmental Protection Agency analytical methods.

This study was conducted in accordance with OPPTS Number 850.2100 and OECD guideline number 205.

The objective of this study was to compare the acute toxicity of Chloropicrin as the technical material or as the microencapsulated material administered orally to the northern bobwhite (*Colinus virginianus*). The test consisted of a Chloropicrin technical group and a Chloropicrin microencapsulated group. Ten northern bobwhite, five males and five females were assigned to each group by indiscriminate draw. At experimental start, a single dose of the test substances was administered orally in a gelatine capsule to each bird. Each bird was individually weighed and dosed on the basis of 62 milligrams of Chloropicrin active ingredient per kilogram of body weight (62 mg a.s/kg). The test dosage (62 mg a.s/kg) and the LD50 value was established from a previous study with technical Chloropicrin ([REDACTED] Project Number: 650-101).

Based in the determined purity of 28.8 % for the microencapsulated Chloropicrin the nominal dosage for the microencapsulated Chloropicrin was 68 mg. a.s./kg. The birds were fasted for approximately 17 hours prior to dosing.

A record was maintained of all mortality, signs of toxicity and abnormal behaviour. Body weights were measured individually one day prior to the test to allow for capsule preparation and on Days 3, 7 and 14 of the test. Average feed consumption was determined by pen for each test group for Days 0-3, 4-7 and 8-14.

Results:

There were 60 % mortalities in the group receiving technical Chloropicrin and 100% mortality in the group receiving microencapsulated Chloropicrin. By the last observation on Day 0, no mortalities had occurred in the group receiving technical Chloropicrin while 30% mortality occurred in the group receiving microencapsulated Chloropicrin. On Day 1, the mortality rate was 40 % for the technical group and 90 % for the microencapsulated group. The final two mortalities in the group receiving technical Chloropicrin occurred on Day 11, when two females were euthanized due to their condition and body weight. Total mortality (100%) occurred in the group receiving microencapsulated Chloropicrin on Day 7, when the remaining bird was euthanized due to condition and body weight loss.

Onset and severe signs of toxicity were comparable between the two groups. Signs of toxicity were first noted in the group receiving technical Chloropicrin 65 minutes after dosing and for the group receiving microencapsulated Chloropicrin 55 minutes after dosing. On day 4 of the test the surviving birds in the technical level were noted to have very little excreta on dropping pans. The only surviving bird in the microencapsulated level on Day 5 was noted with having no evidence of feeding. Signs of toxicity in the technical group were ruffled appearance, wing droop, loss of coordination, lower limb weakness, reduced reaction to external stimuli (sound and movement), lacrimation, salivation, lethargy and minor muscle fasciculation. Signs of toxicity in the microencapsulated group were ruffled appearance, wing droop, loss of coordination, lower limb weakness, emaciation, lacrimation, lethargy, prostate posture and convulsions. Surviving birds in the technical group were normal in appearance and behaviour from Day 12 until termination of the study on Day 14.

For the group receiving technical Chloropicrin, there was a loss in mean body weight among surviving male (-28g) and females (-29g) from day 0 to Day 3. For the group receiving microencapsulated Chloropicrin, the only surviving bird lost 50 grams during each body weight period (Day 0 to 3 and Day 3 to 7). Body weight data after for the microencapsulated group could not be compared due to total mortality on Day 7. Among the surviving birds in the technical group, four gained weight and two continued to lose weight from Day 3 to Day 7. Among the surviving birds in the technical group, four gained weight and two continued to lose weight from Day 3 to Day 7. From Day 7 to Day 14, the four surviving birds in the technical group gained body weight. However, their overall change in body weight from Day 0 to Day 14 was still negative.

Gross necropsies were performed on the two birds in the group receiving technical Chloropicrin that were euthanized on Day 11 due to their body weight and condition and on the male from the technical group at test termination. The lesions seen at necropsy would indicate that the technical Chloropicrin had a corrosive impact on the gastro-intestinal tract not seen with the microencapsulated material. The initial assault to the lining of the G-I tract may have had an impact upon absorption of the test substance at the time of dosing and may have influenced the slight difference in mortality pattern observed.

Conclusions:

The acute toxicities of Chloropicrin in the technical and microencapsulated forms were compared by evaluating mortality, time of onset of clinical sign, nature and severity of clinical signs and impact upon body weight.

Based upon the endpoints evaluated, the toxicity of the microencapsulated form of Chloropicrin was equivalent to, if not greater than, that of the technical form of Chloropicrin.

This study is considered to be acceptable.

Bartolomé J., 2009. Chloropicrin: Determination of Residues Levels in Non-Target Insects, Plants and Earthworms Following Commercial Application of Chloropicrin to Soil in the UK in 2009. Huntingdon Life Sciences, UK. Unpublished report No.: SXC0027. Owner European Chloropicrin Group. (IIA 8.16.2/01)

GLP: Yes.

There were no relevant guidelines for this study.

A study was performed to evaluate the levels of residues of chloropicrin in non-target insects, plants (as seed and as seedling plants), and earthworms following a commercial application of chloropicrin under field conditions. The species selected were chosen as being non-target and to represent a wide range of taxonomic groups, and based on their availability and amenability to handle in this type of study. The insects were selected to represent foliar dwelling and feeding (aphids); soil dwelling, foliar feeding species (crickets) and soil dwelling, burrowing, non-foliar feeding species (beetles). The mealworms were representative of soil dwelling larval insect stages, and the blow fly pupae of insect pupae stages.

The species were placed within the plots in four different locations:

Location 1: Underneath the virtually impermeable film (VIF)

Location 2: On top of the VIF

Location 3: Immediately adjacent to treated areas (between the film covered plots) and at a distance of 0.5 m from the VIF

Location 4: 1 m from the border of the VIF in the outside rows

Samples of each species were taken before application to act as controls. Chloropicrin was applied at the field rate of 272 l/ha (nominal) using commercial application equipment. In order to contain the insect species and the earthworms situated at the soil surface (Location 1) in the field they were placed into mesh containers before they were transported to the field site. The mesh was of a size that ensured the insects and earthworms were contained, whilst allowing free gaseous exchange and permeation of any volatile residues of the test substance. They were placed between the two injectors for the fumigation equipment. Covered pots of aphid infested bean plants and barley seedlings with adult crickets and uncovered pots of seedling barley and pea plants were placed immediately after the application (Location 2). The insects, earthworms and barley and pea seeds in mesh containers were buried with the top of the container level with the soil surface. Covered pots of aphid infested bean plants and barley seedlings with adult crickets were half buried. Seedling barley and pea plants were transplanted into the soil. All were placed immediately after the application and covered with sheets of nylon mesh to avoid disruption from the wind and any local wildlife (Location 3 and 4).

Three replicate samples of each insect species, earthworms and barley and pea foliage and seeds were collected from all locations at up to 2 hours, 6 hours and 1, 3, 7, 14 and 21 days after application. Quantitation of the chloropicrin residues in insects, plants and earthworms was performed using gas chromatography with electron capture detection (GC-ECD).

Results

For all the species situated under the agricultural film the residue levels increased to a maximum at 1 DAT for meal worms, earthworms, beetles and crickets and at 3 DAT for blowfly pupae and seeds (barley and peas) and then declined steadily to close to <LOQ at 21 DAT. The highest level of residues was detected in the mealworms where a maximum of 5253.33 mg/kg (average of 3 replicates) was detected at 1 DAT. No residues were detected in any of the control samples. An overview of the results is given in Table B.9.9-6

Residues in feed items:

Day	Earthworms	Blow Fly pupae	Carabid beetles	Meal worms	Crickets	Pea seeds	Barley seeds
0 (2 h)	36.33	120.33	752	325.67	277	14	41.33
0 (6 h)	85.33	452	2490	1069.33	623.67	34.67	29.33
1	233.67	1343	3198.67	5253.33	532.67	89.33	82.67
3	216.33	1526	276.67	3077	520.67	95	202.33
7	3.67	204.67	357.67	598.33	70	1.3	22.5
14	0.4	9	9.67	33.33	12	1.03	5.23
21	0.08	n.a.	1.53	1.9		0.05	2.02

No residues were detected in the samples collected from on top of the agricultural film or at distances of 0.5 and 1 m from it, for up to 3 days after application indicating that there was no movement of the chloropicrin from the site of application into these areas.

Residues in feed items on day 8 (tarp removal)

Species	Chloropicrin residues (mg/kg) detected at day		
Day	7	8 *	14
<i>Earthworms</i>	3.67	2.67*	0.4

Species	Chloropicrin residues (mg/kg) detected at day		
Day	7	8 *	14
<i>Blow Fly pupae</i>	204.67	131.0	9
<i>Carabid beetles</i>	357.67	213.5	9.67
<i>Meal worms</i>	598.33	396.1	33.33
<i>Crickets</i>	52.5	42.51	12.0
Overall Insects	303.3	195.8*	16.0

Species	Chloropicrin residues (mg/kg) detected at day		
Day	7	8 *	14
<i>Pea seeds</i>	1.3	1.257	1.03
<i>Barley seeds</i>	22.5	18.27	5.23
Overall Seeds	11.9	9.76*	3.13

* interpolated, assuming first-order degradation

Calculated DT50 for different food items

Species	Type of test	DT ₅₀ (mean) (days)	Reference	Report No.
<i>Carabid Beetle</i>	Field	0.6	Bartolome (2009)	SXC0027
<i>Crickets</i>	Field	2.2	Bartolome (2009)	SXC0027

<i>Mealworms</i>	Field	2.2	Bartolome (2009)	SXC0027
<i>Blowfly pupae</i>	Field	1.4	Bartolome (2009)	SXC0027
Overall mean (insects)	1.667			
<i>Earthworms</i>	Field	0.7	Bartolome (2009)	SXC0027

Conclusions:

For all the species situated under the agricultural film the residue levels increased to a maximum at 1 DAT for meal worms, earthworms, beetles and crickets and at 3 DAT for blowfly pupae and seeds (barley and peas) and then declined steadily to close to <LOQ at day 21. The highest level of residues was detected in the mealworms where a maximum of 5253.33 mg/kg (average of 3 replicates) was detected at 1 DAT.

No residues were detected in any of the control samples.

No residues were detected in the samples collected from locations 2, 3 and 4 till 3 DAT although all have declined at the next sampling event (7 DAT) indicating that there was a slight movement of the chloropicrin from the site of application into these areas.

This study is considered to be acceptable.

B.9.1.2 References relied on

ACTIVE SUBSTANCE

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 8.1.1/01	Hubbard, P.M., Beavers, J.B.	2009a	Chloropicrin: An Acute Oral Toxicity Study with the Northern Bobwhite Using a Sequential Testing Procedure. [REDACTED] Report no.: 650-101 GLP, Unpublished	Y	European Chloropicrin Group
IIA 8.1.1/02	Weinberg, J.T.	2008	A 5-day Whole-Body Inhalation Toxicity Study of Chloropicrin in Northern Bobwhite Quail [REDACTED] Report no.: [REDACTED] 697002. GLP, Unpublished	Y	European Chloropicrin Group
IIA 8.1.2/01	Hubbard, P.M., Martin, K.H., and Beavers, J.B.	2009a	Chloropicrin: A Dietary LC ₅₀ Study with the Mallard [REDACTED] Report no.: 650-106. GLP, Unpublished	Y	European Chloropicrin Group
IIA 8.1.3/01	Hubbard, P. M., Martin, H. K., Beavers, B. J	2009 b	Chloropicrin: A Dietary LC ₅₀ Study With The Northern Bobwhite [REDACTED] Report no. 650-105 GLP, Unpublished	Y	European Chloropicrin Group
IIA 8.1.4/01	Frey, L.T., Martin, K.H., Beavers, J.B. and Jaber, M.	2009	Chloropicrin: A Reproduction Study with the Northern Bobwhite [REDACTED] report no.: 650-104. GLP, Unpublished	Y	European Chloropicrin Group
IIA 8.2.4/01	Lee M.R.	2009	Chloropicrin - Early Life-Stage Toxicity Test with Rainbow Trout (<i>Oncorhynchus mykiss</i>) Following OECD Guideline #210 and OPPTS Draft Guideline 850.1400 [REDACTED] Report No. 13939.6100 GLP, Unpublished	Y	European Chloropicrin Group
IIA 8.3.2.1/01	Jenkins, C.A.	2009	Chloropicrin Prolonged Toxicity to <i>Daphnia magna</i> , Huntingdon Life Sciences Ltd Report No. SXC0011 GLP, Unpublished	Y	European Chloropicrin Group
IIA 8.4/02	Wilby, J.J	2009a	Chloropicrin Algal Growth Inhibition Assay Huntingdon Life Science Ltd. Report no.: SXC0022 GLP, Unpublished	Y	European Chloropicrin Group

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 8.6/01	Wilby, J.J	2009 b	Chloropicrin Higher Plant (<i>Lemna</i>) Growth Inhibition Test. Huntingdon Life Science Ltd. Report no.: SXC0023 GLP, Unpublished	Y	European Chloropicrin Group
IIA 8.7/01	Porch, J et al.	2009	Chloropicrin: An acute vapour exposure toxicity study with the honey bee Wildlife international report no: 650-107 GLP, Unpublished	Y	European Chloropicrin Group
IIA 8.9.2/01	Rogers. M.H.	2009a	Chloropicrin To Determine the Effects on Reproduction and Growth of the Earthworm, <i>Eisenia fetida</i> . Huntingdon Life Sciences Ltd. GLP. Unpublished	Y	European Chloropicrin Group
IIA 8.9.2/02	Rogers. M.H.	2009 b	Chloropicrin To Determine the Effects of Aged Soil Residues on Reproduction and Growth of the Earthworm, <i>Eisenia fetida</i> . Huntingdon Life Sciences Ltd. GLP, Unpublished	Y	European Chloropicrin Group
IIA 8.10.1/02	Carter, J.N	2008	Chloropicrin: Effects on Soil Non-Target Micro-Organisms: Nitrogen Transformation. Huntingdon Life Sciences Ltd Report No.: SXC0019 GLP, Unpublished	Y	European Chloropicrin Group
IIA 8.10.1/03	Carter, J.N	2009	Chloropicrin: Evaluation of Effects on Soil Non-Target Micro-Organisms following Commercial Application of Chloropicrin to Soil in the UK in 2009 Huntingdon Life Sciences Ltd. Report No.: SXC0028 GLP, Unpublished	Y	European Chloropicrin Group
IIA 8.12/01	Weinberg, J.T.	2009	Toxicity of Chloropicrin to six plant species: corn, soybean, ryegrass, oilseed rape, cucumber and sunflower. WIL Research Laboratories, Report No.: WIL- 697004. GLP, Unpublished	Y	European Chloropicrin Group
IIA 8.16.1-01	Hubbard P.M., Beavers, J.B	2009 b	A Comparison of Technical Chloropicrin vs Microencapsulated Chloropicrin in an Acute Oral Toxicity Study with the Northern Bobwhite Report No.: 650-102 GLP, Unpublished	Y	European Chloropicrin Group

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 8.16.2/01	Bartolme, J	2009	Chloropicrin: Determination of Residues Levels in Non-Target Insects, Plants and Earthworms Following Commercial Application of Chloropicrin to Soil in the UK in 2009 Huntingdon Life Sciences Report No.: SXC0027 GLP, Unpublished.	Y	European Chloropicrin Group

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