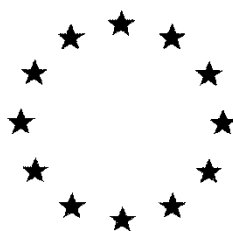


European Commission

**Second Programme for the Renewal of the Inclusion of Active Substances
in Annex I of Council Directive 91/414/EEC**



**Draft Renewal Assessment Report prepared according to the Commission
Regulation (EU) N° 1141/2010**

Amitrole

Volume 3 – Annex B.9

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B.9. ECOTOXICOLOGY**SUMMARY OF USES AND PROPERTIES****Table B 9-1: Proposed maximum use of amitrole within the European Union**

Crop and/or situation	Group of pests controlled	Formulation	Application			Application rate per treatment	
			Method	Growth stage	Number	Water L/ha	kg a.s./ha
Orchard and olives	Non selective control of annual and perennial monocotyledonous and dicotyledonous weeds	CA2735	Spraying under the row at foot of trees and vine plants	Jan.-April or mid-Oct.-mid-Dec	2	300-500	2.977 max per season corresponding to 13 L/ha of CA2735*
Vineyard				Feb.-April or mid-Oct.-mid-Dec			
Non-cropped areas			Spraying onto soil	Not applicable			

* It should be noted that **CA2735** applications are not directed to the crops either in orchards or in vineyard. The spray is applied under the row (at the foot of the plants or of the trees). It was thus considered that only one third of the total crop surface is really treated. Therefore only 1/3 of the total dose (0.993 kg a.s./ha) were actually applied onto the soil.

ENVIRONMENTAL DATA AND WORST-CASE SCENARIOS**Table B9-2: Metabolite concerned by ecotoxicological risk assessment**

Metabolite name	Maximum of parent compound (%)	Compartment of formation
1,2,4-triazole	9.9	soil

1,2,4-triazole was included in the risk assessment for soil and surface water by the applicant for all intended uses. RMS notes that according to the sustained GAP for agricultural uses (application period), photodegradation is not expected to be a major route of degradation of amitrole in soil and therefore 1,2,4-triazole is not expected to be formed in significant amounts (see Vol 3 B.8). Therefore, to the RMS opinion, a risk assessment was not strictly required. However, RMS considers that if further agricultural uses occurring when photodegradation may be important would be sustained, it should be discussed whether 1,2,4-triazole may need to be included in the risk assessment or not. Therefore, risk assessment for 1,2,4-triazole are presented for information. It is highlighted that photodegradation and microbial degradation will be concomitant and concurrent processes. Therefore, if photodegradation occurs in field, the maximum occurrence reached is expected to be lower than 9.9%.

TEST MATERIALS USED IN ECOTOXICOLOGY ASSAYS

Analysis of batches used in ecotoxicological test are reported in Volume 4 (confidential part).

SUBMISSION OF THE EU DOSSIER TO SUPPORT THE ANNEX I RENEWAL OF AMITROLE

Amitrole was first annex I included on March, 5th, 2001 (Directive 2001/21/EC). The directive came into force on January, 1st 2002. In support to this inclusion was submitted a complete dossier according to the requirements of the Directive 91/414/EEC.

This section presents only the new data available since Amitrole was included in annex I as well as the data that were specifically required by the RMS or identified in the monograph and /or in the review report as points to be addressed at national level.

Regarding ecotoxicology, in level 4 of the monograph the following studies were requested:

- effects on algal growth with Aminotriazole on *S. subspicatus*,
- effects on aquatic plants with aminotriazole,
- effects on N mineralisation.

Regarding the effects on algal growth, a second study done on *Anabaena flos-aquae* was submitted (Otten, R.J, 1997). It was evaluated and summarized in the addendum to the monograph and was accepted to address the point raised in level 4 of the monograph.

Three more GLP studies were conducted on *Selenastrum capricornotum*, *Navicula pelliculosa* and *Skeletonema costatum*. They are summarized in the present EU dossier for annex I renewal, section B.9.2.9.

With respect to the effects on aquatic plants, a GLP study on *Lemna gibba* was conducted and is summarized in the addendum to the monograph (Palmer, S.J., 1998). It was considered as acceptable.

To address the point on the effects on N-mineralization, a GLP activated sludge, respiration inhibition test was submitted and is summarized in the addendum to the monograph (Gossman, A. 1999). This test was done according to OECD guideline n° 209. This study was accepted to address the point.

No more data was requested.

In the review report (6839/VI/97-final) and directive 2001/21/EC it was asked to the member states to pay particular attention to:

- Protection of beneficial arthropods
- Protection of birds and mammals: the use of amitrole during the breeding season may only be authorized when an appropriate risk assessment has demonstrated that there is no unacceptable impact and when the conditions of authorization include, where appropriate, risk mitigation measures.

These points were addressed for this re-registration process:

- New extended laboratory tests were conducted at 3600 g a.s./ha on *Typhlodromus pyri* and *Aphidius rhopalosiphii*. They are summarized in this section under point B.9.5. On the basis of all available data (*Typhlodromus pyri*, *Aphidius rhopalosiphii*, *Poecilus cupreus* and *Orius insidiosus*) as well as literature data, the risk was considered to be acceptable with no spray buffer zone as mitigation measures.
- The risk assessments for birds and mammals were refined using results of residue studies conducted on weeds and insects. The risk was shown to be acceptable for both birds and mammals.

In addition, according to the provisional criteria set in annex II article 3.6 of regulation (EC) N° 1107/2009/EC, amitrole might be considered as a potential endocrine disruptor. In agreement with the Rapporteur Member State, the potential consequences for ecotoxicology of these potential endocrine disrupting properties are addressed with:

- a 2-generation study in birds (Mallard duck),
- a multigeneration test in fish (Medaka).

One non-GLP study was performed in order to assess the effects of Weedazol TL on seed germination and vegetative vigor. One GLP study was conducted to assess the effects of Weedazol TL on seedling emergence. Both studies are summarized under B.9.9.

The representative formulation for the first annex I inclusion was a soluble concentrate (CA0123) containing 229 g/L of amitrole and 215 g/L of ammonium thiocyanate (specific synergist). Further to publication of Directive 2003/53/EC dealing with the restrictions on the marketing and use of nonylphenol and nonylphenol ethoxylate, a SL formulation without NPE was developed (CA2210). For this current annex I renewal process the representative formulation is still a soluble concentrate containing 229 g/L of amitrole and 215 g/L of

ammonium thiocyanate (CA2735). Compared with the weedazol TL (CA 2210), minor changes were made in formulation CA2735 (please refer to the detailed composition submitted in the confidential part). A comparison of detailed compositions for CA0123 (containing NPE), CA2210 and CA2735 is included in the confidential part.

Therefore the ecotoxicological studies that were conducted on CA 2210 remain valid to cover the ecotoxicological profile of CA2735.

The risk assessments were redone according to the last guidance documents in force^{1,2,3}.

The endpoints that were agreed at EU level are presented in the corresponding points.

B.9.1. EFFECTS ON BIRDS (ANNEX IIA 8.1, ANNEX IIIA 10.1)

This point was addressed in the dossier submitted and reviewed for the inclusion of amitrole in the annex I. Therefore, there is no need for summarizing again the studies. The main endpoints are shown in table 9.1.8.1.1. Please also refer to review report SANCO/ 6839/VI/97-final, 22/03/2001.

Because amitrole might be considered as a potential endocrine disruptor, the consequences for non-target organisms are addressed with a 2-generation study in birds (Mallard duck)

B.9.1.1. Acute oral toxicity (annex IIA 8.1.1, annex IIIA 10.1.1)

Please refer to point 9.1.

B.9.1.2. Subchronic toxicity and reproduction (annex IIA 8.1.2, annex IIIA 8.1.3)

Please refer to point 9.1.

B.9.1.2.1. Two generation reproduction of amitrole to *Anas platyrhynchos*

- ☐ **Reference:** KIIA 8.16/03
- ☐ **Author(s); year:** [REDACTED] 2012
- ☐ **Title:** A two generation reproduction study with the Mallard using pulsed exposure.
- ☐ **Report No:**
- ☐ **Guidelines:** U.S. EPA OPPTS Number 850.2300, FIFRA Subdivision E, Section 71-4, OECD 206
- ☐ **Deviations:**
- ☐ **GLP:** Yes
- ☐ **Executive summary:**

The objective of this study was to evaluate the effects upon the mallard (*Anas platyrhynchos*) of a pulsed dietary exposure to amitrole over two generations to aid in the evaluation of the possible consequences on the population of potential endocrine disruptive effects of amitrole. Mallards were exposed in a pulsed manner over two generations to a diet containing the test substance at concentrations chosen by the sponsor to reflect environmental levels after application of 6L/ha, 10L/ha and 13 L/ha of CA2735. Amitrole is applied once a year either at the end of winter or in autumn. The pulsed exposure in this study mimicked an exposure twice a year at the end of winter and in autumn (maximized exposure).

The F0 generation was exposed to treated diets from photostimulation through an egg laying cycle of seven weeks and selected F1 offspring were exposed to treated diets for the first eight weeks of rearing. Both exposures correspond to end of winter application of the test substance. The F1 offspring were exposed to treated diets for an eight-week period during grow-out, beginning at approximately 23 weeks of age, to correspond to a fall

¹ EFSA Journal 2009; 7 (12): 1438. Guidance of Efsa. Risk assessment for birds and Mammals.

² SANCO/3268/2001 rev 4 (final). 17 October 2002. Guidance document on Aquatic ecotoxicology.

³ SANCO 10329/2002 rev2 final. 17 October 2002. Guidance document on terrestrial ecotoxicology under Council Directive 91/414/EEC.

application of the test substance. F1 birds were paired and exposed to appropriate treated diets again at approximately 45 weeks of age and monitored through a six week reproduction cycle. F2 hatchlings were reared to 14-days of age on untreated diet. Given the impact seen on adults in the F0 generation, 10 pairs of mallards each from the F0 156 and 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw (W) phase. Offspring from these birds (F1W) were handled comparably to the F1 exposure phase birds. Effects on bird health, weight gain and feed consumption were evaluated. In addition, the effects of exposure to amitrole on the number of eggs laid, normal development of eggs, embryo viability, hatchability, offspring survival and egg shell thickness were evaluated for both the F0 and F1 generations.

❑ Materials and methods:

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, "Amitrole: A Two-Generation Reproduction Study with the Mallard Using Pulsed Exposure" (Appendix XV). The protocol was based, in part, upon procedures outlined in the Environmental Protection Agency's Registration Guidelines Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Subsection 71-4; OECD Guideline 206; ASTM "Standard Practice for Conducting Reproductive Studies with Avian Species"; and U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.2300 (1,2,3 & 4).

Test Substance

Amitrole for use as a reference standard was received from Nufarm S.A.S. on November 9, 2010 and was assigned Wildlife International, Ltd. identification number 9791 upon receipt. The test substance was a solid and was identified as: Lot 80821. The test material had a reported purity of 97.5% amitrole, with an expiration date of August, 2014 (Appendix XII). The reference substance was held under ambient conditions in locked storage at the Wildlife International, Ltd. facilities in Easton, Maryland. The test substance, amitrole, was received from Nufarm S.A.S. on November 11, 2010 and was assigned Wildlife International, Ltd. identification number 9793 upon receipt. The test substance was a liquid and was identified as: CA 2735, Batch No.: RD-877-142C. The test material had a reported purity of 229 g/L amitrole (Appendix XII). The test substance was held under ambient conditions in locked storage at the Wildlife International, Ltd. facilities in Easton, Maryland. Additional test material was received from Nufarm S.A.S. on April 11, 2011 and was assigned Wildlife International, Ltd. identification number 9980 upon receipt. The test substance was a liquid, identified as: CA 2735, Lot No.: 310123555, with a reported purity of 229 g/L amitrole. Both Wildlife International, Ltd. Identification numbers 9793 and 9980 were used for preparation of test diets and matrix fortifications. Concentrations of the test substance in the diet were adjusted to 100% active ingredient. Therefore, dietary concentrations are expressed as parts per million active ingredient (ppm a.i.) in the diet.

Test Organisms

Approximately 91 pairs of pen-reared mallard were purchased from Whistling Wings, Inc., 113 Washington Street, Hanover, IL 61041-0509, U.S.A. At the start of acclimation, the mallard were apparently healthy and phenotypically indistinguishable from wild type. The birds were from the same hatch, approaching their first breeding season and had not been used in any previous testing. At the start of acclimation, a random number generating function in a spreadsheet program was used to randomize pen assignment for each bird. Immediately prior to test initiation, all potential study birds were examined for physical injuries and general health. Birds that did not appear healthy (either due to injury or inability to acclimate to laboratory conditions) or were outside the weight range were excluded from the study. All birds were 31 weeks of age at test initiation (first day of exposure to test diet) and ranged in weight from 863 to 1295 grams. Sex of the birds was determined by a visual examination of the plumage.

Identification

Adult F0 birds were identified by individual leg bands, all F1 and F1W ducklings were identified by wing bands, F1 and F1W ducklings selected for rearing were further identified by microchip implants and leg bands and all F2 and F2W ducklings were identified by wing bands. Each pen was identified with a unique number, and groups of pens were identified by project number and concentration. All eggs laid during the study were marked with the pen number using a permanent ink marking pen for identification.

Avian Feed and Water

All adult birds and their offspring were given feed and water *ad libitum* during acclimation and testing. The basal diet was formulated to Wildlife International, Ltd. specifications by Cargill Animal Nutrition, Shippensburg, PA (Appendix I). The basal ration contained at least 27% protein and 2.5% crude fat, and no more than 3.8% crude fiber. The basal diet contained approximately 1.10% calcium, derived from feedstuffs and the limestone used in the formulation of the basal diet by Cargill. While this level of calcium is sufficient for growth and maintenance rations, additional calcium is required in the ration of breeding birds for egg shell formation. Therefore, an additional 5% (w/w) of limestone (approximately 38.6% Ca) was added to the basal diet for the adults at photostimulation. This raised the calcium level in the diet for the breeding birds to approximately 3%, slightly above the 2.75% minimum recommended for mallard (5). Water was supplied by the town of Easton public water supply. Neither the adults nor offspring received any form of medication in their feed during the test. Feed and water were analyzed periodically in accordance with Wildlife International, Ltd. Standard Operating Procedures.

Diet Preparation

During exposure pulses test diets were prepared by mixing amitrole into a premix that was used for weekly preparation of the final diet. Dietary concentrations were adjusted for purity of the test substance and are presented as parts per million active ingredient (ppm a.i.). Details of the weekly preparation of test and control diets are shown in Appendix II.

Diet Sampling

Homogeneity of the test substance in the diet was evaluated by collecting six samples from each of the treated diets and one sample from the control diet on Day 0 of Week 1. Samples were collected from the top, middle and bottom of the left and right sections of the mixing vessel. Control and treatment group diet samples were also collected from the bin feeders on Day 7 of Week 1 to assess stability of the test substance under actual test conditions. Additionally, samples were collected from the control and treatment group diets during Weeks 5, 9, 12, 17, 36, 57 and 63 of the test to measure/verify test concentrations. The diet samples were transferred immediately to the Wildlife International, Ltd. analytical chemistry facility and stored frozen prior to analysis.

Analytical Method

The method used for the analysis of amitrole in avian diet was based upon methodology developed by Wildlife International, Ltd. Samples were extracted with 10 millimolar potassium bromide in methanol (10 mM KBr in methanol). A method outline is provided in Appendix XIII, Figure 1. Concentrations of amitrole in extracts of the samples were determined by high performance liquid chromatography using a Waters Alliance High Performance Liquid Chromatograph (HPLC) equipped with a Waters 486 variable wavelength detector (VWD). High performance liquid chromatograph separations were achieved using a Zorbax SB-Phenyl analytical column (250 mm x 4.6 mm I.D., 5 µm particle size). The instrument parameters are summarized in Appendix XIII, Table 1. Calibration standards of amitrole, ranging in concentration from 0.100 to 2.50 µg a.i./mL, were analyzed with each sample set. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. An example of a calibration curve is presented in Appendix XIII, Figure 2. The concentration of test substance in the samples was determined by substituting the peak area responses of the samples into the applicable linear regression equation. Typical chromatograms of low-level and high-level calibration standards are shown in Appendix XIII, Figures 3 and 4, respectively. Examples of equations used in calculations are presented in Appendix XIII, Table 2. The method limit of detection (LOD) was set at the lowest analytical standard analyzed 0.100 µg a.i./mL amitrole (7.5 ng a.i. on column). The method limit of quantitation (LOQ) for these analyses was set at 25 ppm a.i. based upon product of the lowest analytical standard 0.100 µg a.i./mL and the dilution factor (250) of the matrix blank extract. Measured values greater than or equal to the LOQ were reported. Along with the sample analyses, nine matrix blanks were analyzed to determine possible interferences. No interferences were observed at or above the LOQ standard during the sample analyses (Appendix XIII, Table 3). A typical chromatogram of a matrix blank is presented in Appendix XIII, Figure 5.

Avian diet samples were fortified at 50 and 500 ppm a.i. and analyzed concurrently with the samples to determine the mean procedural recovery. The method yielded mean procedural recoveries of 93%, 91%, 96%, 99%, 102%, 105%, 111%, 93%, and 94%. These values correspond to each sample set analyzed during the definitive study (Appendix XIII, Table 3). Sample measured concentrations were not corrected for the mean procedural recoveries from each sample set. A typical chromatogram of a matrix fortification is presented in Appendix XIII, Figure 6.

Study Phases

The primary phases of the study and their approximate durations were:

Week 1	1. F0 Acclimation - 8 weeks
	2. F0 Photostimulation – Start of exposure
	3. F0 Pre-egg laying (with photostimulation) - 2 weeks
	4. F0 Egg laying - 7 weeks
Week 10	5. F0 Adult termination – (0, 95, 10 pair - 156, 10 pair - 256 ppm a.i.) following Lot F egg set, Withdraw birds (10 pair - 156, 10 pair - 256 ppm a.i.) placed on untreated diet to continue
	6. F1 Offspring rearing to 14 days of age on treated diets (Lots A – D) - approximately 6 weeks
Wks 13-21	7. F1 Offspring (Lots E & F) – Reared on treated diets – 8 weeks
Week 21	8. F1 Grow out – Return to basal diet – 14 weeks
Week 35	9. F1 Grow out – Begin treated diet – 8 weeks
Week 43	10. F1 Grow out – Return to basal diet
Week 56	11. F1 Adult – Paired housing
Week 57	12. F1 Photostimulation and return to treated diet
	13. F1 Pre-egg laying (with photostimulation) – 2 weeks
	14. F1 Egg laying – 6 weeks
Week 64	15. F1 Adult termination – Lot F eggs set
Week 70	16. F2 Post-adult termination (final incubation, hatching and 14-day offspring rearing period) - 6 weeks

The phases of the study for the withdraw groups and their approximate durations were:

Week 10	1. F0 Primary Adult termination – F0W Withdraw birds (10 pair - 156, 10 pair - 256 ppm a.i.) placed on untreated diet to continue egg production.
	2. F0W Egg laying - 5 weeks
Week 15	3. F0W Withdraw adult termination – following Lot WE egg set
	4. F1W Withdraw Offspring reared to 14 days of age on treated diets (Lots WA – WB) - approximately 3 weeks
Wks 18-26	5. F1W Withdraw Lots WC, WD & WE reared on treated diets – 8 weeks
Week 26	6. F1W Grow out – Return to basal diet – 14 weeks
Week 40	7. F1W Grow out – Begin treated diet – 8 weeks
Week 48	8. F1W Grow out – Return to basal diet
Week 62	9. F1W Adult – Paired housing, photostimulation and return to treated diets
	10. F1W Pre-egg laying (with photostimulation) – 2 weeks
	11. F1W Egg laying – 6 weeks
Week 69	12. F1W Adult termination – Lot WF eggs set
Week 74	13. F2W Post-adult termination (final incubation, hatching and 14-day offspring rearing period) – approximately 6 weeks

Housing and Environmental Conditions

Housing and husbandry practices were conducted so as to adhere to the guidelines established by the National Research Council (6). During the reproductive portions of the study, the adult birds (F0, F0W, F1 and F1W) were housed indoors in batteries of pens manufactured by Safeguard Products, Inc. (Model No. 5355), measuring approximately 75 X 90 X 45 cm high. The pens were constructed of vinyl-coated wire mesh. Sisal ropes were added to each pen for animal enrichment. Diagrams of the test layouts are presented in Appendix XIV. Each pen was equipped with a bin feeder. Weekly, sufficient feed for the feeding period was placed in the bin feeder for each pen and presented to the birds. During the feeding period additional feed was weighed and added to the bin feeders as needed. Water was supplied by nipple-type waterers.

Hatchlings (F1, F1W, F2 and F2W) were housed in batteries of brooding pens manufactured by Safeguard Products, Inc. Each pen measured approximately 62 X 92 X 25.5 cm high. The walls, floors and ceilings of each pen were constructed of vinyl-coated wire mesh. Thermostats in the brooding compartment of each pen were set to maintain a temperature of approximately 38° C from the time of hatching until the birds were five to seven days of age, when the temperature was adjusted to maintain a temperature of approximately 29°C. Each pen was equipped with a bin feeder. Weekly, sufficient feed for the feeding period was placed in the bin feeder for each pen and presented to the birds. During the feeding period additional feed was weighed and added to the bin feeders as needed. Water was supplied by automatic cup-type waterers. F1 and F1W hatchlings selected for grow-out were maintained in safeguard brooders or battery pens manufactured by Safeguard Products, Inc. (Model No. 5355) until transfer to grow out floor pens. Each pen was equipped with a bin feeder. Weekly, sufficient feed for the feeding period was placed in the bin feeder for each pen and presented to the birds. During

the feeding period additional feed was weighed and added to the bin feeders as needed. Water was supplied by automatic cup-type waterers. At approximately 5 to 6 weeks of age the F1 and F1W juveniles, to be grown out, were transferred to floor pens.

During the grow-out period of rearing the F1 birds were housed in a 28 x 28 foot study room divided into four approximately equal sections divided into two approximately equal sections with X-pen dividers to maintain study groups separately. F1W birds were housed in a 29 x 13 foot study room, also with X-pen dividers to maintain study groups separately. Concrete floors were covered with Dura-tuff plastic sow floor and woodchip shavings. Water was supplied to 17.5 gallon tubs that were positioned to drain into the floor gutters along the room perimeter. Feed was provided in 50 gallon Kuhl plastic poultry feeders. Only birds associated with this study were maintained in the study rooms in order to avoid excessive disturbances. The average temperature in the F0 adult mallard study room during the course of the initial portion of the test was $20.8 \pm 0.5^{\circ}\text{C}$ (SD) with an average relative humidity of $38 \pm 9\%$ (SD). For F1 hatchlings, the average ambient room temperature was $23.6 \pm 0.9^{\circ}\text{C}$ (SD) with an average relative humidity of $38 \pm 14\%$ (SD). The average temperature in the F1 grow-out study room was $23.2 \pm 1.0^{\circ}\text{C}$ (SD) with an average relative humidity of $64 \pm 15\%$ (SD) and the average temperature in the F1 adult study room was $20.9 \pm 0.5^{\circ}\text{C}$ (SD) with an average relative humidity of $42 \pm 10\%$ (SD). For F2 hatchlings, the average ambient room temperature was $23.8 \pm 1.0^{\circ}\text{C}$ (SD) with an average relative humidity of $47 \pm 13\%$ (SD). The average temperature in the F0W adult mallard study room during the course of that portion of the test was $21.8 \pm 0.5^{\circ}\text{C}$ (SD) with an average relative humidity of $35 \pm 9\%$ (SD). For F1W hatchlings, the average ambient room temperature was $25.6 \pm 1.7^{\circ}\text{C}$ (SD) with an average relative humidity of $75 \pm 11\%$ (SD). The average temperature in the F1W grow-out study room was $22.7 \pm 1.2^{\circ}\text{C}$ (SD) with an average relative humidity of $58 \pm 17\%$ (SD) and the average temperature in the F1W adult study room was $21.8 \pm 0.6^{\circ}\text{C}$ (SD) with an average relative humidity of $48 \pm 12\%$ (SD). For F2W hatchlings the average ambient room temperature was $25.3 \pm 1.3^{\circ}\text{C}$ (SD) with an average relative humidity of $66 \pm 11\%$ (SD). The air handling system in each of the study rooms was designed to vent up to 15 room air volumes every hour and replace them with fresh air. The photoperiod in each of the study rooms was maintained by a time clock. The photoperiod during F0 adult bird acclimation was eight hours or less of light per day and was increased to 17 hours of light per day at test initiation and first exposure to the treated diet. The photoperiod was maintained at 17 hours of light per day until adult termination. The photoperiod for the F1 and F1W hatchlings was maintained at 16 hours of light per day. During the grow-out phase of the F1 and F1W offspring, the photoperiod was initially maintained at 16 hours of light or less, with a gradual reduction in photoperiod designed to mimic the fall months of the year. The photoperiod for the F1 and F1W adults was increased to 17 hours of light to induce egg laying for the reproductive phase and last exposure to treated diet and was maintained at that length until the F1 or F1W adult birds were euthanized. The photoperiod for the F2 and F2W hatchlings was maintained by a time clock at 16 hours of light per day. During the test, the F0 adult birds and withdraw group birds received a mean of approximately 200 lux (~ 19 ft. candles), with the F0W adult birds receiving a mean of approximately 217 lux (~ 20 ft. candles) during the withdraw period. During the grow-out periods the F1 offspring received a mean of approximately 549 lux (~ 51 ft. candles) and the F1W offspring received a mean of approximately 223 lux (~ 21 ft. candles). The F1 adult birds received a mean of approximately 327 lux (~ 30 ft. candles) and the F1W adult birds received a mean of approximately 378 lux (~ 35 ft. candles). Illumination in all study rooms was provided by fluorescent lights that closely approximated the color spectrum of noonday sunlight.

Observations

The F0 test birds were acclimated to the facilities and study pens for eight weeks prior to initiation of the test. During acclimation, all birds were observed daily. Birds exhibiting abnormal behavior or debilitating physical injuries were not used for the test. During the 75 week study, all birds present were observed daily for signs of toxicity or abnormal behavior. A record was maintained of all mortalities and clinical observations.

Necropsy and Tissue Collection

Adult birds that died or were euthanized during the course of the study were subjected to a gross necropsy. At the conclusion of the F0 and F0W study periods and at the conclusion of the F1 and F1W study periods, all surviving adult birds were euthanized by cervical dislocation and necropsied. Thyroid glands of each bird were collected, fixed in Davidson's solution and preserved in formalin for potential histopathological evaluation. In addition, up to two offspring (when available) per parental pen of origin from Lot D of the F1 offspring and Lot WC of the F1W offspring were euthanized at hatch and thyroid glands were collected, fixed and preserved in formalin for potential histopathological evaluation. At 14 days of age, thyroids were collected from up to two additional offspring per pen (when available) from the Lot D, F1 and Lot WC, F1W. Similarly, thyroids of up to two offspring per pen (when available) from the Lot D and E F2 offspring and Lot WD and WE F2W offspring were collected at hatch and 14-days of age, fixed and preserved for potential evaluation. Young adult mallards

that were not used for the F1 or WF1 portion of the test were euthanized by cervical dislocation, necropsied and disposed of by incineration.

Body Weight Measurements

Individual F0 adult body weights were measured at test initiation and at primary adult termination. In addition, body weights of withdraw F0W adult birds were measured at the start of the withdraw phase and at adult termination. For all generations, individual body weights of live offspring were collected at Day 0 and Day 14. F1 offspring being grown out were individually weighed at approximately 9, 23, 31 and 45 weeks of age to correspond with transitions to and from treated diets. F1 adults were also weighed at the termination of the egg laying portion of the study. Similarly, body weights of F1W withdraw offspring being grown out were individually weighed at approximately 8, 23, 31 and 45 weeks of age to correspond with diet transitions and at termination of the egg laying portion of the study. Body weights were not measured during egg laying because of the possible adverse effects handling may have had on egg production.

Animal Diet and Feed Consumption

All test birds were fed a game bird ration formulated to Wildlife International, Ltd. Specifications (Appendix 1). Five percent (w/w) limestone was added to the ration of adult birds in breeding condition to provide a calcium source. F0 and F1 adults received limestone in the diet beginning at the time of photostimulation.

The F0 adult birds received the appropriate treated or control diet from the time of photostimulation until primary adult termination. The F1 hatchlings were also fed the appropriate treated or control game bird ration (Appendix I), but without the addition of limestone. Offspring received the appropriate treated diets from the time of hatching. The last two groups of F1 offspring (Lots E and F) and F1W offspring (Lots WD and WE) received the appropriate treated or control diets for the first eight weeks of rearing, a second eight-week period beginning at approximately 23 weeks of age and a third period of approximately seven weeks, beginning at photostimulation and running for the remainder of the study. F2 offspring were fed untreated diet without the addition of 5% supplemental limestone. Feed consumption was measured for the adults and offspring for a seven-day period each week throughout the test when treated diet was presented. Feed consumption was determined by weighing the freshly filled feeder on Day 0, recording the amount of any additional diet added during the week and weighing the feeder and remaining feed at the end of the feeding period (Day 7). The amount of feed wasted by the birds was not quantified, since the wasted feed was normally scattered and mixed with water and excreta. Therefore, feed consumption is presented as an estimate of total feed consumption.

Egg Collection and Storage

F0, F0W, F1 and F1W eggs were collected daily from all pens, when available. Eggs were washed to reduce the possibility of pathogen contamination before storing them in the cold room. Eggs were washed in a commercial egg washer (Kuhl Egg Washer) with a chlorine based detergent (Kuhl Super CD). Water in the washer was warmed to approximately 45°C. The eggs were placed in the wash water and soaked for approximately 15 seconds. The washer's circulation motor was then turned on for approximately three minutes. The eggs were removed from the washer, rinsed with fresh water and allowed to cool to approximately room temperature. The eggs were then stored in a cold room until incubation. For the F0 eggs, the cold room was maintained at a mean temperature of $14.1 \pm 0.0^\circ\text{C}$ (SD) with a mean relative humidity of approximately $71 \pm 5\%$ (SD) and F0W eggs were stored at a mean temperature of $14.1 \pm 0.0^\circ\text{C}$ (SD) with a mean relative humidity of approximately $78 \pm 5\%$ (SD). Storage conditions for the F1 eggs were maintained at a mean temperature of $14.1 \pm 0.1^\circ\text{C}$ (SD) with a mean relative humidity of approximately $76 \pm 6\%$ (SD) and WF1 eggs were stored at a mean temperature of $14.1 \pm 0.1^\circ\text{C}$ (SD) with a mean relative humidity of approximately $81 \pm 6\%$ (SD). Groups of eggs were identified by an alphabetic lot code. All eggs laid in a weekly interval were considered as one lot.

Candling and Incubation

At the end of the weekly interval, all eggs were removed from the cold room, counted and eggs selected by indiscriminate draw for egg shell thickness measurement. The remaining eggs were candled with a Speed King (Model No. 32) egg-candling lamp to detect egg shell cracks or abnormal eggs. Cracked or abnormal eggs were recorded and discarded.

All eggs not discarded or used for egg shell thickness measurements were placed in a NatureForm Incubator (Model No. NMC 4000, NMC 7000 or Model # 2340). For the F0, F0W, F1 and F1W eggs, the incubator temperature was maintained at an average $37.4 \pm 0.0^\circ\text{C}$ (SD) with an average relative humidity of approximately

55 ± 0% (SD). The incubator was equipped with a pulsator fan and blades that produced a mild breathing air movement designed to eliminate intracabinet temperature and humidity variation during incubation. In order to prevent adhesion of the embryo to the shell membrane, the incubator was also equipped with an automatic egg rotation device, designed to rotate the eggs from 45° off of vertical in one direction to 45° off of vertical in the opposite direction (total arc of rotation was 90°) every hour through Day 24 of incubation. Eggs were candled on Day 14 of incubation to determine embryo viability and on Day 21 to determine embryo survival.

Hatching and Brooding

On Day 24 of incubation, the eggs were placed in a NatureForm Incubator (Model No. NMC 4000) configured for hatching, and allowed to hatch. Pedigree baskets constructed of galvanized steel wire mesh were used to keep hatchlings separated by parental pen of origin. Eggs were not rotated in the hatcher. For the F0, F0W, F1 and F1W hatchlings the average temperature in the hatching compartment was 37.3 ± 0.0°C (SD) with an average relative humidity of 60 ± 0% (SD). All hatchlings, unhatched eggs and egg shells were removed from the hatcher on Day 27 or 28 of incubation. The individual body weights of live hatchlings were recorded and the group body weight by pen was determined. Hatchlings were wing banded for identification by pen of origin and a blood spot was collected from all offspring, except F1 Lot A, to determine genetic sex. All offspring, except for those euthanized at hatch for thyroid collection, were routinely housed according to the appropriate parental concentration grouping in brooding pens until at least 14 days of age. At 14 days of age, the individual body weights of surviving F1, F1W, F2 and F2W hatchlings were recorded and the average body weight by parental pen of all surviving ducklings was determined. At 14 days of age, ducklings not selected for grow out or thyroid collection were euthanized with carbon dioxide and disposed of by incineration.

Egg Shell Thickness Measurements

Weekly throughout the egg laying periods, one egg was collected, when available, from each of the odd numbered pens during odd numbered weeks (1,3,5,7) and from each of the even numbered pens during the even numbered weeks (2,4,6). The eggs were opened at the waist, the contents removed, and the shells thoroughly rinsed with water. The shells were then allowed to air dry for at least one week at room temperature. The average thickness of the dried shell plus the membrane was determined by measuring five points around the waist of the egg using a micrometer. Measurements were made to the nearest 0.002 mm.

Statistical Analyses

Upon completion of the test, an analysis of variance (ANOVA) was performed to determine statistically significant differences between groups. Dunnett's multiple comparison procedure (7, 8) was used to compare the three treatment means with the control group mean and assess the statistical significance of the observed differences. Sample units were the individual pens within each experimental group, except adult body weights where the sample unit was the individual bird. Percentage data were examined using Dunnett's method following arcsine square root transformation (see Appendix III) for reproductive parameters. Each of the following parameters was analyzed statistically.

1. Adult Body Weight - Individual body weight was measured for the F0 adults at test initiation and adult termination. Individual body weight was measured for the F1. In addition, body weights of F1 offspring being grown out were individually weighed at 9, 23, 31 and 45 weeks of age. F1 adults were also weighed at the termination of the egg laying portion of the study.
2. Adult Feed Consumption - Feed consumption expressed as grams of feed per bird per day was examined by pen weekly during the test during periods when test diet was presented. When possible (birds not group housed) statistical comparisons were made between the control and each treatment group.
3. Eggs Laid of Maximum Laid - The number of eggs laid per female divided by the largest number of eggs laid by any one female. This transformation was used to convert the number of eggs laid to a percentile value less than or equal to 100.
4. Eggs Cracked of Eggs Laid - The number of eggs determined by candling to be cracked divided by the number of eggs laid, per pen.
5. Viable Embryos of Eggs Set - The number of viable embryos at the Day 14 candling was divided by the number of eggs set, per pen.
6. Live 3-Week Embryos of Viable Embryos - The number of live embryos at the Day 21 candling was divided by the number of viable embryos, per pen.
7. Hatchlings of 3-Week Embryos - The number of hatchlings removed from the hatcher was divided by the number of live 3-week embryos, per pen.

8. 14-Day Old Survivors of Hatchlings - The number of 14-day old survivors was divided by the number of hatchlings, per pen.
9. Hatchlings of Eggs Set - The number of hatchlings was divided by the number of eggs set, per pen.
10. 14-Day Old Survivors of Eggs Set - The number of 14-day old survivors was divided by the number of eggs set per pen.
11. Hatchlings of Maximum Set - The number of hatchlings per female divided by the largest number of eggs set from any one female. This transformation was used to convert the number of hatchlings to a percentile value equal to or less than 100.
12. 14-Day Old Survivors of Maximum Set - The number of 14-day old survivors per pen divided by the largest number of eggs set.
13. Egg Shell Thickness - The average egg shell thickness of indiscriminately selected eggs per pen was measured.
14. Offspring's Body Weight - The group body weights of surviving hatchlings and 14-day old survivors were measured by parental pen group.

❑ **Findings:**

RESULTS AND DISCUSSION

Mallards received amitrole at nominal dietary concentrations of 95, 156 and 256 ppm a.i. at intervals during a two generation reproduction study. The F0 generation was exposed to treated diets from photostimulation through an egg laying cycle of seven weeks and selected F1 offspring were exposed to treated diets for the first eight weeks of rearing. The F1 offspring were exposed to treated diets for an eight-week period during grow-out and were paired and exposed to appropriate treated diets again at approximately 45 weeks of age and monitored through a six week reproduction cycle. F2 hatchlings were reared to 14-days of age on untreated diet. Due to the impact of the test substance upon mallards in the 156 and 256 ppm a.i. treatment groups for the F0 generation, 10 pairs of mallards from each of those groups were transferred to untreated diet for a five week withdraw (W) phase. Offspring from those birds (F1W) were handled comparably to the F1 exposure phase birds.

Daily Dietary Dose

Estimated test substance intakes, daily dietary dose, for mallards were calculated by treatment group and for each of the exposure periods, using the following formula: Daily Dietary Dose = Test Concentration (mg a.i./kg) x Daily Feed Consumption (g/bird/day) (mg a.i./kg body weight/day) Body Weight (g/bird)

The mean body weight value is the mean of both male and female body weights. The accuracy of the estimated mean daily dietary dose may be impacted by differences in individual feed consumption, both within and between pens, and feed wastage. The estimated daily dietary doses are presented in the following table and in Appendices F0 XI, F1 XI and F1W XI.

Calculated Daily Dietary Dose (mg amitrole/kg body weight/day) from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure

F0 - One Exposure Interval F1 and WF1 - Three Exposure Intervals								
Experimental Group	Pulse 1		Pulse 2		Pulse 3		Average ¹	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
F0								
Control	0.00	0.00						
95 ppm a.i.	12.47	2.30					12.47	2.30
156 ppm a.i.	21.20	4.13					21.20	4.13
256 ppm a.i.	29.38	4.51					29.38	4.51
F1								
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
95 ppm a.i.	26.02	20.59	11.65	0.91	14.32	2.66	17.46	13.38
156 ppm a.i.	40.68	28.69	16.09	1.38	22.24	3.92	26.52	19.62
256 ppm a.i.	62.21	44.75	28.86	2.64	35.72	8.34	42.55	29.70
Withdraw F1 (Treated Diets)								
156 ppm a.i.	44.59	27.80	18.85	3.88	20.62	2.63	28.37	20.00
256 ppm a.i.	70.47	50.62	34.78	3.03	32.64	6.38	46.54	33.90
Average for All Generations, Groups and Pulses								
Control							0.00	0.00
95 ppm a.i.							15.95	11.40
156 ppm a.i.							26.33	17.98
256 ppm a.i.							41.83	29.22
¹ Average of all exposure intervals within generational group. Calculated daily dietary dose assumes uniformity in body weight change and feed consumption between birds in pens and pens within groups. Please see Appendix XI for F0, F1 and F _W values by week.								

Analytical Results

None of the control samples showed any indication of the presence of the test substance or of the presence of a co-eluting substance at the characteristic retention time of the test substance. Diet samples were collected from the 95, 156 and 256 ppm a.i. test concentrations, and were analyzed to evaluate the homogeneity of the test substance in the diet. Means and standard deviations for the three test concentrations were 89.3 ± 2.96 ppm a.i., 144 ± 5.49 ppm a.i., and 243 ± 10.8 ppm a.i., respectively. The coefficients of variation were 3.31%, 3.81% and 4.44%, respectively (Appendix XIII, Table 4). Samples collected during the test to verify test substance concentrations for the 95, 156 and 256 ppm a.i. diets had means and standard deviations of 91.2 ± 6.95 ppm a.i., 153 ± 8.84 ppm a.i. and 238 ± 10.0 ppm a.i., respectively. The coefficients of variation were 7.62%, 5.78% and 4.20%. These values represented 96%, 98% and 93% of nominal concentrations (Appendix XIII, Table 5). Analysis of diet samples collected from feeders after being held at ambient temperature for 7 days averaged 95%, 102%, and 93% of the Day 0 values for the 95, 156, and 256 ppm a.i. test concentrations, respectively (Appendix XIII, Table 6). A typical chromatogram of a test sample is shown in Appendix XIII, Figure 7.

Primary Phase

Summary

No treatment related mortalities were observed during any generation or at any test concentration. At the 95 ppm a.i. test concentration no overt signs of toxicity were observed and there were no treatment related effects upon body weight, feed consumption or any of the reproductive parameters measured at any generation. At the 156 ppm a.i. test concentration there were reductions in F1 14-day old survivor body weight and feed consumption during brooding and early grow out, but subsequent recovery. Additionally, there were treatment related effects

upon egg production for both the F0 and F1 generations. At the 256 ppm a.i. test concentration there were impacts upon offspring health during brooding and grow-out and impacts upon body weight and feed consumption for both the F0 and F1 generations. Additionally, for the F0 generation there were marked impacts upon the reproductive parameters measured and an impact upon hatchability for the F1 generation. At all necropsy intervals and across generations, primarily concentration responsive impacts upon thyroid gland size were noted at the 95, 156 and 256 ppm a.i. concentrations.

Mortalities

F0 No F0 adult birds died during the course of the study.

F1 During rearing to 14 days of age, 1% or less of the offspring (F1) in any treatment group were found dead (Appendix F1 IV). During the F1 grow-out period from Week 15 to Week 56, no mortalities occurred in the control group or in the 95 ppm a.i. treatment group. At the 156 ppm a.i. test concentration, one bird was found dead and one bird was euthanized because of its debilitated condition during Week 17 of the study. Both mortalities were considered to be the result of physical injury, resulting lameness and inability to move. No mortalities occurred during grow-out at the 256 ppm a.i. test concentration. Once paired during Week 56, no mortalities occurred during the F1 reproductive portion of the study.

F2 During rearing to 14 days of age, less than 1% of the F2 offspring in any treatment group were found dead (Appendix F2 IV).

Clinical Observations

F0 For the F0 adult birds, incidental clinical observations noted during the test included those that normally are associated with injuries and pen wear. Such signs included foot and/or leg lesions or injury, associated lameness and feather loss.

F1 During brooding of the F1 ducklings, three offspring from the control group were noted as abnormally small during brooding, while one offspring from the 95 ppm a.i. treatment group was noted as lame. Two offspring from the 156 ppm a.i. treatment group were noted as abnormally small and two additional offspring were noted as weak prior to being found dead. In the 256 ppm a.i. treatment group, one offspring was noted with lethargy and reduced reaction to external stimuli (sound and movement) for two days during brooding, but subsequently recovered. Clinical observations noted during brooding were not considered to be treatment related. During grow-out of the F1 mallards, from Week 15 to Week 56, one bird from the control group was noted as small for approximately one and one-half weeks, one bird was noted with right wing droop for approximately one week and one bird was noted as lame for several days. In the 95 ppm a.i. treatment group, two birds were noted with leg injury/lameness for approximately one week, one bird was noted as small for one week and an additional bird was noted with twisted primary feathers on one wing. In the 156 ppm a.i. treatment group, feather loss was noted in three birds and one bird was noted as slightly lame for approximately one and one-half weeks. At the 256 ppm a.i. test concentration, seven birds were noted as small to very small and/or with abrasions and scratches, particularly on the back, until several weeks following the initial exposure period. Additionally one of these birds was noted with an eye lesion, one bird exhibited lameness and a bird was noted with twisted primary feathers on one wing. While clinical observations noted during rearing at the 95 and 156 ppm a.i. test concentrations were not considered to be treatment related, the increased incidence of small and/or abraded birds in the 256 ppm a.i. treatment group was considered to be related to treatment. During the F1 reproductive phase, foot lesions were noted in all treatment groups and were not considered to be treatment related. **F2** During brooding for the F2 offspring (untreated diet), two ducklings from the control group were noted to be abnormally small and one duckling was noted with dorsal head curl. While no clinical signs were noted in F2 offspring from the 95 ppm a.i. treatment group, one duckling from the 156 ppm a.i. treatment group was noted to be wet and weak, while another was noted as abnormally small. Clinical observations noted at the 95 and 156 ppm a.i. test concentrations during brooding were not considered to be treatment related. At the 256 ppm a.i. test concentration, two F2 offspring were noted as wet and weak, while five additional offspring were noted to be weak. While only a few offspring were impacted, the increased numbers of weak ducklings at the 256 ppm a.i. test concentration were considered to be related to treatment. Except for findings noted, all birds appeared normal throughout the study. Daily clinical observations are presented in Appendices F0 IV, F1 IV and F2 IV.

Gross Necropsy

All surviving adults were subjected to gross necropsy following adult termination. When compared to the control group, primarily concentration responsive enlarged thyroids were evidenced in the F0 and F1 adult birds of the 95, 156 and 256 ppm a.i. treatment groups. Additionally, during collection of thyroids from the 156 and 256 ppm

a.i. F1 and F2 offspring for possible histopathological evaluation, thyroid enlargement was noted. Necropsy findings are reported in Appendices F0 X and F1 X.

Body Weight Measurements

F0 For the F0 adults there were no apparent treatment-related effects upon adult body weights at the 95 and 156 ppm a.i. test concentrations and no statistically significant differences between the control group and the 95 and 156 ppm a.i. treatment groups were observed. At the 256 ppm a.i. test concentration there were statistically significant ($p < 0.01$) reductions in weight gain and terminal body weight of F0 hens over the treatment period.

Summary of results are reported below:

Experimental Group	Male					Female				
	TREATED				Withdrawal ¹	TREATED				Withdrawal ¹
	Week 1	Δ	Week 10			Week 1	Δ	Week 10		
	12/28/10		3/4/11		4/8/11	12/28/10		3/4/11		4/8/11
Control 0 ppm a.i.	Mean	1122	-53	1069		1033	148	1182		
	SD	75	57	79		102	87	125		
95 ppm a.i.	Mean	1135	-29	1106		1027	131	1158		
	SD	86	67	93		84	96	104		
156 ppm a.i.	Mean	1129	-42	1087		1022	109	1131		
	SD	98	62	86		78	99	118		
	Withdraw Mean			1125 ¹	73			1129 ¹	-55	1074 *
	SD			68 ¹	54			111 ¹	80	113
256 ppm a.i.	Mean	1127	-36	1091		1030	62 *	1091 *		
	SD	87	65	94		82	90	124		
	Withdraw Mean			1073 ¹	69			1106 ¹	-24	1083
	SD			77 ¹	85			121 ¹	107	85

The mean values for body weights and body weights changes were calculated and rounded separately.
 *Statistically different from the control group (concurrent or closest interval) at $p < 0.05$ (Dunnett's t-test).
 **Statistically different from the control group (concurrent or closest interval) at $p < 0.01$ (Dunnett's t-test).
¹ Ten pair from the 156 & 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw phase.

F1 For the F1 offspring (receiving treated diets) there were no apparent treatment related effects upon or statistically significant differences in hatchling (Day 0) body weight any of the concentrations tested or 14-day old survivor body weight at the 95 ppm a.i. test concentration. When compared to the control group, there were statistically significant ($p < 0.01$) reductions in the body weight of 14-day old survivors from the 156 and 256 ppm a.i. treatment groups. There were no statistically significant differences in body weight at any concentration tested at the Week 21 body weight interval (end of Pulse 1). At the start of Pulse 2, there was a statistically significant increase in the body weight of males from the 256 ppm a.i. treatment group, but no other statistically significant differences between the control group and any treatment group were noted in F1 body weights at any other interval.

Summary of results are reported below

F1 Table 1 - Page 1

Mean Body Weights (g) from a Two-Generation Mallard
Reproduction Study with Amitrole Using Pulsed Exposure
First Filial Generation - F1 Hatch to 14-Day, Brooding

TREATED Diets

Experimental Group	2/24/11 to 4/1/11			3/10/11 to 4/14/11		
	Hatchlings			14-Day Old Survivors		
	Wt. (g)	SD	#	Wt. (g)	SD	#
Control	34.0 ± 3.3		372	305 ± 21		334
0 ppm a.i.						
95 ppm a.i.	36.1 ± 3.3		404	293 ± 27		367
156 ppm a.i.	35.4 ± 2.6		311	260 ± 36 **		281
256 ppm a.i.	34.4 ± 2.6		124	198 ± 24 **		109

** Statistically different from the control group value at $p < 0.01$ (Dunnett's t-test).
All offspring received the appropriate treated diet during rearing.

F1 Table 1 - Page 2

Mean Body Weights (g) from a Two-Generation Mallard
Reproduction Study with Amitrole Using Pulsed Exposure
First Filial Generation - F1 Hatch to Termination

Three Exposure Intervals

Experimental Group	Sex	Pulse 1						Pulse 2				Pulse 3			
		Start	Δ		End	Δ	Start	Δ		End	Δ	Start	Δ		Adult Term
		Hatch ¹		2 Wks ¹			5/19/11	8/25/11		10/20/11		1/26/12		3/14/12	
Control	Male	34.0	271	305	729	1101	108	1209	34	1243	-195	1048	-4	1045	
	Female					966	134	1100	75	1175	-140	1035	152	1187	
95 ppm a.i.	Male	36.1	257	293	737	1105	96	1201	35	1236	-199	1037	-9	1029	
	Female					954	144	1098	102	1200	-170	1030	145	1175	
156 ppm a.i.	Male	35.4	225	260 **	756	1094	114	1208	10	1218	-197	1021	31	1052	
	Female					938	164	1102	45	1147	-168	979	181	1161	
256 ppm a.i.	Male	34.4	164	198 **	779	1044	244	1288 **	11	1299	-202	1097	-26	1071	
	Female					909	206	1115	83	1198	-240	958	196	1155	

¹ Weights represent all offspring from all lots and are not separated by sex.

** Statistically different from the control group value at $p < 0.01$ (Dunnett's t-test).

F2 For the F2 offspring (receiving untreated diets) there were no apparent treatment related effects upon hatchling or 14-day old survivor body weight any of the concentrations tested. When compared to the control group, there were no statistically significant differences in offspring body weight at the 95, 156 and 256 ppm a.i.

treatment groups. Mean body weight measurements are presented in Table F0 1, F1 1 and F2 1. Individual body weight measurements are presented in Appendices F0 V, F1 V and F2 V.

Summary of results are reported below:

F2 Table 1						
Mean Offspring Body Weight (g) from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure Second Filial Generation - F2 Hatch to 14-Day, Brooding						
Untreated Diets						
Experimental Group	3/8/12 to 4/12/12 Hatchlings			3/22/12 to 4/26/12 14-Day Old Survivors		
	Wt. (g)	SD	#	Wt. (g)	SD	#
Control	37.9 ± 1.9		398	298 ± 19		338
95 ppm a.i.	38.0 ± 1.9		373	310 ± 22		310
156 ppm a.i.	38.7 ± 2.8		330	312 ± 24		269
256 ppm a.i.	39.0 ± 2.7		216	308 ± 17		173
The above differences from the control group were not statistically significant. All offspring reared on untreated diet.						

Feed Consumption Measurements

F0 During the F0 exposure phase, there were no apparent treatment-related effects upon feed consumption at the 95 and 156 ppm a.i. test concentrations and no statistically significant differences were observed at any of the feed consumption intervals. At the 256 ppm a.i. test concentration, statistically significant reductions ($p < 0.05$) in feed consumption were observed during Weeks 7, 8 and 9 ($p < 0.05$) and Week 10 ($p < 0.01$). Summary of results are reported below:

F0 Table 2										
Mean Adult Feed Consumption (g/bird/day) from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure Parental Generation - F0 Adult Repro Units										
	TREATED									
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10
	12/28/10	1/4/11	1/11/11	1/18/11	1/25/11	2/1/11	2/8/11	2/15/11	2/22/11	3/1/11
Control 0 ppm a.i.										
Mean	79	116	135	145	159	157	148	152	163	183
SD	23	25	30	34	33	31	26	27	25	49
95 ppm a.i.										
Mean	74	126	148	148	161	166	150	150	165	167
SD	28	29	27	38	37	27	25	31	35	33
156 ppm a.i.										
Mean	74	130	146	148	163	165	151	158	168	184
SD	26	31	32	48	45	45	47	45	48	69
256 ppm a.i.										
Mean	76	121	128	141	142	139	117 *	120 *	129 *	132 **
SD	22	25	36	57	50	45	30	35	42	43
*Statistically different from the F0 control group at p < 0.05 (Dunnett's t-test).										
**Statistically different from the F0 control group at p < 0.01 (Dunnett's t-test).										

F1 While F1 birds were housed in groups by treatment during brooding and rearing, a statistical comparison of feed consumption values could not be performed. However, at the 156 and 256 ppm a.i. treatment groups there was an apparent treatment-related reduction in feed consumption during brooding and at least the first two weeks of grow-out, a time when offspring were exposed to treated diets. There was no apparent treatment related effect upon feed consumption at any test concentration during the second exposure pulse (Weeks 35 through 42) or during the third exposure pulse (Weeks 57 through 63, reproduction). No statistically significant differences were observed between the control group and any treatment group during the third pulse. Mean feed consumption measurements are shown in Tables F0 2 and F1 2. Feed consumption measurements by pen are presented in Appendices F0 VI and F1 VI.

Summary of results are reported below:

F1 Table 2 - Page 1

Mean Feed Consumption (g/bird/day) from a
Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure
First Filial Generation - F1 Brooders to Floor Pens Hatch to 56 Days of Age Pulse 1

		TREATED							
		Brooding		Week 15	Week 16	Week 17	Week 18	Week 19	Week 20
		1st Wk	2nd Wk	4/7/11	4/14/11	4/21/11	4/28/11	5/5/11	5/12/11
Control 0 ppm a.i.	Mean	24	82	115	118	78	64	99	63
95 ppm a.i.	Mean	23	78	122	121	97	76	53	59
156 ppm a.i.	Mean	19	65	109	96	95	77	81	67
256 ppm a.i.	Mean	17	49	82	53	113	58	66	71

F1 Table 2 - Page 2

Mean Feed Consumption (g/bird/day) from a
Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure
First Filial Generation - F1 Floor Pens 22 to 30 Weeks of Age Pulse 2

		TREATED							
		Week 35	Week 36	Week 37	Week 38	Week 39	Week 40	Week 41	Week 42
		8/25/11	9/1/11	9/8/11	9/15/11	9/22/11	9/29/11	10/6/11	10/13/11
Control 0 ppm a.i.	Mean	103	127	141	138	149	157	137	125
95 ppm a.i.	Mean	128	135	164	156	150	140	145	142
156 ppm a.i.	Mean	124	119	121	118	117	143	116	107
256 ppm a.i.	Mean	125	128	135	133	140	169	137	139

F1 Table 2 - Page 3							
Mean Feed Consumption (g/bird/day) from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure First Filial Generation - F1 44 to 51 Weeks of Age Repro Units Pulse 3							
	TREATED						
	Week 57	Week 58	Week 59	Week 60	Week 61	Week 62	Week 63
	1/26/12	2/2/12	2/9/12	2/16/12	2/23/12	3/1/12	3/8/12
Control 0 ppm a.i.							
Mean	89	167	174	168	168	169	171
95 ppm a.i.							
Mean	91	162	177	177	175	171	175
156 ppm a.i.							
Mean	88	158	164	167	163	151	163
256 ppm a.i.							
Mean	68	151	164	168	157	166	174
The above differences from the control group were not statistically significant.							

Reproductive Results

F0 For the F0 generation, there was no apparent treatment related impact upon reproductive success at the 95 ppm a.i. test concentration and no statistically significant differences were noted for any reproductive parameter measured. At the F0 156 ppm a.i. test concentration, while not statistically significant, there was a reduction in egg production that was considered to be related to treatment. At the F0 256 ppm a.i. test concentration there were profound effects upon reproductive performance, including egg production and hatchability. Statistically significant differences ($p < 0.01$) were observed in eggs laid of maximum laid, hatchlings of live 3-week embryos and hatchlings and 14-day old survivors of both eggs set and maximum eggs set.

Summary of results are reported below

Reproductive Parameter	Experimental Group (ppm a.i.)			
	Control	95	156	256
Number Surviving Replicates	20	20	20	20
Total Eggs Laid	595	608	488	286
Eggs Cracked	3	0	3	3
Eggs Set	529	552	429	243
Viable Embryos	498	518	404	232
Live 3-Week Embryos	495	508	399	232
Hatchlings	372	404	311	124
14-Day Old Survivors	334	367	281	109
Eggs / Hen	30	30	24	14
Eggs Laid / Hen / Day ¹	0.57	0.58	0.47	0.28
14-Day Old Survivors / Hen	17	18	14	5

Reproductive Parameter	Experimental Group (ppm a.i.)			
	Control	95	156	256
Number Surviving Replicates	20	20	20	20
Eggs Laid	595	608	488	286
Eggs Laid / Maximum Laid (%)	57	58	47	28 **
Eggs Cracked / Eggs Laid (%)	1	0	2	1
Viable Embryos / Eggs Set (%)	94	93	93	90
Live 3-Week Embryos / Viable Embryos (%)	99	98	99	100
Hatchlings / Live 3-Week Embryos (%)	77	78	80	51 **
14-Day Old Survivors / Hatchlings (%)	98	99	99	99
Hatchlings / Eggs Set (%)	72	72	74	46 **
14-Day Old Survivors / Eggs Set (%)	68	69	72	44 **
Hatchlings / Maximum Set (%)	42	46	35	14 **
14-Day Old Survivors / Maximum Set (%)	38	42	32	12 **

¹ Based on 52 days of egg production.
 ** Statistically different from the control group at p < 0.01 (Dunnett's t-test).

F1 For the F₁ generation, there was no apparent treatment related impact upon reproductive success at the 95 ppm a.i. test concentration and no statistically significant differences were noted for any reproductive parameter measured. At the F₁ 156 ppm a.i. test concentration there was a slight, but statistically significant (p < 0.05) reduction in egg production that was considered to be related to treatment. At the F₁ 256 ppm a.i. test concentration, while not statistically significant, there was a reduction in hatchability that was considered to be related to treatment. Summaries of the reproductive data are presented in Tables F0 3 and F1 3. Reproductive parameters by pen are presented in Appendices F0 VII, F0 VIII, F1 VII and F1 VIII.

Summary of results are reported below:

F1 Table 3				
Summary of Reproductive Results from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure First Filial Generation - F1 Adult Repro Units TREATED Diets				
Reproductive Parameter	Experimental Group (ppm a.i.)			
	Control	95	156	256
Number Surviving Replicates	17	18	17	11
Total Eggs Laid	580	561	501	349
Eggs Cracked	2	2	5	1
Eggs Set	525	506	444	315
Viable Embryos	471	464	396	297
Live 3-Week Embryos	464	458	394	295
Hatchlings	398	374	330	216
14-Day Old Survivors	338	310	269	173
Eggs / Hen	34	31	29	32
Eggs Laid / Hen / Day ¹	0.73	0.66	0.63	0.68
14-Day Old Survivors / Hen	20	17	16	16
Reproductive Parameter	Experimental Group (ppm a.i.)			
	Control	95	156	256
Number Surviving Replicates	17	18	17	11
Eggs Laid	580	561	501	349
Eggs Laid / Maximum Laid (%)	79	72	69 *	74
Eggs Cracked / Eggs Laid (%)	0	0	1	0
Viable Embryos / Eggs Set (%)	89	92	89	95
Live 3-Week Embryos / Viable Embryos (%)	99	99	99	99
Hatchlings / Live 3-Week Embryos (%)	86	81	83	74
14-Day Old Survivors / Hatchlings (%)	99	99	99	99
Hatchlings / Eggs Set (%)	76	74	73	70
14-Day Old Survivors / Eggs Set (%)	74	71	68	66
Hatchlings / Maximum Set (%)	59	52	49	49
14-Day Old Survivors / Maximum Set (%)	50	43	40	39
¹ Based on 47 days of egg production.				
* Statistically different from the control group at p < 0.05 (Dunnett's t-test).				

Egg Shell Thickness

F0 There were no apparent treatment related effects upon F0 egg shell thickness at the 95 or 156 ppm a.i. test concentrations and, when compared to the control group, there were no statistically significant differences in egg

shell thickness in the 95 or 156 ppm a.i. treatment groups. However, at the 256 ppm a.i. test concentration there was a reduction in mean egg shell thickness that was statistically significant at $p < 0.05$.

Summary of results are reported below:

F0 Table 4 Mean Egg Shell Thickness (mm) from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure Parental Generation - F0 Lots A to F			
Treated Diets			
Experimental Group	Mean	SD	Number of Eggs Measured
Control	0.387 ± 0.022		50
95 ppm a.i.	0.378 ± 0.024		51
156 ppm a.i.	0.370 ± 0.025		43
256 ppm a.i.	0.360 ± 0.032 *		34
* Statistically different from the control group value at $p < 0.05$ (Dunnett's t-test).			

F1 For the F1 generation, there were no treatment-related effects upon egg shell thickness at any of the concentrations tested. When compared to the control group, there were no statistically significant differences in egg shell thickness in any treatment group. Egg shell thickness data are presented in Table F0 4 and F1 4 and in Appendix F0 IX and F1 IX.

Summary of results are reported below

F1 Table 4			
Mean Egg Shell Thickness (mm) from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure First Filial Generation - F1 Lots A to F			
Treated Diets			
Experimental Group	Mean	SD	Number of Eggs Measured
Control	0.387 ± 0.022		48
95 ppm a.i.	0.380 ± 0.025		49
156 ppm a.i.	0.390 ± 0.021		47
256 ppm a.i.	0.393 ± 0.027		31
The above differences from the control group value were not statistically significant.			

Withdraw Phase

Summary

No treatment related mortalities were observed during any generation or at any test concentration. At the 156W ppm a.i. test concentration there were impacts upon offspring health during grow-out and reductions in F1W body weight and feed consumption during brooding and early grow out. Additionally, there were treatment related effects upon egg production for the F1W generation. At the 256W ppm a.i. test concentration there were impacts upon offspring health during brooding and grow-out, and impacts upon body weight and feed consumption for F1W generation adults. Additionally, there was an impact upon F1W generation egg production and hatchability. At all necropsy intervals and across generations, impacts upon thyroid gland size were noted at the 156W and 256W ppm a.i. concentrations.

Withdraw Mortalities

F0W Given the impact seen on adults in the F0 generation, 10 pairs of mallards each from the F0 156 and 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw (W) phase. No F0W adult birds died during the course of the study.

F1W During rearing to 14 days of age, less than 1% of the offspring (F1W) in either treatment group were found dead (Appendix F1W IV). During the F1W grow-out period from Week 20 to Week 63, at the 156W ppm a.i. test concentration one bird was euthanized due to its poor condition and at the 256W ppm a.i. test concentration one bird was euthanized due to an injury. Once paired during Week 56, no mortalities occurred during the F1W reproductive portion of the study.

F2W During rearing to 14 days of age, only 1% of the F2W offspring in any treatment group were found dead (Appendix F2W IV).

Withdraw Clinical Observations

F0W For the F0W adult birds, incidental clinical observations noted during the test included those that normally are associated with injuries and pen wear. Such signs included foot lesions and/or associated lameness, feather loss and head or neck lesions and were considered to be unrelated to treatment.

F1W During brooding of the F1W ducklings, one offspring from the 156W ppm a.i. treatment group was noted with a cranial abnormality and euthanized and another was noted as weak before being found dead. In the 256W ppm a.i. treatment group, one offspring was noted as abnormally small. Clinical observations were not considered to be related to treatment. All other F1W offspring were normal in appearance and behavior during brooding. During grow-out of the F1W mallards, from Week 20 to Week 63, one bird from the 156W ppm a.i. treatment group was noted as cold and weak, up to four birds were noted with feather loss and/or with abrasions and scratches, particularly on the back, one bird was noted as abnormally small and one bird was noted with slight wing droop of one wing. At the 256W ppm a.i. test concentration, four birds were noted as abnormally small and five birds were noted with feather loss. The increased incidences of small birds and birds with feather loss/abrasions at both the 156W and 256W ppm a.i. test concentrations were considered to be related to treatment. Additionally one bird in the 256W ppm a.i. treatment group was noted with loss of coordination, lower limb rigidity and head tremors on only one day, possibly the result of head injury. During the F1W reproductive phase, foot lesions were noted in up to four birds in the 156W ppm a.i. treatment group and up to three birds in the 256W ppm a.i. treatment group.

F2W During brooding for the F2W offspring (untreated diets), one duckling from the 156W ppm a.i. treatment group was noted as lame, and one duckling was noted as abnormally small. Additionally, one duckling was noted as weak before being found dead and one duckling was noted with lateral head curl prior to death. At the 256W ppm a.i. test concentration, two WF2 offspring were noted as weak. Except for findings noted, all birds appeared normal throughout the study. Daily clinical observations are presented in Appendices F0W IV, F1W IV and F2W IV.

Withdraw Gross Necropsy

All withdraw group F0 adult mallards (untreated diet for six weeks) were subjected to gross necropsy following adult termination. Enlarged thyroids ($>$ corresponding exposure phase control group mean value plus one standard deviation) were evidenced in three males and four females from the 156W ppm a.i. treatment group and six males and two females from the 256W ppm a.i. treatment group. At terminal necropsy for the F1W adult birds, enlarged thyroids were observed in all but one bird from the 156W ppm a.i. treatment group and all adults from the 256W ppm a.i. treatment group. Necropsy findings are reported in Appendices F0W X and F1W X.

Withdraw Body Weight Measurements

F0W For the F0W adults, following six weeks on untreated diets, at the 156W ppm a.i. test concentration there was a statistically significant ($p < 0.01$) increase in the mean body weight of males and a statistically significant ($p < 0.05$) decrease in the mean body weight of females when compared to the corresponding closest control group values. There were no apparent treatment-related effects upon adult body weights at the 256W ppm a.i. test concentration and no statistically significant differences between the closest control group values and the 256W ppm a.i. treatment group were observed. Summary of results were already reported below:

F0W Table 1

Adult Body Weights (g) from a Two-Generation Mallard
Reproduction Study with Amitrole Using Pulsed Exposure
Parental Generation - F0W Adult Repro Units

Experimental Group		Male					Female				
		TREATED			Withdrawal ¹		TREATED			Withdrawal	
		Week 1 12/28/10	Δ	Week 10 3/4/11	Δ	Week 15 4/8/11	Week 1 12/28/10	Δ	Week 10 3/4/11	Δ	Week 15 4/8/11
Control 0 ppm a.i.	Mean	1122	-53	1069			1033	148	1182		
	SD	75	57	79			102	87	125		
95 ppm a.i.	Mean	1135	-29	1106			1027	131	1158		
	SD	86	67	93			84	96	104		
156 ppm a.i.	Mean	1129	-42	1087			1022	109	1131		
	SD	98	62	86			78	99	118		
	Withdraw Mean			1125 ¹	73	1198 **			1129 ¹	-55	1074 *
	SD			68 ¹	54	76			111 ¹	80	113
256 ppm a.i.	Mean	1127	-36	1091			1030	62 *	1091 *		
	SD	87	65	92			82	90	124		
	Withdraw Mean			1073 ¹	69	1141			1106 ¹	-24	1083
	SD			77 ¹	85	100			121 ¹	107	85

The mean values for body weights and body weights changes were calculated and rounded separately.

*Statistically different from the control group (concurrent or closest interval) at $p < 0.05$ (Dunnett's t-test).

**Statistically different from the control group (concurrent or closest interval) at $p < 0.01$ (Dunnett's t-test).

¹ Ten pair from the 156 & 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw phase.

F1W For the F1W offspring (receiving treated diets) there were no apparent treatment related effects upon or statistically significant differences in hatchling (Day 0) body weight at either concentration. However, there were statistically significant ($p < 0.01$) reductions in the body weights of 14-day old survivors from the 156W and 256W ppm a.i. treatment groups when compared to the closest control group value. While only statistically significant ($p < 0.05$) for males from the 156W ppm a.i. treatment group, there was a reduction in body weight for both treatment groups and sexes at the Week 26 body weight interval (end of Pulse 1). In the 156W ppm a.i. treatment group at the start of Pulse 2 there was a statistically significant ($p < 0.01$) decrease in the body weight of females and statistically significant decreases in the body weights of males ($p < 0.05$) and females ($p < 0.01$) at the end of Pulse 2. While not statistically significant, there was a reduction in body weight of males and females in the 256W ppm a.i. treatment group at the end of Pulse 1, and a continued reduction in the body weight of females through Pulse 2. No other statistically significant differences were noted in F1W body weights at any other interval. Summary of results are reported below:

F1W Table 1 - Page1							
Mean Body Weights (g) from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure First Filial Generation - F1W Hatch to 14-Day, Brooding							
Withdraw Groups - F0 Adults on Untreated Diets ¹							
TREATED Diets							
Experimental Group	4/7/11 to 5/5/11			4/21/11 to 5/19/11			
	Hatchlings			14-Day Old Survivors			
	Wt. (g)	SD	#	Wt. (g)	SD	#	
156 ppm a.i.	33.9 ± 2.8		154	241 ± 37 **		137	
256 ppm a.i.	34.8 ± 3.2		118	204 ± 25 **		100	

¹ Ten pair from the 156 & 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw phase. Offspring received treated diet.

** Statistically different from the exposure phase control group value at p < 0.01 (Dunnett's t-test).

F1W Table 1 - Page 2													
Mean Body Weights (g) from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure First Filial Generation - F1W Hatch to Termination													
Withdraw Group - F0 Adults on Untreated Diets ¹													
Three Exposure Intervals													
Experimental Group	Sex	Pulse 1						Pulse 2				Pulse 3	
		Start Hatch ²	Δ	2 Wks ³	Δ	End 6/23/11	Δ	Start 9/28/11	Δ	End 11/23/11	Δ	Start 3/1/12	Adult Term 4/17/12
156 ppm a.i.	Male	33.9	207	241 **	701	999 *	169	1168	-24	1144 *	-94	1050	19
	Female					884	139	1023 *	35	1058 **	-74	984	176
256 ppm a.i.	Male	34.8	169	204 **	750	1020	236	1256	-23	1233	-136	1097	3
	Female					888	189	1077	26	1103	-56	1047	163

¹ Ten pair from the 156 & 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw phase.

² Weights represent all offspring from all lots and are not separated by sex.

* Statistically different from the exposure phase control group value at p < 0.05 (Dunnett's t-test).

** Statistically different from the exposure phase control group value at p < 0.01 (Dunnett's t-test).

F2W For the F2W offspring (receiving untreated diets) from the 156W and 256W treatment groups, there were no apparent treatment related effects upon hatchling or 14-day old survivor body weight any of the

concentrations tested. When compared to the closest control group values, there were no statistically significant differences in offspring body weight at the 156W and 256W ppm a.i. treatment groups. Mean body weight measurements are presented in Table F0 1, F1W 1 and F2W 1. Individual body weight measurements are presented in Appendices F0W V, F1W V and F2W V. Summary of results are reported below:

F2W Table 1						
Mean Offspring Body Weights (g) from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure Second Filial Generation - F2 Hatch to 14-Day, Brooding						
Withdraw Groups - F0 Adults on Untreated Diets ¹						
Untreated Diets						
Experimental Group	4/12/12 to 5/17/12			4/26/12 to 5/31/12		
	Hatchlings			14-Day Old Survivors		
	Wt. (g)	SD	#	Wt. (g)	SD	#
156 ppm a.i.	36.9 ± 3.4		186	318 ± 31		151
256 ppm a.i.	39.1 ± 1.6		119	318 ± 19		94

¹ Ten F0 pair from the 156 & 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw phase. F2 offspring reared on untreated diet.
The above differences from the exposure phase control group were not statistically significant (Dunnett's t-test).

Withdraw Feed Consumption Measurements

F0W During the F0W withdraw phase (untreated diet), there were no apparent treatment-related effects upon feed consumption at the 156W ppm a.i. test concentration and, when compared to the closest control group values, no statistically significant differences were observed at any of the feed consumption intervals. At the 256W ppm a.i. test concentration there was a slight, but statistically significant ($p < 0.05$) reduction in feed consumption during Week 12. No statistically significant differences were observed at any other feed consumption interval. Summary of results are reported below:

F0W Table 2					
Mean Feed Consumption (g/bird/day) from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure Parental Generation - F0W Adult Repro Units Withdraw Group - F0 Adults on Untreated Diets ¹					
	Untreated Diets				
	Week 11	Week 12	Week 13	Week 14	Week 15
	3/4/11	3/11/11	3/18/11	3/25/11	4/1/11
156 ppm a.i.					
Mean	181	171	183	179	157
SD	87	54	50	56	64
256 ppm a.i.					
Mean	135	138 *	168	160	141
SD	46	29	26	32	33
¹ Ten pair from the 156 & 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw phase. Statistically different from the F0 control group (closest interval) at p < 0.05 (Dunnett's t-test).					

F1W While F1W birds were housed in groups by treatment during brooding and rearing, a statistical comparison of feed consumption values could not be performed. However, at the 156W and 256W ppm a.i. treatment groups there was an apparent treatment-related reduction in feed consumption during the last week of brooding and at least the first two weeks of grow-out, a time when offspring were exposed to treated diets. There was no apparent treatment related effect upon feed consumption at any test concentration during the second exposure pulse (Weeks 40 through 47) or during the third exposure pulse (Weeks 62 through 68, reproduction). No statistically significant differences were observed between the control group and any treatment group during the third pulse. Mean feed consumption measurements are shown in Tables F0W 2 and F1W 2. Feed consumption measurements by pen are presented in Appendices F0W VI and F1W VI. Summary of results are reported below

F1W Table 2 - Page 1								
Mean Feed Consumption (g/bird/day) from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure First Filial Generation - F1W Brooders to Floor Pens Hatch to 56 Days of Age Pulse 1 Withdraw Groups - F0 Adults on Untreated Diets ¹								
TREATED								
Brooding		Week 20	Week 21	Week 22	Week 23	Week 24	Week 25	
1st Wk	2nd Wk	5/12/11	5/19/11	5/26/11	6/2/11	6/9/11	6/16/11	
156 ppm a.i.								
Mean	21	64	89	70	105	125	114	114
256 ppm a.i.								
Mean	22	56	81	72	72	115	105	57
¹ Ten pair from the F0 156 & 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw phase. F1 received treated diets.								

F1W Table 2 - Page 2								
Mean Feed Consumption (g/bird/day) from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure First Filial Generation - F1W Floor Pens 22 to 30 Weeks of Age Pulse 2 Withdraw Groups - F0 Adults on Untreated Diets ¹								
TREATED								
Week 40	Week 41	Week 42	Week 43	Week 44	Week 45	Week 46	Week 47	
9/28/11	10/5/11	10/12/11	10/19/11	10/26/11	11/2/11	11/9/11	11/16/11	
156 ppm a.i.								
Mean	174	156	152	109	123	102	104	141
256 ppm a.i.								
Mean	176	169	134	164	150	168	160	148
¹ Ten pair from the F0 156 & 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw phase. F1 received treated diets.								

F1W Table 2 - Page 3							
Mean Feed Consumption (g/bird/day) from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure First Filial Generation - F1W 44 to 51 Weeks of Age Repro Units Pulse 3 Withdraw Groups - F0 Adults on Untreated Diets ¹							
	TREATED						
	Week 62	Week 63	Week 64	Week 65	Week 66	Week 67	Week 68
	3/1/12	3/8/12	3/15/12	3/22/12	3/29/12	4/5/12	4/12/12
156 ppm a.i.							
Mean	99	143	133 *	152	156	160	151
256 ppm a.i.							
Mean	80	154	131 *	150	160	163	159
¹ Ten pair from the F0 156 & 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw phase. F1 received treated diets.							
* Statistically different from the F1 exposure phase control group (closest interval) at p < 0.05 (Dunnett's t-test).							

Withdraw Reproductive Results

F0W For the F0W generation, while on untreated diet, there were no apparent treatment related impacts upon reproductive success at the 152W or 256W ppm a.i. test concentrations when compared to the exposure phase control group values. No statistically significant differences were noted for any reproductive parameter measured. Summary of results are reported below.

F0W Table 3 - Page 1

Summary of Reproductive Results from a Two-Generation Mallard
Reproduction Study with Amitrole Using Pulsed Exposure
Parental Generation - F0W Adult Repro Units

Withdraw Phase - Untreated Diets¹

Reproductive Parameter	Experimental Group (ppm a.i.)			
	Exposure Control	Adjusted ² Control	156W	256W
Number Surviving Replicates	20	10	10	10
Total Eggs Laid	595	200	215	191
Eggs Cracked	3	1	1	1
Eggs Set	529	178	190	164
Viable Embryos	498	168	177	155
Live 3-Week Embryos	495	167	175	153
Hatchlings	372	125	155	118
14-Day Old Survivors	334	112	138	100
Eggs / Hen	30	20	22	19
Eggs Laid / Hen / Day	0.57 ³		0.61 ⁴	0.55 ⁴
14-Day Old Survivors / Hen	17	11	14	10

¹ Ten pair from the 156 & 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw phase.

² Count values adjusted for length of laying period (52 vs 35) and number of replicates (20 vs 10).

³ Based on 52 days of egg production.

⁴ Based on 35 days of egg production.

F0W Table 3 - Page 2				
Summary of Reproductive Results from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure Parental Generation - F0W Adult Repro Units Withdraw Phase - Untreated Diets ¹				
Reproductive Parameter	Experimental Group (ppm a.i.)			
	Exposure Control	Adjusted ² Control	156W	256W
Number Surviving Replicates	20		10	10
Eggs Laid	595	200	15	191
Eggs Laid / Hen / Day	0.57 ³		0.61 ⁴	0.55 ⁴
Eggs Laid / Maximum Laid (%)	57		67	60
Eggs Cracked / Eggs Laid (%)	1		0	0
Viable Embryos / Eggs Set (%)	94		94	95
Live 3-Week Embryos / Viable Embryos (%)	99		99	99
Hatchlings / Live 3-Week Embryos (%)	77		88	78
14-Day Old Survivors / Hatchlings (%)	98		100	99
Hatchlings / Eggs Set (%)	72		81	73
14-Day Old Survivors / Eggs Set (%)	68		78	69
Hatchlings / Maximum Set (%)	42		53	41
14-Day Old Survivors / Maximum Set (%)	38		48	34
¹ Ten pair from the 156 & 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw phase.				
² Count values adjusted for length of laying period (52 vs 35) and number of replicates (20 vs 10).				
³ Based on 52 days of egg production.				
⁴ Based on 35 days of egg production.				
The above differences from the exposure phase control group were not statistically different.				

F1W At the F1W 156W ppm a.i. test concentration, while not statistically significant, there was a slight reduction in egg production that was considered to be related to treatment. At the F1W 256W ppm a.i. test concentration, while not statistically significant, there were reductions in egg production and hatchability that were considered to be related to treatment. Summaries of the reproductive data are presented in Tables F0W 3 and F1W 3. Reproductive parameters by pen are presented in Appendices F0W VII, F0W VIII, F1W VII and F1W VIII. Summary of results are reported below:

F1W Table 3 - Page 1				
Summary of Reproductive Results from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure First Filial Generation - F1W Adult Repro Units Withdraw Group - F0 Adults on Untreated Diets ¹ 156 ppm a.i. Group - TREATED Diet				
Reproductive Parameter	Experimental Group (ppm a.i.)			
	Exposure Control	Adjusted ² Control	156W	256W
Number Surviving Replicates	17		9	8
Total Eggs Laid	580	290	249	190
Eggs Cracked	2	1	1	0
Eggs Set	525	263	217	163
Viable Embryos	471	236	207	157
Live 3-Week Embryos	464	232	207	156
Hatchlings	398	199	186	119
14-Day Old Survivors	338	169	151	94
Eggs / Hen	34		28	24
Eggs Laid / Hen / Day	0.73 ³		0.59 ³	0.51 ³
14-Day Old Survivors / Hen	20		17	12
¹ Ten pair from the F0 156 & 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw phase. F1 offspring received treated diet.				
² Count values adjusted for number of replicates (17 vs 8.5).				
³ Based on 47 days of egg production.				

F1W Table 3 - Page 2				
Summary of Reproductive Results from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure First Filial Generation - F1W Adult Repro Units Withdraw Group - F0 Adults on Untreated Diets ¹ 156 ppm a.i. Group - TREATED Diet				
Reproductive Parameter	Experimental Group (ppm a.i.)			
	Exposure Control	Adjusted ² Control	156W	256W
Number Surviving Replicates	17		9	8
Eggs Laid	580	290	249	190
Eggs Laid / Hen / Day	0.73 ³		0.59 ³	0.51 ³
Eggs Laid / Maximum Laid (%)	79		77	66
Eggs Cracked / Eggs Laid (%)	0		0	0
Viable Embryos / Eggs Set (%)	89		96	97
Live 3-Week Embryos / Viable Embryos (%)	99		100	99
Hatchlings / Live 3-Week Embryos (%)	86		88	77
14-Day Old Survivors / Hatchlings (%)	99		97	99
Hatchlings / Eggs Set (%)	76		83	74
14-Day Old Survivors / Eggs Set (%)	74		79	69
Hatchlings / Maximum Set (%)	59		65	46
14-Day Old Survivors / Maximum Set (%)	50		52	37
¹ Ten pair from the F0 156 & 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw phase. F1 offspring received treated diet.				
² Count values adjusted for number of replicates (17 vs 8.5).				
³ Based on 47 days of egg production.				
The above differences from the F1 exposure phase control group were not statistically significant (Dunnett's t-test).				

Withdraw Egg Shell Thickness

There were no apparent treatment related effects upon F0W egg shell thickness at the 156W or 256W ppm a.i. test concentrations. When compared to the exposure phase control group, there were no statistically significant differences in egg shell thickness in the 156W or 256W ppm a.i. treatment groups. Additionally, there were no apparent treatment related effects upon F1W egg shell thickness at the 156W or 256W ppm a.i. test concentrations and no statistically significant differences were observed. Egg shell thickness data are presented in Tables F0W 4 and F1W 4 and in Appendices F0W IX and F1W IX. Summary of results are reported below:

F0W Table 4			
Mean Egg Shell Thickness (mm) from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure Withdraw Groups - F0 Adults on Untreated Diets ¹ Parental Generation - F0W Lots AW to EW			
Untreated Diets			
Experimental Group	Mean	SD	Number of Eggs Measured
156 ppm a.i.	0.374 ± 0.036		22
256 ppm a.i.	0.369 ± 0.028		19
¹ Ten pair from the 156 & 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw phase. The above differences from the exposure phase control group value were not statistically significant (Dunnett's t-test).			

F1W Table 4			
Mean Egg Shell Thickness (mm) from a Two-Generation Mallard Reproduction Study with Amitrole Using Pulsed Exposure Withdraw Groups - F0 Adults on Untreated Diets ¹ First Filial Generation - F1W Lots AW to EW			
Treated Diets			
Experimental Group	Mean	SD	Number of Eggs Measured
156 ppm a.i.	0.383 ± 0.021		21
256 ppm a.i.	0.375 ± 0.029		17
¹ Ten pair from the 156 & 256 ppm a.i. treatment groups were transferred to untreated diet for a five week withdraw phase. The above differences from the exposure phase control group value were not statistically significant (Dunnett's t-test).			

□ **Conclusion:**

No impact upon growth or reproductive success was noted at the 95 ppm a.i. test concentration. Multiple, and typically concentration responsive, effects were noted upon growth and reproduction at the 156 and 256 ppm a.i. test concentrations, including the corresponding withdraw groups. At all necropsy intervals, primarily concentration responsive impacts upon thyroid gland size were noted at the 95, 156 and 256 ppm a.i. concentrations. The reproductive no-observed-effect concentration was 95 ppm a.i., approximately equivalent to a calculated average daily dietary dose of 16 mg/kg bw/day during the exposure phases.

❑ **Comment (RMS): Study acceptable**

No treatment related mortalities were observed during any generation or at any test concentration.

At the 95 ppm a.i. test concentration no overt signs of toxicity were observed and there were no treatment related effects upon body weight, feed consumption or any of the reproductive parameters measured at any generation.

At the 156 ppm a.i. test concentration there were reductions in F1 14-day old survivor body weight and feed consumption during brooding and early grow out, and subsequent recovery. However, there were no reductions in F2 14-day survivor body weight. Additionally, there were treatment related effects upon egg production for both the F0 and F1 generations.

At the 256 ppm a.i. test concentration there were impacts upon offspring health during brooding and grow-out and impacts upon body weight and feed consumption for both the F0 and F1 generations. However, there were no reductions in F2 14-day survivor body weight. Additionally, for the F0 generation there were marked impacts upon the reproductive parameters measured and an impact upon hatchability for the F1 generation.

At all necropsy intervals and across generations, primarily concentration responsive impacts upon thyroid gland size (higher size than in the control) were noted at the 95, 156 and 256 ppm a.i. concentrations.

An amendment to the final study report was submitted at the end of December 2012. In this amendment, it is reported that at collection of thyroids for potential histopathology from selected (up to two per pen) F2 hatchlings and untreated 14-day old survivors, thyroids appeared comparable in size to the control group. However, no measured data were submitted to the RMS. In these conditions, due to this uncertainty no NOEC based on effects on thyroid can be set.

In conclusion, a NOEC based on reproductive performance parameters can be set at 95 ppm. However, no overall NOEC can be set. In F0 and F1 there are impacts upon thyroid glands size at all tested concentrations. It is well known that increase or decrease of thyroid gland size may influence vertebrates behaviour. There is therefore an uncertainty on the impact of such effects on wild animals (e.g. parental care, nesting behaviour, territoriality and mounting behaviour). The value of 95 ppm a.s corresponding to 16 mg/kg bw/day should therefore be considered as a LOEC.

A bibliographic search was conducted by the notifier in order to try to find a relationship between an increase of thyroid size and an impact on parental care, nesting behaviour, territoriality and mounting behavior. A list of references was submitted at the end of December 2012, but no summary of these papers was done by the notifier. The notifier concludes that nothing could be found. However, considering the late submission of this bibliography, it was not possible for the RMS to check. Moreover, even if nothing could be found, it doesn't mean that there are no uncertainties about such effect on thyroid gland size. The notifier states that a field residue study, to measure the level of amitrole remaining on insects and weeds used as feed for birds after application of amitrole-containing product, showed that the residues of amitrole in feed ranged at maximum from 0.08 to 3.09 mg/kg of feed (maximum values measured on the day or the day after the application). This represents 0.0003% of feed.

In Regulation (EC) n° 1107/2009 it is specified in Annex II under point 3.8.2 that "An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines, it is not considered to have endocrine disrupting properties that may cause adverse effects on non-target organisms unless the exposure of non-target organisms to that active substance in a plant protection product under realistic proposed conditions of use is negligible."

The notifier concludes that a level of exposure at 3.9 ppm maximum over one day can be considered as negligible. The above statement is the notifier's interpretation of the regulation. However, it does not modify RMS' conclusion on the risk assessment.

B.9.13. Metabolites

Please refer to point 9.1.

B.9.1.4. Toxicity of plant protection product

Please refer to point 9.1.

B.9.1.5. Supervised cage or field trials (annex IIIA 10.1.2)

Supervised cage or field tests are not required because the TER_a and TER_{st} are >10 , and the TER_{lt} is >5 for the active substance.

B.9.1.6. Acceptance of bait, granules or treated seeds by birds (annex IIIA 10.1.3)

This test is not required because formulated product WEEDAZOL TL is not intended for use as bait, granules, or seed treatments and the TER_a for the active substance is >10 .

B.9.1.7. Effects of secondary poisoning (annex IIIA 10.1.4)

Amitrole log K_{ow} is < 3 (2.49) indicating that a secondary poisoning risk assessment is not necessary.

B.9.1.8. Risk assessment for birds**B.9.1.8.1. Toxicity endpoints for birds**

The relevant toxicity data are summarised in the table below.

Table 9.1.8.1.1: Toxicity data for amitrole

Species	Time scale	Test substance	LD ₅₀ /LC ₅₀ (nominal)	NOEL/NOEC (nominal)	Reference
Bobwhite quail	Acute (oral)	amitrole	$> 2150 \text{ mg/kg b.w.}$	-	review report 6839/VI/97-final, 22/03/2001.
Mallard duck	Short-term (5d)	amitrole	$> 5000 \text{ ppm a.s., equivalent to } > \text{ca. } 829 \text{ mg a.s./kg b.w./day}$	-	review report 6839/VI/97-final, 22/03/2001.
Bobwhite quail	Reproduction (22w)**	amitrole	-	Medium herbivorous bird relevant NOEC = 450 ppm a.s., equivalent to ca. 38.1 mg a.s./kg b.w./day	review report 6839/VI/97-final, 22/03/2001.
Mallard duck	Reproduction (22w)**	amitrole	-	Lowest NOEC = 100 ppm a.s., equivalent to ca. 12.1 mg a.s./kg b.w./day	review report 6839/VI/97-final, 22/03/2001.
Mallard duck	2 generation Reproduction (15 months)	amitrole	-	NOEC reproductive = 95 ppm a.s., equivalent to ca. 16 mg a.s./kg b.w./day At all necropsy intervals, impacts upon thyroid size were noted at all concentrations. Overall LOEC = 95 ppm a.s., equivalent to ca. 16 mg a.s./kg b.w./day Overall NOEC: none	Temple D., 2012

*: value in **bold** are used into TER calculations.

** Toxicity data not sufficient for assessing potential effects on development and reproduction due to endocrine disrupting properties of the active substance (see explanation below)

The one-generation reproduction test on mallard duck (NOEC=12.1 mg a.s./kg b.w./day) was not sufficient for assessing potential effects on development and reproduction due to endocrine disrupting properties of the active substance. This is due to the fact that the one-generation avian reproduction study did not include exposure during all relevant stages of the bird's development. These potential consequences on development and reproduction were studied with the 2-generation study in birds (Mallard duck). No NOEC could be established as there were impacts upon thyroid gland size (higher size than in the control) at all concentrations tested in F0 and F1. An amendment to the final study report was submitted at the end of December 2012. In this amendment, it is reported that at collection of thyroids for potential histopathology from selected (up to two per pen) F2 hatchlings and untreated 14-day old survivors, thyroids appeared comparable in size to the control group. However, no measured data were submitted to the RMS. In these conditions, due to this uncertainty no NOEC based on effects on thyroid can be set.

An amendment to the final study report was submitted at the end of December 2012. In this amendment, it is reported that at collection of thyroids for potential histopathology from selected (up to two per pen) F2 hatchlings and untreated 14-day old survivors, thyroids appeared comparable in size to the control group. However, no measured data were submitted to the RMS. In these conditions, due to this uncertainty no NOEC based on effects on thyroid can be set.

In conclusion, a NOEC based on reproductive performance parameters can be set at 95 ppm. However, no overall NOEC can be set. In F0 and F1 there are impacts upon thyroid gland size at all tested concentrations. It is well known that increase or decrease of thyroid gland size may influence vertebrates behaviour. There is therefore an uncertainty on the impact of such effects on wild animals at population level in natural conditions (e.g. parental care, nesting behaviour, territoriality and mounting behaviour). The value of 95 ppm a.s corresponding to 16 mg/kg bw/day should therefore be considered as a LOEC. Therefore, long term risk assessment for birds could not be finalized.

Short-term LC₅₀ and reproduction NOEC values were converted on daily dose as follows:

Daily dose (mg/kg bw/d) = food concentration (mg/kg food) x daily food consumption (g/bird/d)/body weight (g)

The acute oral LD₅₀ of >2150 mg a.s./kg bw will be applied in the Tier 1 assessment for birds presented below.

According to the guidance (EFSA 2009) there is no need for an assessment of the short-term (dietary) risk to birds.

An acute toxicity study was performed with WEEDAZOLE TL in Mallard ducks. The toxicity of formulated product was not shown to be higher than that of the active substance.

Table 9.1.8.1.2: Toxicity data for WEEDAZOLE TL

Species	Time scale	Test substance	LD ₅₀ /LC ₅₀ (nominal)	NOEL/NOEC (nominal)	Reference
Mallard duck	Short-term (5d)	WEEDAZOL TL	> 2000 mg /kg b.w	-	EU (DAR)

*: value used into TER calculations

The LD₅₀ of WEEDAZOL TL is > 2000 mg /kg b.w. Therefore, the risk assessments were based on the active substance endpoints mentioned above.

B.9.1.8.2. Calculations of acute DDD and TER for birds

For non-cropped areas, risk is covered by risk assessment for cropped-areas. Therefore, the potential exposure of birds to Amitrole was estimated following one application of CA2735 at 13 L/ha in orchards and vineyards. This represents the maximum dose rate used per season.

However CA2735 can be applied once (full dose) or twice per season but the dose rate will never exceed 13 L/ha corresponding to 2.977 kg a.s./ha.

For the 2 crops, the worst case was considered, 1 application at the full dose rate. For a detailed summary of the GAP of **CA2735**, please refer to **table B.9.1**, "Table of Intended Uses".

It should be noted that **CA2735** applications are not directed to the crops either in orchards or in vineyard. The spray is applied on the row (at the foot of the plants or of the trees). It was thus considered that only one third of the total crop surface is really treated. Therefore only 1/3 of the total dose (0.993 kg a.s./ha) were actually applied onto the soil.

The exposure was estimated according to the EFSA Journal (2009)⁴.

Screening assessment

The first step in the EFSA modelling is the screening assessment. The daily dietary dose (DDD) for a single application was calculated with the following formula:

DDD = application rate (kg/ha) × shortcut value

With:

Shortcut value = default parameter combining food intake rate, body weight, concentration of the substance in the diet (based on the 90th percentile residues) and the fraction of diet obtained in the treated area for the avian indicator species/crop combination in question.

Since one single application per season at the full dose rate of **CA2735** was considered as the worst case, the MAF (multiple application factor) was not included in the calculations. The TER value was calculated by dividing the acute endpoint (LD₅₀) by the daily dietary dose (DDD).

Table 9.1.8.2.1: Acute screening for birds according to EFSA Journal (2009)

Crop	Application rate ¹	Indicator species	Shortcut Value ²	DDD ³	LD ₅₀ [mg/kg b.w./day]	TER (10) ⁴
Orchard	0.993 kg Amitrole/ha	Small insectivorous bird	46.8	41.65	2150	46.3
Vineyard		Small omnivorous bird	95.3	94.63		22.7

¹ corresponding to 1/3 of the full dose rate due to the application made under the row

² 90th percentile residues

³ daily dietary dose

⁴ Toxicity-to-exposure ratio (Trigger)

The screening step after application of **CA2735** in orchard and vineyard indicates no acute risk to birds (the TER_A value is above the trigger of 10). Therefore, no Tier 1 risk assessment is required.

Therefore, if **CA2735** is used according to the manufacturer recommendations, it does not represent any significant acute risk to birds.

B.9.1.8.3. Calculation of long term DDD and TER for birds

⁴ European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

Long term DDD and TER for birds were calculated by the applicant based on the one-generation NOEL of 12.1 mg/kg b.w./day but as no overall NOEC could be established from the two-generation bird study, long term risk assessment for birds couldn't be finalized.

Based on the one-generation NOEL of 12.1 mg/kg b.w./day the risk was shown to be acceptable in Tier 1 for some species in orchard and vineyard, but not for others and a higher tier (based on focal species and measured residue values) was presented. Even if calculations are not reported, new studies have been assessed and are presented below for information.

Higher tier risk assessment for birds

✓ Vineyard

If an active substance, and its associated product and use, fails Tier 1, it is possible to further refine the exposure element of risk via the use of a 'focal species'.

a) Refining PT and PD

Report:	KIIIA1 10.1.2.1/01, [REDACTED] 2006
Title:	Bird species in vineyards in France and Italy: Field data for the determination of focal species
Document No:	[REDACTED]
Guidelines:	Bibby et al. 1992. Bird census techniques. Academic Press, London
GLP:	Yes

Executive Summary

The objectives of this generic study were to determine the qualitative and quantitative composition of the bird community employing the parameters, frequency of occurrence (FO_{field} and FO_{survey}) and dominance both as overall and as grapevine growth stage specific descriptors. Another objective was then to allocate the selected species to defined foraging guilds, diet guilds and size classes. The study was performed in Champagne-Ardenne/Bourgogne regions of France and the Tyrol and Apulia regions of Italy, encompassing 30, 17 and 15 vineyards, respectively selected to represent average vineyards, vineyard size and the structure of the landscape. In order to cover different vine growth stages, three line transect surveys were conducted in 2005 for each vineyard during dormancy and/or bud development (survey 1), leaf development or inflorescence emergence (survey 2) and inflorescence emergence and/or development of fruits (survey 3). A standard line transect consisted of an "in-crop transect band" (a 50 m wide recording band of 25 m to each side of the observer moving along a longitudinal in-crop field transect). For the assessment of the bird community, frequency of occurrence (FO_{field} and FO_{survey}) and dominance were determined. Data were analysed using the "ECOLOGY RESEARCH DATABASE SYSTEM" (ERDS). The ranking of species within the list of focal species candidates was carried out in decreasing order of importance, i.e. FO_{field} > FO_{survey} > dominance. This list of candidates of focal species was then used to allocate the respective species to defined foraging guilds, diet guilds and size classes.

A total of 722, 739 and 184 individual bird contacts, comprising 36, 33 and 18 different species, was recorded throughout all surveys within the 'in-crop transect bands' in the Champagne-Ardenne/Bourgogne region of France, and the Tyrol and Apulia regions of Italy, respectively. The species that were recorded with a frequency of occurrence (FO_{field}) ≥ 20% were thus determined as the main candidates for focal bird species in the respective country.

The recorded candidates of focal bird species were assigned to the following guilds in accordance with the SANCO guidance document (ranked by their respective frequency of occurrence and dominance):

France

- o Small insectivore great tit (ground/foliage) > black redstart (ground)
- o Small granivorous linnet >> goldfinch (all ground/foliage)

- o Small omnivore wood lark (ground) > skylark (ground) > chaffinch (ground/foilage) > cirl bunting (ground) > greenfinch (ground/foilage)
- o Medium omnivore blackbird (ground/foilage)
- o Large omnivore carrion crow (ground)

Northern Italy

- o Small insectivore spotted flycatcher (foilage) > great tit (ground/foilage) > wryneck (ground/foilage) > wheatear (ground)
- o Small granivorous serine > goldfinch (all ground/foilage)
- o Small omnivore chaffinch > greenfinch > tree sparrow (all ground/foilage)
- o Medium insectivore hoopoe (ground)
- o Medium omnivore blackbird > song thrush > fieldfare (all ground/foilage)

Southern Italy

- o Small granivorous serine > goldfinch > linnet (all ground/foilage)
- o Small omnivore crested lark (ground) > tree sparrow > house sparrow (all ground/foilage),
- o Medium insectivore hoopoe (ground)
- o Medium omnivore magpie (ground/foilage)

The linnet and woodlark were the most characteristic and stable elements of the bird community in vineyards in France across all vine growth stages. Analogous results were detected for the blackbird, cirl bunting and carrion crow, but with lower FO_{field} values. Other species, showing peak FO values $\geq 20\%$ for individual vine growth stages only were the skylark (for surveys 2 and 3), chaffinch, great tit and greenfinch (for survey 1). The blackbird and chaffinch were the most characteristic and stable elements of the bird community in vineyards in Northern Italy (Tyrol) across all vine growth stages. Similar results were obtained for the greenfinch and tree sparrow, but with lower FO_{field} values. Other species, showing peak FO values $\geq 20\%$ for individual vine growth stages only, were the serine and spotted flycatcher (for surveys 2 and 3), song thrush (for surveys 1 and 3), fieldfare (for survey 1), wryneck, great tit and wheatear (for survey 2). The goldfinch was the most characteristic and stable element of the bird community in vineyards in Southern Italy (Apulia) across all vine growth stages. Analogous results were detected for the tree sparrow, but with lower FO_{field} values. Other species, showing peak FO values $\geq 20\%$ for individual vine growth stages only were the serine (for surveys 1 and 3), crested lark (for surveys 1 and 2), linnet, magpie and house sparrow (for survey 1). All the species listed in the tables above can be considered as potential candidates for focal bird species in a refined risk assessment for plant protection products in vineyards in France and Italy, respectively

I. MATERIALS AND METHODS

1. In life dates: 15/03 – 03/07/2005

2. Experimental design

The objectives of this generic study were to determine the qualitative and quantitative composition of the bird community employing the parameters, frequency of occurrence (FO_{field} and FO_{survey}) and dominance both as overall and as grapevine growth stage specific descriptors. Another objective was then to allocate the selected species to defined foraging guilds, diet guilds and size classes.

The study was performed in Champagne-Ardenne/Bourgogne regions of France and the Tyrol and Apulia regions of Italy, encompassing 30, 17 and 15 vineyards, respectively selected to represent average vineyards, vineyard size and the structure of the landscape. In order to cover different vine growth stages, three line transect surveys were conducted in 2005 for each vineyard during dormancy and/or bud development (survey 1), leaf development or inflorescence emergence (survey 2) and inflorescence emergence and/or development of fruits (survey 3). A standard line transect consisted of an "in-crop transect band" (a 50 m wide recording band of 25 m to each side of the observer moving along a longitudinal in-crop field transect).

3. Observations:

Vineyards were visited between the middle of March and the beginning of July 2005. Three surveys were conducted in each field within a three-month period. A standardised vineyard form and a bird survey form were developed to record a number of parameters during each field survey. The avifauna of the vineyards was surveyed by the line transect method for which general information can be found in Bibby et al. (1992). To meet

the specific methodological requirements of this report, the line transect method described by Bibby et al. (1992) was adapted for this study in order to be compatible with the ERDS software.

4. Statistics

For the assessment of the bird community, frequency of occurrence (FO_{field} and FO_{survey}) and dominance were determined. Data were analysed using the "ECOLOGY RESEARCH DATABASE SYSTEM" (ERDS). The ranking of species within the list of focal species candidates was carried out in decreasing order of importance, i.e. FO_{field} > FO_{survey} > dominance. This list of candidates of focal species was then used to allocate the respective species to defined foraging guilds, diet guilds and size classes.

II. RESULTS AND DISCUSSION

A total of 722, 739 and 184 individual bird contacts, comprising 36, 33 and 18 different species, was recorded throughout all surveys within the 'in-crop transect bands' in the Champagne-Ardenne/Bourgogne region of France, and the Tyrol and Apulia regions of Italy, respectively. The following species listed in the tables below were recorded with a frequency of occurrence (FO_{field}) ≥ 20% and were thus determined as the main candidates for focal bird species in the respective country. In most cases the findings were supported by FO_{survey} and dominance data (tables 10.1.2.1-1, 10.1.2.1-3 and 10.1.2.1-5).

France

Eleven species were found to be characterised by a FO_{field} of at least 20% (Table 16). Most of these species were grouped into the small bird category (< 50 g) and only one each was medium-sized (blackbird) or large (carrion crow).

In France the linnet showed high FO values throughout the season with a maximum of 50.0% during the first survey period; the skylark, blackbird and woodlark also showed high FO values during the third survey with peaks of 50.0%, 46.7% and 43.3%, respectively. FO values of the chaffinch, great tit, curl bunting, carrion crow and greenfinch showed small maxima (23.3 – 36.7%) during the first survey period, though levels in some cases were lower during the second and third survey periods.

Table 10.1.2.1-1: List of candidates of focal species in vineyards in France

Species	FO _{field} n = 29 [%] ^{a,b}	FO _{survey} n = 83 [%] ^{a,c}	Dominance n = 29 [%] ^{a,d}	Body weight [g] ^e	Stratum use ^{f)}	Diet guild ^{g)}
Linnet (<i>Carduelis cannabina</i>)	76.7	42.2	23.3	15	ground/foilage	granivorous
Wood lark (<i>Lullula arborea</i>)	66.7	40.0	11.2	26.9	ground	omnivorous
Skylark (<i>Alauda arvensis</i>)	63.3	34.4	6.0	37.2	ground	omnivorous
Carrion crow (<i>Corvus corone</i>)	60.0	22.2	5.0	570.0	ground	omnivorous
Blackbird (<i>Turdus merula</i>)	56.7	33.3	7.6	113.0	ground/foilage	omnivorous
Chaffinch (<i>Fringilla coelebs</i>)	40.0	18.9	3.6	20.9	ground/foilage	granivorous
Greenfinch (<i>Carduelis chloris</i>)	76.5	39.2	5.3	27.8	ground/foilage	omnivorous
Curl bunting (<i>Emberiza cirrus</i>)	36.7	25.6	8.9	23.1	ground	omnivorous
Great tit (<i>Parus major</i>)	36.7	17.8	3.3	19.0	ground/foilage	insectivorous
Greenfinch (<i>Carduelis chloris</i>)	36.7	14.4	3.3	27.8	ground/foilage	omnivorous

Black redstart (<i>Phoenicurus ochruros</i>)	30.0	12.2	2.5	16.5	ground	insectivorous
Goldfinch (<i>Carduelis carduelis</i>)	20.0	6.7	1.4	15.6	ground/foilage	granivorous

- a) Across the complete study period (3 survey periods)
b) Based on 30 study plots (vineyards)
c) Based on 90 surveys
d) Based on the arithmetic mean number of individuals per study plot of one species in relation to the mean number of individuals per study plot of all species for a total number of 30 vineyards
e) According to Dunning (1993). In the case of sex-specific values, the lower value was chosen
f) Predominant foraging stratum during growing season according to Perrins (1998)
g) Predominant diet composition during growing season according to Perrins (1998)

The species listed above were grouped according to their dietary guilds and foraging strata. Details for each species are presented in table 10.1.2.1-2.

Table 10.1.2.1-2: Characteristics of focal bird species candidates in vineyards in France

SPECIES	CHARACTERISTICS
Small granivorous birds	
Linnet (<i>Carduelis cannabina</i>)	Lowest body weight regarding group; highest FO figures, slightly lower values during second survey; foraging on ground and on weeds
Goldfinch (<i>Carduelis carduelis</i>)	Largest body weight of group; low overall FO figures, not recorded during second survey but increasing towards end of survey period; forages on ground and on weeds
Small insectivorous birds	
Great tit (<i>Parus major</i>)	Highest body weight of group; high FO values at beginning of season, declining towards end; feeds chiefly on insects during breeding season but is generally omnivorous; predominantly foliage-foraging;
Black redstart (<i>Phoenicurus ochruros</i>)	Lowest body weight of group; FO numbers below 20% during individual surveys; predominantly ground-foraging
Small omnivorous birds	
Chaffinch (<i>Fringilla coelebs</i>)	Lowest body weight within group; FO figures show a sharp decline during course of season; forages on ground and in foliage
Greenfinch (<i>Carduelis chloris</i>)	Second highest body weight; low FO figures particularly towards the end of the season; forages on ground and in foliage
Cirl bunting (<i>Emberiza cirlus</i>)	Second lowest body weight of group; low variation of FO values across survey period; forages chiefly on ground
Wood lark (<i>Lullula arborea</i>)	Body weight intermediate within group; constantly high FO figures throughout season, slightly lower FO numbers during second survey; forages on ground
Skylark (<i>Alauda arvensis</i>)	Highest body weight in group; FO values show constant increase during season; forages on ground
Medium omnivorous birds	
Blackbird (<i>Turdus merula</i>)	Only representative of group; FO moderate, but much lower during second survey period; forages on ground and in foliage
Large omnivorous birds	
Carion crow (<i>Corvus corone</i>)	Only representative of group; FO rather low with highest values during first survey period; forages mainly on ground

Northern Italy

Thirteen species were found to be characterised by an FO_{field} of at least 20% (10.1.2.1-3). Most of these species were grouped into the small bird category (< 50 g), while four were medium-sized (blackbird, song thrush, fieldfare and hoopoe).

In Northern Italy the blackbird showed moderate to high FO values throughout the season with maxima of 100 % during the second and third survey periods; the chaffinch also showed high FO values with a peak of 88.2 % during both the first and second surveys. FO values for the tree sparrow, serine, wryneck and great tit reached their maxima (23.5 – 52.9%) during the second survey period. The spotted flycatcher, greenfinch and song thrush showed increasing FO values with peaks of 35.3 – 52.9% during the third survey, whereas the fieldfare showed a high FO value (41.2%) only during the first survey.

Table 10.1.2.1-3: List of candidates of focal species in vineyards in Northern Italy

Species	FO _{field} n = 29 [%] ^{a,b}	FO _{survey} n = 83 [%] ^{a,c}	Dominance n = 29 [%] ^{a,d}	Body weight [g] ^e	Stratum use ^f	Diet guild ^g
Blackbird (<i>Turdus merula</i>)	100.0	98.0	36.4	113.0	ground/foilage	omnivorous
Chaffinch (<i>Fringilla coelebs</i>)	100.0	82.4	15.2	20.9	ground/foilage	granivorous
Greenfinch (<i>Carduelis chloris</i>)	76.5	39.2	5.3	27.8	ground/foilage	omnivorous
Serine (<i>Serinus serinus</i>)	76.5	31.4	3.2	11.2	ground/foilage	granivorous
Tree sparrow (<i>Passer montanus</i>)	70.6	39.2	8.5	22.0	ground/foilage	omnivorous
Spotted flycatcher (<i>Muscicapa striata</i>)	64.7	27.5	4.6	14.6	foilage	insectivorous
Song thrush (<i>Turdus philomelos</i>)	41.2	23.5	6.6	66.6	ground/foilage	granivorous
Fieldfare (<i>Turdus pilaris</i>)	41.2	17.6	3.1	104.0	ground/foilage	omnivorous
Great tit (<i>Parus major</i>)	41.2	13.7	1.2	19.0	ground/foilage	insectivorous
Wryneck (<i>Jynx torquilla</i>)	35.3	11.8	0.8	33.5	ground/foilage	insectivorous
Goldfinch (<i>Carduelis carduelis</i>)	23.5	9.8	1.4	15.6	ground/foilage	granivorous
Wheatear (<i>Oenanthe oenanthe</i>)	23.5	7.8	1.1	22.3	ground	insectivorous
Hoopoe (<i>Upupa epops</i>)	23.5	7.8	1.1	146.0	ground	insectivorous

a) Across the complete study period (3 survey periods)

b) Based on 17 study plots (vineyards)

c) Based on 51 surveys

d) Based on the arithmetic mean number of individuals per study plot of one species in relation to the mean number of individuals per study plot of all species for a total number of 17 vineyards

e) According to Dunning (1993). In the case of sex-specific values, the lower value was chosen

f) Predominant foraging stratum during growing season according to Perrins (1998)

g) Predominant diet composition during growing season according to Perrins (1998)

The species listed above were grouped according to their dietary guilds and foraging strata. Details for each species are presented in 10.1.2.1-4.

Table 10.1.2.1-4: Characteristics of focal bird species candidates in vineyards in Northern Italy

SPECIES	CHARACTERISTICS
Small granivorous birds	
Goldfinch (<i>Carduelis carduelis</i>)	Largest body weight of group; low overall FO figures, not recorded during first survey but increasing towards end of study period; forages on ground and weeds
Serine (<i>Serinus serinus</i>)	Lowest body weight regarding group; high FO figures but missing during first survey; foraging on ground and on weeds
Small insectivorous birds	
Spotted flycatcher (<i>Muscicapa striata</i>)	Lowest body weight in group; high FO values during second and third surveys but not present during first survey (migratory species); forages in foliage and aerial (fly catching from exposed perch)
Wryneck (<i>Jynx torquilla</i>)	Second highest body weight in group; moderate FO only during second survey, otherwise missing (first survey) or low values (third survey); forages on ground and in foliage
Wheatear (<i>Oenanthe oenanthe</i>)	Intermediate body weight and only present during second survey with rather low FO values; forages on ground
Great tit (<i>Parus major</i>)	Second-lowest body weight of group; moderate to low FO values during first two surveys and not present during last survey; forages chiefly in foliage
Medium insectivores	
Hoopoe (<i>Upupa epops</i>)	Only representative of group; missing during first survey and low FO values during the second and third surveys; forages on ground
Small omnivorous birds	
Chaffinch (<i>Fringilla coelebs</i>)	Lowest body weight of group; FO figures very high throughout season; forages in foliage and on ground
Greenfinch (<i>Carduelis chloris</i>)	Highest body weight; constant FO figures throughout with a slight increase towards end; forages in foliage and on ground
Tree sparrow (<i>Passer montanus</i>)	Body weight intermediate; FO figures were highest at second survey period and slightly lower during third survey; forages in foliage and on ground
Medium omnivorous birds	
Song thrush (<i>Turdus philomelos</i>)	Lowest body weight in group; low to moderate FO values with peaks during first and third surveys; forages chiefly on ground
Fieldfare (<i>Turdus pilaris</i>)	Intermediate body weight in group; moderate FO only during first survey, otherwise very low FO figures; forages on ground and in foliage
Blackbird (<i>Turdus merula</i>)	Highest body weight in group; FO very high throughout; forages on ground and in foliage

Southern Italy

Eight species were found to be characterised by an FO_{field} of at least 20%. Six of these species were grouped into the small bird category (< 50 g) and two were medium-sized (50 – 500 g).

In Southern Italy the goldfinch and tree sparrow showed homogeneous FO values throughout the season with maxima of 46.7% and 33.3% during the first and second survey periods, respectively. The serine displayed high FO values during the first and third surveys with a peak of 53.3% during the first survey, whereas the linnet, crested lark, magpie and house sparrow showed decreasing FO values with peaks of 20.0 – 60.0% during the first survey.

Table 10.121-5: List of candidates of focal species in vineyards in Southern Italy

Species	FO _{field} n = 29 [%] ^{a,b}	FO _{survey} n = 83 [%] ^{a,c}	Dominance n = 29 [%] ^{a,d}	Body weight [g] ^e	Stratum use ^f	Diet guild ^g
Serine (<i>Serinus serinus</i>)	93.3	33.3	12.5	11.2	ground/foilage	granivorous

Goldfinch (<i>Carduelis carduelis</i>)	73.3	40.0	14.1	15.6	ground/foilage	granivorous
Crested lark (<i>Galerida cristata</i>)	73.3	31.1	9.8	39.0	ground	omnivorous
Tree sparrow (<i>Passer montanus</i>)	60.0	28.9	11.4	22.0	ground/foilage	omnivorous
Linnet (<i>Carduelis cannabina</i>)	60.0	24.4	22.8	15	ground/foilage	granivorous
Magpie (<i>Pica pica</i>)	53.3	22.2	9.2	166	ground/foilage	omnivorous
House sparrow (<i>Passer domesticus</i>)	40.0	15.6	7.6	27.4	ground/foilage	omnivorous
Hoopoe (<i>Upupa epops</i>)	20.0	6.7	1.6	146	ground	insectivorous

a) Across the complete study period (3 survey periods)

b) Based on 15 study plots (vineyards)

c) Based on 45 surveys

d) Based on the arithmetic mean number of individuals per study plot of one species in relation to the mean number of individuals per study plot of all species for a total number of 15 vineyards

e) According to Dunning 1993. In the case of sex-specific values, the lower value was chosen

f) Predominant foraging stratum during growing season according to Perrins (1998)

g) Predominant diet composition during growing season according to Perrins (1998)

The species listed above were grouped according to their dietary guilds and foraging strata. Details for each species are presented in 10.1.2.1-6.

Table 10.1.2.1-6: Characteristics of focal bird species candidates in vineyards in Southern Italy

SPECIES	CHARACTERISTICS
Small granivorous birds	
Linnet (<i>Carduelis cannabina</i>)	Second lowest body weight of group; marked decline in frequency of occurrence from first to second survey, not present during third survey; forages on ground and on weeds
Serine (<i>Serinus serinus</i>)	Lowest body weight of group; low FO during second survey but otherwise high values; forages on ground and on weeds
Goldfinch (<i>Carduelis carduelis</i>)	Highest body weight of group; high FO figures throughout season but values decline towards end of survey period; forages on ground and on weeds
Small omnivorous birds	
Tree sparrow (<i>Passer montanus</i>)	Lowest body weight of group; FO figures more or less constant throughout season with small peak during second survey; forages on ground and in foliage
House sparrow (<i>Passer domesticus</i>)	Second highest body weight; low FO figures particularly during second and third surveys; forages on ground and in foliage
Crested lark (<i>Galerida cristata</i>)	Highest body weight of group; FO values show a constant decrease during season and not present during last survey; forages on ground
Medium insectivores	
Hoopoe (<i>Upupa epops</i>)	Only representative of group; FO figures are moderate during the third survey period but obviously lower during second survey and missing completely during first survey; foraging on ground
Medium omnivorous birds	
Magpie (<i>Pica pica</i>)	Only representative of group; FO figures moderate during first survey period but obviously lower during second and third surveys; foraging on ground and in foliage

III.

CONCLUSIONS

The recorded candidates of focal bird species were assigned to the following guilds in accordance with the SANCO guidance document (ranked by their respective frequency of occurrence and dominance):

France

- o Small insectivore great tit (ground/foilage) > black redstart (ground)
- o Small granivorous linnet >> goldfinch (all ground/foilage)
- o Small omnivore wood lark (ground) > skylark (ground) > chaffinch (ground/foilage) > cirl bunting (ground) > greenfinch (ground/foilage)
- o Medium omnivore blackbird (ground/foilage)
- o Large omnivore carrion crow (ground)

Northern Italy

- o Small insectivore spotted flycatcher (foilage) > great tit (ground/foilage) > wryneck (ground/foilage) > wheatear (ground)
- o Small granivorous serine > goldfinch (all ground/foilage)
- o Small omnivore chaffinch > greenfinch > tree sparrow (all ground/foilage)
- o Medium insectivore hoopoe (ground)
- o Medium omnivore blackbird > song thrush > fieldfare (all ground/foilage)

Southern Italy

- o Small granivorous serine > goldfinch > linnet (all ground/foilage)
- o Small omnivore crested lark (ground) > tree sparrow > house sparrow (all ground/foilage),
- o Medium insectivore hoopoe (ground)
- o Medium omnivore magpie (ground/foilage)

The linnet and woodlark were the most characteristic and stable elements of the bird community in vineyards in France across all vine growth stages. Analogous results were detected for the blackbird, cirl bunting and carrion crow, but with lower FO_{field} values. Other species, showing peak FO values ≥ 20% for individual vine growth stages only were the skylark (for surveys 2 and 3), chaffinch, great tit and greenfinch (for survey 1). The blackbird and chaffinch were the most characteristic and stable elements of the bird community in vineyards in Northern Italy (Tyrol) across all vine growth stages. Similar results were obtained for the greenfinch and tree sparrow, but with lower FO_{field} values. Other species, showing peak FO values ≥ 20% for individual vine growth stages only, were the serine and spotted flycatcher (for surveys 2 and 3), song thrush (for surveys 1 and 3), fieldfare (for survey 1), wryneck, great tit and wheatear (for survey 2). The goldfinch was the most characteristic and stable element of the bird community in vineyards in Southern Italy (Apulia) across all vine growth stages. Analogous results were detected for the tree sparrow, but with lower FO_{field} values. Other species, showing peak FO values ≥ 20% for individual vine growth stages only were the serine (for surveys 1 and 3), crested lark (for surveys 1 and 2), linnet, magpie and house sparrow (for survey 1). All the species listed in the tables above can be considered as potential candidates for focal bird species in a refined risk assessment for plant protection products in vineyards in France and Italy, respectively

(Dietzen et al, 2006)

RMS comment: Study acceptable. Agree with the choice of focal species.

Based on the results of the above-summarized study, PT and PD were determined for the focal bird species of interest. A study was conducted in France in 2005. It is summarized below.

Report:	KIIIA1 10.1.2.1/02, Selbach, A., 2007
Title:	Generic field monitoring of birds in vineyards in France
Document No:	RA05223-2
Guidelines:	Not applicable; the test was especially designed for the purpose of this study
GLP:	Yes

Executive Summary

The aim of this generic study was to obtain estimates of diet (PD) and the proportions taken from vineyards (PT) by four focal bird species, circl bunting, great tit, linnet and woodlark. The study was conducted during spring and summer 2006 in the Burgundy region around the municipalities of Santenay, Dezize-lès-Maranges and Cheilly-lès-Maranges (France), a typical wine growing district, and encompassed a study area of 1220 ha. Twenty individuals of each species were trapped with mist nets in or adjacent to vineyards, marked with a metal and colour ring, and radio tagged. Each individual was tracked continuously over one whole daily activity period (from dusk till dawn), during which time it was aimed to keep the bird under continuous visual observation.

In the case of two circl buntings, one linnet and three woodlarks, individuals were tracked for 2 daily activity periods. For analysis the respective sessions were pooled. The proportion of time foraging in vineyards (compared with the total potential foraging time) was estimated from the data obtained by radio-tracking and visual observation. These values are regarded as equivalent to the proportion of diet obtained from the treated area (PT). To estimate the proportion of different food types in the diet (PD), faeces and/or stomach contents were sampled and analysed. Correction factors derived from the literature were applied to estimate the number of items actually ingested by the birds from the number of arthropods and seeds recognised within the food samples. Estimations of the prey length provided information on the food size selection of the birds. Length-weight regressions of invertebrates and seeds identified via the literature were used to calculate the proportion of dry weight of each food type in the diet actually ingested by the birds.

Based on the study results, the mean PT value derived for circl buntings was 0.43 (90th percentile = 0.77), for great tits the mean PT value was 0.05 (90th percentile = 0.08), for linnets the mean PT was 0.78 (90th percentile = 0.97) and for woodlarks 0.86 (90th percentile = 1). It is important to emphasise that these values represent the total time spent potentially foraging in vineyards. These values include times when birds may not actually be foraging but information is not available to exclude this possibility. Therefore, the calculated PT values for all species can be regarded as a conservative assumption.

Circl buntings ingested invertebrate items and plant seeds in similar proportions (0.499 and 0.501, respectively) related to the total dry weight of their diet. Insect adults and Poaceae seeds were the most important taxa with PD values of 0.297 and 0.334, respectively. Great tits fed mainly on invertebrates (PD value of 0.955), whereas the PD value for seeds was only 0.045. Insect adults and larvae were the most important food items, providing together a PD of 0.788. In contrast, linnets ingested mainly seeds, with a PD of 0.973. Most important were Brassicaceae seeds, providing a PD value of 0.508. The diet of woodlarks consisted basically of invertebrates (PD of 0.921) with insect adults as the most important food items (PD of 0.527).

I. MATERIALS AND METHODS

1. In life dates:

03/03 – 02/12/2006

2. Experimental design

The aim of this generic study was to obtain estimates of diet (PD) and the proportions taken from vineyards (PT) by four focal bird species, circl bunting, great tit, linnet and woodlark. The study was conducted during spring and summer 2006 in the Burgundy region around the municipalities of Santenay, Dezize-lès-Maranges and Cheilly-lès-Maranges (France), a typical wine growing district, and encompassed a study area of 1220 ha.

3. Observations:

Twenty individuals of each species were trapped with mist nets in or adjacent to vineyards, marked with a metal and colour ring, and radio tagged. Each individual was tracked continuously over one whole daily activity period (from dusk till dawn), during which time it was aimed to keep the bird under continuous visual observation. In the case of two circl buntings, one linnet and three woodlarks, individuals were tracked for 2 daily activity periods. For analysis the respective sessions were pooled. The proportion of time foraging in vineyards (compared with the total potential foraging time) was estimated from the data obtained by radio-tracking and visual observation. These values are regarded as equivalent to the proportion of diet obtained from the treated area (PT).

To estimate the proportion of different food types in the diet (PD), faeces and/or stomach contents were sampled and analysed. Correction factors derived from the literature were applied to estimate the number of items actually ingested by the birds from the number of arthropods and seeds recognised within the food samples. Estimations of the prey length provided information on the food size selection of the birds. Length-weight

regressions of invertebrates and seeds identified via the literature were used to calculate the proportion of dry weight of each food type in the diet actually ingested by the birds.

II. RESULTS AND DISCUSSION

PT values

The combination of radio-tracking with visual appraisal and the trapping scheme as presented here, allowed an accurate and representative assessment of potential foraging times in given home ranges, thus making it possible to calculate reliable PT values. All birds were closely associated with vineyards and had the opportunity to use these fields as a foraging habitat. The results can thus be considered a worst case in terms of potential exposure.

Table 10.1.2.1-7: PT of 20 radiotracked birds/species

Individual n°	PT values			
	linnets	woodlarks	Cirl buntings	Great tits
1	0.93	1.00	0.97	0.03
2	0.85	0.89	0.37	0.02
3	0.91	0.49	0.44	0.00
4	0.58	0.88	0.35	0.05
5	0.49	0.96	0.76	0.01
6	0.48	0.76	0.42	0.01
7	0.84	1.00	0.84	0.03
8	0.52	0.71	0.41	0.01
9	1.00	1.00	0.16	0.11
10	0.84	0.98	0.16	0.08
11	0.97	0.41	0.13	0.35
12	0.84	0.45	0.37	0.06
13	0.72	0.65	0.38	0.01
14	0.16	0.99	0.16	0.06
15	0.86	0.97	0.4	0.02
16	0.97	1.00	0.24	0.01
17	0.82	1.00	0.54	0.00
18	0.96	1.00	0.25	0.01
19	0.99	0.96	0.53	0.03
20	0.92	1.00	0.77	0.05
50%tile	0.85	0.96	0.39	0.02
90%tile	0.97	1.00	0.77	0.09*
mean	0.78	0.86	0.43	0.05*

*excluding PT of 0 from the calculations

Extracted from appendices 20, 24, 27 and 21 of the study report.

Based on the study results (table 10.1.2.1-7), the mean PT value derived for cirl buntings was 0.43 (90th percentile = 0.77), for great tits the mean PT value was 0.05 (90th percentile = 0.08), for linnets the mean PT was 0.78 (90th percentile = 0.97) and for woodlarks 0.86 (90th percentile = 1). It is important to emphasise that these values represent the total time spent potentially foraging in vineyards. These values include times when birds may not actually be foraging but information is not available to exclude this possibility. Therefore, the calculated PT values for all species can be regarded as a conservative assumption.

Table 10.1.2.1-8: Overview of the PT (Vineyards)

Proportion of diet obtained in vineyards determined by combined radiotracking (PT)				
Species	Cirl bunting	Great tit	Linnet	Woodlark
50thtile	0.39	0.02	0.85	0.96
90thtile	0.77	0.09*	0.97	1
Mean	0.43	0.05*	0.78	0.86

Jacobs' preference index [D]

This index evaluates the habitat preference of an individual bird by comparing the PT in vineyards with the proportion (percentage) of vineyards in the home range.

Vineyards were part of every single home range of all four bird species studied. The mean preference index for ciril buntings was [D] = -0.42 (90th percentile = 0.32). For great tits the calculated Jacobs' index constituted [D] = -0.92 (90th percentile = -0.87). The preference index for linnets was [D] = 0.34 (90th percentile = 0.97), and for woodlarks [D] = 0.45 (90th percentile = 1).

Given the relatively high PT values for linnets and woodlarks, the Jacobs' index for the two species demonstrated that individuals of these species showed on average a preference for vineyards as a foraging habitat. Nevertheless, for both species the habitat use by individuals varied for these two species, presumably partly due to the availability of food in the foraged habitats. It might be expected that for linnets and woodlarks the PT will be influenced by the proportion of vineyards in the landscape.

A negative preference index calculated for ciril buntings indicated a slight tendency to avoid vineyards. For ciril buntings, the proportions of non-vineyard in their habitat i.e. bush and grassy marginal habitats is probably the main factor driving their foraging behaviour.

Increased vineyard in the landscape will therefore probably not change their PT significantly, only their abundance. The Jacobs' index for great tits confirmed that the individuals of this species do not prefer vineyards as a feeding habitat, and like the ciril bunting PT will not be influenced by a landscape with more vineyards.

PD values

Ciril buntings ingested invertebrate items and plant seeds in similar proportions (0.499 and 0.501, respectively) related to the total dry weight of their diet. Insect adults and Poaceae seeds were the most important taxa with PD values of 0.297 and 0.334, respectively. Great tits fed mainly on invertebrates (PD value of 0.955), whereas the PD value for seeds was only 0.045. Insect adults and larvae were the most important food items, providing together a PD of 0.788. In contrast, linnets ingested mainly seeds, with a PD of 0.973. Most important were Brassicaceae seeds, providing a PD value of 0.508. The diet of woodlarks consisted basically of invertebrates (PD of 0.921) with insect adults as the most important food items (PD of 0.527). Main results are presented in table 10.1.2.1-8.

Table 10.1.2.1-9: Overview of the PD (Vineyards)

Food type		Ciril bunting Based on 17 faeces and 3 flushing samples	Great tit Based on 16 faeces and 4 flushing samples	Linnet Based on 17 faeces and 3 flushing samples	Woodlark Based on 20 faeces
Invertebrate matter	Insect (adults)	0.297	0.405	0.010	0.527
	Insects (larvae)	0.151	0.383	-	0.209
	Araneida	0.003	0.165	0.017	0.014
	gastropoda	0.048	0.002	-	0.172
	TOTAL	0.499	0.955	0.027	0.921
Plant matter + seeds	Asteraceae	0.002	-	0.042	0.007
	Brassicaceae	-	-	0.508	-
	Caryophyllaceae	0.095	0.0004	0.146	0.0002
	Chenopodiaceae	0.011	-	0.023	-
	Geraniaceae	-	-	0.115	0.001
	Pinaceae	0.005	-	-	0.007
	Poaceae	0.334	0.016	0.084	0.030
	Polygonaceae	0.053	-	-	0.006
	Ranunculaceae	-	-	-	0.001
	Resedaceae	-	-	0.002	-

	Violaceae	0.0001	-	0.004	-
	Labiatae	-	-	-	0.026
	Other seeds	-	0.029	0.050	0.001
	TOTAL	0.501	0.045	0.973	0.079

III.**CONCLUSIONS**

Radio-tracking of circl buntings, great tits, linnets and woodlarks (20 individuals of each species) in a typical wine growing region yielded data on their time budget, habitat selection and home range sizes

The portion of time each bird spent 'potentially foraging' in vineyards was calculated. The PT value's were generated based on the combined results for the corresponding tracking sessions (means/90th percentiles are shown): circl bunting: 0.43 / 0.77; great tit: 0.05 / 0.08; linnet: 0.78 / 0.97; woodlark: 0.86 / 1

The spatial portion of vineyards in the birds' home range was calculated for each tracking session and compared to the corresponding PT value. This comparison (calculated as the Jacobs' index [9]) illustrates the preference of the individual bird for vineyards as a feeding habitat during its tracking session. Linnets and woodlarks show on average a preference for vineyards as a feeding habitat, whereas circl buntings and especially great tits avoid them for foraging.

Analysis of the faeces and stomach flushing samples taken from the trapped birds provided information on their food composition. In summary we conclude that circl buntings feed on 50% large insects and 50% weed seeds; great tits on 100% insects; linnets on 100% weed seeds and woodlarks on 100% ground dwelling insects.

(Selbach, 2007)

RMS comment : Study acceptable.

The study followed 4 focal species in vine (circl bunting, great tit, linnet and woodlark). Woodlark had a mean PT value estimated to be 0.86 while circl bunting and linnet had a mean PT value estimated to be 0.43 and 0.78, respectively. The great had only a mean PT value of 0.05 and this value could be considered as no relevant for a focal species. Therefore, a worst case PT value of 1 is conserved for this focal species.

Individual PT values of 0 have been excluded for the calculation of the mean and 90th percentile PT value, that is relevant and conservative.

The use of the 90th percentile PT value based on radiotracking of 20 individuals could be considered more relevant than the mean PT value in risk assessment in accordance with Guidance of 'EFSA 2009- Risk Assessment for Birds and Mammals'.

In this study PD values have been estimated based on "percentages by weight" that is relevant for risk assessment.

Refined risk assessment based on refined PT and PD

Based on the data obtained in the studies presented above, a new risk assessment was performed taking into account the PT (90th percentile) and PD values experimentally determined as well as on the default RUD and ftwa (EFSA, 2009 appendix A). The risk assessment was done on the following species taking into consideration the following parameters. (table 9.1.8.3.3).

Table 9.1.8.3.3: Characteristics of Focal Bird Species in Vineyards. Determination of Parameters for long-term Risk Assessment

Species	Linnet	Woodlark	Great tit	Circl bunting
FO_{field} (n = 29) [%]	76.7	66.7	36.7	36.7
Body Weight (g)¹	15.3	28.5	19.0	23.1

Stratum	Ground / foliage	Ground	Ground / foliage	Ground
Diet Guild	Granivorous	Omnivorous	Insectivorous	Omnivorous
FIR (weight diet) calculated based on PD values below	4.57	18.25	14.20	12.3
FIR_{diet} / bw	0.299	0.640	0.748	0.532
90th percentile PT value²	0.97	1	1	0.77
AV	1			
Area sprayed per ha	0.3			
Diet Composition & proportion based on dry weight Diet³	PD			
Invertebrate matter (PD 1)	0.027	0.921	0.955	0.499
Plant matter (seeds) (PD 2)	0.973	0.079	0.045	0.501

¹ Source: Appendix A EFSA 2009 guidance document / bird bible 1998 / Dunning (1993) / Crocker et al (2002) DEFRA CSL Report No. PN0908 p. 11^(2c), 17^(2a), 12^(2d)

² Staab, F. & Moosmayer, P. 2007. Multi Field Approach PT values are used for Long Term Risk Assessment

³ Staab, F. & Moosmayer, P. 2007.

According to the working document EFSA Guidance document 2009, the long-term risk to birds can be assessed on the basis of residue concentrations in food items.

The evaluation was therefore refined taking into consideration the maximum residue concentrations determined in a study conducted under realistic conditions.

Refined risk assessment based on more realistic RUD value

A study (Knäbe, S. 2009) for determining the residue level in plants and invertebrates contaminated by a CA2735 application according to the GAP was conducted in orchard. The application rate (13 L/ha) and the way of applying CA2210 (application under the row corresponding to 1/3 of the max. dose rate) are the same ones in orchard and vineyard. Therefore, it can be assumed that the residue levels measured in plants and arthropods in a orchard will not be significantly different from the potential concentrations obtained in the same plants and invertebrates in a vineyard. The study is presented below.

b) Refining RUD

Report:	KIIIA 10.1.2.1/03, 2009 KIIIA 10.1.2.1/04, 2009
Title:	o Residues of Amitrole in Arthropods as Food Sources for Wild Birds and Mammals after Application of Weedazol (Northern Germany 2008) o Analytical Phase Report. Residues of Amitrole in Arthropods as Food Sources for Wild Birds and Mammals after Application of Weedazol (Northern Germany 2008)
Document No:	
Guidelines:	There is no specific guideline. The study was designed for complying with the Guidance Document for Birds and Mammals Under Council Directive, 91/414/EEC (SANC0/4145/2000, 25 September 2002)
GLP:	Yes

Executive Summary

A SL formulation containing 229 g/L amitrole (nominal) was applied to an apple orchard with 2709 trees located in Northern Germany. The orchard was spread over an area of 0.952 ha. One single application was performed according to good agricultural practice at the maximum recommended rate for the product, according to the proposed uses (3.44 kg amitrole/ha, dilution 200 L/ha). Residues of amitrole were measured in different food sources for wild birds and mammals. The trial design consisted of one test item treatment group. The actual

application rate was therefore 16380 g product/ha (based on the measured deviation of the spray solution applied on the field to the spray solution required), corresponding to 3751 g a.s./ha. Samples of potential bird/mammal food were collected from the following three sources: short vegetation from between the apple tree rows in the orchard (grass and weeds) and surface-dwelling arthropods (alive and active animals caught in pitfall traps, alive and dead animals collected using Vortis suction sampling) and active crop- and ground-dwelling arthropods caught with earwig traps. Sampling of the weeds, pitfall traps and Vortis suction samples were carried out on the paths between the rows. The earwig traps were installed in the trees.

Plant samplings were carried out 5 days before the application, and 0, 1, 2, 4, 8, 16, 32, 44 and 56 days after the application of the test item. The pitfall traps were sampled 11 and 5 days before application and 1, 2, 3, 4, 8, 16, 32, 44 and 56 days after application. The Vortis suction sampling took place 5 days before the application, and 0, 1, 2, 4, 8, 16, 32 and 56 days after application. The special traps for earwigs were all set up 12 days before application and sampling took place directly before application as well as 1, 2, 4, 8, 16, 32, 44 and 56 days after the treatment. The collected arthropods of the pitfall traps were separated in four taxonomic groups: spiders (Araneae, including Opiliones), ground beetles (Carabidae, Coleoptera), woodlice (Isopoda), and other arthropods. Arthropods collected with the Vortis suction sampling were separated into spiders (Araneae, including Opiliones), ground beetles (Carabidae, Coleoptera), crickets (Ensifera), and other arthropods. The special earwig traps were only split into two groups earwigs (Forficula) and other arthropods. The four taxonomic groups of the pitfall traps were the only lower taxonomic groupings analysed separately. All other sampling types were analysed as composite samples. Determination was performed by HPLC with MS/MS detection.

In short vegetation, the residues analyses allowed to calculate (with a coefficient of determination of 0.9687) DT₅₀ and DT₉₀ of 5.79 days and 19.23 days, respectively.

In surface-dwelling arthropods (pitfall traps), all 4 taxonomic groups were present in high enough numbers to be analysed. Residue values in arthropods collected varied strongly. The first order kinetic model was appropriate to describe the degradation of amitrole for the spiders and ground beetles. It was not appropriate to describe the degradation in woodlice, other arthropods and in the corrected mean. This was due to the large variation in the residue levels. The DT₅₀ and DT₉₀ values were similar for spiders and ground beetles with around 3 days for the DT₅₀ and 10 days for the DT₉₀. The times of reduction to 50% of the maximum value were 1 day for the woodlice/corrected mean and 5 days for the other arthropods.

In surface-dwelling arthropods (vortis suction), again the highest residue level was not measured in the sampling taken directly after application. Therefore, the model was started with the value measured 1 day after application. The data did not fit the first order degradation model with an r^2 value of 0.4348. Therefore, the model is not appropriate to describe the degradation. The time of reduction to 50% of the maximum value in ground dwelling arthropods was 1 day.

In ground- and crop-dwelling arthropods in special traps (earwig traps), the samplings were the only sampling type where the highest value was recorded on the first sampling after application. The first residue level was probably due to some arthropods moving from the ground vegetation into the trees. The movement occurred most likely due to the disturbance during the application. Because of the fast decline, the results were not usable even with the very good fit of the model to the data. This can be clearly seen on the calculated initial value of 3292 mg/kg. The time of reduction to 50% of the maximum value in ground and plant dwelling arthropods was 1 day.

I. MATERIALS AND METHODS

1. In life dates:

29/02 – 23/04/2008

2. Experimental design

A SL formulation containing 229 g/L amitrole (nominal) was applied to an apple orchard with 2709 trees located in Northern Germany. The orchard was spread over an area of 0.952 ha. One single application was performed according to good agricultural practice at the maximum recommended rate for the product, according to the proposed uses (3.44 kg amitrole/ha, dilution 200 L/ha). Residues of amitrole were measured in different food sources for wild birds and mammals. The trial design consisted of one test item treatment group. The actual application rate was therefore 16380 g product/ha (based on the measured deviation of the spray solution applied on the field to the spray solution required), corresponding to 3751 g a.s./ha.

Samples of potential bird/mammal food were collected from the following three sources: short vegetation from between the apple tree rows in the orchard (grass and weeds) and surface-dwelling arthropods (alive and active animals caught in pitfall traps, alive and dead animals collected using Vortis suction sampling) and active crop- and ground-dwelling arthropods caught with earwig traps. Plant samplings were carried out 5 days before the application, and 0, 1, 2, 4, 8, 16, 32, 44 and 56 days after the application of the test item.

The pitfall traps were sampled 11 and 5 days before application and 1, 2, 3, 4, 8, 16, 32, 44 and 56 days after application. The Vortis suction sampling took place 5 days before the application, and 0, 1, 2, 4, 8, 16, 32 and 56 days after application. The special traps for earwigs were all set-up 12 days before application and sampling took place directly before application as well as 1, 2, 4, 8, 16, 32, 44 and 56 days after the treatment.

The collected arthropods of the pitfall traps were separated in four taxonomic groups: spiders (Araneae, including Opiliones), ground beetles (Carabidae, Coleoptera), woodlice (Isopoda) and other arthropods. Arthropods collected with the Vortis suction sampling were separated into spiders (Araneae, including Opiliones), ground beetles (Carabidae, Coleoptera), crickets (Ensifera), and other arthropods. The special earwig traps were only split into two groups earwigs (Forficula) and other arthropods.

The four taxonomic groups of the pitfall traps were the only lower taxonomic groupings analysed separately. All other sampling types were analysed as composite samples. Determination was performed by HPLC with MS/MS detection.

II. RESULTS AND DISCUSSION

Residue analysis of short vegetation sampling

Samples of short vegetation taken during the trial consisted of above ground growing vegetation as might be grazed by birds and mammals.

Results are presented in tables 10.1.2.1-11 and 10.1.2.1-12.

Table 10.1.2.1-11: Amitrole residue levels determined in short vegetation

Sampling	Sampling date	Residues (mg/kg wet weight)
1	5 DBA	< LOD
2	0 DAA	0.04
3	1 DAA	0.08
4	2 DAA	0.08
5	4 DAA	0.07
6	8 DAA	0.04
	16 DAA	< LOD
8	32 DAA	< LOD
9	44 DAA	< LOD
10	56 DAA	< LOD
TWA*		0.013

* TWA = time weighted average calculated as area under the curve (if the Conc1 was equal Conc0, 0.001 was subtracted from Conc1 for calculation)

LOQ = limit of quantification (LOQ=0.01 mg/kg)

LOD = limit of detection (LOD=0.003 mg/kg)

DBA = days before application; DAA = days after application

The coefficient of determination for amitrole in short vegetation samples was $r^2 = 0.9650$. This is well above the required value of $r^2 > 0.85$. Therefore, the chosen model is appropriate to describe the degradation of residues. The DT₅₀ and DT₉₀ values were 5.52 days and 18.35 days, respectively.

Table 10.1.2.1-12: Amitrole degradation determined for short plant samples

Test item	Amitrole 200 SL
Test object	samples from short plants
Study duration [days]	56
C ₀ mean [mg/kg wet weight]	0.04 (measured) / 0.10 (calculated by model)
Reduction to 50% of the highest value	7 days
DT ₅₀ amitrole	5.52 days
DT ₉₀ amitrole	18.35 days

Residue Analysis of Ground-Dwelling Arthropods (Pitfall traps)

Ground-dwelling arthropods were sampled using dry funnel pitfall traps.

Results are presented in tables 10.1.2.1-13 and 10.1.2.1-14.

Table 10.1.2.1-13: Amitrole residue levels determined in ground-dwelling arthropods (pitfall traps)

Sampling	Residues detected in surface-dwelling arthropods caught with pitfall traps (mg/kg wet weight)				
	Araneae/ Opiliones	Coleoptera	Isopoda	Other arthropods	Mean*
11/5 DBA	< LOD	< LOD	< LOD	< LOD	< LOD
1 DAA	0.0029	0.05	4.6	0.53	0.3200
2 DAA	0.008	0.1	10	0.70	0.6752
3 DAA	0.0029	0.04	0.86	1.16	0.0809
4 DAA	0.81	0.06	1.43	1.07	0.5807
8 DAA	0.31	0.03	3.07	0.06	0.2828
16 DAA	0.06	< LOQ (0.005)	5.47	0.61	0.0785
32 DAA	< LOD	< LOD	0.09	< LOQ (0.005)	0.0037
44 DAA	< LOD	< LOD	0.27	< LOQ (0.006)	0.0042
56 DAA	< LOD	< LOD	0.04	0.07	0.0035
TWA**	0.068	0.011	1.393	0.149	0.079

* weight corrected mean

**TWA = time weighted average calculated as area under the curve (if the Conc1 was equal Conc0, 0.001 was subtracted from Conc1 for calculation)

LOQ = limit of quantification (LOQ=0.01 mg/kg)

LOD = limit of detection (LOD=0.003 mg/kg)

DBA = days before application; DAA = days after application

All 4 taxonomic groups were present in high enough numbers to be analysed. Residue values in arthropods collected varied strongly. The first order kinetic model was appropriate to describe the degradation of amitrole for the spiders and ground beetles. It was not appropriate to describe the degradation in woodlice, other arthropods and in the corrected mean. This was due to the large variation in the residue levels. The DT₅₀ and DT₉₀ values were similar for spiders and ground beetles with around 3 days for the DT₅₀ and 10 days for the DT₉₀. The times of reduction to 50% of the maximum value were below 1 day for the woodlice/corrected mean and 5 days for the other arthropods.

Table 10.1.2.1-14: Amitrole degradation determined for surface-dwelling arthropod samples for the individual taxonomic groups and the corrected mean (pitfall traps)

Test item	Amitrole 200 SL				
Test object	Araneae	Coleoptera	Isopoda	Other arthropods	Corrected mean
Study duration [days]	56				
C ₀ mean [mg/kg wet weight]	< LOD*/2.07**	0.05*/0.13**	4.60*/5.22**	0.53*/2.75**	0.32*/0.64**
Reduction to 50% of the highest value [days]	< 1	< 1	< 1	5	< 1
DT ₅₀ amitrole [days]	2.95	3.06	14.50	2.55	5.89
DT ₉₀ amitrole [days]	9.79	10.17	48.17	8.48	19.58

C₀ - initial concentration; * measured; ** calculated by model**Residue Analysis of Ground-Dwelling Arthropods (Vortis suction sampling)**

Vortis sampling was used as an additional method to collect ground-dwelling arthropods. Results are presented in tables 10.1.2.1-15 and 10.1.2.1-16.

Table 10.1.2.1-15: Amitrole residue levels determined in ground-dwelling arthropods (vortis sampling)

Sampling	Sampling date	Residues (mg/kg wet weight)
1	5 DBA	< LOD
2	0 DAA	0.59
3	1 DAA	0.61
4	2 DAA	0.12
5	4 DAA	0.07
6	8 DAA	0.38
7	16 DAA	0.01
8	32 DAA	< LOQ
9	56 DAA	< LOD
TWA*		0.051

*TWA = time weighted average calculated as area under the curve (if the Conc1 was equal Conc0, 0.001 was subtracted to Conc1 for calculation)

LOQ = limit of quantification (LOQ=0.01 mg/kg)

LOD = limit of detection (LOD=0.003 mg/kg)

DBA = days before application; DAA = days after application

Again the highest residue level was not measured in the sampling taken directly after application. Therefore, the model was started with the value measured 1 day after application. Again the data did not fit the first order degradation model with an r² value of 0.4336. Therefore, the model is not appropriate to describe the degradation. The time of reduction to 50% of the maximum value in ground dwelling arthropods was below 1 day.

Table 10.1.2.1-16: Amitrole degradation determined for surface-dwelling arthropods sampled with Vortis suction sampling

Test item	Amitrole 200 SL
Test object	Surface –dwelling arthropods
Study duration [days]	56
C ₀ mean [mg/kg wet weight]	0.59 (measured) 0.44 (calculated by model)
Reduction to 50% of the highest value [days]	< 1
DT ₅₀ amitrole [days]	4.99

DT90 amitrole [days]

16.59

Residue Analysis of Ground-and Crop-Dwelling Arthropods in special Traps (Earwig Traps)

Earwig are highly mobile arthropods living on the ground and in the trees. The traps consisted of a clay flowerpot filled with straw and hung upside down in the apple tree canopy.

A summary of the residue levels found in the earwig trap samples is shown in tables 10.1.2.1-17 and 10.1.2.1-18.

Table 10.1.2.1-17: Amitrole residue levels determined in ground- and crop-dwelling arthropods (earwig trap sampling)

Sampling	Sampling date	Residues (mg/kg wet weight)
1	0 DBA	< LOD
2	1 DAA	3.09
3	2 DAA	< LOD
4	4 DAA	< LOD
5	8 DAA	< LOD
6	16 DAA	0.05
7	32 DAA	< LOD
8	44 DAA	< LOD
9	56 DAA	< LOD
TWA*		0.013

*TWA = time weighted average calculated as area under the curve (if the Conc1 was equal Conc 0, 0.001 was subtracted to Conc1 for calculation)

LOQ = limit of quantification (LOQ=0.01 mg/kg)

LOD = limit of detection (LOD=0.003 mg/kg)

DBA = days before application; DAA = days after application

The earwig samplings were the only sampling type where the highest value was recorded on the first sampling after application. The first residue level was probably due some arthropods moving from the ground vegetation into the trees.

The movement occurred most likely due to the disturbance during the application. Because of the fast decline the results were not usable even with the very good fit of the model to the data. This can be clearly seen on the calculated initial value of 299785 mg/kg.

The time of reduction to 50% of the maximum value in ground and plant dwelling arthropods was below 1 day.

Table 10.1.2.1-18: Amitrole degradation determined for crop- and ground-dwelling arthropods sampled with earwig traps

Test item	Amitrole 200 SL
Test object	Crop- and ground-dwelling arthropods
Study duration [days]	56
C0 mean [mg/kg wet weight]	3.09 (measured) / 299785.00 (calculated by model)
Reduction to 50% of the highest value [days]	< 1
DT50 amitrole [days]	0.06
DT90 amitrole [days]	0.20

III.

CONCLUSIONS

The highest residue levels of the composite samples were recorded for the arthropods in the earwig traps (3.09 mg amitrole/kg arthropods). The value was about five-fold higher than in the other two arthropod sampling methods and 38-fold compared to the plants.

Only the arthropod samples of the pitfall traps were analysed separately for residues. The highest residue value in the individual taxonomic groups analysed in the pitfall traps was found for the woodlice (Isopoda) with 10 mg/kg 2 days after application. The high residue level in the woodlice can be explained by their feeding habit and ecology. Woodlice are land living crustaceans and move only during night over damp ground. The bare earth of the herbicide applied stripe close to the trees would be an attractive area for the specimens to move. Additionally, woodlice feed on decaying or dead plant material. Plants dying through the application of the herbicide would be a good food source for the woodlice, increasing the contamination.

However, if the mean residues are corrected for the proportion of the taxonomic groups sampled, the residue level at this sampling date would be only 0.68 mg amitrole/kg arthropods.

Generally, residue levels showed a high variability throughout the study period for all sampling types. The earwig traps showed the highest residue level directly after application. Residue levels in other sampling types increased to the highest level only in later samplings.

A first order degradation model fitted only for the residues in the short vegetation samplings, the spiders in the pitfall traps and the ground beetles in the pitfall traps.

The initial concentrations measured were always smaller than the initial concentrations calculated by the first order degradation model. It was 0.04 mg amitrole/kg plant (measured) to 0.10 mg amitrole/kg plant (calculated) for the short vegetation samplings.

The concentration was below LOD (measured) up to 2.07 mg amitrole/kg for spiders (calculated) and 0.05 mg amitrole/kg to 0.13 mg amitrole/kg for ground beetles (measured).

The DT50 values were 5.52 days for the short plants, 2.95 days for the spiders and 3.06 days for the ground beetle. The first order degradation model did not fit the data for the woodlice, other arthropods and the corrected mean for the pitfall traps. Therefore, only the 50% reduction from the highest level of residues could be observed. The time for the 50% reduction of the highest value observed was below 1 day for the Isopoda and the corrected mean of the pitfall traps and 5 days for the other arthropods caught with pitfall traps. The Vortis suction samples and earwig trap sample residue data did also not fit a first model degradation. For those residues a 50% reduction of the highest value observed was below 1 day.

(Knäbe, S. 2009)

RMS comment: Study acceptable with the following comments:

- Two over 56 days study period was calculated. However, the residue values were below LOD much earlier. It is therefore not considered to be feasible to determine the twa of such a long period. Moreover, data below LOD should be excluded in the calculation.
- Calculation of DT50 is not reliable when there are too few data points (<5 sampling points) as it is the case for measured residues in vegetation, araneae and coleoptera.
- Calculation of DT50 is not reliable when the determination coefficient (r^2) is too low (<0.85) as it is the case for the DT50 calculated for isopoda, other arthropods, surface-dwelling arthropods, crops- and ground-dwelling arthropods.
- Another draw-back of the study is that all samples have been put together. The variance between the samples is unknown.

Considering the previous comments, it is more relevant to refine risk assessment using only the maximum residue levels of the different food item groups and not the Twa over 56 days. Moreover, long term risk assessment has to be conducted based on initial residue levels as degradation of the active substance may not be appropriate for use with endocrine disrupting compound.

Moreover, considering the application rate of 3751 g a.s./ha and the mode of application (under the row) in this study, it was thus considered that only one third of the total crop surface is really treated. Therefore only 1/3 of the total dose (1250 g a.s./ha) was applied and the measured residues correspond to this third of the total dose. DDD could be calculated considering directly measured residue values that is worst case (1250 g a.s./ha in the study covering 1/3 of the application rate what is equal to 993 g as/ha).

According to the working document EFSA Guidance document, 2009, the long-term risk to birds can be assessed on the basis of residue concentrations in food items. The evaluation was therefore refined taking into consideration the maximum residue concentrations determined in a study conducted under realistic conditions.

The different scenarios considered are presented in table 9.1.8.3.5

Table 9.1.8.3.5: residue concentration scenarios (vineyards)

Diet Composition & Portion of Diet	Residue			Cirl bunting	Great Tit	Linnet	Woodlark
	Scenario 1	Scenario 2	Scenario 3	PD	PD	PD	PD
Animal matter (RUD 1)	0.61^a	3.09 ^b	0.67 ^c	0.499	0.955	0.027	0.921
Plant matter (RUD 2)	0.08 ^d	0.08 ^d	0.08 ^d	0.501	0.045	0.973	0.079

^a Ground Dwelling Arthropods (Vortis sampling) $R_{\max}$ – see table 10.1.2.1-15 (Knäbe, S. 2009)

^b Ground & Crop dwelling Arthropods (Earwig traps) $R_{\max}$ – see table 10.1.2.1-19 (Knäbe, S. 2009)

^c Ground dwelling Arthropods (Pitfall traps) Mean $R_{\max}$ – see table 10.1.2.1-13 (Knäbe, S. 2009). Here the highest mean residue was chosen as a realistic estimate. Residue in Isopoda was found to be higher (and more variable), but it is unlikely that Isopoda would form a high % of the diet of the indicator bird species in pome orchards. Isopoda only move during the night when the air humidity is high. Isopoda hide during the day and would not easily (or not more easily than any other arthropod prey) be spotted and consumed by the indicator bird species

^d Short vegetation $R_{\max}$ – see table 10.1.2.1-11 (Knäbe, S. 2009)

✓ Orchards

a) Refining PT and PD

If an active substance, and its associated product and use, fails Tier 1, it is possible to further refine the exposure element of risk via the use of a 'focal species'.

Report:	KIHA 10.1.2.2/01, [REDACTED] 2006
Title:	Bird species in pome fruit orchards in Poland and Italy: Field data for the determination of focal species
Document No:	[REDACTED]
Guidelines:	Bibby et al. 1992. Bird census techniques. Academic Press, London
GLP:	Yes

Executive Summary

The objectives of this generic study were to determine the qualitative and quantitative composition of the bird community employing the parameters, frequency of occurrence (FOfield and FOsurvey) and dominance both as overall and as pome fruit growth stage specific descriptors. Another objective was then to allocate the selected species to defined foraging guilds, diet guilds and size classes. The Lubelskie, Mazowieckie and Swietokrzyskie regions of Poland, the Tyrol and the Veneto regions of Italy served as study areas, encompassing 21 and 29

pome fruit orchards, respectively, selected to represent average pome fruit orchards, pome fruit orchard size and the structure of the landscape. In order to cover different pome fruit growth stages, three line transect surveys were conducted in 2005 for each pome fruit orchard during sprouting, flowering and early stages of fruit development (survey 1), early stages of fruit development (survey 2) and fruit development (survey 3). A standard line transect consisted of an 'in-crop transect band' (a 50/100 m wide recording band of 25/50 m to each side of the observer moving along a longitudinal in-crop field transect). For the assessment of the bird community, frequency of occurrence (FO_{field} and FO_{survey}) and dominance were determined. Data were analysed using the "ECOLOGY RESEARCH DATABASE SYSTEM" (ERDS). The ranking of species within the list of focal species candidates was carried out in decreasing order of importance, i.e. FO_{field} > FO_{survey} > dominance. This list of candidates of focal species was then used to allocate the respective species to defined foraging guilds, diet guilds and size classes.

A total of 1,393 and 1,619 individual bird contacts, comprising 42 and 30 different species, was recorded throughout all surveys within the 'in-crop transect bands' in Poland and Italy, respectively. The following species listed in the tables below were recorded with a frequency of occurrence (FO_{field}) ≥ 20% and were thus determined as the main candidates for focal bird species in the respective country.

The recorded candidates of focal bird species were assigned to the following guilds in accordance with the SANCO guidance document (ranked by their respective frequency of occurrence and dominance):

Poland

- o Small granivorous serine > linnet > goldfinch (all ground/foliage),
- o Small omnivore chaffinch (ground/foliage) > skylark (ground) > greenfinch (ground/foliage) > yellowhammer (ground),
- o Medium omnivore fieldfare > song thrush > blackbird > starling (all ground/foliage).

Italy

- o Small insectivore red-backed shrike (ground) > spotted flycatcher (foliage) > wryneck (ground/foliage),
- o Small granivorous goldfinch > serine > linnet (all ground/foliage),
- o Small omnivore greenfinch > chaffinch > tree sparrow (all ground/foliage),
- o Medium herbivore turtle dove (ground),
- o Medium insectivore green woodpecker (ground/foliage) > hoopoe (ground),
- o Medium omnivore blackbird > song thrush > fieldfare > starling (all ground/foliage).

The chaffinch and serine were the most characteristic and stable elements of the bird community in pome fruit orchards in Poland across all pome fruit growth stages. Other species, showing peak FO values ≥ 20% for individual pome fruit growth stages only, were the fieldfare, song thrush, linnet, skylark, blackbird, yellowhammer, greenfinch and starling.

The blackbird, greenfinch, goldfinch, song thrush and chaffinch were the most characteristic and stable elements of the bird community in pome fruit orchards in Italy across all pome fruit growth stages. Other species, showing peak FO values ≥ 20% for individual pome fruit growth stages only, were the serine, goldfinch, tree sparrow, linnet, red-backed shrike, hoopoe, wryneck, green woodpecker, turtle dove and spotted flycatcher.

All the species listed in the tables above can be considered as potential candidates for focal bird species in a refined risk assessment for plant protection products in pome fruit orchards in Poland and Italy, respectively.

I. MATERIALS AND METHODS

1. In life dates: 16/03 – 06/07/2005

2. Experimental design

The objectives of this generic study were to determine the qualitative and quantitative composition of the bird community employing the parameters, frequency of occurrence (FO_{field} and FO_{survey}) and dominance both as overall and as pome fruit growth stage specific descriptors. Another objective was then to allocate the selected species to defined foraging guilds, diet guilds and size classes. The Lubelskie, Mazowieckie and Swietokrzyskie regions of Poland, the Tyrol and the Veneto regions of Italy served as study areas, encompassing 21 and 29 pome fruit orchards, respectively, selected to represent average pome fruit orchards, pome fruit orchard size and the structure of the landscape. In order to cover different pome fruit growth stages, three line transect surveys were conducted in 2005 for each pome fruit orchard during sprouting, flowering and early stages of fruit development (survey 1), early stages of fruit development (survey 2) and fruit development (survey 3). A

standard line transect consisted of an 'in-crop transect band' (a 50/100 m wide recording band of 25/50 m to each side of the observer moving along a longitudinal in-crop field transect).

3. Observations:

Pome fruit orchards were visited between the middle of March and the beginning of July 2005. Three surveys were conducted in most orchards within a three month period. In the Veneto region some orchards could not be accessed during the first survey. A standardised pome fruit orchard form and a bird survey form were developed to record a number of parameters during each field survey. The avifauna of the vineyards was surveyed by the line transect method for which general information can be found in Bibby et al. (1992). To meet the specific methodological requirements of this report, the line transect method described by Bibby et al. (1992) was adapted for this study in order to be compatible with the ERDS software.

4. Statistics

For the assessment of the bird community, frequency of occurrence (FO_{field} and FO_{survey}) and dominance were determined. Data were analysed using the "ECOLOGY RESEARCH DATABASE SYSTEM" (ERDS). The ranking of species within the list of focal species candidates was carried out in decreasing order of importance, i.e. FO_{field} > FO_{survey} > dominance. This list of candidates of focal species was then used to allocate the respective species to defined foraging guilds, diet guilds and size classes.

II. RESULTS AND DISCUSSION

A total of 1,393 and 1,619 individual bird contacts, comprising 42 and 30 different species, was recorded throughout all surveys within the 'in-crop transect bands' in Poland and Italy, respectively. The following species listed in the tables below were recorded with a frequency of occurrence (FO_{field}) ≥ 20% and were thus determined as the main candidates for focal bird species in the respective country. In most cases the findings were supported by FO_{survey} and dominance data (tables 10.1.2.2-1 and 10.1.2.2-2).

Italy

Sixteen species were found to be characterised by an FO_{field} of at least 20% (Table 10.1.2.2-1). Most of these species were grouped into the small bird category (< 50 g).

Table 10.1.2.2-1: Candidate Focal Bird Species in Pome Orchards in Italy (Species are ranked by decreasing FO_{field} > FO_{survey} > dominance > size)

Species	FO _{field} n = 29 [%]	FO _{survey} n = 83 [%]	Dominance n = 29 [%]	Body weight [g]	Stratum use	Diet guild
Blackbird (<i>Turdus merula</i>)	93.1	73.5	35.1	113.0	ground/foilage	omnivorous
Goldfinch (<i>Carduelis carduelis</i>)	58.6	36.1	4.6	15.6	ground/foilage	granivorous
Greenfinch (<i>Carduelis chloris</i>)	55.2	44.6	6.3	27.8	ground/foilage	omnivorous
Song thrush (<i>Turdus philomelos</i>)	51.7	48.2	18.3	medium	ground/foilage	omnivorous
Chaffinch (<i>Fringilla coelebs</i>)	51.7	41.0	8.5	20.9	ground/foilage	omnivorous
Fieldfare (<i>Turdus pilaris</i>)	48.3	38.6	8.3	104.0	ground/foilage	omnivorous
Serine (<i>Serinus serinus</i>)	48.3	26.5	3.8	11.2	ground/foilage	granivorous
Tree sparrow (<i>Passer montanus</i>)	34.5	19.3	3.0	22.0	ground/foilage	omnivorous
Linnet (<i>Carduelis cannabina</i>)	34.5	14.5	1.5	15.3	ground/foilage	omnivorous
Green woodpecker (<i>Picus viridis</i>)	34.5	13.3	0.7	medium	ground/foilage	insectivorous

Red-backed shrike (<i>Lanius collurio</i>)	27.6	10.8	1.1	small	ground	insectivorous
Turtle dove (<i>Streptopelia turtur</i>)	27.6	10.8	0.8	medium	ground	herbivorous
Spotted flycatcher (<i>Muscicapa striata</i>)	27.6	9.6	1.3	small	foliage	insectivorous
Hoopoe (<i>Upupa epops</i>)	24.1	9.6	0.9	medium	ground	insectivorous
Wryneck (<i>Jynx torquilla</i>)	20.7	8.4	0.6	small	ground/foilage	insectivorous
Starling (<i>Sturnus vulgaris</i>)	20.7	7.2	1.4	79.9	ground/foilage	omnivorous

In Italy the blackbird and greenfinch showed moderate to high and increasing FO values throughout the season with maxima of 86.6% and 48.3% during the third survey period. The goldfinch displayed equally high FO values with a peak of 48.3% during the third survey, but FO values were lower during the first survey. The chaffinch, song thrush and fieldfare also displayed constantly moderate FO values with peaks of 44.8%, 52.0% and 44.0% during the second, first and first survey, respectively. All other species showed FO values in excess of 20.0% only during one or two survey periods with peaks of 20.7% – 41.4% during the first (tree sparrow), second (serine, linnet, wryneck) or third survey (redbacked shrike, spotted flycatcher, turtle dove, hoopoe).

The species listed above were grouped according to their dietary guilds and foraging strata. Details for each species are presented in table 10.1.2.1-2.

Table 10.1.2.1-2: Characteristics of focal bird species candidates in orchards in Italy

SPECIES	CHARACTERISTICS
Small granivorous birds	
Goldfinch (<i>Carduelis carduelis</i>)	Highest body weight of group; high FO throughout with a peak during third survey; forages on ground and weeds
Serine (<i>Serinus serinus</i>)	Lowest body weight of group; FO figures > 20% with peak during the second survey; forages on ground and weeds
Linnet (<i>Carduelis cannabina</i>)	Intermediate body weight in group; FO figures > 20% with peak during the second survey; forages on ground and weeds
Small insectivorous birds	
Red-Backred shrike (<i>Lanius collurio</i>)	FO peak during third survey; forages on ground
Spotted flycatcher (<i>Muscicapa striata</i>)	FO peak during third survey; forages chiefly in foliage
Wryneck (<i>Jynx torquilla</i>)	FO peak during second survey; forages chiefly in foliage but also on ground
Medium insectivores	
Green woodpecker (<i>Picus viridis</i>)	Intermediate body weight; intermediate FO throughout with peak during first survey; forages on ground and in foliage
Hoopoe (<i>Upupa epops</i>)	low FO figures throughout, with small peak during third survey; forages on ground
Small omnivorous birds	
Chaffinch (<i>Fringilla coelebs</i>)	Lowest body weight of group; moderate FO figures with peak during second survey; forages on ground and in foliage
Greenfinch (<i>Carduelis chloris</i>)	Highest body weight of group; Moderate to high FO throughout with maxima during the third survey period; forages on ground and in foliage
Tree sparrow (<i>Passer montanus</i>)	Intermediate body weight; moderate FO values with peak during first survey; tendency for flocking behaviour; forages on ground and in foliage
Medium herbivorous birds	
Turtle dove (<i>Streptopelia</i>)	Only representative of group; moderate FO values throughout particularly during

turtur)	first and second surveys; forages on ground
Medium omnivorous birds	
Blackbird (<i>Turdus merula</i>)	Intermediate body weight; Moderate to high FO throughout with maxima during third survey; forages on ground and in foliage
Song thrush (<i>Turdus philomelos</i>)	Intermediate body weight of group; moderate FO values with peak during first survey; forages on ground and in foliage
Fieldfare (<i>Turdus pilaris</i>)	Intermediate body weight; moderate FO values with peak during first survey; forages on ground and in foliage
Starling (<i>Sturnus vulgaris</i>)	Highest body weight of group; FO values low except for small peak during first survey; forages on ground and in foliage

Poland

Eleven species were found to be characterised by an FO_{field} of at least 20% (Table 10.1.2.2-3). Most of these species were grouped into the small bird category (< 50 g) except 2 species, the carrion crow and the blackbird.

Table 10.1.2.1-3: List of candidates of focal species in orchards in Poland

Species	FO _{field} n = 29 [%]	FO _{survey} n = 83 [%]	Dominance n = 29 [%]	Body weight [g]	Stratum use	Diet guild
Chaffinch (<i>Fringilla coelebs</i>)	100	98.4	25.6	20.9	ground/foliage	granivorous
Serine (<i>Serinus serinus</i>)	95.2	68.3	12.4	11.2	ground/foliage	granivorous
Fieldfare (<i>Turdus pilaris</i>)	85.7	79.4	20	104.0	ground/foliage	omnivorous
Skylark (<i>Alauda arvensis</i>)	85.7	69.8	7.3	37.2	ground	omnivorous
Song thrush (<i>Turdus philomelos</i>)	85.7	55.6	4.6	medium	ground/foliage	omnivorous
Linnet (<i>Carduelis cannabina</i>)	81	52.4	9.8	15	ground/foliage	granivorous
Blackbird (<i>Turdus merula</i>)	61.9	44.4	3.9	113.0	ground/foliage	omnivorous
Greenfinch (<i>Carduelis chloris</i>)	61.9	33.3	2.1	27.8	ground/foliage	omnivorous
Starling (<i>Sturnus vulgaris</i>)	47.6	17.5	3.1	79.9	ground/foliage	omnivorous
Yellowhammer (<i>Emberiza citrinella</i>)	42.9	23.8	2.1	26.5	ground	omnivorous
Goldfinch (<i>Carduelis carduelis</i>)	33.3	14.3	1.5	15.6	ground/foliage	granivorous

In Poland the chaffinch showed high FO values throughout the season with a maximum of 100.0% during the first and the second survey period.

The fieldfare, serine, song thrush and linnet also displayed high FO values with peaks between 57.1 – 81.0% during the second and/or the third survey, whereas the skylark reached their maxima during the first survey (76.2%). FO values of the blackbird, greenfinch, yellowhammer and starling showed small maxima (23.8 – 47.6%) during the second or third survey period but reached lower levels during the first survey period.

The species listed above were grouped according to their dietary guilds and foraging strata. Details for each species are presented in table 10.1.2.1-4.

Table 10.1.2.1-4: Characteristics of focal bird species candidates in orchards in Poland

SPECIES	CHARACTERISTICS
Small granivorous birds	
Goldfinch (<i>Carduelis carduelis</i>)	Highest body weight of group; high FO throughout with a peak during third survey; forages on ground and weeds
Serine (<i>Serinus serinus</i>)	Lowest body weight of group; FO figures > 20% with peak during the second survey; forages on ground and weeds
Linnet (<i>Carduelis cannabina</i>)	Intermediate body weight in group; FO figures > 20% with peak during the second survey; forages on ground and weeds
Small omnivorous birds	
Chaffinch (<i>Fringilla coelebs</i>)	Lowest body weight of group; moderate FO figures with peak during second survey; forages on ground and in foliage
Greenfinch (<i>Carduelis chloris</i>)	Highest body weight of group; Moderate to high FO throughout with maxima during the third survey period; forages on ground and in foliage
Yellowhammer (<i>Emberiza citrinella</i>)	Second lowest body weight of group; low variation of FO values across survey period with peak during second survey; forages chiefly on ground
Skylark (<i>Alauda arvensis</i>)	Highest body weight of group; FO values show constant decrease during season; forages mainly on ground
Medium omnivorous birds	
Blackbird (<i>Turdus merula</i>)	Intermediate body weight; Moderate to high FO throughout with maxima during third survey; forages on ground and in foliage
Song thrush (Turdus philomelos)	Intermediate body weight of group; moderate FO values with peak during first survey; forages on ground and in foliage
Fieldfare (4) (<i>Turdus pilaris</i>)	Intermediate body weight; moderate FO values with peak during first survey; forages on ground and in foliage
Starling (Sturnus vulgaris)	Highest body weight of group; FO values low except for small peak during first survey; forages on ground and in foliage

III.

CONCLUSIONS

The recorded candidates of focal bird species were assigned to the following guilds in accordance with the SANCO guidance document (ranked by their respective frequency of occurrence and dominance):

Italy

- o Small insectivore red-backed shrike (ground) > spotted flycatcher (foliage) > wryneck (ground/foliage)
- o Small granivorous goldfinch > serine > linnet (all ground/foliage)
- o Small omnivore greenfinch > chaffinch > tree sparrow (all ground/foliage)
- o Medium herbivore turtle dove (ground)
- o Medium insectivore green woodpecker (ground/foliage) > hoopoe (ground)
- o Medium omnivore blackbird > song thrush > fieldfare > starling (all ground/foliage)

Poland

- o Small granivorous serine > linnet > goldfinch (all ground/foliage),
- o Small omnivore chaffinch (ground/foliage) > skylark (ground) > greenfinch (ground/foliage) > yellowhammer (ground),
- o Medium omnivore fieldfare > song thrush > blackbird > starling (all ground/foliage).

The chaffinch and serine were the most characteristic and stable elements of the bird community in pome fruit orchards in Poland across all pome fruit growth stages. Other species, showing peak FO values ≥ 20% for individual pome fruit growth stages only, were the fieldfare, song thrush, linnet, skylark, blackbird, yellowhammer, greenfinch and starling.

The blackbird, greenfinch, goldfinch, song thrush and chaffinch were the most characteristic and stable elements of the bird community in pome fruit orchards in Italy across all pome fruit growth stages. Other species, showing peak FO values ≥ 20% for individual pome fruit growth stages only, were the serine, goldfinch, tree sparrow, linnet, red-backed shrike, hoopoe, wryneck, green woodpecker, turtle dove and spotted flycatcher.

All the species listed in the tables above can be considered as potential candidates for focal bird species in a refined risk assessment for plant protection products in pome fruit orchards in Poland and Italy, respectively.

(2006)

RMS comment: Study acceptable. Agree with the choice of focal species. However, it has to be kept in mind that other areas of study depending on the country of application could be proposed for determining potential candidates for focal species.

Based on the results of the above summarized study, PT and PD were determined for the focal bird species of interest. A study was conducted in Southern Germany in 2005. It is summarized below.

Report:	KIIIA 10.1.2.2/02, [REDACTED] P. 2007
Title:	Generic field monitoring of selected bird species in orchards in southern Germany
Document No:	[REDACTED]
Guidelines:	Not applicable; the test was especially designed for the purpose of this study
GLP:	Yes

Executive Summary

The aim of this generic study was to obtain estimates of diet (PD) and the proportions taken from orchards (PT) by four focal bird species, *Turdus merula* (blackbird), *Parus major* (great tit), *Fringilla coelebs* (chaffinch), *Carduelis cannabina* (linnet) and *Serinus serinus* (serine). The study was conducted in and around eight pome fruit orchards (= study plots) and two additional pome fruit orchards in southern Germany. Six orchards were located in the Palatinate region of Rhineland-Palatinate and two in the Odenwald-Neckartal region of Baden-Wuerttemberg, Germany. A combine approach was used: radio-tracking and visual observations. Radio-tracking has the advantage to access the location of the bird almost consistently without influencing its behaviour and hence to monitor its habitat selection continuously. Visual observation offers the opportunity to know the behaviour of a bird directly. The combination of both approaches offered the optimum of information currently obtainable in behavioural observation projects of songbirds. Bird trapping, radio-tracking, visual observations, plus faeces and stomach content analyses were the methods used to estimate the importance of pome fruit orchards as foraging habitat within the home range and to determine the food choice of these five species. The radio-tracking technique was used to locate the bird in order to observe it and to follow the bird continuously over a defined period in order to determine site and behavioural changes. Each tracking session lasted for a whole activity period from dawn to dusk (at least for 12 hours). Faeces samples and stomach flushings were carried out to determine the food items ingested by birds.

PT values: A representative assessment of potential foraging times of birds in given home ranges and subsequent calculation of reliable PT-values were obtained by using radio-tracking in combination with visual observation. The trapped and radio-tracked birds were closely associated with pome fruit orchards and had the opportunity to use them as a major foraging habitat. Therefore the results can be considered to be a worst case in terms of potential dietary exposure. Based on the study results considering the multi field approach, the 50th percentile PT-value derived for the five focal bird species living within or in close vicinity of pome fruit orchards in spring/summer was: 0.081 (90th percentile = 0.547) for the blackbird, 0.161 (90th percentile = 0.697) for the great tit, 0.586 (90th percentile = 0.947) for the chaffinch, 0 (90th percentile = 0.560) for the linnet and 0.156 (90th percentile = 0.637) for the serine. It is important to emphasize that these values represent the total time a bird spent 'potentially foraging' in all pome fruit orchards. These values include times when foraging was not evident but could not be excluded. Therefore, the calculated PT-values for all five species can be regarded as a conservative assumption.

Based on the study results considering the single field approach, the 50th percentile PT-value derived for the five focal bird species living within or in close vicinity of pome fruit orchards in spring/summer was: 0.051 (90th percentile = 0.466) for the blackbird, 0.094 (90th percentile = 0.250) for the great tit, 0.267 (90th percentile = 0.748) for the chaffinch, 0 (90th percentile = 0.284) for the linnet and 0.064 (90th percentile = 0.390) for the serine. These values represent the total time a bird spent 'potentially foraging' in the predominantly used pome fruit orchard during any given tracking session.

Diet sample contents: Major content in the diet sample volume of blackbirds and great tits were invertebrates. In contrast plant material (seeds, fruits or other plant matter) played a minor role. The samples of chaffinches and linnets were mainly composed of plant material, while the diet of serines was exclusively made up of plant matter. With respect to their number, arthropods below or equal to 5 mm were more numerous in the diet samples of great tits and chaffinches but scarcely found in the samples of blackbirds. Among the plant matter found in the diet samples, seeds were most numerous in the diet of blackbirds, chaffinches, linnets and serines, whereas in the diet of great tits fruits were found to be the larger portion.

I. MATERIALS AND METHODS

1. In life dates: 22/03 – 31/10/2005

2. Experimental design

The aim of this generic study was to obtain estimates of diet (PD) and the proportions taken from orchards (PT) by four focal bird species, *Turdus merula* (blackbird), *Parus major* (great tit), *Fringilla coelebs* (chaffinch), *Carduelis cannabina* (linnet) and *Serinus serinus* (serine). The study was conducted in and around eight pome fruit orchards (= study plots) and two additional pome fruit orchards in southern Germany. Six orchards were located in the Palatinate region of Rhineland-Palatinate and two in the Odenwald-Neckartal region of Baden-Wuerttemberg, Germany.

3. Observations:

A combine approach was used: radio-tracking and visual observations. Radio-tracking has the advantage to access the location of the bird almost consistently without influencing its behaviour and hence to monitor its habitat selection continuously. Visual observation offers the opportunity to know the behaviour of a bird directly. The combination of both approaches offered the optimum of information currently obtainable in behavioural observation projects of songbirds.

The five bird species *Turdus merula* (blackbird), *Parus major* (great tit), *Fringilla coelebs* (chaffinch), *Carduelis cannabina* (linnet) and *Serinus serinus* (serine) were chosen as test organisms. All individuals monitored during this study were trapped in pome fruit orchards, which is indicating that the birds may use pome fruit orchards as a foraging habitat.

Bird trapping, radio-tracking, visual observations, plus faeces and stomach content analyses were the methods used to estimate the importance of pome fruit orchards as foraging habitat within the home range and to determine the food choice of these five species.

The radio-tracking technique was used:

- To locate the bird in order to observe it
- to follow the bird continuously over a defined period in order to determine site and behavioural changes

Each tracking session lasted for a whole activity period from dawn to dusk (at least for 12 hours).

Faeces samples and stomach flushings were carried out to determine the food items ingested by birds.

4. Data evaluation:

In this study PT values for pome fruit orchards were derived for five bird species. Due to the selection of individuals for radio-tracking closely associated to pome fruit orchards and the inclusion of all periods of time while foraging could not be excluded, the calculated PT values can be regarded as a conservative assumption. Hence, they illustrate the highest possible portion of time the individual bird spent foraging in pome fruit orchards.

Overall this study provides reliable refinement parameters (PT; diet composition) for the use in higher tier risk assessment for birds foraging in modern spindle bush pome fruit orchards

II. RESULTS AND DISCUSSION

PT values

The combination of radio-tracking with visual appraisal and the trapping scheme as presented here, allowed an accurate and representative assessment of potential foraging times in given home ranges, thus making it possible to calculate reliable PT values. All birds were closely associated with vineyards and had the opportunity to use these fields as a foraging habitat. The results can thus be considered a worst case in terms of potential exposure.

Based on the study results considering the multi field approach (table 10.1.2.2-5), the 50th percentile PT-value derived for the five focal bird species living within or in close vicinity of pome fruit orchards in spring/summer was: 0.081 (90 percentile = 0.547) for the blackbird, 0.161 (90th percentile = 0.697) for the great tit, 0.586 (90th percentile = 0.947) for the chaffinch, 0 (90th percentile = 0.560) for the linnet and 0.156 (90th percentile = 0.637) for the serine. It is important to emphasize that these values represent the total time a bird spent 'potentially foraging' in all pome fruit orchards. These values include times when foraging was not evident but could not be excluded. Therefore, the calculated PT-values for all five species can be regarded as a conservative assumption.

Table 10.1.2.2.4: PT of radiotracked birds/species

Individual n°	PT values				
	Blackbirds	Great tits	Chaffinges	Linnets	Serins
1	0	0.704	0.821		0.930
1/1	-		-	0.366	-
1/2	-		-	0.906	-
2	0.015	0.120	1	0.114	-
2/1	-		-	-	0.263
2/2	-		-	-	0.156
3	0.242	0.192	0.156	0.560	0.310
4	0.382	0.069	0.064	0	0.257
5	0.012	0.126	0	0	0.098
6	0.729	0.240	0.145	-	0.070
6/1	-		-	0.006	-
6/2	-		-	0	-
7	0	0.167	0.722	0	0.032
8	0.020	0.111	0.154	0	-
8/1	-	-	-	-	0.105
8/2	-	-	-	-	0.067
9	0.387	0.171	0.291	0	0.637
10	0.066	0.161	0.740	-	-
11	0.111	0.011	0.137	-	-
12	0.081	-	0.004	-	-
12/1	-	0.285	-	-	-
12/2	-	0.303	-	-	-
13	0.130	0.104	0.091	-	-
14	0	-	0.306	-	-
14/1	-	0.686	-	-	-
14/2	-	0.791	-	-	-
15	0.029	-	-	-	-
15/1	-	0.427	0.914	-	-
15/2	-	0.363	0.986	-	-
16	0.389	0.068	0.510	-	-
17	0.993	0.127	0.018	-	-
18	0.587	0.003	-	-	-
18/1	-	-	0.738	-	-
18/2	-	-	0.661	-	-
19	0	0.912	-	-	-
19/1	-	-	0.763	-	-
19/2	-	-	0.681	-	-
20	0	0.062	0.945	-	-

21	0.166	0.012	0.947	-	-
22	0.330	0.147	-	-	-
23	0.043	-	-	-	-
50%tile	0.081	0.161	0.586	0	0.156
90%tile	0.629*	0.697	0.947*	0.7676*	0.637
mean	0.262*	0.254	0.513*	0.3904*	0.266

*excluding the 0 values

Based on the study results considering the single field approach (table 10.1.2.2-5), the 50th percentile PT-value derived for the five focal bird species living within or in close vicinity of pome fruit orchards in spring/summer was: 0.051 (90th percentile = 0.466) for the blackbird, 0.094 (90th percentile = 0.250) for the great tit, 0.267 (90th percentile = 0.748) for the chaffinch, 0 (90th percentile = 0.284) for the linnet and 0.064 (90th percentile = 0.390) for the serine. These values represent the total time a bird spent 'potentially foraging' in the predominantly used pome fruit orchard during any given tracking session.

Table 10.1.2.2-5: Overview of the PT (orchards)

Proportion of diet obtained in pome fruit orchards determined by combined radiotracking (PT)					
Species	Blackbird	Great tit	Chaffinch	Linnet	Serine
Multi field approach: ('potential foraging' time birds spent in all used pome fruit orchards as a proportion of the total 'potential foraging' time)					
50%tile	0.081	0.161	0.586	0	0.156
90%tile	0.629	0.697	0.947	0.767	0.637
Mean ± sd	0.262 ± 0.268	0.254 ± 0.256	0.513 ± 0.368	0.3904 ± 0.306	0.266 ± 0.279
Single field approach: ('potential foraging' time spent in the predominantly used pome fruit orchard as a proportion of the total 'potential foraging' time)					
50%tile	0.051	0.094	0.267	0	0.064
90%tile	0.466	0.250	0.748	0.284	0.390
Mean ± sd	0.172 ± 0.211	0.122 ± 0.122	0.347 ± 0.301	0.123 ± 0.227	0.147 ± 0.210

Jacobs' preference index [D]

This index evaluates the habitat preference of an individual bird by comparing the PT in orchards with the proportion (percentage) of orchards in the home range.

The index value for [D] ranges from -1 to +1. Index values lower than 0 represent negative choice and above 0 a positive choice ('-1': complete avoidance; '+1': exclusive preference; '0': neutral: neither avoidance nor preference, i.e. a proportional use of the habitat in relation to its proportion in the home range). The following values were obtained for the five species.

Black bird: The mean Jacobs' index [D] was -0.163 . This means that no clear avoidance or preference could be determined.

Great Tit: The mean Jacobs' index [D] was -0.028. This reflects a slight avoidance of the habitat 'pome fruit orchard'.

Chaffinch: The mean Jacobs' index [D] was 0.315. This means a slight preference of the habitat 'pome fruit orchard'.

Linnet: The mean Jacobs' index [D] was -0.419 . This means that pome fruit orchards were on average rather avoided as a feeding habitat by the tracked linnets in comparison to other available habitats within their home ranges.

Serine: The mean Jacobs' index [D] was 0.101 showing neither clear avoidance nor preference for the habitat 'pome fruit orchard'.

PD values

Major content in the diet sample volume of blackbirds and great tits were invertebrates. In contrast plant material (seeds, fruits or other plant matter) played a minor role. The samples of chaffinches and linnets were mainly composed of plant material, while the diet of serines was exclusively made up of plant matter.

With respect to their number, arthropods below or equal to 5 mm were more numerous in the diet samples of great tits and chaffinches but scarcely found in the samples of blackbirds.

Among the plant matter found in the diet samples, seeds were most numerous in the diet of blackbirds, chaffinches, linnets and serines, whereas in the diet of great tits fruits were found to be the larger portion.

Main results are presented in table 10.1.2.2-6.

Table 10.1.2.2-6: Overview of different food types found in the diet samples (orchards)

Proportion of different food types found in the diet (%)											
Species		Blackbird		Great tit		Chaffinch		Linnet		Serine	
		Mean No.*	%	Mean No.*	%	Mean No.*	%	Mean No.*	%	Mean No.*	%
Animal matter	Arthropods ≤ 5 mm	1.8	17.1	4.7	49.5	4.5	21.8	0.2	1.5	-	-
	Arthropods > 5 mm	4.7	44.8	4.0	42.1	3.2	15.5	0.1	0.5	-	-
	Other invertebrates	0.8	7.6	-	-	-	-	-	-	-	-
Plant matter	Seeds	2.1	20.0	0.2	2.1	10.0	48.5	13.0	68.1	6.5	63.7
	Plant material	0.7	6.7	0.2	2.1	0.7	3.4	5.7	29.8	3.5	34.3
	Fruits	0.4	8	0.4	4.2	2.2	10.7	0.1	0.1	0.2	2.0
Total		10.5	100	9.5	100	20.6	99.9	19.1	100	10.2	100
*Mean number of animal and plant items actually eaten by focal species foraging in and around modern pome fruit spindle bush orchards											

Conversion of the number of individuals in the diet samples to their portion of dry weight of the diet actually ingested (PD)

Food type specific correction factors derived from the literature were applied to calculate the number of different food types actually ingested by the birds from the number found in the diet samples. This led to an estimate of the numerical proportion of different food types in the diet actually ingested by the birds. In order to calculate their mass proportions, the length-weight regressions derived from the literature were used for different invertebrate taxa and plant seeds. Hence, the length estimations of the food items found in the diet samples were used to calculate their approximate dry weight. To obtain PD values for each bird species, the dry weights were first calculated for the various food items in each sample. The values for each food item were then pooled for each bird species separately and used for calculating the proportions of each taxon in the diet of the species in question.

Determination of PD as % of dry weight

Bird species	Great tit		Chaffinch		Linnet		Serin		Blackbird	
	Numerical proportion (%)	Dry weight proportion (%) PD	Numerical proportion (%)	Dry weight proportion (%) PD	Numerical proportion (%)	Dry weight proportion (%) PD	Numerical proportion (%)	Dry weight proportion (%) PD	Numerical proportion (%)	Dry weight proportion (%) PD
Animal	99	99	55	87	3	6	0	0	89	99.5

matt er										
Plant matt er	1	1	45	13	97	94	100	100	11	0.5

III.

CONCLUSIONS

In this study PT values for pome fruit orchards were derived for five bird species. Due to the selection of individuals for radio-tracking closely associated to pome fruit orchards and the inclusion of all periods of time while foraging could not be excluded, the calculated PT values can be regarded as a conservative assumption. Hence, they illustrate the highest possible portion of time the individual bird spent foraging in pome fruit orchards.

Overall this study provides reliable refinement parameters (PT; diet composition) for the use in higher tier risk assessment for birds foraging in modern spindle bush pome fruit orchards.

( 2007)

RMS comment: Study acceptable.

The study followed 5 focal species in orchards (blackbird, great tit, chaffinch, linnet and serine). These focal species have been chosen according to the results from previous studies conducted in pome fruit orchards in Poland and Italy. PT values from this study conducted in Germany indicate the relevance of these focal species.

Individual PT values of 0 have been excluded for the calculation of the mean and 90th percentile PT value, that is relevant and conservative.

The use of the 90th percentile PT value based on radiotracking of 20 individuals could be considered more relevant than the mean PT value in risk assessment in accordance with Guidance of 'EFSA 2009- Risk Assessment for Birds and Mammals'.

In this study PD values have been estimated based on "percentages by weight" that is relevant for risk assessment.

Based on the data obtained in the studies presented above, a new risk assessment was performed taking into account the PT (90th percentile) and PD values as well as on the default RUD and ftwa (EFSA, 2009).. The risk assessment was conducting on the following species taking into consideration the following parameters. (table 9.1.8.3.9).

Table 9.1.8.3.9: Characteristics of Focal Bird Species in orchards. Determination of Parameters for long-term Risk Assessment

Species	Great tit	Blackbird	Chaffinch	Linnet	Serine
Body Weight (g) ¹	19.0	113	20.9	15.3	11.2
Stratum	Ground / foliage	Ground / foliage	Ground / foliage	Ground / foliage	Ground / foliage
Diet Guild	Insectivorous	omnivorous	omnivorous	Granivorous	Granivorous
FIR (weight diet) calculated based on PD below	14.54	48.69	14.32	4.85	3.52
FIR_{diet} / BW	0.765	0.431	0.686	0.317	0.314
90thtile value PT ² (Multi Field Approach)	0.697	0.629	0.947	0.767	0.637
AV	1				
Area sprayed per ha	0.3				
Diet Composition &					

Portion based on dry weight diet ³		PD				
Animal matter	PD 1	0.99	0.995	0.87	0.06	0.00
Plant matter	PD 2	0.01	0.005	0.13	0.94	1.00

¹Source: Appendix A EFSA 2009 guidance document / bird bible 1998 / Dunning (1993) / Crocker et al (2002) DEFRA CSL Report No. PN0908 p. 11^(2c), 17^(2a), 12^(2d)

²Staab, F. & Moosmayer, P. 2007. Multi Field Approach PT values are used for Long Term Risk Assessment

³Staab, F. & Moosmayer, P. 2007. Table 10.1.2.2-6 above.

A study for determining the residue level in plants and vertebrates contaminated by a CA2735 application according to the GAP was conducted in orchard in Germany. The application rate (13 L/ha) and the way of applying CA2735 (application under the row corresponding to 1/3 of the max. dose rate) are the same ones in orchard and vineyard. Therefore, it can be assumed that the residue levels measured in plants and arthropods in a German orchard will not be significantly different from the potential concentrations obtained in the same plants and invertebrates in other countries. The study was summarized before (KIIIA 10.1.2.1/03, Knäbe, S. 2009 and KIIIA 10.1.2.1/04, Mende, P. 2009).

According to the Guidance document from EFSA, 2009, the long-term risk to birds can be assessed on the basis of time-weighted-average residue concentrations in food items. The previous evaluation was therefore refined taking into consideration the maximum residue concentrations determined in a study conducted under realistic conditions.

The different scenarios considered are presented in table 9.1.8.3.11

Table 9.1.8.3.11: Maximum residue concentration scenarios (orchards)

Diet Composition & Portion of Diet	Residue			Great tit	Blackbird	Chaffinch	Linnet	Serine
	Scenario 1	Scenario 2	Scenario 3	PD	PD	PD	PD	PD
Animal matter (RUD 1)	0.61 ^a	3.09 ^b	0.67 ^c	0.99	0.995	0.87	0.06	0.00
Plant matter (RUD 2)	0.08 ^d	0.08 ^d	0.08 ^d	0.01	0.005	0.13	0.94	1.00

^a Ground Dwelling Arthropods (Vortis sampling) R_{max} – see table 10.1.2.1-15 (Knäbe, S. 2009)

^b Ground & Crop dwelling Arthropods (Earwig traps) R_{max} – see table 10.1.2.1-19 (Knäbe, S. 2009)

^c Ground dwelling Arthropods (Pitfall traps) Mean R_{max} – see table 10.1.2.1-13 (Knäbe, S. 2009). Here the max mean residue was chosen as a realistic estimate. Residue in Isopoda was found to be higher (and more variable), but it is unlikely that Isopoda would form a high % of the diet of the indicator bird species in pome orchards. Isopoda only move during the night when the air humidity is high. Isopoda hide during the day and would not easily (or not more easily than any other arthropod prey) be spotted and consumed by the indicator bird species

^d Short vegetation R_{max} – see table 10.1.2.1-11 (Knäbe, S. 2009)

B.9.1.8.4. Secondary poisoning risk assessment

B.9.1.8.4.1. TER for earthworm-eating birds

According to the EFSA Guidance Document on Risk Assessment for Birds and Mammals, substances with a log P_{OW} greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in

terrestrial food chains. Bioaccumulation of Amitrole is not expected since the $\log P_{OW}$ (2.49) is below 3. Thus, secondary poisoning through **CA2735** via the food chain (earthworm-eating birds and fish-eating birds) can be excluded.

B.9.1.8.5. Risk from drinking water

Most of the birds can in principle satisfy (at least parts of) their daily water demand via food uptake. However, this potential depends on the water content of the diet items, which is lowest for seeds. Therefore, the assessment methodology for the risk to birds of pesticides in drinking water as provided below, uses a small granivorous bird as focal species at Tier 1.

In the EFSA Guidance (2009)⁵, the drinking water rate (DWR) for the focal bird species with a body weight of 15.3 g is given as 7.0 mL/d (equivalent to 0.46 L/kg b.w./day). This DWR is used in the following risk assessment.

- Uptake from contaminated water collected in pools on plants:

According to EFSA (2009), the leaf scenario clearly reflects a worst-case situation with regard to the risk to birds. It is relevant for spray application only. Formation of pools that would serve as drinking water supply for birds requires a certain plant morphology. Leaves must point upwards and at the same time must be closely pressed against other leaves or the stem at their basis to form cavities that could hold water over a considerable amount of time. Also, these structures must be accessible to birds, i.e. must be able to sit on the plant for drinking.

No uptake from contaminated water collected in pools on plants is expected to occur as **CA2735** is not applied on the green parts of vine or grape but under the row at the foot of trees and/or vine. Any weeds present are not considered to be of any relevance for this form of exposure either in spatial terms or in time.

- Uptake from contaminated water collected in puddles on soil:

Birds may be exposed to residues of **CA2735** by drinking water in puddles on soil within or close to the target area. According to the EFSA Guidance (2009), the concentration in puddles (PEC_{puddle}) is calculated by the following equation:

$$PEC_{puddle} = (AR / 10) / [1000 \times (w + Koc \times s)]$$

where: AR = application rate [g/ha]; divided by 10 to achieve rate in mg/m²,
w = 0.02 (pore water term: volume),
s = 0.0015 (soil term: volume, density, organic carbon content)

The TERa and TERlt are calculated as follows:

$$TERa = LC_{50} / (PEC_{puddle} \times DWR) \text{ and } TERlt = NOEC / (PEC_{puddle} \times DWR) \text{ with } DWR = 0.46.$$

The TER calculation results are presented in the **Table 10.1.9-1**.

Table 9.1.8.5.1: TER calculation resulting from a water intake from a contaminated puddle.

Dose (g/ha)	PEC (mg/L)	Endpoint (mg/kg b.w.)	TERa / TERlt
0.993	0.57*	LC ₅₀ : 2150	7366
		NOEC: N.A	N.A

* based on a Koc of 89 (see vol 3B.8).

The water intake from contaminated puddles by birds does not present any significant risk under the recommended conditions of use of **CA2735**.

⁵ European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

B.9.1.9. RMS conclusion

The risk assessments for birds were conducted in accordance to EFSA guidance (European Food Safety Authority; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438).

Endpoints for the risk assessment are acute LD₅₀ > 2150 mg/kg b.w. and dietary LD₅₀ > 829 mg/kg b.w./d. The one-generation reproduction test on mallard duck (NOEC=12.1 mg a.s./kg b.w./day) is not sufficient for assessing potential effects on development and reproduction due to endocrine disrupting properties of the active substance. This is due to the fact that the one-generation avian reproduction study does not include exposure during all relevant stages of the bird's development. These potential consequences on development and reproduction are studied with the 2-generation study in birds (Mallard duck).

No NOEC could be established as there were impacts upon thyroid gland size (higher size than in the control) at all concentrations tested in F0 and F1. An amendment to the final study report was submitted at the end of December 2012. In this amendment, it is reported that at collection of thyroids for potential histopathology from selected (up to two per pen) F2 hatchlings and untreated 14-day old survivors, thyroids appeared comparable in size to the control group. However, no measured data were submitted to the RMS. In these conditions, due to this uncertainty no NOEC based on effects on thyroid can be set.

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In conclusion, a NOEC based on reproductive performance parameters can be set at 95 ppm. However, no overall NOEC can be set. In F0 and F1 there are impacts upon thyroid gland size at all tested concentrations. It is well known that increase or decrease of thyroid gland size may influence vertebrates behaviour. There is therefore an uncertainty on the impact of such effects on wild animals at population level in natural conditions (e.g. parental care, nesting behaviour, territoriality and mounting behaviour). The value of 95 ppm a.s. corresponding to 16 mg/kg bw/day should therefore be considered as a LOEC. Therefore, long term risk assessment for birds could not be finalized.

A bibliographic search was conducted by the notifier in order to try to find a relationship between an increase of thyroid size and an impact on parental care, nesting behaviour, territoriality and mounting behavior. A list of references was submitted at the end of December 2012, but no summary of these papers was done by the notifier. The notifier concludes that nothing could be found. However, considering the late submission of this bibliography, it was not possible for the RMS to check. Moreover, even if nothing could be found, it doesn't mean that there are no uncertainties about such effect on thyroid gland size.

Therefore, as there is an uncertainty, long term risk assessment for birds cannot be finalized.

In a first screening step risk assessment, the acute TER value is higher than the trigger value of 10 for insectivorous and omnivorous birds in orchards and vineyard, respectively.

Long term TER for birds were calculated by the applicant based on the one-generation NOEL of 12.1 mg/kg b.w./day but as no overall NOEC could be established from the two-generation bird study, long term risk assessment for birds couldn't be finalized.

Concerning risk from drinking water, the TER_{acute} is above the trigger of 5.

Due to the low log K_{OW} (< 3) of amitrole, the risk to birds through secondary poisoning had not to be assessed. The application of CA2735, therefore, does not raise concerns regarding an accumulation in the food chain or secondary poisoning.

The use of CA2735 in accordance to the recommended uses is acceptable to birds.

B.9.2. EFFECT AN AQUATIC ORGANISMS (ANNEX IIA 8.2, ANNEX IIIA 10.2)

This point was addressed in the dossier submitted and reviewed for the inclusion of amitrole in the annex 1. Therefore, there is no need for summarizing again the studies. The main endpoints are shown in table 9.2.17.1.1, 9.2.17.1.2 and 9.2.17.1.3. Please also refer to review report SANCO/ 6839/VI/97-final, 22/03/2001.

Because amitrole might be considered as a potential endocrine disruptor, the consequences for non-target organisms are addressed with a multigeneration test in fish (see point B.9.2.3.2 below).

B.9.2.1. Acute toxicity to fish (annex IIA 8.2.1) – amitrole and 1,2,4-triazole**B.9.2.1.1. Acute toxicity of amitrole to rainbow trout (*Oncorhynchus mykiss*)**

Please refer to initial point B.9.2.

B.9.2.1.2. Acute toxicity of amitrole to *Leuciscus idus*

Please refer to initial point B.9.2.

B.9.2.1.3. Acute toxicity of amitrole to *Lepomis macrochirus*

- ❑ **Reference:** KIIA 8.2.1.3./01
- ❑ **Author(s); year:** [REDACTED] 1985
- ❑ **Title:** Acute Toxicity of aminotriazole to Bluegill sunfish (*Lepomis macrochirus*)
- ❑ **Report No:** [REDACTED]
- ❑ **Guidelines:**

Committee on Methods for Toxicity Tests with Aquatic Organisms (C. E. Stephan, Chairman). 1975. Methods for Acute Toxicity Tests with Fish, Macro invertebrates and Amphibians. Environmental Protection Agency, Ecological Research Series EPA-660/3-75-009, April, 1975. 61 p. American Public Health Association. 1980. Standard Methods for the Examination of Water and Wastewater. 15th ed. Washington, DC. 1134 p

- ❑ **Deviations:**
- ❑ **GLP:**

The study was performed in 1985, there is no GLP certificate however, a quality assurance statement and a study compliance statement are included in the report

- ❑ **Executive summary**

The acute toxicity of Aminotriazole to bluegill sunfish (*Lepomis macrochirus*) was assessed at the following nominal concentrations of Aminotriazole: 62.5, 125, 250, 500 and 1000 mg/L. Ten fish, with a mean weight of 0.21 g (± 0.038) and a mean standard length of 21 mm (± 1.1), were exposed to each test concentration and control water. Water quality parameters of temperature, dissolved oxygen and pH were measured throughout the test and were within acceptable limits.

The LC₅₀ (96 h) was estimated to be > 1000 mg/L. The No Observed Effect Concentration was 1000 mg/L.

- ❑ **Materials and methods:**

I. MATERIALS AND METHODS

A. MATERIALS

- 1. Test Material:** aminotriazole
Description: white powder
Lot/batch #: w.o. 3466; RTS 5833 AB; ATA Tech
Purity of active ingredients: 96.5%
Stability of test compound: Not stated
- 2. Vehicle and/or control:** None
- 3. Test animals -**
Species: *Lepomis macrochirus* (bluegill sunfish)
Length: 2.1 cm (average at start)
Weight: 0.21 g (average at start)
Source: [REDACTED]
Feeding: standard commercial fish food occasionally supplemented with brine shrimp nauplii (*Artemia* species) daily. The test fish were not fed 48-96 hours before the start.
- Acclimatisation period:** At least 14 days
Water: soft reconstituted water: deionised water containing 48 mg NaHCO₃, 30 mg CaSO₄ + 2 H₂O, 30 mg MgSO₄, 2 mg KCl
Dissolved oxygen: 9.2 mg/L at pH 7.2.
Loading rate: 0.14 g fish/litre test solution
- Environmental conditions:**
Temperature: 22° ± 1°C
Photoperiod: daily 16 hours photoperiod

B. STUDY DESIGN AND METHODS:

- 1. In life dates:** 27/09/1985-01/10/1985
- 2. Experimental treatments:**

A range-finding test of 96-hour duration was conducted to determine the concentration range for the definitive study. The preliminary test concentration was set at 100 mg/L. Based on the results of preliminary testing, five concentrations of the test compound ranging in a geometric series from 62.5 to 1000 mg/l, with ten fish per concentration were selected for the definitive bioassay. Also included was a dilution water control. The fish were added to the test chambers by random assignment within 30 minutes after addition of test material.

3. Observations:

All test organisms were observed once every 24 hours for mortality and abnormal (sub-lethal) effects. Any dead individuals were removed from the test chambers after each 24-hour observation.

4. Statistics

Statistical analysis of the concentration vs. effect data (generally mortality) was obtained by employing a computerized LC₅₀ program developed by Stephan et al.. This program calculated the LC₅₀ statistic and its 95-percent confidence limits using the binomial, the moving average, and the probit tests. However, if no mortality occurred or if a dose-response could not be demonstrated over a reasonable range (<37 to >63%) an LC₅₀ and/or its 95-percent confidence limits could not be calculated. Three different methods for analysing the data were used since no one method of analysis is appropriate for all possible sets of data that may be obtained. The method of calculation selected was that which gave the narrowest confidence limits for the LC₅₀ although all three models are valid.

☐ **Findings:**

II. RESULTS AND DISCUSSION

The 96-hour static toxicity test with bluegill sunfish (*Lepomis macrochirus*) exposed to aminotriazole resulted in 24-, 48- and 96-hour LC₅₀ values >1000 mg/L. All results were based on the nominal concentrations of 62.5, 125, 250, 500 and 1000 mg/L. The 96-hour no-observed effect concentration was estimated to be 1000 mg/L, the highest concentration tested, based on the lack of mortality or observed abnormal (sub-lethal) effects. An examination of the fish culture and acclimation records for this test indicated that the fish were in good condition for testing.

The dissolved oxygen concentrations ranged from 6.8 to 9.3 mg/L during the test. These values represented 77 and 107% saturation at 22 and 23°C, respectively, and were considered adequate for testing. The pH values ranged from 6.9 to 7.2.

❑ Conclusion:

The LC₅₀ (96 h) was estimated to be > 1000 mg/L. The No Observed Effect Concentration was 1000 mg/L.

- ❑ Comment (RMS): study acceptable. Concentrations were not measured during the study but results from other studies indicate that amitrole is stable in water system.**

B.9.2.1.4. Acute toxicity of amitrole to *Cyprinodon variegatus*

- ❑ Reference:** KIIA 8.2.1.3./02
- ❑ Author(s); year:** [REDACTED] 1993
- ❑ Title:** AMITROLE TECHNICAL - ACUTE TOXICITY TO SHEEPSHEAD MINNOW (*Cyprinodon variegatus*) UNDER STATIC RENEWAL CONDITIONS
- ❑ Report No:** [REDACTED]
- ❑ Guidelines:** FIFRA GUIDELINE REFERENCE # 72-3
- ❑ Deviations:**
- ❑ GLP:** yes
- ❑ Executive summary**

Five groups of 10 Sheepshead Minnow were exposed to nominal concentrations of 1000, 600, 360, 220 and 130 mg a.s./L for 96 hours under semi-static conditions (medium renewal after 48 hours). A concurrent control group was exposed to natural filtered seawater only. All test vessels were examined at 0-, 2-, 4-, 24-, 48-, 72- and 96-hours of exposure as follows: mortalities were recorded, dead sheepshead minnow were removed, and observations of the sheepshead minnow and the physical characteristics of the test solutions were recorded. All samples were analysed for Amitrole using a high performance liquid chromatographic (HPLC) procedure.

Mean measured concentrations averaged 101% of nominal and defined the concentrations tested as 1000, 620, 370, 220 and 130 mg a.s./L. No visible sign of undissolved test material (i.e., film on the solutions' surface, precipitate) was observed in any of the test solutions during the test period. At test termination, no mortality or sub lethal effects (e.g., loss of equilibrium, lethargy) were observed among fish exposed to any of the concentrations tested. Based on these data, the 96-hour LC₅₀ value for sheepshead minnow exposed to Amitrole Technical was estimated to be > 1000 mg a.s./L, the highest mean measured concentration tested. The No-Observed-Effect Concentration established for this study was determined to be 1000 mg/L.

- ❑ Materials and methods:**

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:	aminotriazole
Description:	white powder
Lot/batch #:	L1-4420
Purity of active ingredients:	98.22%
Stability of test compound:	Not stated. Renewal 48 hours after starting the test.
2. Vehicle and/or control:	None
3. Test animals -	
Species:	<i>Cyprinodon variegatus</i> (Sheepshead Minnow)
Length:	mean 28 mm (range 20-37 mm)
Weight:	mean 0.3 g (range 0.19-0.42 g)
Source:	Zeigler Brothers Inc. Prime Flakes.
Acclimatisation period:	At least 14 days
Water:	Natural filtered seawater
Dissolved oxygen:	4.4-7.8 mg/L.
Loading rate:	0.2 g fish/litre test solution
4. Environmental conditions:	
Temperature:	22 ± 1°C
Photoperiod:	daily 16 hours photoperiod

B. STUDY DESIGN AND METHODS:

- 1. In life dates:** 31/03/1993-04/04/1993
- 2. Experimental treatments:**

Exposure vessels consisted of 18.9-L glass aquaria, which held 15 L of test solution. The exposure solution in each aquarium had a depth of 18.4 cm and a surface area of 819 cm². During this study, a single exposure vessel was maintained for each test concentration and the control. Prior to test initiation, a 1000 mg a.s./L stock solution was prepared. The stock solution was stirred for approximately five minutes after which the solution was observed to be clear and colourless with no visible sign of undissolved test material. Each test concentration was prepared individually by adding the appropriate amount of the stock solution with the appropriate amount of dilution water to total 15 L of test solution in each exposure vessel. The control aquarium contained the same dilution water and was maintained under the same conditions as the test aquaria but contained no Amitrole Technical. Fresh test solutions were prepared at 48-hours following the procedure mentioned above.

An additional set of exposure vessels was established at this time for the renewal of the exposure solutions. At 48-hours, following renewal of the test solutions, organisms were transferred to the newly prepared solutions.

The test was initiated when sheepshead minnow were selected impartially from the holding tank and added to each test vessel (10 fish per treatment level). The resulting test organism loading concentration was 0.20 grams of biomass per litre of test solution. Fish were not fed during the study.

Test vessels were positioned in stratified random order in a water bath designed to maintain test solution temperatures at 22 ± 1°C. Test solutions were not aerated. Light at an intensity of 80 footcandles was provided at the test solutions' surface. The testing area received a regulated photoperiod of 16 hours of light and 8 hours of darkness.

3. Observations:

All test vessels were examined at 0-, 2-, 4-, 24-, 48-, 72- and 96-hours of exposure as follows: mortalities were recorded, dead sheepshead minnow were removed, and observations of the sheepshead minnow and the physical characteristics of the test solutions were recorded. The pH, dissolved oxygen concentration, temperature, and salinity were measured in each treatment level solution and the control at test initiation and each subsequent 24-hour interval of the exposure period. Water quality measurements were performed in both the old and new test

solutions at the 48-hour observation interval. Continuous temperature monitoring of the surrounding water in the water bath was also performed.

During the definitive exposure period, water samples were removed from each treatment level and the control at 0-, 48- and 96-hours of exposure for analysis of Amitrole concentration. Samples analysed at 0- and 48-hours were removed from the newly prepared test solutions while samples analysed at 96-hours were removed from the old test solutions. All samples were analysed for Amitrole using a high performance liquid chromatographic (HPLC) procedure. A method validation study prior to the initiation of the definitive test established an average recovery of $109 \pm 9\%$ for Amitrole from seawater.

4. Statistics

The mean measured concentrations tested (0-, 48- and 96-hour analyses) and the corresponding biological-response data (mortality) derived from the toxicity test were used to estimate 24-, 48-, 72- and 96-hour median lethal concentrations (LC_{50}) and 95% confidence intervals. The LC_{50} is defined as the concentration of the test material in dilution water, which caused mortality of 50% of the test organism population at the stated time interval. During this study $< 50\%$ mortality was observed in all of the concentrations tested, therefore, the LC_{50} value was estimated to be greater than the highest treatment level tested and no statistical analysis was performed. The No-Observed-Effect Concentration (NOEC) during the 96-hour exposure period was also determined.

The NOEC is defined as the highest concentration tested at and below which there were no toxicant-related mortalities or physical and behavioural abnormalities (e.g., lethargy, loss of equilibrium, darkened pigmentation), with respect to the control organisms.

□ Findings:

II. RESULTS AND DISCUSSION

A. FINDINGS

Preliminary test:

Prior to initiating the definitive study, a preliminary range-finding test was conducted. During this range-finding test, sheepshead minnow were exposed under static conditions to nominal concentrations ranging from 400 - 1.0 mg a.s./L. Throughout the exposure period, a decrease in the dissolved oxygen concentration of all exposure solutions was observed. Following 96-hours of exposure, no mortality or sub lethal effects (e.g., loss of equilibrium, lethargy) were observed among fish exposed to any of the concentrations tested. Based on the results of this range-finding test, it was determined that the definitive study would be conducted under static renewal conditions at nominal concentrations of 1000, 600, 360, 220 and 130 mg a.s./L.

Definitive test:

Although a decrease in dissolved oxygen concentration was observed for each treatment level prior to and following the renewal of exposure solutions at 48-hours of exposure, the dissolved oxygen concentration at each treatment level remained above acceptable levels (i.e. 90% saturation at test initiation, and $> 60\%$ of saturation during the remainder of the exposure). The remaining water quality parameters measured (pH, temperature and salinity) were unaffected by the concentrations of Amitrole Technical tested and remained within acceptable ranges for the survival of sheepshead minnow. Continuous monitoring of the temperature of the surrounding water in the water bath established a temperature range of $21 - 23^{\circ}\text{C}$.

Throughout the exposure period, analytical measurements were generally consistent between sampling intervals. Mean measured concentrations averaged 101% of nominal and defined the concentrations tested as 1000, 620, 370, 220 and 130 mg a.s./L. Based on the results of these analyses, it was established that the appropriate quality control was maintained during the analysis of the exposure solutions.

No visible sign of undissolved test material (i.e., film on the solutions' surface, precipitate) was observed in any of the test solutions during the test period. At test termination, no mortality or sub lethal effects (e.g., loss of equilibrium, lethargy) were observed among fish exposed to any of the concentrations tested. Based on these data, the 96-hour LC_{50} value for sheepshead minnow exposed to Amitrole Technical was estimated to be > 1000 mg a.s./L, the highest mean measured concentration tested. The No-Observed-Effect Concentration established for this study was determined to be 1000 mg/L.

☐ **Conclusion:**

The LC₅₀ (96 h) was estimated to be > 1000 mg/L. The No Observed Effect Concentration was 1000 mg/L.

☐ **Comment (RMS): study acceptable**

B.9.2.1.5. Acute toxicity of 1,2,4-triazole for *Oncorhynchus mykiss*

Please refer to initial B.9.2. For 1,2,4-triazole, see the DAR of tebuconazole.

B.9.2.2. Acute toxicity to fish (annex IIIA 10.2.1) – CA2735

B.9.2.2.1. Acute toxicity of CA2735 to *Oncorhynchus mykiss*

Please refer to initial B.9.2.

B.9.2.3. Chronic toxicity to fish (annex IIA 8.2.2) – amitrole and 1,2,4-triazole

B.9.2.3.1. Chronic test with amitrole

Please refer to initial B.9.2.

B.9.2.3.2. Fish two generation life cycle test with amitrole for *Oryzias latipes*

- ☐ **Reference:** K IIA 8.16/04
- ☐ **Author(s); year:** [REDACTED] 2012
- ☐ **Title:** Amitrole: Fish two generation life-cycle test with the Medaka (*Oryzias latipes*)
- ☐ **Report No:** [REDACTED]
- ☐ **Guidelines:** EPA Draft Medaka Multigeneration Test Guideline (19 April 2010)
- ☐ **Deviations:**
- ☐ **GLP:** Yes
- ☐ **Executive summary**

The objective of this study is to evaluate the possible consequences on the population of potential endocrine disrupting effects of amitrole. Medaka are exposed to the test substance at concentrations that are naturally retrieved in the environment after application of 13L/ha, 10L/ha and 6L/ha of CA2735. This exposure mimics the actual exposure of fishes to amitrole that is applied twice a year at 6 months interval.

Medaka (*Oryzias latipes*) were exposed to three test concentrations and a negative (dilution water) control under flow-through conditions.

Water samples were collected at specified intervals for analysis of the test substance.

Each parental generation (F0) treatment and control group had six replicate test chambers. Each replicate contained one reproductive male and female pair. The test started by exposing young reproductively active fish for three weeks. At the beginning of the fourth week, eggs were collected to start the F1 generation. Fish in the F1 generation were reared for a total of 14 weeks post fertilization (wpf). For developmental endpoints, fish were sampled in Test Week 11 (8 wpf); spawning was assessed from Test Week 14 through 15 (11 through 12 wpf). F2 generation could not be assessed due to Low embryo survival for the F2 generation (in the control and treatment groups) attributed to fungusing of embryos. This effect was not related to exposure to Amitrole. Fungusing of the F2 generation embryos may be attributed to incubation of embryos in test components with reproductive pairs. Presence of adult fish and addition of feed to test chambers may have contributed to microbial growth. However, the study does provide a complete set of data for an entire generation and therefore can provide valuable data that addresses the question of thyroid mediated effects during chronic exposures.

Observations of the effects of the test substance on F0 and F1 generations of medaka were used to calculate the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). The endpoints measured to evaluate possible population-level effects included survival, growth, reproduction (both fecundity and fertility), secondary sex characteristics and sex ratio.

Fecundity and fertility of each breeding pair was assessed for the F0 generation at the end of the 3 weeks exposure period and for the F1 generation in test weeks 14 through 16.

There was no apparent treatment related effects on Fecundity and fertility in either the F0 or F1 generation.

There was no statistically significant difference in hatching success for any of the treatment groups for the F1 embryos when compared to the negative control. Hatching success for the F2 generation embryos was low due to fungusing of eggs which is not treatment related. Fungusing of eggs was observed in the control group as well as the treatment groups and is probably due to the fact that in the current design egg compartments are in adults tanks so that feeding of adults may have contributed to the fungus contamination of eggs.

There was no treatment related effect on survival either of the F0 or of the F1 generations.

No treatment-related effect was observed on growth (no statistically significant reduction in either length or weight for either the F0 or F1 generation in any treatment groups).

RESULTS: Based on mean measured test concentrations:

Mean measured concentrations ranged from 4.7 to 10.9 µg a.i./L during the 21-day F0 generation exposure. Mean measured concentrations ranged from 4.3 to 10.2 µg a.i./L during the F1 generation exposure.

The NOEC during the F0 generation exposure for reproduction was 10.9 µg a.i./L. The NOEC during the F1 generation for all endpoints was 10.2 µg a.i./L. The LOEC for this study, based on F0 and F1 generation endpoints was >10.2 µg a.i./L (highest tested concentration). The NOEC was 10.2 µg a.i./L and the MATC was calculated to be >10.2 µg a.i./L. Despite the fact that the F2 generation could not be assessed, further to fungus contamination in all groups (controls and treatment groups), the study does provide a complete set of data for an entire generation and therefore provide valuable data that addresses the question of potential thyroid mediated effects during chronic exposures.

Based on the obtained results, it can be concluded that Chronic exposure to Amitrole did not result in effects on growth and development suggestive of thyroid mediated effects. Therefore, no impact on the fish population is expected further to exposure to amitrole at predictable environmental concentrations

Parameter	Parental Generation		F1 Generation	
	NOEC (µg a.i./L)	LOEC (µg a.i./L)	NOEC (µg a.i./L)	LOEC (µg a.i./L)
Hatching Success	--	--	10.2	>10.2
Survival	--	--	10.2	>10.2
Growth	--	--	10.2	>10.2
Reproduction	10.9	>10.9	10.2	>10.2
Sex Ratio	--	--	10.2	>10.2
Secondary Sex Characteristics	--	--	10.2	>10.2
MATC >10.9	MATC >10.2			

□ **Materials and methods:**

A- MATERIAL

1. Test Material: CA2735**Description:** liquid (SL formulation)**Lot/batch #:** 310123555**Active ingredient content:** 229g/L**Expiry date:** 16/12/2012**2. Control:**

Dilution water

3. Test animals - Medaka (*Oryzias latipes*). Fishes used in the test were sexually dimorphic adults that were actively spawning. Females had a mean weight of 300 mg and males had a mean weight 306 mg based on a subsample of fish weighed at the beginning of the pre-exposure period.

Age: Adults (~22 weeks post fertilization at initiation of parental generation exposure)

Source:

Feeding: The F0 generation fish were fed at least once per day during holding and three times per day during the pre-exposure and during the test with brine shrimp nauplii (*Artemia* sp.). The first feeding began when greater than 90% of the individuals hatched in an F1 generation replicate. Newly hatched larvae were fed an excess amount of newly-hatched brine shrimp nauplii (*Artemia* sp.) supplied by Brine Shrimp Direct, Ogden, Utah, at least twice per day during the first 14 days post-hatch. Fish were not fed at least 12 hours prior to terminal growth measurements to allow the fish to clear their digestive tracts prior to weight measurements.

Pre-exposure period: 17 days

Water: Soft reconstituted water: deionised water containing 48 mg NaHCO_3 , 30 mg $\text{CaSO}_4 + 2 \text{H}_2\text{O}$, 30 mg MgSO_4 , 2 mg KCl

Dissolved oxygen: At or above 7.2 mg a.i./L ($\geq 86\%$ of saturation at 24 °C) at pH-8.1-8.4

Loading rate: 0.042 g of fish per liter of test solution that passed through the test chamber during a 24-hour period. Instantaneous loading (the total wet weight of fish per liter of water in the tank) was 0.33 g of fish per liter of test solution.

4- Environmental conditions:**Temperature:** $24^\circ \pm 1^\circ$ **Photoperiod:** Photoperiod of 16 hours of light and 8 hours of darkness.**5- Test Apparatus**

The toxicity test was conducted using an exposure system consisting of a continuous-flow diluter to deliver each concentration of the test substance and a negative control (dilution water) to test chambers. Syringe pumps (Harvard Apparatus, Massachusetts) were used to deliver appropriate volumes of test stock substance solutions to mixing chambers indiscriminately assigned to each treatment. The stock solution was diluted with well water in the mixing chambers in order to obtain the desired test concentrations prior to delivery to the test chambers. The flow of dilution water into each mixing chamber was controlled using rotameters, and was adjusted to provide approximately 8 volume additions of test solution in each test chamber per day. After mixing, the test solution in each mixing chamber was pumped into the appropriate replicate test chamber using a peristaltic pump (Cole-Parmer Instrument Company, Chicago, Illinois).

Test chambers for each generation were indiscriminately placed in a temperature-controlled environmental chamber designed to maintain the target test temperature throughout the test period.

In both the F0 and F1 generations embryo exposures, incubation of eggs was done in an incubation device consisting of a glass test tube, transected by a stainless steel screen, suspended from the test chamber cover and held in place by the test tube screw top cap. A small stainless tube was inserted through the cap of each

incubation device to provide aeration during incubation. The end of the stainless steel tube was positioned near the rounded incubator bottom to provide gentle bubbling with air for agitation of the eggs.

B. STUDY DESIGN AND METHODS:

1. In life dates: 16/09/2011-24/02/2012

2-Lenght of exposure: Parental Generation: Reproductively active fish exposed for 21 days prior to egg collection
F1 Generation: Embryo to 13 weeks post fertilization

2. Experimental treatments and Observations

Medaka (*Oryzias latipes*) were exposed to three test concentrations and a negative (dilution water) control under flow-through conditions. Nominal test concentrations were based on the worst case expected environmental concentrations following applications of amitrole according to the European Good Agricultural Practices. Nominal test concentrations selected were 4.0, 6.0 and 9.0 µg active ingredient (a.i.)/L. Mean measured concentrations were determined for the parental generation (F0) and for the first filial generation (F1) from samples of test water collected throughout the test period. The analytical method used for the analysis of amitrole in freshwater samples was based upon methodology developed by Wildlife International, Ltd. The analytical method consisted of diluting the samples with freshwater, as necessary, and analyzing the samples by High Performance Liquid Chromatography with mass selective detection (LC/MS/MS). Mean measured concentrations for the F2 generation were not determined due to early termination of the F2 generation.

However, the study does provide a complete set of data for an entire generation and therefore can provide valuable data that addresses the question of thyroid mediated effects during chronic exposures.

Tested concentrations

Nominal (µg a.i./L)	Parental Generation Mean Measured (µg a.i./L)	F1 Generation Mean Measured (µg a.i./L)
Negative Control	<LOQ	<LOQ
4.0	4.3	4.3
6.0	8.4	6.9
9.0	10.9	10.2

Each parental generation (F0) treatment and control group had six replicate test chambers. Each replicate contained one reproductive male and female pair. The test was started by exposing young reproductively active fish for three weeks. At the beginning of the fourth week, eggs were collected to start the F1 generation. During test week 7 (test Day 43), the F1 generation fry were thinned to a maximum of 10 per replicate test chamber, for a maximum total of 60 per experimental group, and the remaining fish were discarded. The exposure of F1 generation fish was continued until test day 80, when breeding pairs were established. On test day 80, the phenotypic sex of each individual was determined. Based on the phenotypic sex data, one male-female breeding pair was impartially established in each of six replicates per experimental group. The remaining F1 generation fish in each replicate were terminated following establishment of breeding pairs, and were analyzed for growth (length/weight) and were preserved for anal fin papillae evaluation.

Beginning on test day 92 and continuing through test day 114, spawned eggs in the F1 replicates were counted and assessed for fertility. Each day, all eggs, either held by the female or deposited in the tank were removed and counted. Exposure of the F1 generation breeding pairs was continued until test day 116, when the fish were terminated and analyzed for growth (length/weight) and were preserved for anal fin papillae evaluation.

On test days 113 and 114, eggs from the F1 generation breeding pairs were collected to initiate the F2 generation using the same procedures as were used to start the F1 generation. The F2 generation was terminated at the end of the hatching period due to low hatching success in the negative control group and all treatment groups.

Observations of the effects of the test substance on the F0 and F1 generations of medaka were used to calculate the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). The endpoints measured to evaluate possible population-level effects include survival, growth, reproduction (both

fecundity and fertility), secondary sex characteristics and sex ratio. All of these endpoints were evaluated in the context of the phenotypic sex of the individual.

Survival and General Observations

Assessments of survival and general observations were made daily in order to assess the health of the fish. Any mortalities or external abnormalities (such as hemorrhage, discoloration) were noted. Abnormal behavior (relative to controls), such as hyperventilation, loss of equilibrium, uncoordinated swimming, atypical quiescence, and feeding abstinence, was also noted. Mortalities were recorded and dead fish removed from test chambers as soon as possible and discarded. Dead fish were not replaced in either the control or treatment vessels.

Growth

Terminal lengths and wet weights of F0 generation fish and of F1 generation fish from test Day 80 (thinning) and Day 116 (termination) were recorded. Fish length was measured to the nearest mm, and fish were blotted dry and weighed to the nearest 0.1 mg.

Fecundity and Fertility

Fecundity and fertility of each breeding pair was assessed for the F0 generation for three days (Days 22, 23 and 24) of the test, and for 23 days (Days 92-114) of the test the F1 generation. Egg clumps were gently removed from netted females or from the aquarium bottom each morning. The number of eggs spawned and the number of fertile eggs was determined for each breeding pair daily during these periods.

Determination of Sex for Selection of F1 Generation Breeding Pairs

An attempt was made to determine the genotypic sex via fin clips from the F1 generation fish in test week 11. However after repeated attempts, the contract laboratory was unable to successfully identify genotypic sex from the fin clips provided using PCR methodology. Therefore, the phenotypic sex of the test fish was determined based upon sex characteristics including the presence (male) or absence (female) of a notched dorsal fin and sail-like and transparent appearance of the anal fin. Phenotypic sex identification is consistent with the Japanese medaka full life-cycle test guideline (3). The phenotypic sex of all fish in the F1 generation was determined on Day 80 of the test, and was used to evaluate sex ratio, defined as the proportion of fish that were males. Following phenotypic sex identification, one male and one female from each replicate was paired and the remaining fish were terminated and processed for growth and anal fin papillae assessment.

Necropsy and Anal Fin Papillae Evaluation

At termination, the fish were euthanized with MS-222. The tail was spread and fixed for assessment of the number of anal fin papillae. The body cavity of each fish was opened and perfused with fixative prior to submersing the entire body in fixative (Davidson's solution). After at least 24 hours of fixation, the samples were rinsed with 70% ethanol and placed in 10% neutral-buffered formalin.

3. Statistics

Statistical analyses were performed to evaluate differences between treatment and control groups for each of the following biological endpoints, if possible:

- Hatch
- Survival
- Growth (length & weight)
- Fecundity (eggs per female reproductive day and cumulative eggs produced)
- Fertility (fertile eggs/eggs collected)
- Anal Fin Papillae
- Sex Ratio

The unit of statistical analysis for the biological observations was the replicate test chamber. Males and females were analyzed separately for each endpoint, when appropriate. Data was analyzed in the context of the phenotypic sex of the specific fish. Each endpoint was analyzed using two complementary statistical approaches.

First, an analysis using a step-down approach based on the Jonckheere-Terpstra trend test was used to evaluate trends in the ranks of replicate means to determine dose responsive trends among the treatment groups. The use of a trend-based test is desirable because such tests have superior power characteristics when the dose response is monotonic. The Jonckheere-Terpstra trend test also has the advantage that it is a non-parametric test, and data do not have to satisfy assumptions of normality and homogeneity of variance.

In addition to evaluating trends of replicate means, pair-wise comparisons were performed to determine which treatment groups differ statistically from the control group. When evaluating pairwise tests one must consider the magnitude of an effect as well as its statistical significance. For example, a 3% difference in weight may be statistically significant, but within a reasonable range of biological variability. The most convincing evidence of a treatment-related effect is one that shows a significant trend, is statistically different from the control, and at a sufficient magnitude to be biologically relevant.

The pair-wise comparisons were performed using Dunnett's Multiple Comparison Test. Data were evaluated for normality (e.g., Shapiro-Wilk's test) and homogeneity of variance (e.g., Bartlett's or Levene's test). Data transformations were employed, when possible, to correct the data for non-normality or heterogeneous variances. If the data for these continuous variables was normally distributed with homogeneous variance, then the pair-wise comparisons were by Dunnett's Multiple Comparison Test. When the conditions of normality and homogeneity of variances were not met, and data transformations were not successful in correcting the conditions, then the pair-wise comparisons were performed using the Wilcoxon Rank-Sum test, if appropriate. Statistical tests were performed at $\alpha = 0.05$, with the exception of tests for normality and homogeneity which will be performed at $\alpha = 0.01$. All statistical tests were performed using a personal computer with commercially available computer software programs such as SAS. Additional analyses of data was conducted if deemed appropriate by the Study Director. The results of the analyses are documented in the raw data and summarized in the final report.

□ Findings:

Measurement of Test Concentrations

Nominal concentrations selected for use in this study were 4.0, 6.0 and 9.0 $\mu\text{g a.i./L}$. The measured concentrations in the test chambers prior to initiation of the F0 generation exposure ranged from 114 to 127% of nominal concentrations. The concentrations of the stock solutions delivered to the diluter system were also confirmed prior to test initiation, with measured concentrations that ranged from 108 to 111% of nominal concentrations.

The measured concentrations of the samples collected during the F0 generation exposure generally ranged from approximately 110 to 150% of nominal concentrations. When the measured concentrations of the samples collected during the F0 generation were averaged for each treatment group, the mean measured concentrations were 4.7, 8.4 and 10.9 $\mu\text{g a.i./L}$, which represented 118, 140 and 121% of nominal concentrations, respectively.

The F1 generation exposure occurred in the same test chambers as the F0 generation, so samples analyzed from the F0 generation confirmed the operation of the diluter. The measured concentrations of the samples collected during the F1 generation exposure generally ranged from approximately 95 to 120% of nominal concentrations, with extremes of 85.0 and 157% of nominal. When the measured concentrations of the samples collected during the F1 generation were averaged for each treatment group, the mean measured concentrations were 4.3, 6.9 and 10.2 $\mu\text{g a.i./L}$, which represented 108, 115 and 113% of nominal concentrations, respectively. The results of the study were based on the mean measured test concentrations during the F0 and F1 generation exposures, as applicable.

Hatching Success

F1 Generation - There were no apparent treatment-related effects on hatching success in the parental-generation exposure. There were no apparent differences in time to hatch between the control groups and the 4.3, 6.9 and 10.2 $\mu\text{g a.i./L}$ treatment groups in the F1 generation. The majority of viable embryos in the control and treatment groups hatched between days 8 and 11 post fertilization. Hatching was completed in all control and treatment replicates by day 11 post fertilization.

The mean hatching success in the negative control, 4.3, 6.9 and 10.2 $\mu\text{g a.i./L}$ treatment groups was 40.1, 63.9, 68.7 and 54.9%, respectively. The Jonckheere-Terpstra trend test indicated there were no statistically significant trends and Dunnett's test indicated that there were no statistically significant decreases in hatching success in any treatment group in comparison to the negative control during any period in the parental-generation exposure ($p > 0.05$). Therefore, the NOEC for hatching success in the F1 generation was 10.2 $\mu\text{g a.i./L}$, the highest concentration tested. A summary of the results are reported in the following table (see below).

Post-Hatch Survival

F1 Generation – There were no apparent treatment-related effects on survival in the F1 generation exposure. Percent survival was evaluated over key periods of development during the F1 generation exposure. These

included the period from hatch to initial thinning (Days 31 to 43 of the test), Day 43 to Day 80 of the test when fish were thinned to form reproductive groups, and the reproductive period from Day 80 of the test through termination of the F1 generation on Day 116 of the test.

The mean larval survival to initial thinning (Day 43 of the test) in the negative control, 4.3, 6.9 and 10.2 µg a.i./L treatment groups was 68.1, 82.3, 88.8 and 86.7%, respectively. The mean survival from Day 43 to formation of reproductive groups on Day 80 of the test in the negative control, 4.3, 6.9 and 10.2 µg a.i./L treatment groups was 84.4, 98.3, 95.0 and 100%, respectively. The mean survival from Day 80 to termination of the F1 generation reproductive pairs on Day 116 of the test in the negative control, 4.3, 6.9 and 10.2 µg a.i./L treatment groups was 100, 83.3, 91.7 and 100%, respectively. The Jonckheere-Terpstra trend test indicated there were statistically significant trends for increased survival in all treatment groups in comparison to the negative control from hatch to Day 43 and from Day 43 to Day 80 ($p < 0.05$). There were no statistically significant trends in survival during the reproductive period. Increased survival in the treatment groups was not considered to be a treatment-related effect and Dunnett's test indicated that there were no statistically significant decreases in survival in any treatment group in comparison to the negative control during any period in the F1 generation exposure ($p > 0.05$). **The NOEC for survival in the F1 generation was 10.2 µg a.i./L, the highest concentration tested.** A summary of the results are reported in the following table (see below).

Mean Measured Concentration (µg a.i./L)	Hatching Success (%)	Larval Survival to Thinning on Day 43 (%)	Survival from Thinning to Pairing on Day 80 (%)	Survival from Pairing on Day 80 to Termination (Day 116) (%)
Negative Control	40.1	68.1	84.4	100
4.3	63.9	82.3	98.3	83.3
6.9	68.7	88.8	95.0	91.7
10.2	54.9	86.7	100	100
There were no statistically significant decreases in hatching success or survival in any treatment group in comparison to the negative control using Jonckheere-Terpstra Trend test and Dunnett's test ($p > 0.05$).				

RMS comment: The mean hatching success in the negative control was 40.1 %. RMS considered that 40.1% hatching success is very low for a negative control. There should be more than 80% hatchability of eggs in the controls according to OECD draft fish two generations test guideline (2002). Considering the low hatching success in the control, even if there were no statistically significant decreases in hatching success in any treatment group in comparison to the negative control, it is not possible to conclude on the potential impact of amitrole on hatchability of eggs. The notifier informed the RMS late in december 2012 that a new experimental phase is being conducted in order to conclude on potential effects on hatchability of eggs.

Growth

F0 Generation – Growth for the F0 generation was not an endpoint for this study. F0 generation fish were actively spawning adults at the beginning of the exposure. However, length and weight measurements taken at the end of the F0 exposure period demonstrate the relative uniformity of size between the control and treatment group fish. There were no statistical differences ($p > 0.05$) between the control group and treatment groups for either male or female length or weight at termination of the F0 generation according to Dunnett's test. With the exception of male length, the Jonckheere-Terpstra trend test indicated no statistically significant trends for length or weight for F0 generation treatment group fish in comparison to the control group ($p > 0.05$). There was a significant trend for increased length for males in the 10.9 µg a.i./L group. This difference was slight and was attributed to indiscriminate assignment of pairs to the test groups. A summary of the results are reported in the following table (see below).

Summary of Growth Measurements at Termination of F0 generation Medaka Exposed to Amitrole				
Mean Measured Concentration (µg a.i./L)	Mean ± SD of Growth Parameters in Parental-Generation Fish			
	Terminated Following Spawning ¹			
	Male Total Length (mm)	Male Wet Weight (mg)	Female Total Length (mm)	Female Wet Weight (mg)
Negative Control	35.5 ± 1.05	381.8 ± 25.88	33.6 ± 1.52	359.2 ± 48.46
4.7	35.8 ± 1.17	410.8 ± 49.63	33.0 ± 1.10	327.4 ± 25.49
8.4	36.2 ± 0.98	414.8 ± 41.09	34.7 ± 1.37	352.4 ± 41.44
10.9	36.7 ± 0.52	414.3 ± 71.20	34.7 ± 2.42	352.1 ± 45.16

¹ Total length and wet weight were measured by sex in all fish at termination of the F0 generation on Day 30, following the adult reproductive exposure period. There were no statistically significant decreases in length or weight in any treatment group in comparison to the negative controls using the Jonckheere-Terpstra trend test or Dunnett's test ($p > 0.05$).

F1 Generation – There were no apparent treatment-related effects on growth in the F1 generation exposure. Growth (total length and wet weight) was evaluated on Day 80 of the test from the fish thinned during the formation of reproductive groups, and at termination of the reproductive period on Day 116 of the test. Due to the differences in length and weight of adult male and female fish caused by sexual dimorphism (i.e., the difference in size between males and females in the species), the fish measured on Day 80 and at termination were statistically compared by sex. A summary of the results are reported in the following table (see below).

Test Day 80 (Thinned F1 Fish)

Males: The mean total length of male fish thinned at formation of reproductive groups on Day 80 of the test in the negative control, 4.3, 6.9 and 10.2 µg a.i./L treatment groups was 26.1, 26.0, 26.7 and 26.7 mm, respectively, while wet weight measurements were 186.9, 171.0, 176.1 and 189.3 mg, respectively. The Jonckheere-Terpstra trend test indicated there were no statistically significant trends and Dunnett's test indicated there were no statistically significant decreases in total length or weight among males in any treatment group in comparison to the negative control in the F1 generation at sexual maturity ($p > 0.05$).

Females: The mean total length of female fish thinned at formation of reproductive groups on Day 80 of the test in the negative control, 4.3, 6.9 and 10.2 µg a.i./L treatment groups was 24.8, 27.1, 28.9 and 28.0 mm, respectively, while wet weight measurements were 179.4, 223.0, 283.4 and 249.6 mg, respectively. Statistical analyses of lengths and weights taken from fish thinned on Day 80 indicated that there were no statistically significant decreases in total length or wet weight in females in any treatment group in comparison to the negative control in the F1 generation at sexual maturity ($p > 0.05$). The Jonckheere-Terpstra trend test did indicate there were statistically significant trends for increased length and weight among females in all treatment groups when compared to the negative control ($p \leq 0.05$). Dunnett's test also indicated a statistically significant increase in total length for females in all treatment groups, and a statistically significant increase in wet weight for females in the 6.9 and 10.2 µg a.i./L treatment groups on Day 80 ($p \leq 0.05$). The slight increase in growth among females in the treatment groups in comparison to the negative control females was not considered to be an adverse effect and is not considered to be a treatment-related effect.

Test Day 116 (F1 Adult Fish at Termination)

Males: The mean total length of male fish at termination on Day 116 of the test in the negative control, 4.3, 6.9 and 10.2 µg a.i./L treatment groups was 32.2, 30.8, 31.4 and 31.7 mm, respectively, while wet weight measurements were 344.7, 269.7, 295.8 and 294.3 mg, respectively. The Jonckheere-Terpstra trend test indicated there were no statistically significant trends in total length or wet weight among males in any treatment group, and Dunnett's test indicated there were no statistically significant decreases in total length among males in any treatment group in comparison to the negative control at termination of the F1 generation ($p > 0.05$). Dunnett's test indicated a statistically significant decrease in wet weight for males in the 4.3 µg a.i./L treatment group ($p \leq 0.05$), but this decrease was not apparent in the 6.9 and 10.2 µg a.i./L treatment groups and was not considered to be treatment related.

Females: The mean total length of female fish at termination on Day 116 of the test in the negative control, 4.3, 6.9 and 10.2 µg a.i./L treatment groups was 34.0, 35.0, 35.3 and 34.5 mm, respectively, while wet weight

measurements were 477.7, 522.0, 494.2 and 476.6 mg, respectively. The Jonckheere-Terpstra trend test indicated there were no statistically significant trends and Dunnett's test indicated there were no statistically significant decreases in total length or weight among females in any treatment group in comparison to the negative control at termination of the F1 generation ($p > 0.05$).

Since there were no statistically significant treatment-related effects on growth at any measured interval during the F1 generation exposure, **the NOEC for growth in the F1 was 10.2 µg a.i./L, the highest concentration tested.**

Mean Measured Concentration (µg a.i./L)	Mean ± SD of Growth Parameters in F1-Generation Fish							
	Thinned on Day 80 ¹				At Termination on Day 116			
	Male Total Length (mm)	Male Wet Weight (mg)	Female Total Length (mm)	Female Wet Weight (mg)	Male Total Length (mm)	Male Wet Weight (mg)	Female Total Length (mm)	Female Wet Weight (mg)
Negative Control	26.1 ± 0.81	186.9 ± 18.40	24.8 ± 1.61	179.4 ± 42.90	32.2 ± 1.33	344.7 ± 52.48	34.0 ± 2.10	477.7 ± 119.52
4.3	26.0 ± 0.75	171.0 ± 17.78	27.1 ± 0.92	223.0 ± 27.12	30.8 ± 1.30	262.7 ± 31.29*	35.0 ± 1.41	522.0 ± 107.40
6.9	26.7 ± 0.63	176.1 ± 18.85	28.9 ± 0.69	283.5 ± 28.29	31.4 ± 1.34	295.8 ± 41.23	35.3 ± 2.25	494.2 ± 61.82
10.2	26.7 ± 0.57	189.3 ± 17.01	28.0 ± 0.60	249.6 ± 15.69	31.7 ± 2.16	294.3 ± 53.54	34.5 ± 0.84	476.6 ± 46.59

¹ Total length and wet weight were measured by phenotypic sex in all fish not selected for use in the adult reproductive exposure period.
There were no statistically significant decreases in length or weight in any treatment group in comparison to the negative control using Jonckheere-Terpstra Trend test ($p > 0.05$).
* There was a statistically significant decrease in comparison to the negative control group using Dunnett's test ($p < 0.05$).

Clinical Observations

F0 Generation - All fish in the negative control group and in all treatment groups appeared normal throughout the 21-day exposure prior to collection of spawns and during the three days of spawn collection (Days 22, 23 and 24) for the F0 generation. With the exception of one female in the negative control noted with a folded operculum following spawn collection, all F0 generation fish were normal in appearance prior to termination. There were no observations during the F1 generation that were considered to be treatment-related.

F1 Generation - The majority of fish in the control and treatment groups appeared normal throughout the F1 generation exposure period. Observations of larvae lying on the chamber bottom, surfacing or as weak were observed in the treatment groups during the first week following release of larvae into the test chambers. Small fish were also noted in the 6.9 and 10.2 µg a.i./L treatment groups prior to thinning for formation of reproductive pairs on Day 80 of the test. One female in the 4.3 µg a.i./L treatment groups was noted as lying on the bottom, hyperventilating and as pale during the reproductive phase. However, observations in the treatment groups were generally comparable to observations in the control groups, and were not considered to be treatment related.

Reproduction

Effects on reproduction in adult fish were assessed through analysis of fecundity and fertility in the F0 and F1 generation spawning groups. Fecundity and fertility for the F0 and F1 generations are summarized in the next two Tables. Reproduction in medaka is known to be highly variable. Current endocrine screens start with an excess of 25 to 33% more reproductive groups to ensure an adequate number of proven breeders are available for initiating exposure because of poor performance in some groups that occurs solely by chance.

✓ F0 Generation

Fecundity F0 Generation

While spawning activity was noted for the F0 generation during the 21-day exposure prior to egg collection, fecundity and fertility was not assessed during this period. Fecundity and fertility information for the F0 generation is based upon the three spawns collected after the 21-day exposure for initiation of the F1 generation. Fecundity was defined as the total number of eggs produced per replicate and the number of eggs per female per reproductive day during the reproductive period. The mean number of eggs per replicate in the negative control, 4.7, 8.4 and 10.9 µg a.i./L treatment groups was 33.3, 30.8, 36.0 and 28.2 eggs, respectively. The mean number of eggs per female per reproductive day in the negative control, 4.3, 8.4 and 10.9 µg a.i./L treatment groups was 11.1, 10.3, 12.0 and 9.4, respectively. The Jonckheere-Terpstra trend test indicated there were no statistically significant trends and Dunnett's test indicated that there were no statistically significant decreases in fecundity in any treatment group in comparison to the negative control ($p > 0.05$). **The NOEC for fecundity in the F0**

generation was 10.9 µg a.i./L, the highest concentration tested during the F0 generation exposure. A summary of the results are reported in the following table (see below).

Fertility F0 Generation

Percent fertility was defined as the number fertile eggs per the number of eggs spawned. The mean percent fertility in the negative control, 4.7, 8.4 and 10.9 µg a.i./L treatment groups was 45.7, 93.7, 85.7 and 79.1% respectively. The Jonckheere-Terpstra trend test indicated there were no statistically significant trends and Dunnett's test indicated that there were no statistically significant decreases in fertility in any treatment group in comparison to the negative control during the F0 generation exposure ($p > 0.05$). **The NOEC for fertility in the F0 generation was 10.9 µg a.i./L, the highest concentration tested.** A summary of the results are reported in the following table (see below).

Summary of Fecundity and Fertility of F0 Generation Medaka Exposed to Amitrole			
Mean Measured Concentration (µg a.i./L)	Reproductive Parameters		
	Number of Eggs (Mean ± SD)	Eggs/Female/Reproductive Day (Mean ± SD)	Fertility (%) (Mean ± SD)
Negative Control	33.3 ± 20.4	11.1 ± 6.80	45.7 ± 41.5
4.7	30.8 ± 23.1	10.3 ± 7.72	93.7 ± 8.8
8.4	36.0 ± 23.0	12.0 ± 7.67	85.7 ± 22.6
10.9	28.2 ± 14.3	9.4 ± 4.75	79.1 ± 39.0
There were no statistically significant decreases in egg production or fertility in any treatment group in comparison to the negative control using the Jonckheere-Terpstra trend test or Dunnett's test ($p > 0.05$).			

The study was done according to US-EPA guideline draft Medaka Multigenerational Test Guideline. In this guideline there is no validity criteria set for the % of fertility in F0.

The low fertility is observed only in the negative control group and it is likely that due to the methodology used (reproductive replicates in the F0 and F1 generations consisted of a breeding pair, and not a breeding group of 5 males and 5 females) one pairing was not properly done. Over the 6 pairs, 2 did not produce eggs at all. This is a localized problem limited to the untreated batch which is physically separate from the treated batches.

The fertility in all treated groups in F0 is well higher than 80%. In the F1 generation the fertility in BOTH untreated and treated fish remains at a high 90%.

So even if the fertility was low in the control group it can be concluded that no impact of amitrole on fertility is expected.

RMS comment: The fertility in the negative control was 45.7 %. RMS considered that 45.7 % fertility is very low for a negative control. Even if it is not a validity criteria in the US-EPA guideline draft, the % of fertility should be more than 80% fertility in the controls according to OECD draft fish two generations test guideline (2002). The fertility in all treated groups in F0 is higher than 80% (except 79.1% at 10.9 µg a.s./L. In the F1 generation the fertility in both untreated and treated fish remains at a high 90%. Even if the fertility was low in the control group, no impact of amitrole on fertility is expected.

✓ F1 Generation

Fecundity F1 Generation

The number of eggs spawned and the number of fertile eggs for the F1 generation was determined for each breeding pair daily for 23 days beginning on test day 92. Any breeding pair with a mortality during the reproductive phase was excluded from statistical calculations. The mean number of eggs per replicate in the negative control, 4.3, 6.9 and 10.2 µg a.i./L treatment groups was 795.3, 992.0, 936.4 and 828.7 eggs, respectively. The mean number of eggs per female per reproductive day in the negative control, 4.3, 6.9 and 10.2 µg a.i./L treatment groups was 34.6, 43.1, 40.7 and 36.0, respectively. The Jonckheere-Terpstra trend test indicated there were no statistically significant trends and Dunnett's test indicated that there were no statistically significant decreases in fecundity in any treatment group in comparison to the negative control ($p > 0.05$). **The**

NOEC for fecundity in the F1 generation was 10.2 µg a.i./L, the highest concentration tested. A summary of the results are reported in the following table (see below).

Fertility F1 Generation

The mean percent fertility in the negative control, 4.3, 6.9 and 10.2 µg a.i./L treatment groups was 93.3, 91.7, 92.7 and 91.3%, respectively. The Jonckheere-Terpstra trend test indicated there were no statistically significant trends and Dunnett's test indicated that there were no statistically significant decreases in fertility in any treatment group in comparison to the negative control during the F1 generation exposure ($p > 0.05$). **The NOEC for fertility in the F1 generation was 10.2 µg a.i./L, the highest concentration tested.** A summary of the results are reported in the following table (see below).

Mean Measured Concentration (µg a.i./L)	Reproductive Parameters		
	Number of Eggs (Mean ± SD)	Eggs/Female/Reproductive Day (Mean ± SD)	Fertility (%) (Mean ± SD)
Negative Control	795.3 ± 270.29	34.6 ± 11.75	93.3 ± 3.7
4.3	992.0 ± 222.86	43.1 ± 9.69	91.7 ± 3.7
6.9	936.4 ± 160.51	40.7 ± 7.01	92.7 ± 6.2
10.2	828.7 ± 83.34	36.0 ± 3.60	91.3 ± 9.3
There were no statistically significant decreases in egg production or fertility in any treatment group in comparison to the negative control using the Jonckheere-Terpstra trend test or Dunnett's test ($p > 0.05$).			

Sex Ratio

The phenotypic sex of all fish in the F1 generation was determined on day 80 of the test, and was used to evaluate sex ratio, defined as the proportion of fish that were males.

The percentages of male fish in the negative control, 4.3, 6.9 and 10.2 µg a.i./L treatment groups on day 80 were 65.3, 54.5, 50.6 and 55.0%, respectively. There were no apparent treatment related effects upon sex ratio in any treatment group, and none of the differences from the control group sex ratio were statistically significant according to the Jonckheere-Terpstra trend test and Dunnett's test ($p > 0.05$). The percentages of males in the control and all treatment groups found at the end of the study were within the acceptability criteria of the test guideline (30-70%). The observed differences in sex ratios were attributed to the indiscriminate allocation of embryos to treatment groups at test initiation and indiscriminate thinning of fish, and were not believed to be the result of treatment. **The NOEC for sex ratio in the F1 generation was 10.2 µg a.i./L, the highest concentration tested.** A summary of the results are reported in the following table (see below).

Mean Measured Concentration (µg a.i./L)	Proportion of Males of Fish Sexed on Day 80
	(Mean ± SD)
Negative Control	0.653 ± 0.200
4.3	0.545 ± 0.182
6.9	0.506 ± 0.172
10.2	0.550 ± 0.243
There were no statistically significant differences in the proportion of males in any treatment group in comparison to the negative control using the Jonckheere-Terpstra Trend test or Dunnett's test ($p > 0.05$).	

Anal Fin Papillae Evaluation

Following establishment of reproduction pairs on Day 80 of the test and at termination of the F1 generation breeding pairs, the remaining fish were euthanized with MS-222. The tail was spread and fixed for assessment of the number of anal fin papillae. With the exception of a single female in the 6.9 µg a.i./L treatment group noted with a single papillae on Day 80, no anal fin papillae were noted on females on Day 80 or Day 116 (termination). Therefore, no statistical analyses were conducted for females for the number of anal fin papillae.

The mean number of anal fin papillae for males thinned on Day 80 in the negative control, 4.3, 6.9 and 10.2 µg a.i./L treatment groups was 119.5, 134.1, 149.3 and 157.2, respectively. The mean number of anal fin papillae for breeding males at termination of the F1 generation in the negative control, 4.3, 6.9 and 10.2 µg a.i./L treatment groups was 176.8, 148.0, 162.4 and 176.5 respectively. The Jonckheere-Terpstra trend test indicated there were no statistically significant trends and Dunnett's test indicated that there were no statistically significant differences in mean number of anal fin papillae in any treatment group in comparison to the negative control ($p > 0.05$). **The NOEC for number of anal fin papillae in the F1 generation was 10.2 µg a.i./L, the highest concentration tested.** A summary of the results are reported in the following table (see below).

Summary of Anal Fin Papillae Counts for Males Thinned on Day 80 of the Test and at Termination of the F1 Generation Medaka Exposed to Amitrole		
Mean Measured Concentration (µg a.i./L)	Males - Mean ± Standard Deviation	
	Day 80 (Thinned)	Day 116 (Termination)
Negative Control	119.5 ± 50.10	176.8 ± 29.63
4.3	134.1 ± 23.48	148.0 ± 37.36
6.9	149.3 ± 5.57	162.4 ± 23.49
10.2	157.2 ± 33.02	176.5 ± 35.27
There were no statistically significant decreases in anal fin papillae counts for males in any treatment group in comparison to the negative control using the Jonckheere-Terpstra Trend test or Dunnett's test ($p > 0.05$).		

□ **Conclusion:**

Medaka (*Oryzias latipes*) were exposed to Amitrole over a full life-cycle to evaluate effects on hatch, survival, growth, reproduction, sex ratio and secondary sex characteristics. Mean measured concentrations ranged from 4.7 to 10.9 µg a.i./L during the 21-day F0 generation exposure.

Mean measured concentrations ranged from 4.3 to 10.2 µg a.i./L during the F1 generation exposure. The NOEC during the F0 generation exposure for reproduction was 10.9 µg a.i./L. The NOEC during the F1 generation for all endpoints was 10.2 µg a.i./L. The LOEC for this study, based on F0 and F1 generation endpoints was >10.2 µg a.i./L (highest tested concentration). The NOEC was 10.2 µg a.i./L and the MATC was calculated to be >10.2 µg a.i./L. Despite the fact that the F2 generation could not be assessed, further to fungus contamination in all groups (controls and treatment groups), the study does provide a complete set of data for an entire generation and therefore provide valuable data that addresses the question of potential thyroid mediated effects during chronic exposures.

Based on the obtained results, it can be concluded that Chronic exposure to Amitrole did not result in effects on growth and development suggestive of thyroid mediated effects. Therefore, no impact on the fish population is expected further to exposure to amitrole at predictable environmental concentrations

Parameter	Parental Generation		F1 Generation	
	NOEC (µg a.i./L)	LOEC (µg a.i./L)	NOEC (µg a.i./L)	LOEC (µg a.i./L)
Hatching Success	--	--	10.2	>10.2
Survival	--	--	10.2	>10.2
Growth	--	--	10.2	>10.2
Reproduction	10.9	>10.9	10.2	>10.2
Sex Ratio	--	--	10.2	>10.2
Secondary Sex	--	--	10.2	>10.2

Characteristics

MATC >10.9 MATC >10.2

Even if the F2 generation could not be evaluated because of fungus contamination of eggs, the generation F0 was exposed over 4 weeks, and the F1 generation was exposed from the eggs stage until the generation of F2. An entire data set was generated for F1. This design was selected to maximize exposure to young fish and ensure that worst case conditions would be tested. Indeed the study starts by the exposure with adult fish to ensure that the test material or potential metabolites have the opportunity to undergo maternal transfer from the adult into the egg at the time it is formed in the female. This has also been referred to as loading of the egg by the female. Additionally, eggs are exposed to the test substance immediately after release during a spawning event ensuring continual exposure to the egg. This ensures that exposure of the test material in water occurs to the eggs instantaneously instead of approximately 18 to 24 hours later after hardening of membranes has occurred.

The study as conducted :

- meets the validity criteria of the US-EPA draft guideline,
- shows that an exposure of fish to amitrole at environmental concentrations has no impact on the reproduction parameters. The results clearly show no effects on growth, development, or fecundity of F1 fish exposed to Amitrole at 4.7, 8.4, and 10.9 µg a.i./L and that evidence of a thyroid affect was not apparent at these test concentrations.

F1 : Fertility (DRAR Vol3B9 p.90-91) → NOEC 10.2 µg/L

F0 & F1 : Fecundity : → NOEC 10.2 µg/L

F1 : Growth : → NOEC 10.2 µg/L

F1 : Sex ratio : → NOEC 10.2 µg/L

F1 : Anal Fin Papillae : → NOEC 10.2 µg/L

However the notifier informed the RMS that a supplemental experimental phase is being conducted in order to confirm that amitrole has no impact on hatchability of eggs.

❑ **Comment (RMS): Study not acceptable**

Available data are not reliable to conclude on the potential impact of amitrole on hatchability of eggs in the F1 generation (from parental exposure). Moreover, no data are available to conclude on potential impact in the F2 generation. In these conditions, it is concluded that data are not enough to completely address the question of thyroid mediated effects during long term exposures. No endpoint (NOEC) can be derived from this study.

B.9.2.3.3. Literature review report: Changes in thyroid hormone levels and related gene expression in Chinese rare minnow (Gobiocypris rarus) during 3-amino-1,2,4 triazole exposure and recovery

The review was made in order to identify scientific peer-reviewed open literature on the toxicology of the active substance amitrole which may affect the assessment on human health and/or animal health. Publications meeting the relevance criteria are those showing new/unknown effects or information potentially contradictory to the regulatory data package for the active substance, its relevant metabolites and/or the plant protection products on human health, and/or animal health, which could impact the endpoints or the risk assessment parameters. Here after are listed the bibliographic databases searched: ToxCenter: Effects of drugs & other chemicals, BIOSIS Previews/RN - since 1969, CAPLUS- since 1947, IPA (international Pharmaceutical Abstracts) since 1970, MEDLINE since 1958. Results of the study selection process concerning aquatic toxicology section is presented below:

- ❑ **Reference:** Aquatic Toxicology 92:50-57
- ❑ **Author(s); year:** Li, W., Zha, J., Spear, P.A., Li, Z., Yang, L., Wang, Z. 2009
- ❑ **Title:** Changes in thyroid hormone levels and related gene expression in Chinese rare minnow (Gobiocypris rarus) during 3-amino-1,2,4-triazole exposure and recovery
- ❑ **Report No:** none

- ❑ **Guidelines:** Not precised
- ❑ **Deviations:**
- ❑ **GLP :** No
- ❑ **Executive summary:**

The aim is to investigate whether the Thyroid Hormones-related genes can serve as biomarkers to screen the effects of thyroid disruption chemicals in rare minnow.

The rare minnow (*Gobiocypris rarus*) is exposed to different levels of amitrole and is allowed to recover in clean water.

Plasma Thyroid Hormones (THs) levels and the expression of TH-related genes including transthyretin (ttr), desiodinases (d1 and d2) and the thyroid hormone receptor (tr) from the liver and brains were evaluated.

- ❑ **Material and methods:**

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: amitrole

Source: Sigma (Chemical Co. USA)

Lot/batch #: Not stated

Purity of active ingredients: >99%

Stability of test compound: Not stated

2. Vehicle and/or control: water

3. Test animals :

Species: *Gobiocypris rarus* (Rare minnow)

Age 3 months old

Length: 32.5+/-1.6mm (average at start)

Weight: 0.39+/-0.06g (average at start)

Source: Not stated

Feeding: standard commercial fish food pellet (Trea, germany) at a rate of 0.1% body weight per day and newly hatched brine shrimp (*Artemia naupii*) twice daily.

Acclimatisation period: Not precised

Water: Not precised

Dissolved oxygen: Not precised

Loading rate: Not precised

Environmental conditions:

Temperature: 25° ± 1°C

Photoperiod: 16:8 hours photoperiod (light/dark)

4. System:

Flow through system with dechlorinated tap water (pH 7.2-7.6; hardness 44.0-61.0 mgCaCO₃/l)

B. STUDY DESIGN AND METHODS:

1. In life dates: not mentioned

2. Experimental treatments:

240 healthy juvenile rare minnows were randomly divided into 6 groups. Fishes were exposed under flow through conditions to various concentrations of amitrole: 0, 1, 10, 100, 1000 and 10000 ng/L nominal concentrations.

10 males and 10 females in each group were sacrificed after 28 days exposure. Blood was collected from each fish in a heparinised microcapillary tube. T3 and T4 were measured using commercial radioimmunoassay kits.

The livers and brains were collected. Total RNA were isolated from brains and livers of male and female fishes using Trizol agent and subjected to reverse transcription reaction. The cDNA fragment of ttr, d1, d2 and tr were cloned by RT-PCR. All the samples were analyzed in triplicate using real time quantitative PCR. The mean value of the triplicate measurements were used to calculate the mRNA expressions.

3. Observations:

Plasma TH levels (T3 and T4) and the expression of TH-related genes including transthyretin (ttr), desiodinases (d1 and d2) and the thyroid hormone receptor (tr) from the liver and brains were evaluated. Histopathology : Tissues of gonad and liver were subjected to histological analysis.

These observations (Plasma TH levels, the expression of TH-related genes and histopathology) were also done after a 28 day recovery period in clean water.

4. Statistics

Statistical analyses were performed with the SPSS using analysis of variance followed by Bonferroni's Multiple Comparison Test. All quantitative data are expressed as the mean $\pm$ S.E. of the mean (S.E.M). A probability of $p < 0.05$ was considered as statistically significant.

□ Findings:

II. RESULTS AND DISCUSSION

A. FINDINGS

No mortality and no differences in either lengths or body weight between treatment groups and controls were observed.

After exposure, the plasma TH levels did not change. Histopathological observations showed that livers were degenerated at 10000ng/L and these damages could be recovered by the withdrawal of amitrole. However the ttr, d1 and d2 mRNA levels in the livers of males were significantly up-regulated in all exposure groups.

The ttr and d2 mRNA levels were significantly up-regulated at 10000ng/L and 10, 100 and 1000 ng/L in the livers of females respectively.

In the brains of males, a 2 fold increase of d2 mRNA levels at ≥ 100 ng/L and a 5 fold decrease in tr mRNA levels at ≥ 10 ng/L were observed whereas no significant differences were observed in the expression of d2 and tr in the brains of females.

After a recovery period, the ttr, d1 and d2 mRNA levels in the livers of males returned to control levels, but the tr mRNA levels were irreversibly decreased at all treatments.

For the females, ttr mRNA levels from the livers were significantly decreased only at 1 ng/L and d2 mRNA levels were significantly induced at ≥ 1000 ng/L. The d1 and tr mRNA levels showed no significant differences between exposure and control groups.

In the brains, 2fold increases of the d2 mRNA levels at 10000 ng/L and tr mRNA levels at 1000 ng/L were observed in males. For females, the d2 mRNA levels at 10000ng/L and the tr mRNA levels at 10 ng/L were significantly up-regulated.

B- DISCUSSION

The results suggest that amitrole has slight or no effect on the growth of rare minnow and that the effects of amitrole observed at the highest dose on liver are reversible.

The plasma THs indicate that exposure to amitrole did not block the production of THs in rare minnow but alterations of ttr, d1, d2 and tr gene expressions in liver and brain were noted.

TH related gene expression of males were more sensitive to amitrole than the gene expressions in females. Recovery in clean water was associated with selective regulation of TH-related genes.

□ Conclusion:

The ttr, d1, d2 and tr genes could be used as sensitive biomarkers for thyroid disruption in fish exposed to environmental contaminants and future work should be focused on the regulation of the thyroid axis.

Little is known about the effects on thyroid function in fishes at environmentally relevant concentrations of amitrole long term exposure.

Needs to be confirmed in other species reproducibility long term exposure.

The reproductive parameters as well as the clinical signs were not assessed in this study. The thyroid hormones levels were not impacted and some of the observed effects were shown to be reversible after a recovery period.

We can legitimately wonder what the biological significance of such findings can be.

It is suggested that the impact on ttr expression could be to balance the effect of THs levels in fish by amitrole. But it is known that ttr levels can be impacted simply by effects on liver (independently from effect on thyroid). Ttr is a carrier protein that does not play the role of reservoir as does TBG. What could be the cause-effect relationship between a decrease of TH production and the increase of expression of mRNA of ttr?

The authors concluded that fish have a compensatory mechanisms for handling the exposure to amitrole and that further work was needed.

RMS comment: Study reliable with restriction. Not GLP. Not conducted according to OECD guidelines but the investigations are well documented and scientifically acceptable. This literature review report brings some information about the mechanism of action of amitrole in fish metabolism

and confirm thyroid disruption. The plasma Thyroid Hormones indicate that exposure to amitrole did not block the production of THs in rare minnow but alterations of Thyroid Hormones-related genes expressions in liver and brain were noted at 10 to 10000 ng/L.

B.9.2.3.4. Chronic test with 1,2,4-triazole

Please refer to initial B.9.2. For 1,2,4-triazole, see the DAR of tebuconazole.

B.9.2.4. Bio-concentration in fish (annex IIA 8.2.3)

B.9.2.4.1. Bio-concentration of amitrole in fish

Amitrole has a log P_{ow} below 3. Therefore, no risk of bioaccumulation exists. However, a BCF of 2.38 (whole fish) in 7 days was determined, please refer to review report SANCO/ 6839/VI/97-final/22/03/2001.

B.9.2.5. Acute toxicity to aquatic invertebrates (annex IIA 8.2.4) – amitrole and 1,2,4-triazole

B.9.2.5.1. Acute toxicity of amitrole to *Daphnia magna*

Please refer to initial B.9.2.

B.9.2.5.2. Acute toxicity of amitrole to *Mysidopsis bahia*

- ❑ **Reference:** KIIA 8.3.1.3./01
- ❑ **Author(s); year:** Collins M. K. 1993
- ❑ **Title:** AMITROLE TECHNICAL - ACUTE TOXICITY TO MYSIDS (*Mysidopsis bahia*) UNDER STATIC CONDITIONS
- ❑ **Report No:** 93-4-4736
- ❑ **Guidelines:** FIFRA GUIDELINE REFERENCE # 72-3
- ❑ **Deviations:**
- ❑ **GLP:** yes
- ❑ **Executive summary**

Five groups of 10 mysid shrimp were exposed to nominal concentrations of 0.30, 0.50, 0.85, 1.4, 2.4 and 4.0 mg a.s./L for 96 hours under static conditions. A concurrent control group was exposed to natural filtered seawater only. All test vessels were examined at 0-, 24-, 48-, 72- and 96-hours of exposure as follows: mortalities were recorded, dead shrimps were removed, observations of the shrimps and the physical characteristics of the test solutions were recorded. All samples were analysed for Amitrole using a high performance liquid chromatographic (HPLC) procedure.

Mean measured concentrations averaged 103% of nominal and defined the concentrations tested as 0.29, 0.48, 0.89, 1.5, 2.4 and 4.2 mg a.s./L. No visible sign of undissolved test material (i.e., film on the solutions' surface, precipitate) was observed in any of the test solutions during the test period. No visible sign of undissolved test material (i.e., film on the solutions' surface, precipitate) was observed in any of the test solutions during the test period. At test termination, 95% mortality was noted among mysids exposed to the highest treatment level, 4.2 mg a.s./l. Mortality of 25% was seen among mysids exposed to the 2.4 mg a.s./L treatment level. Sub lethal effects (e.g., lethargy) were observed among several of the surviving mysids exposed to both 4.2 and 2.4 mg a.s./L. No mortalities or sub lethal effects were observed among any of the mysids exposed to any of the remaining concentrations tested (0.29, 0.48, 0.89 and 1.5 mg a.s./L).

The IC_{50} (96 h) was estimated to be 2.8 mg a.s./L (corresponding 95% confidence interval of 2.5 - 3.3 mg a.s./L). The No Observed Effect Concentration was 1.5 mg a.s./L.

- ❑ **Materials and methods:**

A. MATERIALS

- 1. Test Material:** aminotriazole
Description: white powder
Lot/batch #: L1-4420
Purity of active ingredients: 98.22%
Stability of test compound: Not stated. Assumed to be stable for the test duration.
- 2. Vehicle and/or control:** None
- 3. Test animals -**
Species: *Mysidopsis bahia*
Source: Springborn Laboratories' culture facility, Massachusetts, USA
Feeding: Zeigler Brothers Inc. Prime Flakes.
Acclimatisation period: At least 14 days
Water: Natural filtered seawater
Dissolved oxygen: 5.1-7.4 mg/L.
Loading rate: not stated
- Environmental conditions:**
Temperature: $25 \pm 1^\circ\text{C}$
pH: 7.9-8.1
Photoperiod: daily 16 hours photoperiod

B. STUDY DESIGN AND METHODS:

- 1. In life dates:** 08/04-12/04/1993

2. Experimental treatments:

The toxicity test was conducted in 1.6-L glass vessels, which contained 1.0 L of test solution. The test solution in each aquarium had a depth of 3.5 cm with a surface area of 280 cm^2 . Two replicate test vessels were established for each treatment level and the control. Prior to test initiation, a 10 mg a.s./mL stock solution was prepared. The resultant stock solution was observed to contain no visible undissolved test material (e.g., precipitate, film on solution's surface). Treatment level solutions were prepared by adding the appropriate amount of the stock solution directly to 2.0 L of dilution water, which resulted in the desired test concentration. Following the addition of the stock solution, each exposure solution was mixed for approximately 30 seconds. A set of control vessels was also established which contained the same dilution water and was maintained under the same conditions as the treatment level vessels but contained no Amitrole Technical.

Test vessels were placed in stratified random order in a temperature controlled water bath designed to maintain test solution temperatures at $25 \pm 1^\circ\text{C}$. The test area was illuminated at an intensity of 80 foot-candles at the solutions' surface. The definitive study was initiated when ten mysid shrimp were selected impartially from the holding tank and placed in each replicate test vessel following exposure solution preparation. Mysids were fed brine shrimp nauplii (*Artemia salina*) once daily during the exposure period.

3. Observations:

All test vessels were examined at 0-, 24-, 48-, 72- and 96-hours of exposure as follows: mortalities were recorded, dead mysids were removed, and observations of the mysids and the physical characteristics of the test solutions were recorded. The pH, dissolved oxygen concentration, temperature, and salinity were measured in each treatment level solution and the control at test initiation and each subsequent 24-hour interval of the exposure period. Continuous temperature monitoring of the surrounding water in the water bath was also performed.

During the definitive exposure period, water samples were removed from each treatment level and the control at 0 and 96-hours of exposure for analysis of Amitrole concentration. All samples were analysed for Amitrole using a high performance liquid chromatographic (HPLC) procedure. A method validation study prior to the initiation of the definitive test established an average recovery of $100 \pm 4\%$ for Amitrole from seawater.

4. Statistics

The mean measured concentrations tested (0 and 96-hour analyses) and the corresponding biological-response data (mortality) derived from the toxicity test were used to estimate 24-, 48-, 72- and 96-hour median lethal concentrations (LC_{50}) and 95% confidence intervals. The LC_{50} is defined as the concentration of the test material in dilution water, which caused mortality of 50% of the test organism population at the stated time interval. The No-Observed-Effect Concentration (NOEC) during the 96-hour exposure period was also determined. The NOEC is defined as the highest concentration tested at and below which there were no toxicant-related mortalities or physical and behavioural abnormalities (e.g., lethargy, loss of equilibrium, darkened pigmentation), with respect to the control organisms.

□ Findings:

II. RESULTS AND DISCUSSION

A. FINDINGS

Preliminary Test

Prior to initiating the definitive study, a preliminary range-finding test was conducted. During this range-finding test, mysids were exposed under static conditions to nominal concentrations ranging from 1.0 - 100 mg a.s./L. Throughout the exposure period, a decrease in the dissolved oxygen concentration of all exposure solutions was observed. Following 96-hours of exposure, 100% mortality was observed among mysids exposed to the 4.0, 10, 40 and 100 mg a.s./L treatment levels while 20% mortality was observed among mysids exposed to the 1.0 mg a.s./L treatment level. No sub lethal effect (e.g., loss of equilibrium, lethargy) was seen among surviving mysids exposed to the lowest concentration tested (1.0 mg a.s./L). Based on the results of this range-finding test, it was determined that the definitive study would be conducted under static conditions at nominal concentrations of 0.30, 0.50, 0.85, 1.4, 2.4 and 4.0 mg a.s./L.

Definitive Test

Although a decrease in dissolved oxygen concentration was observed for each treatment level, the dissolved oxygen concentration at each treatment level remained above acceptable levels (i.e. 90% saturation at test initiation, and > 60% of saturation during the remainder of the exposure). The remaining water quality parameters measured (pH, temperature and salinity) were unaffected by the concentrations of Amitrole Technical tested and remained within acceptable ranges for the survival of *Mysidopsis bahia*. Continuous monitoring of the temperature of the surrounding water in the water bath established a temperature range of 24 - 26°C.

Throughout the exposure period, analytical measurements were generally consistent between sampling intervals. Mean measured concentrations averaged 103% of nominal and defined the concentrations tested as 0.29, 0.48, 0.89, 1.5, 2.4 and 4.2 mg a.s./L. Analysis of the Quality Control samples resulted in measured concentrations, which averaged 102% of the nominal fortified levels (0.500 - 5.00 mg a.s./L) and were consistent with the predetermined recovery range. Summary of analytical measurements are reported in the table below:

Nominal Concentration (mg A.I./L)	0-Hour Measured Concentration (mg A.I./L)	96-Hour Measured Concentration (mg A.I./L)	Mean Measured Concentration ^a (mg A.I./L)
Control	< 0.11	< 0.17	NA ^b
0.30	0.26	0.32	0.29
0.50	0.44	0.51	0.48
0.85	0.84	0.95	0.89
1.4	1.4	1.5	1.5
2.4	2.3	2.6	2.4
4.0	3.9	4.4	4.2
QC #1 ^c	4.93 (5.00) ^d	4.28 (5.00)	NA
QC #2	2.04 (2.00)	2.21 (2.00)	NA
QC #3	0.466 (0.500)	0.492 (0.500)	NA

^a Mean measured concentrations were calculated using the actual analytical results and not the rounded values (two significant figures) presented in this table.
^b NA = Not Applicable.
^c QC = Quality Control sample.
^d Quality Control sample results are presented with the corresponding nominal fortified concentration in parentheses.

No visible sign of undissolved test material (i.e., film on the solutions' surface, precipitate) was observed in any of the test solutions during the test period. At test termination, 95% mortality was noted among mysids exposed to the highest treatment level, 4.2 mg a.s./l. Mortality of 25% was seen among mysids exposed to the 2.4 mg a.s./L treatment level. Sub lethal effects (e.g., lethargy) were observed among several of the surviving mysids exposed to both 4.2 and 2.4 mg a.s./L. No mortalities or sub lethal effects were observed among any of the mysids exposed to any of the remaining concentrations tested (0.29, 0.48, 0.89 and 1.5 mg a.s./L).

Summary of effects are reported in the table below.

Mean Measured Concentration (mg A.I./L)	Replicate	Cumulative Mortality (%)			
		24-Hour	48-Hour	72-Hour	96-Hour
Control	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
0.29	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
0.48	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
0.89	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
1.5	A	0	0	0	0
	B	0	0	0	0
	Mean	0	0	0	0
2.4	A	0	20	20	29 ^a
	B	0	20	30	30 ^b
	Mean	0	20	25 ^a	25 ^b
4.2	A	0	50	90	90
	B	0	90	100	100
	Mean	0	70	95 ^a	95 ^c

^a One of the surviving mysids was observed to be lethargic.
^b Two of the surviving mysids were observed to be lethargic.
^c All surviving mysids were observed to be lethargic.

Based on these results, it was established that the effects observed during this study were clearly concentration-dependent. Based on these data, the 96-hour LC₅₀ value for *Mysidopsis bahia* exposed to Amitrole Technical was calculated to be 2.8 mg a.s./L (corresponding 95% confidence interval of 2.5 - 3.3 mg a.s./L). The No-Observed-Effect-Concentration established for this study was determined to be 1.5 mg a.s./L.

❑ **Conclusion:**

The LC₅₀ (96 h) was estimated to be 2.8 mg a.s./L (corresponding 95% confidence interval of 2.5 - 3.3 mg a.s./L). The No Observed Effect Concentration was 1.5 mg a.s./L.

❑ **Comment (RMS): study acceptable**

B.9.2.5.3. Acute toxicity of 1,2,4-triazole to *daphnia magna*

Please refer to initial B.9.2. For 1,2,4-triazole, see the DAR of tebuconazole.

B.9.2.6. Acute toxicity to aquatic invertebrates (annex IIIA 10.2.1) – CA2735

B.9.2.6.1. Acute toxicity of CA2735 to *Daphnia magna*

Please refer to initial B.9.2.

B.9.2.7. Chronic toxicity to aquatic invertebrates (annex IIA 8.2.5) –amitrole and 1,2,4-triazole

B.9.2.7.1. Chronic toxicity of amitrole to *Daphnia magna*

Please refer to initial B.9.2.

B.9.2.7.2. Chronic toxicity of 1,2,4-triazole to *Daphnia magna*

Please refer to initial B.9.2. For 1,2,4-triazole, see the DAR of tebuconazole.

B.9.2.8. Effects on aquatic molluscs (annex IIA 8.2.6) – amitrole

- ❑ **Reference:** KIIA 8.3.1.4./01
- ❑ **Author(s); year:** Dionne E. 1993
- ❑ **Title:** AMITROLE TECHNICAL - ACUTE TOXICITY TO THE EASTERN OYSTER (*Crassostrea virginica*) UNDER FLOW-THROUGH CONDITIONS
- ❑ **Report No:** 93-6-4816
- ❑ **Guidelines:** FIFRA GUIDELINE REFERENCE # 72-3
- ❑ **Deviations:**
- ❑ **GLP:** yes
- ❑ **Executive summary**

The purpose of this study was to estimate the acute toxicity of Amitrole Technical to Eastern oysters (*Crassostrea virginica*) under flow-through conditions. Acute toxicity is defined as the concentration of test material estimated to cause 50% reduction of shell deposition (EC_{50}) in a test population of Eastern oysters after 96 hours of exposure. Forty oysters were exposed in duplicate test aquaria (20 per aquaria) in a flow-through system to five concentrations of 16, 26, 43, 72 and 120 mg a.s./L of Amitrole Technical and a dilution water (seawater) control for 96 hours. Each replicate solution was sampled and analysed for Amitrole concentration at 0 hour (test initiation) and 96 hours (test termination) of exposure.

Analyses of the test solutions at 0 hour and 96 hours of exposure resulted in mean measured concentrations of 13, 23, 41, 68 and 110 mg a.s./L. At test termination (96 hours), no mortality was observed among oysters exposed to any of the Amitrole Technical concentrations tested or the control, however, several of the oysters exposed to the 110 mg a.s./L test concentration exhibited excessive mucous production. Shell growth among oysters exposed to the concentrations tested ranged from 3.0 to 3.8 mm, which was not significantly different from the growth of the control oysters (3.2 mm).

Based on the mean measured concentrations established and the lack of significant reduction in shell growth, the 96-hour EC_{50} value for Eastern oysters exposed to Amitrole Technical was estimated to be greater than 110 mg a.s./L, the highest mean measured concentration tested. The No-Observed-Effect Concentration (NOEC) established for this study was determined to be 110 mg a.s./L.

- ❑ **Materials and methods:**

1. MATERIALS AND METHODS**A. MATERIALS**

- | | |
|--------------------------------------|---|
| 1. Test Material: | aminotriazole |
| Description: | white powder |
| Lot/batch #: | L1-4420 |
| Purity of active ingredients: | 98.22% |
| Stability of test compound: | Not stated. Assumed to be stable for the test duration. |
| 2. Vehicle and/or control: | None |
| 3. Test animals - | |
| Species: | Eastern oysters (<i>Crassostrea virginica</i>) |
| Source: | P. Cummins Oyster Co., Maryland, USA |
| Feeding: | algal diet, Isochrysis galbana, Aquacultural research corp., Maryland, USA. |
| Acclimatisation period: | At least 11 days |
| Water: | Natural filtered seawater |
| Dissolved oxygen: | 73-94 of saturation. |

Environmental conditions:

Temperature:	20 ± 2°C
pH:	7.2-7.8
Photoperiod:	daily 16 hours photoperiod

B. STUDY DESIGN AND METHODS:

1. In life dates: 04/06-08/06/1993

2. Experimental treatments:

The exposure of oysters was initiated by impartially selecting and placing 20 oysters in each test aquarium (40 per treatment level and the control). Oysters were spaced equidistant from one another with their valve in-flow openings facing toward the flow of water from the Teflon circulator tube. Oysters were exposed in a flow-through system to five concentrations of 16, 26, 43, 72 and 120 mg a.s./L.

3. Observations:

Biological observations of the test organisms (e.g., visible abnormalities, such as excessive mucus production or a failure to siphon and feed, as evidenced by a lack of faeces and pseudofaeces production) and observations of the physical characteristics of the test solutions (i.e., precipitate, surface film of undissolved material) were made at test initiation and at each subsequent 24-hour interval until termination of the test.

Sub lethal effects on the performance and appearance of the exposure oysters were established based on comparison to the performance and appearance of the control oysters. After 96 hours of exposure, the oysters were removed from the test aquaria and the new shell growth was measured microscopically to the nearest 0.1 mm using a calibrated micrometer.

During the definitive exposure, the pH, temperature, salinity and dissolved oxygen concentration were measured daily in each replicate aquarium. In addition, temperature was monitored continuously in one replicate (A) of the dilution water control using a Fisher Scientific Min/Max Thermometer.

During the definitive exposure period, water samples were removed from each treatment level and the control at 0 and 96-hours of exposure for analysis of Amitrole concentration. All samples were analysed for Amitrole using a high performance liquid chromatographic (HPLC) procedure. A method validation study prior to the initiation of the definitive test established an average recovery of 100 ± 4% for Amitrole from seawater.

4. Statistics

The mean measured concentrations and the biological response data derived from the 96-hour definitive test are used to statistically estimate a median effect concentration (EC₅₀) for Amitrole Technical and Eastern oysters. The EC₅₀ is the estimated concentration of test material in seawater, which reduced shell deposition (growth) of the exposed oysters by 50 percent as compared to the growth of the control oysters. Thus, the individual shell growth measurements of 40 oysters for each of the five exposure concentrations are expressed as a percentage of the control oyster growth. In this test, no significant reduction in shell growth was observed, therefore, no statistical analyses were performed. The No-Observed-Effect Concentration (NOEC) during the 96-hour exposure period was also determined. The NOEC is defined as the highest concentration tested at, and below which there was no statistically significant reduction in growth, with respect to the control organisms. This was determined using Williams' Test. All statistical conclusions were made at the 95% level of certainty.

□ Findings:**II. RESULTS AND DISCUSSION****A. FINDINGS****Preliminary Test**

Prior to initiating the definitive study, a preliminary test was conducted. Eastern oysters were exposed under flow-through conditions to nominal Amitrole Technical concentrations ranging from 1.6 to 200 mg a.s./L and a

dilution water control. The concentration range was selected to demonstrate that Amitrole Technical was not acutely toxic to oysters.

At test termination (96 hours), reduction in shell growth among oysters exposed to all of the concentrations tested ranged from 12 - 17%, when compared to the control organisms. Based on these results, nominal concentrations of 16, 26, 43, 72 and 120 mg a.s./L were chosen for the definitive study. Based on the absence of shell growth reduction ~ 50% during preliminary testing, the highest nominal concentration of 120 mg/L was selected in an effort to obtain a measured concentration of at least 100 mg/L.

Definitive Test

Throughout the exposure period, all water quality parameters measured were unaffected by the concentrations of Amitrole Technical tested and remained within satisfactory levels for the survival and growth oysters. Daily monitoring of the exposure solutions established that the temperature ranged from 21 to 22 °C throughout the exposure period. Analyses of the solutions during the pretest period established that the concentrations of Amitrole in the exposure solutions were generally consistent between replicate solutions and that the delivery apparatus maintained the intended concentration gradient. Results of the pretest analyses established measured concentrations, which averaged 102% of nominal. Throughout the exposure period, no visible sign of undissolved test material was observed in the diluter system (e.g., mixing chamber, chemical cells, splitters) or exposure solutions.

Analytical measurements between sampling intervals were generally consistent and established the expected concentration gradient of test material (60% dilutions). Mean measured concentrations averaged 90% of the nominal concentrations and defined the concentrations tested as 13, 23, 41, 68 and 110 mg a.s./L. Coefficients of variation for all mean measured concentrations averaged 7.7%. Analysis of the Quality Control samples resulted in measured concentrations averaged 97.0% of the nominal fortified levels (20.0 to 100 mg/L).

Summary of analytical measurements are reported in the table below:

Nominal Concentration (mg A.I./L)	Measured Concentration (mg A.I./L)				Mean Measured Concentration ^a (mg A.I./L)
	0-hour		96-hour		
	A	B	A	B	
Control	< 3.9	< 3.9	< 2.1	< 2.1	
16	14	15	13	9.7	13 (2)
26	24	25	22	21	23 (2)
43	42	42	38	40	41 (2)
72	71	69	64	69	68 (3)
120	110	110	110	120	110 (4)
QC #1 ^b	21.6 (20.0) ^c		17.3 (20.0)		
QC #2	53.3 (50.0)		46.8 (50.0)		
QC #3	93.4 (100)		94.2 (100)		

^a Mean measured concentrations are presented with the standard deviation in parentheses and were calculated using the actual analytical results and not the rounded numbers (two significant figures) presented in this table.

^b QC = Quality Control Sample.

^c Values in parentheses represent the corresponding nominal fortified concentration for each QC sample result.

Growth among control oysters at test termination averaged 3.2 mm. The Standard Evaluation Procedures (U.S. EPA, 1985, Amended August 1991) for this study state that a minimum of 2 mm of new shell (based on the longest finger of new growth) must be deposited by the end of 96 hours. The growth of control organisms during this study exceeded the required minimum and was within the historical range (0.9 to 4.5 mm). Based on these

data, the average amount of shell deposition observed during this study was considered representative for this species and acceptable for establishing the relative toxicity of Amitrole Technical to Eastern oysters.

At test termination (96 hours), no mortality was observed among oysters exposed to any of the Amitrole Technical concentrations or the control. However, excessive mucous production was observed among several oysters exposed to the 110 mg a.s./L test concentration. Shell growth among oysters exposed to concentrations tested ranged from 3.0 to 3.8 mm, which was not significantly different from the growth of the control organisms (3.2 mm).

Based on the mean measured concentrations and the lack of significant reduction in shell growth, the 96-hour EC₅₀ for Eastern oysters exposed to Amitrole Technical was estimated to be greater than 110 mg a.s./L, the highest mean measured concentration tested.

The No-Observed Effect Concentration (NOEC) established during this exposure was determined to be 110 mg a.s./L.

❑ Conclusion:

The LC₅₀ (96 h) was estimated to be greater than 110 mg a.s./L. The No Observed Effect Concentration was 110 mg a.s./L.

❑ RMS comment: study acceptable

B.9.2.9. Effects on algal growth (annex IIA 9.2.6) – amitrole and 1,2,4-triazole

B.9.2.9.1. Effects of amitrole on *scenedesmus subspicatus*

Please refer to initial B.9.2.

B.9.2.9.2. Effects of amitrole on *Anabaena flos-aquae*

Please refer to initial B.9.2.

B.9.2.9.3. Effects of amitrole on *Selenastrum capricornutum* (5 d)

- ❑ Reference:** KIIA 8.4./01
- ❑ Author(s); year:** Palmer S. J. 1997
- ❑ Title:** AMITROLE: a 5-day toxicity test with freshwater alga (*Selenastrum capricornutum*)
- ❑ Report No:** 461A-101
- ❑ Guidelines:** OECD 201
- ❑ Deviations:**
- ❑ GLP:** yes
- ❑ Executive summary**

The freshwater green alga, *Selenastrum capricornutum*, was exposed to a geometric series of six test concentrations and a negative (culture medium) control under static conditions for five days. Nominal test concentrations used in the study were 0.16, 0.31, 0.63, 1.3, 2.5 and 5.0 mg a.s./L. Concentrations of amitrole in the test solutions were measured at the beginning, middle and end of the test. Mean measured test concentrations were 0.15, 0.31, 0.63, 1.2, 2.5 and 5.1 mg a.s./L, representing 94, 100, 100, 92, 100 and 102% of nominal, respectively.

Temperature measurements were within the acceptable limits ($24 \pm 2^{\circ}\text{C}$) throughout the 5-day exposure period. Light intensity measurements at test initiation were within the range established for the test (4300 ± 650 lux). The pH measurements from the test solutions on Day 0 were 7.4 and ranged from 7.8 to 9.4 on Day 5. The increase of pH relative to algal population was typical for tests conducted with *Selenastrum capricornutum*.

The response of the algae was measured in terms of changes in cell density. An EC_{50} value (i.e., the toxicant concentration that produced a 50% reduction in cell density relative to the control) was determined to be 2.2 mg a.s./L, with 95% confidence limits of 1.6 and 2.7 mg a.s./L. An E_rC_{50} value (i.e., the toxicant concentration that produced a 50% reduction in growth rate relative to the controls) was determined for each 24-hour interval of the test. The Day 5 E_rC_{50} was > 5.1 mg a.s./L, the highest concentration tested. An E_bC_{50} value (i.e., the toxicant concentration that produced a 50% reduction in biomass, expressed as area under the growth curve, relative to the controls) was determined for each 24-hour interval of the test. The Day 5 E_bC_{50} was 2.3 mg a.s./L, with 95% confidence limits of 1.9 and 2.7 mg a.s./L. The no-observed-adverse-effect-concentration (NOAEC), which is the highest test concentration that had no adverse inhibitory effect on algal growth, was determined to be 0.63 mg a.s./L relative to cell density, and 1.2 mg a.s./L relative to growth rate and area under the growth curve.

❑ **Materials and methods:**

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test Material:** aminotriazole
Description: white powder
Lot/batch #: 17051718
Purity of active ingredients: 91.7%
Stability of test compound: expiration date – May 1999.
2. **Vehicle and/or control:** fresh water algal medium
3. **Test animals -**
Species: fresh water green alga (*Selenastrum capricornutum*)
Source: Wildlife International, Maryland, USA
Water: fresh water algal medium
- Environmental conditions:**
Temperature: $24 \pm 2^\circ\text{C}$
pH: 7.5 ± 0.4
Light intensity: 4300 ± 650 lux

B. STUDY DESIGN AND METHODS:

1. **In life dates:** 31/10-05/11/1997
2. **Experimental treatments:**

The freshwater green alga, *Selenastrum capricornutum*, was exposed to a geometric series of six test concentrations and a negative (culture medium) control under static conditions for five days. Three "biological" replicate test chambers were maintained for each treatment and control group. An additional "analytical" test chamber was used to provide test solution for analytical sampling for each treatment and control group. Nominal test concentrations were based upon the results of an exploratory range-finding test. Nominal test concentrations selected were 0.16, 0.31, 0.63, 1.3, 2.5 and 5.0 milligrams of amitrole per litre of test solution (mg a.s./L). Mean measured concentrations were determined from samples of test medium collected from each treatment and control group at the beginning, middle and end of the test.

3. Observations:

At test initiation an inoculum of the algal cells was prepared from the stock culture at a concentration of approximately 3.0×10^5 cells/mL.

The concentration of algal cells was verified and 1.0 mL was added to each test chamber to achieve a nominal concentration of approximately 3.0×10^3 cells/mL.

Samples were collected from each replicate test chamber at approximately 24-hour intervals during the exposure period to determine cell densities. Cell densities were subsequently used to calculate growth rates and areas under the growth curve for each 24-hour interval, as well as percent inhibition values relative to the control for each parameter over the 5-day exposure period. An EC_{50} value (i.e., the theoretical toxicant concentration that

would produce a 50% reduction in cell density relative to the control) was calculated based on the inhibition of growth at Day 5. An ErC_{50} value (i.e., the toxicant concentration that produces a 50% reduction in growth rate relative to the controls) and an E_bC_{50} value (i.e., the toxicant concentration that produces a 50% reduction in biomass, expressed as area under the growth curve, relative to the controls) also were determined for each 24-hour interval of the test. The no-observed-adverse-effect-concentration (NOAEC), defined as the highest test concentration where there were no adverse inhibitory effects on cell growth, was determined relative to each parameter through evaluation of the Day 5 statistical results and the dose-response pattern.

Samples of test medium were collected from each treatment and control group on Days 0, 3 and 5 to measure concentrations of the test substance. Samples collected on Day 0 were taken from the individual batches of test medium prepared at test initiation. Samples collected on Day 3 were taken from the additional "analytical" replicate maintained in each of the treatment and control groups for analytical sampling. Samples collected on Day 5 consisted of composited test medium from each of the three "biological" replicates in each of the respective treatment and control groups. The samples were collected in glass vials and were analysed immediately. Samples collected on Days 3 and 5 were filtered (0.45 μ m) to remove algal cells prior to analysis.

4. Statistics

Cell densities, growth rates, area under the growth curve values and percent inhibition values were calculated using "SAS Release 6.12", while statistical analyses were conducted using "ICPIN Version 2.0" and "TOXSTAT Release 3.2".

Cell densities, growth rates and areas under the growth curve were analysed statistically using the computer program ICPIN to determine the EC_{50} values. This program was designed to calculate the EC_{50} values and 95% confidence limits by linear interpolation. The Day 5 cell densities, growth rates and areas under the growth curve were evaluated for normality and homogeneity of variances using the Chi-square test and either Bartlett's or Hardey's test, respectively. When necessary to correct for unequal variances, a square-root transformation was performed and the treatment groups then were analysed statistically using Dunnett's t-test. The 5-day no-observed-adverse-effect-concentration (NOAEC) was determined through evaluation of the statistical results and the dose-response pattern.

□ Findings:

II. RESULTS AND DISCUSSION

A. FINDINGS

Analytical measurements were performed to verify exposure concentrations of amitrole in the test medium. Nominal concentrations selected for use in this study were 0.16, 0.31, 0.63, 1.3, 2.5 and 5.0 mg a.s./L. Samples collected on Days 0, 3 and 5 ranged from 90 to 106% of nominal. When measured concentrations of samples collected on Days 0, 3 and 5 were averaged, the mean measured concentrations for the test were 0.15, 0.31, 0.63, 1.2, 2.5 and 5.1 mg a.s./L, representing 94, 100, 100.92, 100 and 102% of nominal, respectively. Summary of analytical measurement are reported in the following table:

Nominal Test Concentration (mg a.i./L)	Sampling Time (Days)	Measured ¹ Concentration (mg a.i./L)	Mean Measured Concentration (mg a.i./L)
Negative Control	0 ²	<LOQ	--
	3 ³	<LOQ	
	5 ⁴	<LOQ	
0.16	0	0.144	0.15
	3	0.148	
	5	0.149	
0.31	0	0.318	0.31
	3	0.321	
	5	0.304	
0.63	0	0.647	0.63
	3	0.630	
	5	0.621	
1.3	0	1.29	1.2
	3	1.19	
	5	1.22	
2.5	0	2.64	2.5
	3	2.50	
	5	2.50	
5.0	0	5.06	5.1
	3	5.11	
	5	5.14	

¹ The limit of quantitation (LOQ) was defined as 0.100 mg a.i./L based upon the product of the lowest standard concentration (0.100 mg a.i./L) and the dilution factor of the matrix blanks (1.00).
² Day 0 samples were collected from the individual batches of test solution prepared at test initiation.
³ Day 3 samples were collected from the additional replicate of test solution maintained for analytical sampling.
⁴ Day 5 samples were composites of test solution collected from each of the three replicates per the treatment and control groups.

The temperatures ranged from 24.7 to 25.7°C and were within the $24 \pm 2^\circ\text{C}$ range established for the test. Measurements of pH were 7.4 on Day 0 and ranged from 7.8 to 9.4 for the treatment and control groups on Day 5. The increase of pH relative to algal population was typical for tests conducted with *Selenastrum capricornutum*. The light intensity ranged from 3910 to 4630 lux and was within the desired range of 4300 ± 650 lux.

The toxicity of amitrole to *Selenastrum capricornutum* was measured in terms of cell density. Mean cell densities were used to calculate growth rates and areas under the growth curve [or each 24-hour interval of the 5-day test. As the graphical representations indicate, evaluation of effects upon algal growth based upon percent inhibition can be highly variable prior to Day 3 of the test. Therefore, a better representation of effects on algal growth can be determined through evaluation of the graphical results for Days 3 through 5. Changes in mean cell density indicated that exponential growth occurred in the negative control replicates. There were no statistically significant ($p > 0.05$) differences in cell density, growth rate or area under the growth curve between the control group and the 0.15, 0.31 and 0.63 mg a.s./L treatment groups. By Day 5 of the test, statistically significant differences ($p < 0.05$) were observed in cell density between the negative control and the 1.2, 2.5 and 5.1 mg a.s./L treatment groups. Growth inhibition values for those treatment groups were 25, 58 and 88%, respectively, by Day 5. Statistical analysis of the Day 5 growth rates and areas under the growth curve indicated a significant difference ($p < 0.05$) in the 2.5 and 5.1 mg a.s./L treatment groups when compared to the control group. These reductions in cell density, growth rate and areas under the growth curve were considered to be treatment-related. Summary of effects are reported in the following tables:

Mean Cell Density and Percent Inhibition										
Sponsor:	CFPI Agro									
Test Substance:	Amitrole									
Test Organism:	Freshwater Alga, <i>Selenastrum capricornutum</i>									
Dilution Water:	Freshwater Algal Medium									
Mean Measured Test Concentration (mg a.i./L.)	Cell Densities (Cells/mL) ¹									
	Day 1		Day 2		Day 3		Day 4		Day 5	
	Cell Density	Percent Inhibition ²	Cell Density	Percent Inhibition ²	Cell Density	Percent Inhibition ²	Cell Density	Percent Inhibition ²	Cell Density	Percent Inhibition ²
Negative Control	7,905	--	46,087	--	308,103	--	766,249	--	2,837,799	--
0.15	6,264	21	45,623	1.0	322,001	-4.5	764,178	0.3	2,760,197	2.7
0.31	5,566	30	39,419	14	388,875	-26	731,611	4.3	2,258,561	20
0.63	6,678	16	42,478	7.8	253,710	18	802,591	4.7	2,665,075	6.1
1.2	4,945	37	35,088	24	175,387	43	856,066	-12	2,138,127*	25
2.5	2,996	62	24,444	47	100,453	67	431,873	44	1,204,054*	58
5.1	2,668	66	8,939	81	34,402	89	119,263	84	346,740*	88

¹ The initial cell density of the stock culture was determined and an inoculum volume administered to each test chamber to yield a cell density of approximately 3,000 cells/mL at test initiation (Day 0).

² Percent inhibition was calculated relative to the negative control replicates. Values were obtained using SAS Release 6.12. Manual calculations may vary slightly.

* Statistically different ($p < 0.05$) compared to the negative control replicates.

Mean Growth Rate and Percent Inhibition										
Sponsor:		CFPI Agro								
Test Substance:		Amitrole								
Test Organism:		Freshwater Alga, <i>Selenastrum capricornutum</i>								
Dilution Water:		Freshwater Algal Medium								
Mean Measured Test Concentration (mg a.i./L.)	Growth Rates									
	Day 1		Day 2		Day 3		Day 4		Day 5	
	Mean Growth Rate	Percent Inhibition ¹	Mean Growth Rate	Percent Inhibition ¹	Mean Growth Rate	Percent Inhibition ¹	Mean Growth Rate	Percent Inhibition ¹	Mean Growth Rate	Percent Inhibition ¹
Negative Control	0.040	--	0.057	--	0.064	--	0.058	--	0.057	--
0.15	0.030	24	0.057	6.5	0.065	-0.8	0.058	-0.03	0.057	0.4
0.31	0.026	36	0.054	5.7	0.067	-4.8	0.057	0.8	0.055	3.3
0.63	0.033	17	0.055	3.1	0.062	4.4	0.058	-1.0	0.056	1.1
1.2	0.020	49	0.051	10	0.056	12	0.059	-2.1	0.055	4.1
2.5	0.010	76	0.044	23	0.049	24	0.052	10	0.050*	13
5.1	0.002	94	0.022	61	0.034	47	0.038	33	0.040*	31

¹ Percent inhibition was calculated relative to the negative control replicates. Values were obtained using SAS Release 6.12. Manual calculations may vary slightly.

* Statistically different ($p<0.05$) compared to the negative control replicates.

Mean Area Under the Growth Curve and Percent Inhibition										
Sponsor: CFPI Agro										
Test Substance: Amitrole										
Test Organism: Freshwater Alga, <i>Selenastrum capricornutum</i>										
Dilution Water: Freshwater Algal Medium										
Mean Measured Test Concentration (mg a.i./L)	Areas Under the Growth Curve									
	Day 1		Day 2		Day 3		Day 4		Day 5	
	Mean Area	Percent Inhibition ¹	Mean Area	Percent Inhibition ¹	Mean Area	Percent Inhibition ¹	Mean Area	Percent Inhibition ¹	Mean Area	Percent Inhibition ¹
Negative Control	58,860	--	634,765	--	4,813,043	--	17,633,268	--	60,809,847	--
0.15	39,171	33	589,817	7.1	4,929,307	-2.4	17,891,455	-1.5	60,111,950	1.1
0.31	30,794	48	498,621	21	5,566,154	-16	18,939,990	-7.4	54,750,058	10
0.63	44,137	25	562,005	11	4,044,258	16	16,647,869	6	58,187,864	4.3
1.2	23,337	60	431,732	32	2,885,430	40	15,190,865	14	51,049,187	16
2.5	10,021	83	267,295	58	1,694,058	65	8,009,979	55	27,569,096*	55
5.1	2,067	96	69,351	89	517,441	89	2,289,449	87	7,809,512*	87

¹ Percent inhibition was calculated relative to the negative control replicates. Values were obtained using SAS Release 6.12. Manual calculations may vary slightly.
* Statistically different ($p < 0.05$) compared to the negative control replicates.

After five days of exposure, clumping, flocculation or adherence of the algae to the test chambers were not visually evident in any of the treatment groups. A few enlarged cells were observed in the 5.1 mg a.s./L group. However, there were no other noticeable changes in cell colour, size or morphology in any of the treatment groups, when compared to the control group.

❑ Conclusion:

The Day 5 EC₅₀ value for *Selenastrum capricornutum* exposed to amitrole was calculated in terms of cell densities based on mean measured concentrations and was determined to be 2.2 mg a.s./L. The 95% confidence limits were 1.6 and 2.7 mg a.s./L. The Day 5 ErC₅₀ value calculated in terms of growth rate was greater than 5.1 mg a.s./L, the highest concentration tested. The Day 5 EbC₅₀ value calculated in terms of area under the growth curve was 2.3 mg a.s./L, with 95% confidence limits of 1.9 and 2.7 mg a.s./L. The Day 5 no-observed-adverse-effect-concentration was 0.63 mg a.s./L relative to cell density, and was 1.2 mg a.s./L relative to growth rate and area under the growth curve.

As a part of the studies conducted for investigating the phytotoxic effects on aquatic and terrestrial plants (US-EPA series 122-2 and 123-2, fresh water algae (*Selenastrum capricornutum*) were exposed to 5 concentrations of technical amitrole. The study is summarized below.

❑ Comment (RMS): study acceptable

B.9.2.9.4. Effects of amitrole on *Selenastrum capricornutum* (8 d)

❑ **Reference:** KIIA 8.4./02

❑ **Author(s); year:** Cross J. W. 1993

❑ **Title:** Non-Target Phytotoxicity of Amitrole in Terrestrial Use – Part A - Growth and Reproduction of *Selenastrum capricornutum*

❑ **Report No:** 198501 Part A

❑ **Guidelines:** US-EPA - Pesticide Assessment Guidelines (PAG) Subdivision J - Hazard Evaluation, Non-target Plants - Series 122-2 and Series 123-2 - Tiers I and 2 Combined

❑ **Deviations:**

❑ **GLP:** yes

❑ **Executive summary**

Amitrole technical was dissolved in water to prepare serial dilutions in algal growth medium. The amount of Amitrole added was 6.0 ppm and two-fold dilutions thereof were prepared corresponding to 3, 1.5, 0.75 and 0.38

mg/L. Media were inoculated with an axenic culture of *Selenastrum capricornutum*. Three replicate cultures were prepared for the untreated control and each treatment level. The numbers of algal cells were enumerated by electronic counting after inoculation and 1, 2, 3, 4, 5, 7 and 8 days after addition of test substance.

The results showed no effect of Amitrole on algal growth at any treatment level, when compared to the control.

The concentration of Amitrole, 3-amino-1,2,4-triazole, in treatment solutions and algal media was determined directly by liquid chromatography. Actual concentrations in the algal culture media were found to be within 93 to 96% (avg 94.7% $\pm$ 0.9%) of the target concentrations.

❑ **Materials and methods:**

A. MATERIALS

- 1. Test Material:** aminotriazole
Description: not mentioned
Lot/batch #: L1-4420
Purity of active ingredient: 98.22%
Stability of test compound: not mentioned, assumed to be stable during the experimental phase.

- 2. Vehicle and/or control:** fresh water algal medium

3. Test animals -

- Species:** fresh water green alga (*Selenastrum capricornutum*)
Source: University of Texas, Austin, USA
Water: fresh water algal medium

Environmental conditions:

- Temperature:** 24 $\pm$ 2°C
pH: 7.5 $\pm$ 0.1
Light intensity: photosynthetically active radiation (PAR) 102 $\mu\text{mol}/\text{m}^2/\text{sec}$

B. STUDY DESIGN AND METHODS:

- 1. In life dates:** 18/09/1992-12/02/1993

2. Experimental treatments:

To inoculate the test medium, a starter culture was first inoculated and grown to a suitable cell density. The treatment media were inoculated with a 1/1000 dilution of the starter culture. This dilution gave an initial cell density of 1000 cells/mL, as recommended by the US EPA. Each culture flask contained 100 mL of medium in a 250 mL DeLong flask, closed with a foam plug. The foam plug and the lip of the flask were covered with aluminium foil to prevent entry of contaminants around the glass lip of the flask. The water bath temperature was measured as 24°C on each day during the incubation and the rate of shaking (set at 100 rpm) was measured as 94 cycles/min. The photosynthetically active radiation (PAR) averaged 102 $\mu\text{mol}/\text{m}^2/\text{sec}$ at the level of the culture flasks on the day treatment was initiated.

3. Observations:

A sample of each treated and control culture was taken on the day of inoculation and 1, 2, 3, 4, 5, 7 and 8 days after addition of test substance. Culture samples for counting (5 mL each) were withdrawn from the culture and added to screw-cap culture tubes containing 10 µL formalin solution as a preservative. These samples were stored refrigerated until they were taken to the laboratory for counting. Algal cells were counted with an electronic particle counter. Since electronic particle counters do not selectively count algal cells, contaminated cultures were not counted. At the end of the culture period (8 days) an aliquot of each culture (20 mL) was removed and the cells collected on a pre-weighed 0.45 µm membrane filter. These filters were dried in the oven at 70°C to determine the dry weight of the algal biomass per unit volume of culture.

The Amitrole content of algal media was determined by reversed-phase high performance liquid chromatography with detection by UV absorbance. The samples were diluted in Type I water when necessary. The analyte was separated on an octadecyl silica column eluted with an aqueous/acetonitrile mobile phase containing phosphate buffer and sodium dodecylsulfate as the ion-pair reagent. The mobile phase consisted of 25% acetonitrile (v/v) to 75% of 10 mM phosphoric acid, 10 mM potassium dihydrogen phosphate and 4 mM sodium dodecylsulfate prepared with Type I water.

4. Statistics

Linear regression analysis of $\ln \{ \text{cell concentration (cells/mL)} \}$ versus time (days) was used to obtain the exponential growth rate, k , according to the model: $\ln C = kt + k'$

Where C = cells/mL at time t in days, k = exponential growth rate, k' = a constant. The exponential growth rates were entered into SYSTAT (MS-DOS version 5.0) for ANOVA.

Findings:

The ANOVA showed no significant effect of Amitrole on algal growth ($p=0.21$). Furthermore, linear regression analysis of growth rate (k) versus $\log_{10}(\text{Rate})$ did not indicate a dosage dependent relationship ($R^2=0.23$, $p=0.102$). There was no significant effect of Amitrole on dry weight values.

Summary of effects are reported in the following table:

Treatment Level	Sample Code for Culture	Amitrole Conc'n	Tare Weight	Gross Weight	Net Weight	Cell Concentration	Mass/cell		
		ug/mL	grams	grams	mg	cell/mL /1000	mg/1000 cells	avg.	std dev
Control	198S01CASC08		NA	C	NA	C			
	198S01CASC08		0.0952	0.0963	1.1	6.0	0.18		
	198S01CASC08	0.00	0.0941	0.0959	1.8	4.4	0.41	0.30	0.16
1	198S01TAS1A08		NA	C	NA	C			
	198S01TAS1B08		0.0953	0.0970	1.7	4.1	0.41		
	198S01TAS1C08	0.00	0.0953	0.0968	1.5	3.8	0.39	0.40	0.01
2	198S01TAS2A08		NA	C	NA	C			
	198S01TAS2B08		0.0954	0.0970	1.6	3.7	0.43		
	198S01TAS2C08	3.00	0.0956	0.0972	1.6	5.2	0.31	0.37	0.09
3	198S01TAS3A08		0.0955	0.0977	2.2	6.7	0.33		
	198S01TAS3B08		0.0958	0.0985	2.7	7.6	0.36		
	198S01TAS3C08	1.50	0.0945	0.0963	1.8	5.4	0.33	0.34	0.01
4	198S01TAS4A08		0.0949	0.0969	2.0	5.2	0.38		
	198S01TAS4B08		0.0944	0.0966	2.2	5.5	0.40		
	198S01TAS4C08	0.75	0.0956	0.0973	1.7	5.5	0.31	0.36	0.05
5	198S01TAS5A08		0.0952	0.0983	3.1	7.0	0.44		
	198S01TAS5B08		0.0947	0.0966	1.9	4.1	0.46		
	198S01TAS5C08	0.38	0.0937	0.0953	1.6	3.8	0.42	0.44	0.02

A validation set of samples spiked with known amounts of Amitrole (in Type I water) was successfully run before the study samples. The range of recovery in the validation set was 98 to 101% in the range of 300 to 1300 µg/mL (77.3% at 2048 µg/mL). Actual concentrations in the algal culture media were found to be within 93 to 96% of the target concentration. For algal media, the recoveries were $94.7\% \pm 0.9\%$ (average $\pm$ standard deviation) of the target. These analyses were performed after the last application was performed with a given treatment solution, thereby assuring the stability of the test substance.

☐ **Conclusion:**

The results showed no effect of Amitrole (up to 6 mg/L) on algal growth at any treatment level, when compared to the control.

☐ **Comment (RMS): study acceptable**

B.9.2.9.5. Effects of amitrole on *Skeletonema costatum*

- ☐ **Reference:** KIIA 8.4./03
- ☐ **Author(s); year:** Palmer S. J. 1998
- ☐ **Title: AMITROLE:** a 5-day toxicity test with the marine diatom (*Skeletonema costatum*)
- ☐ **Report No:** 461A-102
- ☐ **Guidelines:** US-EPA FIFRA subdivision J, series 123-2
- ☐ **Deviations:**
- ☐ **GLP:** yes
- ☐ **Executive summary**

The marine diatom, *Skeletonema costatum*, was exposed to a geometric series of five test concentrations and a negative (culture medium) control under static conditions for five days. Nominal test concentrations used in the study were 0.31, 0.63, 1.3, 2.5 and 5.0 mg a.s./L. Concentrations of amitrole in the test solutions were measured at the beginning and end of the test. Mean measured test concentrations were 0.30, 0.68, 1.3, 2.5 and 5.1 mg a.s./L representing 97, 108, 100, 100 and 102% of nominal, respectively.

Temperature measurements were within the acceptable limits ($20 \pm 2^\circ\text{C}$) throughout the 5-day exposure period. Light intensity measurements at test initiation were within the range established for the test (4300 ± 650 lux). The pH measurements from the test solutions on Days 0 and 5 ranged from 8.0 to 8.8, and were typical of pH values obtained in tests conducted with *Skeletonema costatum*.

The response of the algae was measured in terms of changes in cell density. An EC_{50} value was determined for each 24-hour interval of the test. The Day 5 EC_{50} value was 3.1 mg a.s./L, with 95% confidence limits of 1.9 and 3.9 mg a.s./L. An ErC_{50} value was determined for each 24-hour interval of the test. The Day 5 ErC_{50} value was greater than 5.1 mg a.s./L, the highest concentration tested. An EbC_{50} value was determined for each 24-hour interval of the test. The Day 5 EbC_{50} value was 2.1 mg a.s./L, with 95% confidence limits of 1.4 and 2.4 mg a.s./L. The no-observed-adverse-effect-concentration (NOAEC), which is the highest test concentration that had no adverse inhibitory effect on algal growth, was determined to be 1.3 mg a.s./L relative to cell density and growth rate, and was 0.68 mg a.s./L relative to area under the growth curve.

☐ **Materials and methods:**

A. MATERIALS

- 1. Test Material:** aminotriazole
Description: white powder
Lot/batch #: 17051718
Purity of active ingredients: 91.7%
Stability of test compound: expiration date – May 1999.
- 2. Vehicle and/or control:** saltwater culture medium
- 3. Test animals -**
Species: marine diatom (*Skeletonema costatum*)
Source: Wildlife International, Maryland, USA
Water: saltwater culture medium

Environmental conditions:**Temperature:** $20 \pm 2^{\circ}\text{C}$ **pH:** 8.0 to 8.8**Light intensity:** 4300 ± 650 lux**B. STUDY DESIGN AND METHODS:****1. In life dates:** 21/11-10/12/1997**2. Experimental treatments:**

The marine diatom (*Skeletonema costatum*) was exposed to a geometric series of 5 test concentrations and a negative (culture medium) control under static conditions for five days. Three "biological" replicate test chambers were maintained for each treatment and control group. An additional "analytical" test chamber was used to provide test solution for analytical sampling for each treatment and control group. Nominal test concentrations were based upon the results of an exploratory range-finding test. Nominal test concentrations selected were 0.31, 0.63, 1.3, 2.5 and 5.0 milligrams of amitrole per litre of test solution (mg a.s./L). Mean measured concentrations were determined from samples of test medium collected from each treatment and control group at the beginning, middle and end of the test.

3. Observations:

At test initiation an inoculum of the algal cells was prepared from the stock culture at a concentration of approximately 1.0×10^6 cells/mL. The concentration of algal cells was verified and 1.0 mL was added to each test chamber to achieve a nominal concentration of approximately 1.0×10^4 cells/mL. Samples were collected from each replicate test chamber at approximately 24-hour intervals during the exposure period to determine cell densities. Cell densities were subsequently used to calculate growth rates and areas under the growth curve for each 24-hour interval, as well as percent inhibition values relative to the control for each parameter over the 5-day exposure period. An EC_{50} value (i.e., the theoretical toxicant concentration that would produce a 50% reduction in cell density relative to the control) was calculated based on the inhibition of growth at Day 5. An ErC_{50} value (i.e., the toxicant concentration that produces a 50% reduction in growth rate relative to the controls) and an E_bC_{50} value (i.e., the toxicant concentration that produces a 50% reduction in biomass, expressed as area under the growth curve, relative to the controls) also were determined for each 24-hour interval of the test. The no-observed-adverse-effect-concentration (NOAEC), defined as the highest test concentration where there were no adverse inhibitory effects on cell growth, was determined relative to each parameter through evaluation of the Day 5 statistical results and the dose-response pattern.

Samples of test medium were collected from each treatment and control group on Days 0, 3 and 5 to measure concentrations of the test substance. Samples collected on Day 0 were taken from the individual batches of test medium prepared at test initiation. Samples collected on Day 3 were taken from the additional "analytical" replicate maintained in each of the treatment and control groups for analytical sampling. Samples collected on Day 5 consisted of composited test medium from each of the three "biological" replicates in each of the respective treatment and control groups. The samples were collected in glass vials and were analysed immediately. Samples collected on Days 3 and 5 were filtered ($0.45 \mu\text{m}$) to remove algal cells prior to analysis.

4. Statistics

Cell densities, growth rates, area under the growth curve values and percent inhibition values were calculated using "SAS Release 6.12", while statistical analyses were conducted using "ICPIN Version 2.0" and "TOXSTAT Release 3.2".

Cell densities, growth rates and areas under the growth curve were analysed statistically using the computer program ICPIN to determine the EC_{50} values. This program was designed to calculate the EC_{50} values and 95% confidence limits by linear interpolation. The Day 5 cell densities, growth rates and areas under the growth curve were evaluated for normality and homogeneity of variances using the Chi-square test and either Bartlett's or Harney's test, respectively. When necessary to correct for unequal variances, a square-root transformation was performed and the treatment groups then were analysed statistically using Dunnett's t-test. The 5-day no-observed-adverse-effect-concentration (NOAEC) was determined through evaluation of the statistical results and the dose-response pattern.

□ Findings:

Analytical measurements were performed to verify exposure concentrations of amitrole in the test medium. Nominal concentrations selected for use in this study were 0.31, 0.63, 1.3, 2.5 and 5.0 mg a.s./L. Samples collected on Days 0 and 5 ranged from 97 to 108% of nominal. When measured concentrations of samples collected on Days 0 and 5 were averaged, the mean measured concentrations for the test were 0.30, 0.68, 1.3, 2.5 and 5.1 mg a.s./L, representing 97, 108, 100, 100 and 102% of nominal, respectively.

The temperatures ranged from 19.6 to 21.6°C and were within the $20 \pm 2^\circ\text{C}$ range established for the test. Measurements of pH ranged from 8.0 to 8.1 on Day 0 and from 8.2 to 8.8 for the treatment and control groups on Day 5. The increase of pH relative to algal population was typical for tests conducted with *Skeletonema costatum*. The light intensity ranged from 3780 to 4400 lux and was within the desired range of 4300 ± 650 lux.

The toxicity of amitrole to *Skeletonema costatum* was measured in terms of cell density. Mean cell densities were used to calculate growth rates and areas under the growth curve or each 24-hour interval of the 5-day test. As the graphical representations indicate, evaluation of effects upon algal growth based upon percent inhibition can be highly variable prior to Day 3 of the test. Therefore, a better representation of effects on algal growth can be determined through evaluation of the graphical results for Days 3 through 5. Changes in mean cell density indicated that exponential growth occurred in the negative control replicates. There were no statistically significant ($p > 0.05$) differences in cell density between the control group and the 0.30, 0.68 and 1.3 mg a.s./L treatment groups. By Day 5 of the test, statistically significant differences ($p < 0.05$) were observed in cell density between the negative control and the 2.5 and 5.1 mg a.s./L treatment groups, reduced by 38 and 75%, respectively. The reduction in cell density observed in those treatment groups was statistically significant ($p < 0.05$) and was considered to be treatment-related. Summary of effects on cell densities are reported in the following table:

Mean Cell Density and Percent Inhibition										
Sponsor:		CFPI Agro								
Test Substance:		Amitrole								
Test Organism:		Marine Diatom, <i>Skeletonema costatum</i>								
Dilution Water:		Saltwater Algal Medium								
Mean Measured Test Concentration (mg a.i./L)	Cell Densities (Cells/mL) ¹									
	Day 1		Day 2		Day 3		Day 4		Day 5	
	Cell Density	Percent Inhibition ²	Cell Density	Percent Inhibition ²	Cell Density	Percent Inhibition ²	Cell Density	Percent Inhibition ²	Cell Density	Percent Inhibition ²
Negative Control	19,152	--	68,760	--	339,350	--	1,054,069	--	1,620,356	--
0.30	16,097	16	57,509	16	351,410	-3.6	1,023,910	2.9	1,852,479	-14
0.68	18,425	3.8	57,134	17	295,433	13	740,102	30	1,785,127	-10
1.3	14,294	25	45,250	34	159,289	53	507,377	52	1,638,035	-1.1
2.5	13,294	31	38,117	45	122,525	64	313,295	70	1,011,114*	38
5.1	8,763	54	35,562	48	83,970	75	165,767	84	406,759*	75

¹ The initial cell density of the stock culture was determined and an inoculum volume administered to each test chamber to yield a cell density of approximately 10,000 cells/mL at test initiation (Day 0).

² Percent inhibition was calculated relative to the negative control replicates. Values were obtained using SAS Release 6.12. Manual calculations may vary slightly.

* Statistically different ($p < 0.05$) compared to the negative control replicates.

At test termination, there were no statistically significant ($p > 0.05$) differences in mean growth rate between the negative control and the 0.30, 0.68 and 1.3 mg a.s./L treatment groups. However, the mean growth rate in the 2.5 mg a.s./L treatment group was reduced by 9.3% relative to the control group over the same period. While slight, this reduction was statistically significant at $p < 0.05$. A statistically significant ($p < 0.05$) reduction in growth rate (27%) also was evident by Day 5 in the 5.1 mg a.s./L group. Summary of effects on growth rate are reported in the following

table:

Mean Growth Rate and Percent Inhibition										
Sponsor:		CFPI Agro								
Test Substance:		Amitrole								
Test Organism:		Marine Diatom, <i>Skeletonema costatum</i>								
Dilution Water:		Saltwater Algal Medium								
Mean Measured Test Concentration (mg a.i./L.)	Growth Rates									
	Day 1		Day 2		Day 3		Day 4		Day 5	
	Mean Growth Rate	Percent Inhibition ¹	Mean Growth Rate	Percent Inhibition ¹	Mean Growth Rate	Percent Inhibition ¹	Mean Growth Rate	Percent Inhibition ¹	Mean Growth Rate	Percent Inhibition ¹
Negative Control	0.026	—	0.040	—	0.049	—	0.049	—	0.042	—
0.30	0.018	32	0.036	9.9	0.049	-0.48	0.048	0.92	0.043	-2.3
0.68	0.025	4.5	0.036	9.4	0.047	4.1	0.045	7.6	0.043	-1.9
1.3	0.015	44	0.031	22	0.038	21	0.041	46	0.042	-0.21
2.5	0.012	55	0.028	31	0.035	29	0.036	26	0.038*	9.3
5.1 *	0.000	100	0.026	34	0.029	40	0.029	40	0.031*	27
¹ Percent inhibition was calculated relative to the negative control replicates. Values were obtained using SAS Release 6.12. Manual calculations may vary slightly.										
* Statistically different ($p<0.05$) compared to the negative control replicates.										

At test termination, there were no statistically significant ($p > 0.05$) differences in mean area under the growth curve between the negative control group and the 0.30 and 0.68 mg a.s./L treatment groups. However, by Day 5, there was 33, 58 and 80% inhibition of area under the growth curve in the 1.3, 2.5 and 5.1 mg a.s./L groups that was statistically significant ($p < 0.05$) when compared to the control group. Summary of effects on area under the growth curve are reported in the following table:

Mean Area Under the Growth Curve and Percent Inhibition										
Sponsor:		CFPI Agro								
Test Substance:		Amitrole								
Test Organism:		Marine Diatom, <i>Skeletonema costatum</i>								
Dilution Water:		Saltwater Algal Medium								
Mean Measured Test Concentration (mg a.i./L)	Areas Under the Growth Curve									
	Day 1		Day 2		Day 3		Day 4		Day 5	
	Mean Area	Percent Inhibition ¹	Mean Area	Percent Inhibition ¹	Mean Area	Percent Inhibition ¹	Mean Area	Percent Inhibition ¹	Mean Area	Percent Inhibition ¹
Negative Control	109,822	--	924,761	--	5,582,085	--	22,063,119	--	53,916,225	--
0.30	73,169	33	716,442	23	5,383,464	3.6	21,647,296	1.9	55,923,963	-3.7
0.68	101,102	7.9	768,054	17	4,759,100	15	16,945,523	23	47,008,267	13
1.3	51,522	53	526,941	43	2,740,500	51	10,500,483	52	36,005,430*	33
2.5	39,523	64	416,450	55	2,104,151	62	7,093,987	68	22,746,892*	58
5.1	0	100	291,909	68	1,486,298	73	4,243,144	81	10,873,459*	80
¹ Percent inhibition was calculated relative to the negative control replicates. Values were obtained using SAS Release 6.12. Manual calculations may vary slightly.										
* Statistically different ($p<0.05$) compared to the negative control replicates.										

After five days of exposure, clumping, flocculation or adherence of the algae to the test chambers were not visually evident in any of the treatment groups. In addition, there were no noticeable changes in cell colour, size or morphology in the treatment groups, when compared to the control group.

❑ Conclusion:

The Day 5 EC₅₀ value for *Skeletonema costatum* exposed to amitrole was calculated in terms of cell densities based on mean measured concentrations and was determined to be 3.1 mg a.s./L. The 95% confidence limits were 1.9 and 3.9 mg a.s./L. The Day 5 ErC₅₀ value calculated in terms of growth rate was greater than 5.1 mg a.s./L, the highest concentration tested. The Day 5 EbC₅₀ value calculated in terms of area under the growth curve was 2.1 mg a.s./L, with 95% confidence limits of 1.4 and 2.4 mg a.s./L. The Day 5 no-observed-adverse-effect concentration was 1.3 mg a.s./L relative to cell density and growth rate, and was 0.68 mg a.s./L relative to area under the growth curve.

❑ Comment (RMS): study acceptable

B.9.2.9.6. Effects of amitrole on *Navicula pelliculosa*

- ❑ **Reference:** KIIA 8.4./04
- ❑ **Author(s); year:** Palmer S. J. 1997
- ❑ **Title:** AMITROLE: a 5-day toxicity test with the freshwater diatom (*Navicula pelliculosa*)
- ❑ **Report No:** 461A-103
- ❑ **Guidelines:** US-EPA FIFRA subdivision J, series 123-2
- ❑ **Deviations:**
- ❑ **GLP:** yes
- ❑ **Executive summary**

The freshwater diatom, *Navicula pelliculosa*, was exposed to a geometric series of five test concentrations and a negative (culture medium) control under static conditions for five days. Nominal test concentrations used in the study were 0.19, 0.38, 0.75, 1.5 and 3.0 mg a.s./L. Concentrations of amitrole in the test solutions were measured at the beginning and end of the test. Mean measured test concentrations were 0.18, 0.38, 0.76, 1.5 and 3.1 mg a.s./L representing 95, 100, 101, 100 and 103% of nominal, respectively.

Temperature measurements were within the acceptable limits ($24 \pm 2^\circ\text{C}$) throughout the 5-day exposure period. Light intensity measurements at test initiation were within the range established for the test (4300 ± 650 lux). The pH measurements from the test solutions on Days 0 and 5 ranged from 7.4 to 8.0, and were typical of pH values obtained in tests conducted with *Skeletonema costatum*.

The response of the algae was measured in terms of changes in cell density. An EC_{50} value was determined for each 24-hour interval of the test. The Day 5 EC_{50} value was 1.5 mg a.s./L, with 95% confidence limits of 0.53 and 2.7 mg a.s./L.

An ErC_{50} value was determined for each 24-hour interval of the test to be greater than 3.1 mg a.s./L, the highest concentration tested. An EbC_{50} value was determined for each 24-hour interval of the test. The Day 5 EbC_{50} value was 2.1 mg a.s./L, with 95% confidence limits of 0.55 and 2.8 mg a.s./L. The no-observed-adverse-effect-concentration (NOAEC), which is the highest test concentration that had no adverse inhibitory effect on algal growth, was determined to be 0.76 mg a.s./L relative to cell density and growth rate, and was 1.5 mg a.s./L relative to area under the growth curve.

- ❑ **Materials and methods:**

A. MATERIALS

- 1. Test Material:** aminotriazole
Description: white powder
Lot/batch #: 17051718
Purity of active ingredients: 91.7%
Stability of test compound: expiration date – May 1999.
- 2. Vehicle and/or control:** freshwater culture medium

3. Test animals -

Species: freshwater diatom (*Navicula pelliculosa*)
Source: Wildlife International, Maryland, USA
Water: fresh water culture medium

Environmental conditions:

Temperature: $24 \pm 2^\circ\text{C}$
pH: 7.4 to 8.0
Light intensity: 4300 ± 650 lux

B. STUDY DESIGN AND METHODS:

1. In life dates: 06/11-11/11/1997

2. Experimental treatments:

The freshwater diatom (*Navicula pelliculosa*) was exposed to a geometric series of 5 test concentrations and a negative (culture medium) control under static conditions for five days. Three "biological" replicate test chambers were maintained for each treatment and control group. An additional "analytical" test chamber was used to provide test solution for analytical sampling for each treatment and control group. Nominal test concentrations were based upon the results of an exploratory range-finding test. Nominal test concentrations selected were 0.19, 0.38, 0.75, 1.5 and 3.0 milligrams of amitrole per liter of test solution (mg a.s./L).

Mean measured concentrations were determined from samples of test medium collected from each treatment and control group at the beginning, middle and end of the test.

3. Observations:

At test initiation an inoculum of the algal cells was prepared from the stock culture at a concentration of approximately 3.0×10^5 cells/mL. The concentration of algal cells was verified and 1.0 mL was added to each test chamber to achieve a nominal concentration of approximately 3.0×10^3 cells/mL. Samples were collected from each replicate test chamber at approximately 24-hour intervals during the exposure period to determine cell densities. Cell densities were subsequently used to calculate growth rates and areas under the growth curve for each 24-hour interval, as well as percent inhibition values relative to the control for each parameter over the 5-day exposure period. An EC_{50} value (i.e., the theoretical toxicant concentration that would produce a 50% reduction in cell density relative to the control) was calculated based on the inhibition of growth at Day 5. An ErC_{50} value (i.e., the toxicant concentration that produces a 50% reduction in growth rate relative to the controls) and an E_bC_{50} value (i.e., the toxicant concentration that produces a 50% reduction in biomass, expressed as area under the growth curve, relative to the controls) also were determined for each 24-hour interval of the test. The no-observed-adverse-effect-concentration (NOAEC), defined as the highest test concentration where there were no adverse inhibitory effects on cell growth, was determined relative to each parameter through evaluation of the Day 5 statistical results and the dose-response pattern.

Samples of test medium were collected from each treatment and control group on Days 0 and 5 to measure concentrations of the test substance. Samples collected on Day 0 were taken from the individual batches of test medium prepared at test initiation. Samples collected on Day 5 consisted of composited test medium from each of the three replicates in each of the respective treatment and control groups. The samples were collected in glass vials and were analysed immediately. Samples collected on Day 5 were filtered (0.45 μ m) to remove algal cells prior to analysis.

4. Statistics

Cell densities, growth rates, area under the growth curve values and percent inhibition values were calculated using "SAS Release 6.12", while statistical analyses were conducted using "ICPIN Version 2.0" and "TOXSTAT Release 3.2".

Cell densities, growth rates and areas under the growth curve were analysed statistically using the computer program ICPIN to determine the EC_{50} values. This program was designed to calculate the EC_{50} values and 95% confidence limits by linear interpolation. The Day 5 cell densities, growth rates and areas under the growth curve were evaluated for normality and homogeneity of variances using the Chi-square test and either Bartlett's or Hardey's test, respectively.

When necessary to correct for unequal variances, a square-root transformation was performed and the treatment groups then were analysed statistically using Dunnett's t-test. The 5-day no-observed-adverse-effect-concentration (NOAEC) was determined through evaluation of the statistical results and the dose-response pattern.

Findings:

A. FINDINGS

Analytical measurements were performed to verify exposure concentrations of amitrole in the test medium. Nominal concentrations selected for use in this study were 0.19, 0.38, 0.75, 1.5 and 3.0 mg a.s./L. Samples collected on Days 0 and 5 ranged from 94 to 104% of nominal. When measured concentrations of samples

collected on Days 0 and 5 were averaged, the mean measured concentrations for the test were 0.18, 0.38, 0.76, 1.5 and 3.0 mg a.s./L, representing 95, 100, 101, 100 and 103% of nominal, respectively.

The temperatures ranged from 23.7 to 25.6°C and were within the $24 \pm 2^\circ\text{C}$ range established for the test. Measurements of pH were 7.4 on Day 0 and ranged from 7.7 to 8.0 for the treatment and control groups on Day 5. The increase of pH relative to algal population was typical for tests conducted with *Navicula pelliculosa*. The light intensity ranged from 3880 to 4450 lux and was within the desired range of 4300 ± 650 lux.

The toxicity of amitrole to *Navicula pelliculosa* was measured in terms of cell density. Mean cell densities were used to calculate growth rates and areas under the growth curve or each 24-hour interval of the 5-day test. As the graphical representations indicate, evaluation of effects upon algal growth based upon percent inhibition can be highly variable prior to Day 3 of the test. Therefore, a better representation of effects on algal growth can be determined through evaluation of the graphical results for Days 3 through 5.

Changes in mean cell density indicated that exponential growth occurred in the negative control replicates. There were no statistically significant ($p > 0.05$) differences in mean cell density between the negative control and the 0.18, 0.38 and 0.76 mg a.s./L treatment groups. However, by Day 5, mean cell densities in the 1.5 and 3.1 mg a.s./L treatment groups were reduced by 44 and 84%, respectively, compared to the negative control. The reduction in cell density observed in those treatment groups was statistically significant ($p < 0.05$) and was considered to be treatment-related. Summary of effects on cell density are reported in the following table:

considered to be treatment-related. Summary of effects on cell density are reported in the following table.

Mean Cell Density and Percent Inhibition										
Sponsor:	CFPI Agro									
Test Substance:	Amitrole									
Test Organism:	Freshwater Diatom, <i>Navicula pelliculosa</i>									
Dilution Water:	Freshwater Algal Medium with Silica and Selenium									
Mean Measured Test Concentration (mg a.i./L)	Cell Densities (Cells/mL) ¹									
	Day 1		Day 2		Day 3		Day 4		Day 5	
	Cell Density	Percent Inhibition ²	Cell Density	Percent Inhibition ²	Cell Density	Percent Inhibition ²	Cell Density	Percent Inhibition ²	Cell Density	Percent Inhibition ²
Negative Control	6,099	--	22,452	--	100,179	--	276,799	--	1,198,161	--
0.18	5,695	6.6	19,088	15	107,456	-7.3	345,451	-25	1,431,017	-19
0.38	7,608	-25	23,304	-3.8	114,544	-14	309,069	-12	1,035,793	14
0.76	8,372	-37	24,643	-9.8	102,891	-2.7	332,937	-20	920,669	23
1.5	3,246	47	12,447	45	57,739	42	294,027	-6.2	667,722	44
3.1	3,199	48	11,184	50	50,335	50	145,098	48	191,020	84
¹ The initial cell density of the stock culture was determined and an inoculum volume administered to each test chamber to yield a cell density of approximately 3,000 cells/mL at test initiation (Day 0).										
² Percent inhibition was calculated relative to the negative control replicates. Values were obtained using SAS Release 6.12. Manual calculations may vary slightly.										
* Statistically different ($p<0.05$) compared to the negative control replicates										

At test termination, there were no statistically significant ($p > 0.05$) differences in mean growth rate between the negative control and the 0.18, 0.38 and 0.76 mg a.s./L treatment groups. However, the mean growth rate in the 1.5 mg a.s./L treatment group was reduced by 10% relative to the control group over the same period. While slight, this reduction was statistically significant at $p < 0.05$. A statistically significant ($p < 0.05$) reduction in growth rate (30%) also was evident by Day 5 in the 3.1 mg a.s./L group. Summary of effects on growth rate are reported in the following table:

Mean Growth Rate and Percent Inhibition										
Sponsor:		CFPI Agro								
Test Substance:		Amitrole								
Test Organism:		Freshwater Diatom, <i>Navicula pelliculosa</i>								
Dilution Water:		Freshwater Algal Medium with Silica and Selenium								
Mean Measured Test Concentration (mg a.i./L.)	Growth Rates									
	Day 1		Day 2		Day 3		Day 4		Day 5	
	Mean Growth Rate	Percent Inhibition ¹	Mean Growth Rate	Percent Inhibition ¹	Mean Growth Rate	Percent Inhibition ¹	Mean Growth Rate	Percent Inhibition ¹	Mean Growth Rate	Percent Inhibition
Negative Control	0.029	--	0.042	--	0.049	--	0.047	--	0.050	--
0.18	0.026	11	0.039	7.4	0.049	-1.1	0.049	-5.1	0.051	-3.4
0.38	0.038	-32	0.042	-0.85	0.050	-3.7	0.048	-2.5	0.049	2.0
0.76	0.043	-47	0.043	-4.0	0.049	-0.51	0.049	-4.1	0.048	4.1
1.5	0.005	83	0.029	29	0.041	16	0.047	0.81	0.045*	10
3.1	0.004	87	0.025	41	0.039	20	0.040	14	0.035*	30

¹ Percent inhibition was calculated relative to the negative control replicates. Values were obtained using SAS Release 6.12. Manual calculations may vary slightly.
* Statistically different ($p < 0.05$) compared to the negative control replicates.

At test termination, there were no statistically significant ($p > 0.05$) differences in mean area under the growth curve between the negative control group and the 0.18, 0.38, 0.76 and 1.5 mg a.s./L treatment groups. However, by Day 5, there was a 71% inhibition of area under the growth curve in the 3.1 mg a.s./L group that was statistically significant ($p < 0.05$) when compared to the control group. Summary of effects on area under the growth curve are reported in the following table:

Mean Area Under the Growth Curve and Percent Inhibition										
Sponsor:		CFPI Agro								
Test Substance:		Amitrole								
Test Organism:		Freshwater Diatom, <i>Navicula pelliculosa</i>								
Dilution Water:		Freshwater Algal Medium with Silica and Selenium								
Mean Measured Test Concentration (mg a.i./L.)	Areas Under the Growth Curve									
	Day 1		Day 2		Day 3		Day 4		Day 5	
	Mean Area	Percent Inhibition ¹	Mean Area	Percent Inhibition ¹	Mean Area	Percent Inhibition ¹	Mean Area	Percent Inhibition ¹	Mean Area	Percent Inhibition ¹
Negative Control	37,184	--	307,788	--	1,707,351	--	6,159,084	--	23,786,612	--
0.18	32,335	13	257,720	16	1,704,248	0.18	7,067,137	-15	28,312,756	-19
0.38	55,292	-49	354,227	-15	1,936,394	-13	6,947,741	-13	23,014,078	3.2
0.76	64,464	-73	388,646	-26	1,847,059	-8.2	7,004,997	-14	21,976,269	7.6
1.5	4,619	88	126,933	61	891,164	48	5,040,353	18	16,509,336	31
3.1	3,601	90	104,198	66	770,428	55	3,043,626	51	7,005,040	71

¹ Percent inhibition was calculated relative to the negative control replicates. Values were obtained using SAS Release 6.12. Manual calculations may vary slightly.
* Statistically different (*p*<0.05) compared to the negative control replicates.

After five days of exposure, clumping, flocculation or adherence of the algae to the test chambers were not visually evident in any of the treatment groups. In addition, there were no noticeable changes in cell colour, size or morphology in the treatment groups, when compared to the control group.

❑ Conclusion:

The Day 5 EC₅₀ value for *Navicula pelliculosa* exposed to amitrole was calculated in terms of cell densities based on mean measured concentrations and was determined to be 1.5 mg a.s./L. The 95% confidence limits were 0.53 and 2.7 mg a.s./L. The Day 5 ErC₅₀ value calculated in terms of growth rate was greater than 3.1 mg a.s./L, the highest concentration tested. The Day 5 EbC₅₀ value calculated in terms of area under the growth curve was 2.1 mg a.s./L, with 95% confidence limits of 0.55 and 2.8 mg a.s./L. The Day 5 no-observed-adverse-effect-concentration was 0.76 mg a.s./L relative to cell density and growth rate, and was 0.68 mg a.s./L relative to area under the growth curve.

❑ Comment (RMS): study acceptable

B.9.2.9.7. Effects of 1,2,4-triazole on *Selenastrum capricornatum*

Please refer to initial B.9.2. For 1,2,4-triazole, see the DAR of tebuconazole.

B.9.2.10. Effects on algal growth (annex IIIA 10.2.1) – CA2735**B.9.2.10.1. Effects of CA2735 on *Selenastrum capricornatum***

Please refer to initial B.9.2.

B.9.2.11. Effects on sediment dwelling organisms (annex IIA 8.2.7)

According to the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2011 rev4 (final) dated 17 October 2002), for herbicides, *Daphnia* is recognized to be suitably representative for aquatic insects and other invertebrates. In addition according to point 2.3.4.2, a sediment toxicity tests for invertebrates should be required if:

- 1) In a water-sediment study > 10% of applied radioactivity represented by the parent compound is present in the sediment at or after day 14.

AND

- 2) Triggers to identify potential risks to invertebrates for toxicity are met. It is further specified that for testing on sediment-dwelling organisms to be warranted, the NOEC in the chronic *Daphnia* tests must be < 0.1 mg/L.

The NOEC in the chronic *Daphnia* tests performed with amitrole are 0.56 mg/L and 0.32 mg/L for reprotoxicity and body length respectively, so > 0.1 mg/L. The mean radioactivity attributed to the parent compound in the sediment at 30 and 91 days is 7.5% and 5.9% respectively (mean of results obtained in the two tested systems). No assessment was made at 14 days but the radioactivity tends to decrease over 91 days. In addition the Koc for amitrole is low (Koc = 20-117 L/kg) which is consistent with its water solubility and limits its partitioning into the sediment.

For all these reasons and in compliance with the Guidance document SANCO 3268/2001 rev 4 dated 17/10/2002, no study on sediment dwelling organisms is required.

RMS comment: Agree with this position. In the first water/sediment study, amitrole is minor in sediment (<10%) during all the duration of the study with a max of 5.9% at day 0. In the other water/sediment study, amitrole is more present with a max of 14% after 7 days and then the concentration in sediment decreases slowly until the end of the study (8.5% after 91 days). Between day 3 and 60, amitrole is major (>10%) in the sediment (see Vol 3.B.8). Moreover, considering the low value of Koc (20-202 L/kg), amitrole is very soluble in water and a strong adsorption on sediment is not expected (see Vol 3.B.8). Considering results from these two water/sediment studies and the low Koc of amitrole, a high concentration in sediment is not expected (see Vol 3.B.8). Finally, the NOEC value for *Daphnia* is higher than 0.1 mg a.s/L indicating a relatively low toxicity for aquatic invertebrates. Therefore, no study on sediment dwelling organisms is required as amitrole is not expected to adsorb strongly on sediment and it can be considered that toxicity data from *daphnia* is sufficient to cover other aquatic invertebrates as sediment dwelling organisms.

B.9.2.12. Effects on aquatic plants (annex IIA 8.2.8) – amitrole and 1,2,4-triazole**B.9.2.12.1. Effects of amitrole on *Lemna gibba***

Please refer to initial B.9.2.

B.9.2.12.2. Effects of 1,2,4-triazole on *Lemna gibba*

For 1,2,4-triazole, after having made researches it hasn't been found any indication that it could have an herbicidal activity. This is the reason why a test on aquatic plant has not been conducted. However a screening

study testing the potential herbicidal activity of 1,2,4-triazole was performed and submitted in December 2012. It is reported that no herbicidal activity was observed for 1,2,4-triazole.

RMS comment: Considering the late submission of this study (December 2012), it was not possible for the RMS to check. This study should be submitted at national level (level 4).

B.9.2.13. Effects on aquatic plants (annex IIIA 10.2.1) – formulated product

B.9.2.13.1. Effects of CA2735 on *Lemna gibba*

According to the Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2011 rev4 (final) dated 17 October 2002), a test on aquatic plant has to be conducted with the formulated product if *Lemna* is identified as the most sensitive species based on the results obtained with the active ingredient. For amitrole, *Lemna* was not identified as the most sensitive species. Based on the results obtained with the active ingredient, the most sensitive species are algae (*Scenedesmus subspicatus*, *Selenastrum capricornutum*, *Skeletonema costatum*, *Navicula pelliculosa*). The formulation is far less toxic than the active ingredient on the 3 aquatic species tested so the formulation is not expected to be more toxic on *Lemna* anyway.

RMS comment: Based on the results obtained with the active ingredient, toxicity on *Lemna* (EC50=2.5 mg a.s/L) is relatively similar than toxicity on algae (EC50=2.1 mg a.s/L). The formulation is less toxic (factor 3) than the active ingredient on algae so the formulation is not expected to be more toxic on *Lemna*.

B.9.2.14. Effects on other aquatic (rooted) plants (annex IIA 8.2.8)

The mean radioactivity attributed to the parent compound in the sediment at 30 and 91 days is 7.5% and 5.9% respectively (mean of results obtained in the two tested systems). Therefore the radioactivity tends to decrease over 91 days and 90% of Amitrole is retrieved in water phase in the water/sediment study. In addition the Koc for amitrole is low (Koc = 20-117 L/kg) which is consistent with its water solubility and limits its partitioning into the sediment. It was shown that amitrole had around same effects on 5 species of algae (EC50 values of 2.1 mg a.s./L to >6 mg a.s./L) and on *Lemna* (EC50 values of 2.5 mg a.s/L). For all these reasons, no more effects are expected on aquatic rooted plant and therefore no study on aquatic rooted plant is required.

RMS comment: Agree with this position. Considering results from these two water/sediment studies and the low Koc of amitrole, a high concentration in sediment is not expected (see RMS comment in point B.9.2.11). Then, it was shown that amitrole had around same effects on 5 species of algae (EC50 values of 2.1 mg a.s./L to >6 mg a.s./L) and on *Lemna* (EC50 values of 2.5 mg a.s/L). Therefore, no study on aquatic rooted plant is required as amitrole is not expected to adsorb strongly on sediment and it can be considered than toxicity data from *Lemna* is sufficient to cover other aquatic plant as aquatic rooted plant.

B.9.2.15. Microcosm or mesocosm studies (annex IIIA 10.2.2)

When CA2735 is used according to the Good Agriculture Practices, the acute and long-term TER values for aquatic organisms are above the relevant trigger values. Therefore, neither microcosm nor mesocosm studies are required.

B.9.2.16. Residue data in fish (annex IIIA 10.2.3)

Bioaccumulation in fish is not relevant, since log P_{ow} of Amitrole is below 3 (-0.969). A BCF was however determined, 2.38 (whole fish within 7 days). Please refer to Review Report on Amitrole 2001 (6839/VI/97-final)

B.9.2.17. Additional studies (annex IIIA 10.2.4)

B.9.2.18. Risk assessment for aquatic organisms

B.9.2.18.1. Toxicity endpoint for aquatic organisms

Aquatic organisms may be exposed to Amitrole by emissions from treated fields. The provided studies and data allow a risk assessment under practical conditions. The risk assessments will be based on the aquatic endpoints

of the Review Report on Amitrole 2001 (6839/VI/97-final) and on new endpoints from new acceptable studies. The results are presented in the table 9.2.18.1.1.

In the case of Amitrole, 1,2,4-triazole was considered as a major metabolite in soil by photodegradation. RMS notes that according to the sustained GAP for agricultural uses (application period), photodegradation is not expected to be a major route of degradation of amitrole in soil and therefore 1,2,4-triazole is not expected to be formed in significant amounts. Therefore, to the RMS opinion, a risk assessment was not strictly required. However, RMS considers that if further agricultural uses occurring when photodegradation may be important would be sustained, it should be discussed whether 1,2,4-triazole may need to be included in the risk assessment or not. Therefore, risk assessment for 1,2,4-triazole are presented for information. It is highlighted that photodegradation and microbial degradation will be concomitant and concurrent processes. Therefore, if photodegradation occurs in field, the maximum occurrence reached is expected to be lower than 9.9%.

The endpoints are also presented in Table 9.2.18.1.2.

The relevant toxicity endpoints are summarised in the tables below.

Table 9.2.18.1.1: toxicity data for amitrole (only data from studies considered valid are reported)

Group (species)	Time scale	Endpoint	Value (mg a.s./L)	Expressed as *	Reference
Fish (<i>O. mykiss</i>)	Acute 96H (static)	LC ₅₀	>1000	Nominal	EU review report 6839/VI/97-final, 22/03/2001
Fish (<i>Leuciscus idus</i>)	Acute 96H (static)	LC ₅₀	>6000	Nominal	EU review report 6839/VI/97-final, 22/03/2001
Fish (<i>Lepomis macrochirus</i>)	Acute 96H (static)	LC ₅₀	>1000	Nominal	McAllister. 1985
Fish (<i>Cyprinodon variegatus</i>)	Acute 96H (static)	LC ₅₀	>1000	Nominal	Collins M.K. 1993
Fish (<i>O. mykiss</i>)	Chronic 21d (static) ¹	NOEC	100	Nominal	EU review report 6839/VI/97-final, 22/03/2001
Invertebrate (<i>D. magna</i>)	Acute 48H (static)	EC ₅₀	6.1	Nominal	EU review report 6839/VI/97-final, 22/03/2001
Invertebrate (<i>Mysidopsis bahia</i>)	Acute 96H (static)	EC ₅₀	2.8	Mean measured	Collins M.K. 1993
Invertebrate (<i>D. magna</i>)	Chronic 21d (static)	NOEC	0.32	Nominal	EU review report 6839/VI/97-final, 22/03/2001
Aquatic molluscs (<i>Crassostrea virginica</i>)	Acute 96 h	EC ₅₀	>110	Mean measured	Dionne E. 1993
Sediment dwelling organism (<i>C. riparius</i>)	Not necessary (see B.9.2.11) ²				
Algae (<i>Scenedesmus subspicatus</i>)	72H growth test	EC ₅₀	2.3	Nominal	EU review report 6839/VI/97-final, 22/03/2001
Algae (<i>Anabaena flos-aquae</i>)	120H growth test (static)	E _b C ₅₀ EC _{r50}	3.9** >4.8	Nominal	EU (addendum to monograph)
Algae (<i>Selenastrum capricornutum</i>)	5d growth test	EC _{b50} EC _{r50}	2.3 >5.1	Mean measured	Palmer S.J. 1997
Algae (<i>Selenastrum capricornutum</i>)	8d growth test	EC _{b50} /EC _{r50} NOEC	> 6 6	Nominal	Cross J.W. 1993
Algae (<i>Skeletonema costatum</i>)	5d growth test	EC _{b50} EC _{r50}	2.1 >5.1	Mean measured	Palmer S.J. 1998

Group (species)	Time scale	Endpoint	Value (mg a.s./L)	Expressed as *	Reference
Algae (<i>Navicula pelliculosa</i>)	5d growth test	EC _{b50} EC _{r50}	2.1 >3.1	Mean measured	Palmer S.J. 1997
Aquatic plant (<i>Lemna gibba</i>)	14d growth test (static)	EC ₅₀	2.5	Measured (initial concentration)	EU review report 6839/VI/97-final, 22/03/2001
Aquatic rooted plant	Not necessary (see B.9.2.14) ³				

1- Toxicity data not sufficient for assessing potential effects on development and reproduction due to endocrine disrupting properties of the active substance (see explanation in B.9.2.18.3.2)

2- For all the reasons expressed in B.9.2.11, no risk assessment on sediment dwelling organisms is required.

3- For all the reasons expressed in B.9.2.14, no risk assessment on aquatic rooted plant is required.

* RMS comment: endpoint expressed in term of mean measured concentration could be expressed in term of nominal concentration as amitrole was very stable during these tests

** RMS comment: There is a mistake in the review report (EC50 of 2.5 mg a.s/L has been reported whereas this value corresponded to the NOECb in the addendum to monograph)

Table 9.2.18.1.2: Toxicity data of 1,2,4-triazole for aquatic organisms (only data from studies considered valid are reported)

Group (species)	Time scale	Endpoint	Value (mg test substance/L)	Expressed as	Reference*
Fish (<i>O. mykiss</i>)	Acute 96H (static)	LC ₅₀	498	Nominal	EU**
Fish (<i>O. mykiss</i>)	Chronic 28d (semi static)	NOEC	>100 (mortality) 3.2 (sub lethal effects)	Nominal	EU
Invertebrate (<i>D. magna</i>)	Acute 48H (static)	EC ₅₀	>100	Nominal	EU
Algae (<i>Selenastrum capricornutum</i>)	72H growth test	EC _{b50} EC _{r50}	13 >31	Nominal	EU
Algae (<i>Selenastrum capricornutum</i>)	72H growth test	EC _{b50} EC _{r50}	8.2 22.5	Nominal	EU***
Aquatic plant (<i>Lemna gibba</i>)	Not available (see B.9.2.12.2)				

* For 1,2,4-triazole, see DAR of tebuconazole.

** value set during the expert meeting 43 (march 2008)

*** value set during the expert meeting 13 (january 2007)

Additional studies were conducted with the formulation **CA2210**. Further details regarding the tests with this formulation are provided in table **9.2.18.1.3**. The representative formulation is also a soluble concentrate containing 229 g/L of amitrole and 215 g/L of ammonium thiocyanate (specific synergist).

When compared to WEEDAZOL TL (CA0123 then CA2210), which was the representative formulation for the first annex I inclusion, minor changes were made in formulation CA2735.

As only a minor change was brought to this formulation, the ecotoxicological studies that were conducted on CA 2210 remain valid to cover the toxicological profile of CA2735.

Only acute studies were conducted since the formulated product was not shown to be more toxic than the active substance from acute endpoint. The results are presented in the **9.2.18.1.3**.

Table 9.2.18.1.3: Toxicity data for WEEDAZOL TL extrapolated to CA2735 (only data from studies considered valid are reported)

Group (species)	Time scale	Endpoint	Value (mg preparation/L)	Expressed as	Reference
Fish (<i>O. mykiss</i>)	Acute 96H (static)	LC ₅₀	>100 (>22.15 mg a.s/L)	Nominal	EU (DAR)
Invertebrate (<i>D. magna</i>)	Acute 48H (static)	EC ₅₀	12 (2.66 mg a.s/L)	Nominal	EU (DAR)
Algae (<i>Selenastrum capricornutum</i>)	72H growth test (static)	EbC ₅₀ ErC _{r50}	126 (27.91 mg a.s/L) >200 (>44.3 mg a.s/L)	Nominal	EU (DAR)
Aquatic plant (<i>Lemna gibba</i>)	Not necessary (see B.9.2.13.1)				

B.9.2.18.2. Exposure

For agricultural uses (pome fruit, citrus, olives and vineyard) and non-crop areas (grass), the initial PEC_{sw} calculations provided were not considered acceptable. Additional calculations were asked to the applicant, but the new calculations provided still did not address RMS' request and therefore could not be accepted. The applicant finally provided additional calculations at the end of December 2012. However, since it was too late in the process, they were not taken into account. (see vol. 3 B.8)

For non-cropped areas (uses on road and railways), PEC_{sw} for amitrole were calculated in vol. 3.B.8 according to HardSpec. Results of PEC_{sw} values are reported in the table 9.2.18.2.1 below

Table 9.2.18.2.1: amitrole maximum PEC surface water calculated for non-cropped areas (HardSpec).

Crop	Modelling	Critical scenario	PEC _{sw} (µg/L)
Non-cropped areas	HardSpec	Urban stream	454
		Urban pond	43
		Major road stream	233
		Domestic stream	77
		Railway ditch leaching	8
		Railway Ditch runoff	8

The PEC_{sw} for the main amitrole metabolite, 1,2,4-triazole were calculated in vol. 3.B.8. They are presented in table 9.2.18.2.2.

Table 9.2.18.2.2: 1,2,4-triazole maximum PEC surface water calculated for the different critical uses, orchards and vineyards (FOCUS STEP 1) and weeding of non-cropped areas (HardSpec).

Crop	Modelling	Critical scenario	PEC _{sw} (µg/L)
Orchards early	FOCUS STEP 1	n.a.	32.22
Orchards late			28.56
Citrus early&late			28.56
Olives			28.56
Vineyards			25.02
Non-cropped areas	FOCUS STEP 1	grass	27.18
	HardSpec	Urban stream	37.615
		Urban pond	3.589
		Major road stream	19.570
		Domestic stream	8.922
		Railway ditch leaching	0.636
		Railway ditch runoff	0.636

B.9.2.18.3. Risk assessment

B.9.2.18.3.1. TER_A for fish

The acute TER for fish were based on the endpoints given in the Review Report (6839/VI/97-final, 2001) of amitrole.

Table 9.2.18.3-1: Fish acute TER values after application of CA2735

Test organism	Endpoint [µg/L]	Scenario	PEC _{sw} [µg/L]	TER _A	Trigger value
Amitrole					
O. mykiss	LC ₅₀ > 1000000	Agricultural uses and grass	N.A	N.A	100
		Urban stream	454	>2202	
		Urban pond	43		
		Major road stream	233		
		Domestic stream	77		
		Railway ditch leaching	8		
		Railway ditch runoff	8		
1,2,4-triazole					
O. mykiss	LC ₅₀ = 498000	Orchards early	32.22	≥ 13239	100
		Orchards late, citrus & olives	28.56		
		Vineyards:	25.02		
		Urban stream	37.615		
		Urban pond	3.589		
		Major road stream	19.570		
		Domestic stream	8.922		
		Railway ditch leaching	0.636		
		Railway ditch runoff	0.636		
		Grass	27.18		

TER values that exceed the trigger value indicate an acceptable risk for aquatic organisms.

The TER_A values for Amitrole are above the Annex VI trigger value of 100 for non cropped areas.

The TER_A values for 1,2,4-triazole are above the Annex VI trigger value of 100.

Therefore, it can be assumed that there will be no significant acute risk to fish when applying CA2735 under the recommended conditions for non cropped areas.

B.9.2.18.3.2. TER_{LT} for fish**Table 9.2.18.3.2.1: Fish long-term TER values after application of CA2735**

Test organism	Endpoint [µg/L]	Scenario	PEC _{SW} [µg/L]	TER _A	Trigger value
Amitrole					
<i>O. mykiss</i>	NOEC: N.A	Agricultural uses and grass	N.A	N.A	10
		Urban stream	454	N.A	
		Urban pond	43		
		Major road stream	233		
		Domestic stream	77		
		Railway ditch leaching	8		
		Railway ditch runoff	8		

1,2,4-triazole					
<i>O. mykiss</i>	NOEC = 3200 (based on sub-lethal effects)	Orchards early	32.22	≥85	10
		Orchards late, citrus & olives	28.56		
		Vineyards:	25.02		
		Urban stream	37.617		
		Urban pond	3.589		
		Major road stream	19.570		
		Domestic stream	8.922		
		Railway ditch leaching	0.636		
		Railway ditch runoff	0.636		
		Grass	27.18		
TER values that exceed the trigger value indicate an acceptable risk for aquatic organisms.					

The TER_{LT} values for 1,2,4-triazole are above the Annex VI trigger value of 10.

RMS comment: The literature review report brings some information about the mechanism of action of amitrole in fish metabolism and confirms thyroid disruption. Amitrole did not block the production of THs in rare minnow but alterations of Thyroid Hormones-related genes expressions in liver and brain were noted at 10 to 10000 ng/L. The chronic test on juvenile fish (NOEC=100 mg a.s/L) is not sufficient for assessing potential effects on development and reproduction due to endocrine disrupting properties. These potential consequences on development and reproduction *in vivo* should have been addressed with the fish full life-cycle test in fish (Medaka) but this study is not considered valid. Therefore, as there is an uncertainty concerning potential effects on development and reproduction, long term risk assessment for fish cannot be finalized.

B.9.2.18.3.3. TER_A for Daphnia

The acute TER for daphnids were based on the endpoints given in the Review Report (6839/VI/97-final, 2001) of amitrole.

Table 9.2.18.3.3.1: Daphnids acute TER values after application of CA2735

Test organism	Endpoint [µg/L]	Scenario	PEC _{sw} [µg/L]	TER _A	Trigger value
Amitrole					
<i>D. magna</i>	EC ₅₀ = 6100	Agricultural uses and grass	N.A	N.A	100
		Urban stream	454	14	
		Urban pond	43	142	
		Major road stream	233	26	
		Domestic stream	77	79	
		Railway ditch leaching	8	763	
		Railway ditch runoff	8	763	
1,2,4-triazole					

<i>D. magna</i>	EC ₅₀ > 100000	Orchards early	32.22	100
		Orchards late, citrus & olives	28.56	
		Vineyards:	25.02	
		Urban stream	37.617	
		Urban pond	3.589	
		Major road stream	19.570	
		Domestic stream	8.922	
		Railway ditch leaching	0.636	
		Railway ditch runoff	0.636	
		Grass	27.18	

TER values that exceed the trigger value indicate an acceptable risk for aquatic organisms.

The TER_A values for 1,2,4-triazole are above the Annex VI trigger value of 100.

The TER_A values for amitrole and its relevant metabolite are above Annex VI trigger value of 100 for the railway scenario.

Therefore, it can be assumed that there will be no significant acute risk to daphnids when applying CA2735 under the recommended conditions for railways.

Notifier: For three scenarios on non-cropped areas, the TER of the active substance is lower than the trigger value of 100. However, this is only for a transient period (on the first day after application). Then the concentrations in urban stream, major road stream and domestic stream scenarios decrease and the risk is acceptable from the second day onward after application. The actual concentrations of amitrole (6 first days after the application) calculated by HardSpec for the urban, major road and domestic stream scenario are presented in table 10.2.1.3-2.

Table 9.2.18.3.3.2: evolution of amitrole concentrations in urban, major road and domestic streams (TER calculation based on a EC₅₀ of 6100 µ/L).

Time	Conc. (µg/L) urban stream	TER	Conc. (µg/L) major road stream	TER	Conc. (µg/L) Domestic stream	TER
0	27.31	223	19.399	315	0.00	>100
1	452.63	14	232.24	26	98.72	62
2	57.62	106	24.17	252	15.86	385
3	30.75	198	12.91	472	9.05	674
4	16.94	361	5.69	1072	4.91	1242
5	16.17	377	5.93	1028	4.76	1282
6	9.38	664	2.92	2089	2.71	2250

Amitrole reaches peak concentrations 24 hours after the application. The acute TER values are lower than the trigger of 100 (14, 26 and 62 for the urban, major road and domestic stream scenarios, respectively). From the second day onwards, acute TER are above 100, ranging from 106 to 2250. Even though, during a short period after the application, the acute TER are not greater than 100, it can be considered that the risk to daphnids is acceptable. No impact at population level is expected. In streams, the water flow can quickly renew the aquatic invertebrate populations compensating a potential mortality. Two days after the application, the amitrole concentrations do not result in unacceptable mortality (TER > 100). In ponds, where this renewal is "slower", the risk is acceptable even at the peak concentration reached 8 days after the application.

Therefore, it can be assumed that there will be no significant acute risk to daphnids when applying CA2735 under the recommended conditions.

RMS comment: Acute risk assessment for *daphnia* is refined based on the hypothesis of the possible renewal of populations following degradation of the active substance few days after application of the

product compensating the mortality at initial predicted surface water concentrations. As endpoints are based on acute effects, the risk assessment might only be based on initial exposure, and should not take into account a possible degradation of the active substance. Acute risk assessment based on mortality has therefore to be conducted based on initial predicted surface water concentrations.

Considering these comments, a new refined risk assessment has been asked. The risk assessment for the urban, major road and domestic streams scenario has not been further refined and should be considered at member state level.

B.9.2.18.3.4. TER_{LT} for daphnia

No chronic study on daphnids was conducted with the formulation CA2210 or CA2735. The formulation was acutely less toxic than the active substance and therefore, the long-term risk will be based on the amitrole endpoints.

Table 9.2.18.3.4.1: Daphnid long-term TER values after application of CA2735

Test organism	Endpoint [µg/L]	Scenario	PEC _{SW} [µg/L]	TER _A	Trigger value
<i>D. magna</i>	NOEC = 320	Agricultural uses and grass	N.A	N.A	10
		Urban stream	454	0.7	
		Urban pond	43	7.5	
		Major road stream	233	1.4	
		Domestic stream	77	4.1	
		Railway ditch leaching	8	40	
		Railway ditch runoff	8	40	

TER values that exceed the trigger value indicate an acceptable risk for aquatic organisms.

The TER_{LT} value for Amitrole is above the Annex VI trigger value of 10, indicating no significant long-term risk to daphnids when CA2735 is used in railways.

Notifier: In non-cropped areas, the TER is below the trigger value for the urban and major road streams, domestic stream and for urban ponds. However, in these scenarios, daphnids are only acutely exposed to such concentrations.

In the same way as for the acute exposure, the TER were calculated within the period 0-6 days. They are presented in table 9.2.18.3.4.2

Table 9.2.18.3.4.2: evolution of amitrole concentrations in urban, major road and domestic streams (TER calculation based on a EC₅₀ of 320 µ/L).

Time	Conc. (µg/L) urban stream	TER	Conc. (µg/L) major road stream	TER	Conc. (µg/L) Domestic stream	TER
0	27.31	12	19.399	16.5	0.00	>10
1	452.63	0.7	232.24	1.37	98.72	3
2	57.62	6	24.17	13	15.86	20
3	30.75	10.4	12.91	25	9.05	35
4	16.94	19	5.69	56	4.91	65
5	16.17	20	5.93	54	4.76	67
6	9.38	34	2.92	110	2.71	118

Due to the rapid decrease of amitrole concentrations in the stream, daphnids are only acutely exposed to the active substance. Long-term TER are lower than the trigger value of 10 only within a 2-day period. Therefore, even though the TER are lower than 10 during this short period, no significant impact on the population is expected and the long-term exposure of daphnids to amitrole does not result in an unacceptable risk.

In urban pond where the water renewal is "slower", the risk is acceptable 17 days after application (TER >10).

No long-term study is available for the metabolite 1,2,4-triazole. However, it was shown of a very low acute toxicity in daphnids. Therefore, it can be assumed that its chronic toxicity to daphnids will be low and that the compound will not raise any significant concern at the levels it will occur in surface waters.

Therefore, it can be assumed that there will be no significant long-term risk to daphnids when applying CA2735 under the recommended conditions.

RMS comment: Long term risk assessment for *daphnia* is refined based on the hypothesis of the possible renewal of populations following degradation of the active substance few days after application of the product compensating the mortality at initial predicted surface water concentrations. Long term risk assessment has to be conducted based on initial predicted surface water concentrations as degradation of the active substance in surface water may not be appropriate for use with endocrine disrupting compound. Moreover, even if the NOEC of 320 µg a.s/L is based on effects on growth at the LOEC of 560 µg a.s/L and therefore risk assessment could eventually be refined with PECTwa (twa duration depending on the time-to-onset-of-effects), effects on reproduction have been observed in this study at the LOEC of 1000 µg a.s/L concluding to a NOEC reproduction of 560 µg a.s/L that would be used initial predicted surface water concentrations. Considering these comments, a new refined risk assessment has been asked. The risk assessment for the urban stream and pond, major road and domestic streams scenario has not been further refined and should be considered at member state level.

B.9.2.18.3.5. TER_A for aquatic insect

A risk assessment for the sediment dwelling organisms has been proposed considering the acute and chronic toxicity data obtained on *Daphnia* (EC₅₀-48h = 4.342 mg/L, NOAEC 21 jours = 0.321 mg/L) and the predictable concentrations in sediment (PEC_{sed}) as calculated according to FOCUS and HardSPEC.

Assuming an even distribution of the compound within a soil layer of 5 cm depth and a bulk density of 1.5 g/cm³, the acute and toxicity data obtained on *Daphnia* are EC₅₀-48h = 2.894 mg/kg, NOAEC 21 jours = 0.214 mg/kg in sediment.

RMS comment: doesn't agree with the above argumentation. However, for all the reasons expressed in B.9.2.11, no risk assessment on sediment dwelling organisms is required.

B.9.2.18.3.6. TER_{LT} for aquatic insect

See B.9.2.18.3.5

B.9.2.18.3.7. TER_A for aquatic crustaceans

The acute TER for aquatic crustaceans were based on a study performed with amitrole and summarized in AII, 8.3.1.3. The main results are shown in table 9.2.18.3.7.1.

Table 9.2.18.3.7.1: Crustacean acute TER values after application of CA2735

Test organism	Endpoint [µg/L]	Scenario	PEC _{SW} [µg/L]	TER _A	Trigger value
Amitrole					
<i>Mysidopsis bahia</i>	EC ₅₀ = 2800	Agricultural uses and grass	N.A	N.A	100
		Urban stream	454	6	
		Urban pond	43	65	
		Major road stream	233	12	
		Domestic stream	77	36	
		Railway ditch leaching	8	350	
		Railway ditch runoff	8	350	

TER values that exceed the trigger value indicate an acceptable risk for aquatic organisms.

For non-cropped areas, the TER are above 100 for the railways scenario.

The TER are lower than 100 when considering urban, major road and domestic streams as well as for urban pond scenario.

Notifier: As for daphnids, the exposure can be considered as acceptable (please see 10.2.1.3) except for the urban pond scenario for which the risk becomes acceptable after 23 days.

Therefore, it can be assumed that there will be no significant acute risk to *Mysidopsis bahia* when applying CA2735 under the recommended conditions.

RMS comment: Acute risk assessment for *Mysidopsis bahia* is refined based on the hypothesis of the possible renewal of populations following degradation of the active substance few days after application of the product compensating the mortality at initial predicted surface water concentrations. As endpoints are based on acute effects, the risk assessment might only be based on initial exposure, and should not take into account a possible degradation of the active substance. Acute risk assessment based on mortality has therefore to be conducted based on initial predicted surface water concentrations.

Considering these comments, a new refined risk assessment has been asked. The risk assessment for the urban stream and pond, major road and domestic streams scenario has not been further refined and should be considered at member state level.

B.9.2.18.3.8. TER_{LT} for aquatic crustacean

TER_{LT} values for additional aquatic crustacean species are not required since the risk assessments for *Daphnia magna* (Point IIIA 10.2.1.4) is considered to be sufficiently protective to other aquatic invertebrates.

B.9.2.18.3.9. TER_A for gastropod molluscs

No study was conducted on gastropod molluscs with either the active substance or CA2210 or CA2735. However, an acute toxicity study on *Crassostrea virginica*, a bivalve mollusc is available (please refer to AII 8.3.1.4).

Table 9.2.18.3.9.1: Mollusc acute TER values after application of CA2735

Test organism	Endpoint [µg/L]	Scenario	PEC _{SW} [µg/L]	TER _A	Trigger value
Amitrole					
		Agricultural uses and grass	N.A	N.A	100

<i>Crassostrea virginica</i> (96 h)	EC ₅₀ > 100000	Urban stream	454	> 221	
		Urban pond	43	> 2325	
		Major road stream	233	> 429	
		Domestic stream	77	> 1298	
		Railway ditch leaching	8	>12500	
		Railway ditch runoff	8	> 12500	

TER values that exceed the trigger value indicate an acceptable risk for aquatic organisms.

The TER_A values for Amitrole are above the Annex VI trigger value of 100 for non cropped areas.

No test is available for the metabolite 1,2,4-triazole. However, it was shown of a very low acute toxicity in aquatic organisms. Therefore, it can be assumed that its toxicity to molluscs will be low and that the compound will not raise any significant concern at the levels it will occur in surface waters.

Therefore, it can be assumed that there will be no significant acute risk to aquatic molluscs when applying CA2735 under the recommended conditions for non cropped areas.

B.9.2.18.3.10. TER_{LT} for gastropod molluscs

No long-term study is available with such species. However, when CA2735 is used as recommended, it does not present any significant acute risk to molluscs. Therefore, it can be assumed that no long-term risk to aquatic molluscs is expected.

B.9.2.18.3.11. TER_{LT} for algae

The long-term TER for algae were based on the endpoints given in the ReviewReport (6839/VI/97-final, 2001) of amitrole.

Table 9.2.18.3.11.1: Algae TER values after application of CA2735

Test organism	Endpoint [µg/L]	Scenario	PEC _{SW} [µg/L]	TER _{LT}	Trigger value
Amitrole					
Navicula pelliculosa	E _b C ₅₀ = 2100 (lowest EC ₅₀)	Agricultural uses and grass	N.A	N.A	10
		Urban stream	454	5	
		Urban pond	43	49	
		Major road stream	233	9	
		Domestic stream	77	27	
		Railway ditch leaching	8	262	
		Railway ditch runoff	8	262	
1,2,4-triazole					
Selenastrum capricornutum	E _b C ₅₀ = 8200	Orchards early STEP1	32.22	254	10
		Orchards late, citrus & olives	28.56	287	
		STEP1	25.02	327	
		Vineyards: STEP 1	37.617	217	
		Urban stream	3.589	2284	
		Urban pond	19.570	419	
		Major road stream	8.922	919	
		Domestic stream	0.636	12893	
		Railway ditch leaching	0.636	12893	
		Railway ditch runoff	27.18	301.7	
		Grass			

TER values that exceed the trigger value indicate an acceptable risk for aquatic organisms.

The TER_{LT} values for Amitrole are above the Annex VI trigger value of 10 for railways scenario, domestic streams and urban pond.

The TER_{LT} values for 1,2,4-triazole are above the Annex VI trigger value of 10.

The TER are lower than 10 for urban and major stream scenario but algae are only acutely exposed to such concentrations.

Notifier: The TER were calculated within the period 0-6 days. They are presented in table 9.2.18.3.11.2.

Table 9.2.18.3.11.2: Evolution of amitrole concentrations in urban and major road streams (TER calculation based on a EC₅₀ of 2100 µ/L).

Time	Conc. (µg/L) urban stream	TER	Conc. (µg/L) major road stream	TER
0	27.31	77	19.399	108
1	452.63	5	232.24	9
2	57.62	36	24.17	87
3	30.75	68	12.91	163
4	16.94	124	5.69	369
5	16.17	130	5.93	354
6	9.38	223	2.92	719

Due to the rapid decrease of amitrole concentrations in the stream, algae are only acutely exposed to the active substance. Long-term TER are lower than the trigger value of 10 only the day after application. Therefore, even though the TER are lower than 10 during this short period, no significant impact on the population is expected and the long-term exposure of algae to amitrole does not result in an unacceptable risk.

Therefore, it can be assumed that there will be no significant long term risk to algae when applying CA2735 under the recommended conditions.

RMS comment: Risk assessment for *algae* is refined based on the hypothesis of the possible renewal of populations following degradation of the active substance few days after application of the product compensating the inhibition of growth at initial predicted surface water concentrations. As endpoints are based on growth effects and considering that the active substance was very stable in the test, risk assessment could be refined on two exposure (two duration depending on the time-to-onset-of-effects) but it has'nt been done as no PEC_{two} are available in vol 3 B.8. Otherwise a higher tier *Selenastrum capricornutum* study could be conducted in order to mimic a realistic worst-case exposure profile based on the proposed use of amitrole and PEC surface water modelling. The aim of the study would be to assess the effects under a more realistic exposure regime and also demonstrate recovery of *Selenastrum capricornutum* for initial concentrations (able to cover those expected from modelling) to conclude that any effect on *Selenastrum capricornutum* will be reversible and populations of algae have potential for recovery. The submission of this study should be considered at member state level.

B.9.2.18.3.12. TER_{LT} for aquatic plant

The formulation is less toxic (factor 3) than the active ingredient on algae so the formulation is not expected to be more toxic on *Lemna* anyway. The risk will be based on the amitrole endpoints.

Table 9.2.18.3.12.1: Aquatic plant long-term TER values after application of CA2735

Test organism	Endpoint [µg/L]	Scenario	PEC _{SW} [µg/L]	TER _A	Trigger value
Amitrole					
<i>Lemna gibba</i>	E _b C ₅₀ = 2500	Agricultural uses and grass	N.A	N.A	10

	Urban stream	454	6	
	Urban pond	43	58	
	Major road stream	233	11	
	Domestic stream	77	32	
	Railway ditch leaching	8	312	
	Railway ditch runoff	8	312	

TER values that exceed the trigger value indicate an acceptable risk for aquatic plants.

The TER_{LT} values for Amitrole are above the Annex VI trigger value of 10 for railways scenario, domestic and major road stream, and urban pond.

The TER are lower than 10 for urban stream scenario.

Notifier: The TER are lower than 10 for urban stream scenario but aquatic plants are only acutely exposed to such concentrations.

The TER were calculated within the period 0-6 days. They are presented in table 9.2.18.3.12.2.

Table 9.2.18.3.12-2: Evolution of amitrole concentrations in urban streams (TER calculation based on a EC₅₀ of 2500 µ/L).

Time	Conc. (µg/L) urban stream	TER
0	27.31	91
1	452.63	6
2	57.62	43
3	30.75	81
4	16.94	147
5	16.17	155
6	9.38	266

Due to the rapid decrease of amitrole concentrations in the stream, aquatic plants are only acutely exposed to the active substance. Long-term TER are lower than the trigger value of 10 only the day after application. Therefore, even though the TER are lower than 10 during this short period, no significant impact on the population is expected and the long-term exposure of aquatic plants to amitrole does not result in an unacceptable risk.

Therefore, it can be assumed that there will be no significant long term risk to aquatic plants when applying CA2735 under the recommended conditions.

No study is available for the metabolite 1,2,4-triazole. However, it was shown to be of a very low toxicity in aquatic organisms. Therefore, it can be assumed that its effects on aquatic plants will be low and that the compound will not raise any significant concern at the levels it will occur in surface waters.

Therefore, it can be assumed that there will be no significant long-term risk to aquatic plants when applying CA2735 under the recommended conditions.

RMS comment: Risk assessment for *Lemna gibba* is refined based on the hypothesis of the possible renewal of populations following degradation of the active substance few days after application of the product compensating the inhibition of growth at initial predicted surface water concentrations. As endpoints are based on growth effects and considering that the active substance was stable in the test, the risk assessment could be refined on two exposure (two duration depending on the time-to-onset-of-effects) but it has'nt been done as no PEC_{two} are available in vol 3 B.8. Otherwise, a higher tier *Lemna gibba* study could be conducted in order to mimic a realistic worst-case exposure profile based on the proposed use of amitrole and PEC surface water modelling. The aim of the study would be to assess the effects under a more realistic exposure regime and also demonstrate recovery of *Lemna gibba* for initial concentrations (able to cover those expected from modelling) to conclude that any effect on *Lemna gibba*

will be reversible and populations of aquatic plants have potential for recovery. The submission of this study should be considered at member state level.

B.9.2.18.3.13. TER_{LT} for aquatic rooted plants

A risk assessment for aquatic rooted plants has been proposed considering the chronic toxicity data obtained on *Lemna gibba* ($E_bC_{50}=2500\mu\text{g/L}$) and the predictable concentrations in sediment (PEC_{sed}) as calculated according to FOCUS and HardSPEC. Assuming an even distribution 134ft he compound within a soil layer of 5 cm depth and a bulk density of 1.5 g/cm^3 , the $E_bC_{50}=1667\mu\text{g/kg}$ in sediment

RMS comment: Doesn't agree with the above argumentation. However, for all the reasons expressed in B.9.2.14, no risk assessment on aquatic rooted plant is required.

B.9.2.19. RMS conclusion

The risk assessments for aquatic organisms (fish, aquatic invertebrates, algae and aquatic plants) were conducted in accordance to SANCO/3268/2001 rev. 4 final (Guidance Document on Aquatic Ecotoxicology).

For agricultural uses (pome fruit, citrus, olives and vineyard) and non-crop areas (grass), the initial PEC_{sw} calculations provided were not considered acceptable. Additional calculations were asked to the applicant, but the new calculations provided still did not address RMS' request and therefore could not be accepted. The applicant finally provided additional calculations at the end of December 2012. However, since it was too late in the process, they were not taken into account. (see vol. 3 B.8)

Therefore, risk assessment for aquatic organism cannot be finalized.

For impermeable non-cropped areas (uses on road and railways), PEC_{sw} were calculated in vol. 3.B.8 according to HardSpec. The risk assessment for the active substance and the metabolites was conducted with the HardSpec PEC_{sw}.

For uses on railway, TER calculated for the active substance and the metabolite are higher than the trigger values for all aquatic organisms but the long term risk for fish could not be finalized.

The literature review report brings some information about the mechanism of action of amitrole in fish metabolism and confirms thyroid disruption. Amitrole did not block the production of THs in rare minnow but alterations of Thyroid Hormones-related genes expressions in liver and brain were noted at 10 to 10000 ng/L. The chronic test on juvenile fish (NOEC=100 mg a.s/L) is not relevant for assessing potential effects on development and reproduction due to endocrine disrupting properties. These potential consequences on development and reproduction *in vivo* should have been addressed with the fish full life-cycle test in fish (Medaka). Available data from this new study were not reliable to conclude on the potential impact of amitrole on hatchability of eggs in the F1 generation (from parental exposure). The notifier informed the RMS late in december 2012 that a new experimental phase is being conducted in order to conclude on potential effects on hatchability of eggs.

Moreover, no data were available to conclude on potential impact in the F2 generation. In these conditions, it was concluded that data were not enough to address completely the question of thyroid mediated effects during long term exposures. No endpoint (NOEC) can be derived from this study. Therefore, as there is an uncertainty concerning potential effects on development and reproduction, long term risk assessment for fish cannot be finalized

For uses on road, long term risk assessment for fish cannot be finalized for the same reasons.

No risk assessment is required for sediment dwelling organisms and aquatic rooted plants as amitrole is not expected to adsorb strongly on sediment. Moreover, it can be considered that toxicity data from *daphnia* and *Lemna* are sufficient to cover other aquatic invertebrates as sediment dwelling organisms and other aquatic plant as aquatic rooted plant, respectively.

Concerning other organisms, TER calculated for the active substance and the metabolite are higher than the trigger values for fish and gastropod molluscs for acute exposure. For *daphnia*, aquatic crustaceans, algae and aquatic plants, TER are lower than the trigger values for some scenarios (or for all scenario depending on the species).

Acute risk assessment for *Daphnia* and *Mysidopsis bahia* is refined based on the hypothesis of the possible renewal of populations following degradation of the active substance few days after application of the product compensating the mortality at initial predicted surface water concentrations. As endpoints are based on acute effects, the risk assessment might only be based on initial exposure, and should not take into account a possible degradation of the active substance. Acute risk assessment based on mortality has therefore to be conducted based on initial predicted surface water concentrations. Considering these comments, a new refined risk assessment has been asked. The risk assessment for the urban, major road and domestic streams scenario has not been further refined and should be considered at member state level.

Long term risk assessment for *Daphnia* is refined based on the same hypothesis of the possible renewal of populations following degradation of the active substance. Long term risk assessment has to be conducted based on initial predicted surface water concentrations as degradation of the active substance in surface water may not be appropriate for use with endocrine disrupting compound. Moreover, even if the NOEC of 320 µg a.s/L is based on effects on growth at the LOEC of 560 µg a.s/L and therefore risk assessment could eventually be refined with PECTwa (twa duration depending on the time-to-onset-of-effects), effects on reproduction have been observed in this study at the LOEC of 1000 µg a.s/L concluding to a NOEC reproduction of 560 µg a.s/L that would be used with initial predicted surface water concentrations. Considering these comments, a new refined risk assessment has been asked. The risk assessment for the urban, major road and domestic streams scenario has not been further refined and should be considered at member state level.

Risk assessment for *Algae* and *Lemna gibba* is refined based on the same hypothesis of the possible renewal of populations following degradation of the active substance few days after application of the product compensating the inhibition of growth at initial predicted surface water concentrations. As endpoints are based on growth effects and considering that the active substance was stable in the tests, risk assessment could be refined on twa exposure (twa duration depending on the time-to-onset-of-effects) but it hasn't been done as no PECTwa are available in vol 3 B.8. Otherwise a higher tier *Selenastrum capricornutum* and *Lemna* studies could be conducted in order to mimic a realistic worst-case exposure profile based on the proposed use of amitrole and PEC surface water modelling. The aim of the study would be to assess the effects under a more realistic exposure regime and also demonstrate recovery of *Selenastrum capricornutum* and *Lemna* for initial concentrations (able to cover those expected from modelling) to conclude that any effect on *Selenastrum capricornutum* and *Lemna* will be reversible and populations of algae and aquatic plants have potential for recovery. The submission of these studies or further refinement should be considered at member state level.

B.9.3. EFFECT ON OTHER TERRESTRAL VERTEBRATES (ANNEX IIIA 10.3)

B.9.3.1. Toxicity endpoints

The relevant toxicity data for mammals are summarised in the table below.

Table 9.3.1.1: Toxicity data (nominal value)

Test substance	Study	Species	LD ₅₀	NOAEL	Reference
Amitrole	Acute oral	Rat	5000 mg a.s./kg b.w.	-	review report 6839/VI/97-final, 22/03/2001
Amitrole	Multi-generations study	Rat	-	0.9 mg a.s/kg b.w./d	review report 6839/VI/97-final, 22/03/2001

B.9.3.2. Risk assessment for mammals

According to EFSA Journal (2009), only acute and long-term risk assessment modules are considered for the use of sprayed products. Therefore, no assessment is required.

B.9.3.2.1. Calculations of acute DDD and TER for mammals

Mammals may be exposed to amitrole mainly by the consumption of contaminated feed. The evaluation of the risk for mammals was performed in accordance with EFSA guidance document (2009)⁶.

Screening assessment

The first step in the assessment, according to the EFSA Journal (2009) is the screening assessment. The daily dietary dose (DDD) was calculated with the following formula:

$$\text{DDD} = \text{application rate (kg/ha)} \times \text{shortcut value}$$

With:

- Shortcut value = default parameter combining food intake rate, body weight, concentration of the substance in the diet (based on the 90th percentile residues) and the fraction of diet obtained in the treated area for the mammals indicator species/crop combination in question.
- Since **CA2735** will be applied once per season, the MAF (multiple application factor) was not included in the calculations. The TER value was calculated by dividing the acute endpoint (LD₅₀(mix)) by the daily dietary dose (DDD).

Table 9.3.2.1.1: Acute toxicity screening for mammals exposed to CA2735

Crop	Application rate	Indicator species	Shortcut value	DDD ¹	LD ₅₀ [mg/kg b.w.]	TER ² (10)
Orchards	0.993 kg a.s./ha	Small herbivorous mammal	136.4	121.4	5000	41
Vineyards						

¹ daily dietary dose

² Toxicity-to-exposure ratio (Trigger)

The screening step after application of **CA2735** in orchard and vineyard indicates no acute risk for mammals (the TER_A value is above the trigger of 10). Therefore, no Tier 1 risk assessment is required.

Therefore, if **CA2735** is used according to the manufacturer recommendations, it does not represent any significant acute risk to small mammals.

B.9.3.2.1. Calculations of long term DDD and TER for mammals**Screening assessment**

The first step in the assessment, according to the EFSA Journal (2009) is the screening assessment. The daily dietary dose (DDD) was calculated with the following formula:

$$\text{DDD} = \text{application rate (kg/ha)} \times \text{shortcut value} \times \text{twa} \times \text{MAF}$$

With:

- Shortcut value = default parameter combining food intake rate, body weight, concentration of the substance in the diet (based on the mean residues) and the fraction of diet obtained in the treated area for the mammals indicator species/crop combination in question.
- twa = time-weighted average factor.
- MAF = multiple application factor.

⁶ European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

Since CA2735 will be applied once per season, the MAF (multiple application factor) was not included in the calculations. The TER values were calculated by dividing the endpoint (lowest NOEL/NOEC from the reproduction study) by the daily dietary dose (DDD).

Table 9.3.2.1.1: Long-term screening for mammals according to EFSA Journal (2009)

Crop (Scenario)	Application rate	Indicator species	twa	Shortcut value ¹	DDD ²	NOEL [mg a.s./kg b.w./d]	TER ³ (5)
Orchard	0.993 kg a.s./ha	Small herbivorous mammal	0.53	72.3	38.05	0.9	0.02
Vineyard							

¹ mean percentile residues

³ Toxicity-to-exposure ratio (Trigger)

² daily dietary dose

The TER value for Amitrole are below the trigger of 5 and therefore, the chronic risk to mammals is to be refined.

Tier 1 risk assessment

For refining the risk, the generic focal mammal species were considered. The risk assessment is presented in table 9.3.2.1.2.

Table 9.3.2.1.2: Reproductive risk assessment based on generic focal mammal species (application rate 0.993 kg a.s./ha)

Crop	Generic focal bird species	Shortcut value ¹	ftwa ²	DDD ³	NOEC/NOEL [mg a.s./kg b.w./day]	TER ⁴ (5)
Orchard	Small insectivorous mammal "shrew"	1.9	0.53	1	0.9	0.9
	Small herbivorous mammal "vole"	72.3		34.1		0.03
	Large herbivorous mammal "lagomorph"	14.3		11.32		0.1
	Wood mouse (<i>Apodemus sylvaticus</i>): 25% weeds / 50% weed seeds / 25% ground arthropods	7.8		3.68		0.2
	Frugivorous mammals Dormouse*	22.7		9		0.08
Vineyard	Large herbivorous mammal "lagomorph"	11.1	0.53	5.24	0.9	0.2
	Small herbivorous mammal "vole"	72.3		34.1		0.03
	Small insectivorous mammal "shrew"	4.2		2.25		0.4
	Wood mouse (<i>Apodemus sylvaticus</i>): 25% weeds / 50% weed seeds / 25% ground arthropods	7.8		3.68		0.2

¹ Mean percentile residues

³ Daily dietary dose

² Time-weighted average factor

⁴ Toxicity-to-exposure ratio (Trigger)

* Amitrole is applied from BBCH 94 to 75 (so it is not applied during the fruiting or ripening period). In addition in residue trials no amitrole has ever been retrieved in fruits at t=0 even when applied at 4800 gai/ha at BBCH 72-77. Therefore, the scenario "Frugivorous mammals Dormouse" is not relevant.

A higher tier (based on focal species) is needed. Such an evaluation is presented below.

Higher Tier risk assessment

✓ Vineyard

If an active substance, and its associated product and use, fails Tier 1, it is possible to further refine the exposure element of risk via the use of a ‘focal species’.

Report:	KIIIA 10.3.1.4/01, [REDACTED] 2007
Title:	Generic field monitoring of mammals in vineyards in France
Document No:	[REDACTED]
Guidelines:	Not applicable; the test was especially designed for the purpose of this study.
GLP:	Yes

Executive Summary

The objectives of this study were i) to determine the species composition and abundance of small mammals in vineyards compared to other habitats and to define the focal species, ii) to determine the proportion of diet obtained in vineyards (PT estimate) by radiotracking of individual wood mice (the focal species) based on the assumption that the time a small mammal spends ‘active’ or, more specifically, foraging in a habitat, is a reliable measure of the proportion of food obtained in this defined area and iii), to determine the proportion of different food types in the diet by analysing stomach contents.

The study was conducted in the Burgundy region around the municipality of Santenay (France), a typical wine growing district, and encompassed a study area of 487 ha. The study was carried out during spring and summer 2006. Small mammals were trapped in four vineyards and the adjacent surroundings to identify the species present and to define the focal species. Twenty individuals of the focal species, the wood mouse (*Apodemus sylvaticus*), were radio-tracked over their whole activity period (from dusk till dawn). From the radio-tracking data, the proportion of time potentially foraging was compared for vineyards and all habitats in the individuals home range. To estimate the proportion of different food types in the diet, stomach contents of wood mice were sampled and analysed. For each sample the proportions of different food items contributing to the volume of the diet samples were estimated.

The results of trapping indicate that the wood mouse (*Apodemus sylvaticus*) is the only species observed to regularly use vineyards and as such can be considered to be the focal species. Besides a few exceptions, yellow necked mice (*Apodemus flavicollis*) and bank voles (*Clethrionomys glareolus*) were confined to hedgerows and woodland only. There was no shrew and only four captures of common vole (*Microtus arvalis*) in the vineyard out of 7680 trap nights. Live trapping revealed that the surrounding habitats were rather more attractive to wood mice than vineyards (captures/100 trapnights: Surroundings 20.55, vineyard 3.75).

Based on the study results, the mean PT value derived for wood mice living within or in close vicinity to vineyards in spring/summer was 0.14 (90th percentile = 0.41). It is important to emphasise that these values represent the estimated proportion of time spent ‘potentially foraging’ in vineyards. All values of ‘time potentially foraging’ include times when foraging was not evident but could not be precluded. The calculated PT value can therefore be regarded as a conservative estimate. The calculated mean Jacob’s Index was -0.83, implies that vineyards were selected to a lesser extent for foraging than would be expected at random from the proportion of this habitat available within their home range.

The stomach samples of wood mice were mainly composed of plant material. Five out of nine samples were exclusively made up of plant matter. Among the plant matter found in the diet samples, seeds were most numerous in the diet of wood mice.

Of all small mammals, only the wood mouse (*Apodemus sylvaticus*) was found to be in significant numbers in vineyards. Other species were essentially confined to the surrounding, largely woodland habitat. The wood mouse is the dominant species in vineyards.

Live trapping revealed that the surrounding habitats were more attractive for this species than vineyards. Radio-tracked wood mice spent only a small proportion of time foraging in vineyards (time potentially foraging). From a distribution generated from 20 individuals, the PT estimates for 50th and 90th percentiles were 0.15 and 0.41, respectively. Wood mice avoid vineyard habitats, preferring surrounding habitats and vineyards are of marginal importance as feeding habitat for wood mice in spring and summer. The main components of all sampled stomachs were seeds (80.1%) and fruits (14.0%).

I. MATERIALS AND METHODS

1. In life dates: 03/03 – 07/10/2006

2. Experimental design

The study was conducted in and around four different vineyards in the municipality of Santenay, Burgundy, in the eastern part of France. This region is an agrarian landscape characterised by a high number of vineyards, where the spindle-bush cropping system is used. This is the typical system for wine growing in central and southern Europe. The four study vineyards were selected to provide field sizes and a range of edge habitats typical of the region and suitable for the intended study methods.

All small mammals, which occur under natural conditions in vineyards and the adjacent surrounding structure, with special attention being given the most abundant (focal species). Prior information and expectations suggested this was likely to be the wood mouse (*Apodemus sylvaticus*). To assure the availability of vineyards within the home range of the tracked individuals, the mammals were trapped in or in the vicinities of vineyards.

The PT value is the proportion of diet obtained in a treated area. In most cases data to calculate PT values are difficult to obtain directly from field observations. However, it can be assumed that the time a small mammal spends foraging or being active in a certain habitat is a conservative indication of the proportion of diet obtained in a treated area and can thus be used to calculate PT estimates (Finch & Payne, 2006). Therefore PT values were calculated from data obtained on the time periods the mammal spent being active or, more specifically, spent foraging as detected by radio-tracking.

Diet sample contents reflect the proportion of different food types in the diet sample. The proportion (volume) of different food categories was determined by analysing stomach contents of wood mice.

3. Observations:

Live trapping of small mammals with individual marking and subsequent recapture was conducted on four study plots over the whole study period to identify species that might use vineyards as part of their natural home range. In each field a rectangular trapping grid of 64 'Ugglar' multiple capture live traps was installed in a suitable part of the field (adjacent to a habitat with potentially high mammal abundance, such as woodland, shrubs or set-aside areas).

Two lines of eight traps were placed in this woodland habitat and six lines in the vineyard. On one plot, due to the shape of the vineyard, one row of 16 traps covered the surroundings, and three rows with 16 traps each were set up in the vineyard. The total area of the grid was 0.64 ha with an inter-trap spacing of 10 m and a margin of 5 m around the outermost traps. Regular trapping was performed on each study plot on two consecutive trap nights every week.

During the study, suitable adult individuals of the dominant species (wood mouse) caught in the live traps were equipped with radio-collars. Animals were regarded as suitable if the weight of the collar did not exceed 10% of the animal's body mass. During tracking sessions unidirectional Yagi-antennas allowed the determination of the direction of individuals. The signal strength enabled an estimation of the distance from the wood mouse. Each recognisable change in behaviour or location (habitat) together with time and GPS position was recorded. In addition, GPS position was taken normally every 10 to 15 minutes, even if no change was noticed. These data allowed calculation of time budgets (PT), spatial use (proportions of different habitat types, size of the total home range) and habitat preferences (Jacobs' Indices, Jacobs, 1974) on that day. Since the actual behaviour of the tracked animal can only rarely be defined, the PT values refer to the 'time potentially foraging', i.e. the time when an individual is active and the behaviour 'foraging' cannot be proven.

The actual food choice of wood mice in vineyards was based on investigations of stomach contents of individuals foraging in vineyards. To obtain the stomach samples, snap traps were set within the vineyard at night and checked early in the morning throughout the study period. In order to avoid trapping of radio-tagged mice the traps were set in the field as far away as possible from the trapping grid and no snap trapping took place on study fields where a mouse was already radio-tagged but not radio-tracked yet. Prepared stomachs were conserved in alcohol. The investigation of the food items was done via microscopic analysis.

II. RESULTS AND DISCUSSION

The wood mouse (*Apodemus sylvaticus*) was the dominant species trapped on all four study plots. They were captured both in the surrounding habitat and in the vineyards on all plots. A small proportion of yellow necked mice were trapped in vineyards. With the exception of a single capture, no vole was trapped in vineyards.

The main results are presented in table 10.3.1.4-1. The trapping effort was three times higher in vineyards than in the surrounding areas, due to the greater numbers of traps used. Total trapping effort (all plots) amounted to 10240 trap nights. This resulted in a total of 1216 captures (185 marked individuals) of four species (*Apodemus sylvaticus*, *Apodemus flavicollis*, *Microtus arvalis*, *Clethrionomys glareolus*). In order to get comparable results for different habitats the number of captures was related to trap catches per 100 trap nights. The highest trapping rate was obtained with wood mice in traps set up in the surrounding vegetation (20.55 captures per 100 trapnights). In the vineyard, the mean trapping rate of wood mice was lower with 3.75 captures/100 trapnights. Thus the trapping rate in the surroundings was more than five times higher than in the vineyards. In addition to trapped wood mice four common voles were trapped on plot 2 with a mean trapping rate in the vineyard [captures/100 trapnights] of 0.05. Twelve yellow-necked mice were caught, three on plot 1 and nine on plot 3. Mean trapping rate for yellow necked mouse in the vineyard [captures/100 trapnights] was 0.16.

Table 10.3.1.4-1: PT of 20 radiotracked mammals/species

Indicidual n°	PT in vineyards
1	0.01
2	0.05
3	0.21
4	0.00
5	0.41
6	0.25
7	0.00
8	0.27
9	0.04
10	0.07
11	0.41
12	0.12
13	0.02
14	0.01
15	0.04
16	0.12
17	0.28
18	0.44
19	0.01
20	0.13
50th*	0.12
90th*	0.41
Mean**	0.19

*excluding the 0 values from the calculation

**excluding the 0 and 0.01 values from the calculation

Table 10.3.1.4-2: Overview of small mammal abundance, PT and diet analysis

Relevant species in the vineyard (based on live-trapping)		
Species	Mean trapping rate (captures/100 trapnights)	
	vineyard (based on 7680 trapnights)	surrounding (based on 250 trapnights)
<i>Apodemus sylvaticus</i>	3.75	20.55
<i>Apodemus flavicollis</i>	0.16	6.99
<i>Microtus arvalis</i>	0.05	0.00
<i>Clethrionomys glareolus</i>	0.00	8.09
Shrews (<i>Crocidura</i> sp., <i>Sorex</i> sp)	0.00	0.00
Proportion of diet obtained in vineyards determined by radio-tracking (PT)		
Potential foraging time in vineyards as a proportion of the total potential foraging time equals the proportion of diet obtained	based on 20 radio-tracking sessions of 20 wood mouse individuals	
50%tile	0.12	
90%tile	0.41	
mean	0.19	
Proportion of different food types in the diet		
Arthropods and plant items actually eaten by 20 individuals foraging in and around vineyards (mean portion)	based on stomach samples of 20 wood mouse individuals	
Animal matter	3%	
Plant matter	97% (fruits 14.0%; weed seeds 80.1%; others 2.9%)	

Time intervals between each trapping session/population census were similar. Data for all regular trapping session were included in the calculation of the Minimum Number Alive (MNA) for a conservative estimate of population size.

Wood mice were present on all study plots but the abundance varied. MNA on all plots increased at the beginning and declined at the end of the trapping period. This species was most abundant on plot 1, where by the end of March the MNA maximum estimate was 24 individuals. The MNA on study plot 2 reached a maximum of 15 individuals at the end of March, and on study plot 3 with 14 individuals in the middle of May. Only a relatively low number of wood mice were captured on study plot 4 (MNA maximum of 7 individuals). This corresponds to a density of 0 and 37.5 individuals/ha.

The yellow-necked mouse, which is closely related to the wood mouse, was only captured on study plots 1, 2 (one individual) and 3. This species showed varying MNAs with a maximum of 8 individuals on plots 1 and 3, respectively. In contrast to the wood mouse the yellow necked mouse was captured almost exclusively in the traps in the wooded surrounding habitat.

Bank voles were only captured on study plot 2, where they were regularly trapped over the study period. This species showed a maximum MNA at the beginning and in the middle of June with 12 individuals. Bank voles were captured solely in the traps located in the wooded surrounding habitat.

The radio-tracking data of 20 wood mice (7 females, 13 males) were used to calculate home range sizes and the portion of time individuals spent potentially foraging in vineyards and surrounding habitats. Each individual wood mouse was continuously tracked during the whole activity period (from dusk till dawn).

The PT of the wood mouse in vineyards was calculated from the proportion of time spent 'potentially foraging' in vineyards during 20 tracking sessions of 20 individuals. The PT ranged from 0 to 0.44. The mean, median and 90th percentiles were 0.14, 0.09 and 0.41, respectively.

III.

CONCLUSIONS

Of all small mammals, only the wood mouse (*Apodemus sylvaticus*) was found to be in significant numbers in vineyards. Other species were essentially confined to the surrounding, largely woodland habitat. The wood mouse is the dominant species in vineyards. Live trapping revealed that the surrounding habitats were more attractive for this species than vineyards. Radio-tracked wood mice spent only a small proportion of time foraging in vineyards (time potentially foraging). From a distribution generated from 20 individuals, the PT estimates for 50th and 90th percentiles were 0.15 and 0.41, respectively. Wood mice avoid vineyard habitats, preferring surrounding habitats and vineyards are of marginal importance as feeding habitat for wood mice in spring and summer. The main components of all sampled stomachs were seeds (80.1%) and fruits (14.0%).

2007)

RMS comment: Study acceptable. Agree with the choice of focal species.

The study followed the wood mouse (*Apodemus sylvaticus*). Individual PT values of 0 have been excluded for the calculation of the mean and 90th percentile PT value that is relevant and conservative. The mean PT value of 0.14 is considered to be too low for a focal species. In addition, even if the 5 of the 20 wood mice radio-tracked (2 with a PT value of 0 and 3 with a PT value of 0.01) have been excluded, the mean PT value of 0.19 is once again considered to be low for a focal species. Then 90th percentile PT value of 0.41 is more relevant for the risk assessment.

The proportion of different food types in the diet is not based on stomach samples of 20 wood mice. Indeed the content of only ten stomach samples has been analysed, with one empty. The PD estimate is then based on nine samples, and no information is given on the season of the trapping of each individual (trapping dates ranged between the first of March and the 31 of July). Therefore the PD estimate based on low sample size is poorly reliable and the diet can not be interpreted in light of season changes. This dietary regime is considered to be not acceptable. Then, the omnivorous standard dietary regime estimated for the wood mouse (25% weeds/50% weed seeds/25% ground arthropods) according to appendix A of EFSA 2009 guidance document will be used.

In this study PT values for wood mice in vineyards were derived from the portion of their time spent foraging in vineyards and not the actually proportion of time of ascertained foraging *i.e.*, active food uptake. The selected trapping scheme can be regarded as a "worst case" choice in terms of a high probability for the wood mice tracked to use vineyards during their daily activity period. Following the conservative worst case approach, the times of unspecified behaviour without visual contact were considered as foraging times.

Hence, the calculated PT values can be regarded as a conservative assumption and they illustrate the highest possible portion of time the individual wood mouse spent foraging in vineyards.

Based on the data obtained in the studies presented above, a new risk assessment was performed taking into account the PT value (Städler T. 2007). The risk assessment was done on the following species taking into consideration the following parameters. (table 9.3.2.1.3).

Table 9.3.2.1.3: Characteristics of Focal mammal Species in Vineyards. Determination of Parameters for long-term Risk Assessment

Species	Wood mouse
Body Weight (g) ¹	21.7
Stratum	Ground
Diet Guild	omnivorous
FIR _{diet} / BW	0.27
90 th percentile value ²	0.41
AV	1

¹ Source: EFSA 2009 guidance document

² Städtler. 2007. Generic field monitoring of mammals in vineyards in France, PT values are used for Long Term Risk Assessment

Refined risk assessment

a) Refined PT

The refined chronic risk assessment is presented on in table 9.3.2.1.4. It is based on the default FIR/b.w., the PT experimentally determined as well as on the default PD values, RUD and ftwa (EFSA, 2009).

Table 9.3.2.1.4: Refined long-term mammal Risk Assessment for Amitrole in Vineyards based on the maximum dose rate, 13 L/ha corresponding to 0.993 kg a.s./ha (application under the row).

Species	Size & Diet Guild	FIR/BW	kg as/ha	AV	PT	RUD	ftWA	PD1	PD2	DDD*	TER (5)
Wood mouse (focal species)	Omnivorous mammal	0.27	0.993	1	0.41	29.2	0.53	0.25	0.75	1.7	0.5
Vole (generic focal species)	small herbivorous mammal	1.33	0.993	1	1	54.2	0.53	-	1	37.94	0.02
Lagomorph (generic focal species)	large herbivorous mammal	0.39	0.993	1	1	28.7	0.53	-	1	5.89	0.2
Shrew (generic focal species)	Small insectivorous mammal	0.55	0.993	1	1	7.5	0.53	1	-	2.17	0.4

* DDD = FIRfield/bw x application rate x PT x RUD x ((ftwa x PD1) + (ftwa x PD2))

On the basis of a NOEL of 0.9 mg/kg b.w. in rats, the risk is not acceptable and therefore a refinement based on more realistic RUD value is needed.

A study for determining the residue level in plants and vertebrates contaminated by a CA2735 application according to the GAP was conducted in orchard in Germany. The application rate (13 L/ha) and the way of applying CA2735 (application under the row corresponding to 1/3 of the max. dose rate) are the same ones in orchard and vineyard.

Therefore, it can be assumed that the residue levels measured in plants and arthropods in orchard will not be significantly different from the potential concentrations obtained in the same plants and invertebrates in vineyard. The study was summarized in point 10.1.2.1 (KIIIA 10.1.2.1/03, Knäbe, S. 2009 and KIIIA 10.1.2.1/04, Mende, P. 2009).

a) Refined RUD

According to the working document EFSA, 2009 Guidance document for birds and mammals risk assessments, the long-term risk to mammals can be assessed on the basis of maximum residue concentrations in food items. The previous evaluation was therefore refined taking into consideration the maximum residue concentrations determined in a study conducted under realistic conditions. The different scenarios considered are presented in table 9.3.2.1.5.

Table 9.3.2.1.5: Different scenarios considered in vineyards.

	Residue_{MAX}	Wood mouse
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Diet Composition & Portion of Diet	Scenario 1	Scenario 2	Scenario 3	PD
Animal matter (RUD 1)	0.61 ^a	3.09 ^b	0.67 ^c	0.25 (PD 1)
Plant matter (RUD 2)	0.08 ^d	0.08 ^d	0.08 ^d	0.75 (PD 2)

^a Soil Dwelling Arthropods (Vortis sampling) (Knäbe, S. 2009)^b Crop & Soil dwelling Arthropods (Earwig traps) (Knäbe, S. 2009)^c Soil dwelling Arthropods (Pitfall traps) (Knäbe, S. 2009).^d Short vegetation (Knäbe, S. 2009)

The refined chronic risk assessment is presented in tables 9.3.2.1.6, 9.3.2.1.7 and 9.3.2.1.8. It is based on the Tier 1 standard FIR/b.w and PD values, and PT (90th percentile) experimentally determined as well as maximum field residue concentrations.

Table 9.3.2.1.6: Refined long-term mammal Risk Assessment for Amitrole in vineyards (scenario 1: soil-dwelling arthropods and short vegetation) based on the maximum dose rate, 13 L/ha corresponding to 0.89 kg a.s./ha (application under the row).

Focal Species	Size & Diet Guild	FIR/BW	AV	PT	RUD 1	PD1	RUD 2	PD2	ftWA	DDD	TER (5)
Wood mouse (focal species)	Omnivorous mammal	0.27		0.41	0.61	0.25	0.08	0.75	0.53	0.0125	72.2
Vole (generic focal species)	small herbivorous mammal	0.33	1	1	0.61	-	0.08	1	0.53	0.0564	16.0
Lagomorph (generic focal species)	large herbivorous mammal	0.39	1	1	0.61	-	0.08	1	0.53	0.0164	54.4
Shrew (generic focal species)	Small insectivorous mammal	0.55	1	1	0.61	1	0.08	-	0.53	0.1778	5.1

*DDD = FIRfield/bw x PT x ((RUD 1 x PD 1) + (RUD 2 x PD 2)) x ftwa

Table 9.3.2.1.7: Refined long-term mammal Risk Assessment for Amitrole in orchards (scenario 2: crop- and soil-dwelling arthropods [earwig traps] + short vegetation) based on the maximum dose rate, 13 L/ha corresponding to 0.89 kg a.s./ha (application under the row).

Species	Size & Diet Guild	FIR/BW	AV	PT	RUD 1	PD1	RUD 2	PD2	ftWA	DDD*	TER (5)
Wood mouse (focal species)	Omnivorous mammal	0.27	1	0.41	3.09	0.25	0.08	0.75	0.53	0.0489	18.4

Vole (generic focal species)	small herbivorous mammal	1.33	1	1	3.09	-	0.08	1	0.53	0.0564	16.0
Lagomorph (generic focal species)	large herbivorous mammal	0.39	1	1	3.09	-	0.08	1	0.53	0.0164	54.4
Shrew (generic focal species)	Small insectivorous mammal	0.55	1	1	3.09	1	0.08	-	0.53	0.901	1.0

*DDD = FIRfield/bw x PT x ((RUD 1 x PD 1) + (RUD 2 x PD 2)) x ftwa

Table 9.3.2.1.8: Refined long-term mammal Risk Assessment for Amitrole in orchards (scenario 3: soil-dwelling arthropods [pitfall traps] + short vegetation) based on the maximum dose rate, 13 L/ha corresponding to 0.89 kg a.s./ha (application under the row).

Species	Size & Diet Guild	FIR/BW	kg as/ha	AV	PT	RUD 1	PD1	RUD 2	PD2	ftWA	DDD*	TER (5)
Wood mouse (focal species)	Omnivorous mammal	0.27	0.993	1	0.41	0.67	0.25	0.08	0.75	0.53	0.0133	67.9
Vole (generic focal species)	small herbivorous mammal	1.33	0.993	1	1	0.67	-	0.08	1	0.53	0.0564	16.0
Lagomorph (generic focal species)	large herbivorous mammal	0.39	0.993	1	1	0.67	-	0.08	1	0.53	0.0164	54.4
Shrew (generic focal species)	Small insectivorous mammal	0.55	0.0993	1	1	0.67	1	0.08	-	0.53	0.194	4.6

*DDD = FIRfield/bw x PT x ((RUD 1 x PD 1) + (RUD 2 x PD 2)) x ftwa

RMS comment: Based on the lowest NOEL (0.91 mg/kg b.w.) in rats, the TER range from 18.4 to 72.2 for the focal species (wood mouse) when the default value from the EFSA guidance document for FIR/bw, and 90th percentile of PT and max residue concentration determined in the field are considered. Therefore, risk is acceptable for the focal species determined experimentally (Wood mouse).

For the additional generic focal species (Vole, Lagomorph and shrew listed in the EFSA guidance document) the risk is acceptable for lagomorphs and vole for all scenarios but not acceptable for shrew for scenario 2 and 3. However for these species all the other parameters (PT and PD) used in the calculation are default parameters.

Therefore, when CA2735 is used according to the good Agricultural Practice, it is not expected to present any significant long-term risk to herbivorous and omnivorous mammals foraging in vineyards. However, long term risk for insectivorous mammals should be refined at national level (level 4)

✓ Orchard

If an active substance, and its associated product and use, fails Tier 1, it is possible to further refine the exposure element of risk via the use of a 'focal species'.

Report:	KIIIA 10.3.1.5/01, v. [REDACTED] 2006
Title:	Small mammal monitoring in modern pome orchards of Baden-Württemberg and Thuringia
Document No:	[REDACTED]
Guidelines:	Gurnell and Flowerdew (1990)

GLP:	Yes
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WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

Executive Summary

A capture-mark-recapture study was conducted in two typical pome growing regions in Germany, Thuringia (administrative district Gotha) and Baden-Württemberg (administrative district Bodenseekreis). For each region, three study plots were investigated where the trapping grids were set up in modern orchards and adjacent traditional meadows and one control plot where the trapping grid was only set up in modern orchard. Trapping was conducted from mid March until the end of August. Each plot was equipped with traps at least for two nights every two weeks.

The most abundant species in modern pome orchards was found to be the common vole. Apart from the common vole, the yellow-necked mouse was also captured within modern pome orchards but to a far smaller extent. All other species were captured only occasionally.

The common vole's colonisation of traditional meadows and modern pome orchards did not seem to depend primarily on the different habitat type but rather on ground vegetation height. Populations decreased when the ground vegetation was mowed, mulched or pastured in both habitat types. In undisturbed traditional meadow habitats recapture rates and the rate of non-adult individuals were in general higher which indicates a stable population and reproduction. High ground vegetation provides better protection against predators and shelter. Therefore, traditional meadows with high ground vegetation were probably preferred when spring reproduction started. Regular mulched modern pome orchards provide less protection against avian predators and consequently less shelter. Therefore, they were probably not very attractive in spring and only used on a small scale. In summer when primary habitats like traditional meadows were well occupied the modern pome orchards were used as secondary habitat to a larger extent – including reproduction.

Common vole populations seem to depend on the presence of primary habitats. Hence, common vole populations in agricultural landscape characterized by modern pome orchards are probably not at risk by plant protection products because the population resources are located mainly in primary habitats like e.g. meadows.

I. MATERIALS AND METHODS

1. In life dates: 14/03 – 25/08/2005

2. Experimental design

A capture-mark-recapture study was conducted in two typical pome growing regions in Germany, Thuringia (administrative district Gotha) and Baden-Württemberg (administrative district Bodenseekreis). For each region, three study plots were investigated where the trapping grids were set up in modern orchards and adjacent traditional meadows and one control plot where the trapping grid was only set up in modern orchard. Trapping was conducted from mid March until the end of August. Each plot was equipped with traps at least for two nights every two weeks.

In each region three study plots and one control plot were selected. On each plot a trapping grid of 100 traps was set up. In the study plots 50 traps were placed at every second tree row of the modern orchard with a trap distance of 10 m. In the adjacent meadow 50 traps were set up with the same distance as has been done in the orchard. In the control plots 100 traps were placed at every second tree row with a distance of 10 m. Due to logistic reasons only 60 traps, 30 in modern orchard and 30 in traditional meadow, were built up and analysed on study plot 2 (Baden-Württemberg).

3. Observations:

The trapping was conducted with multicapture Ugglan special mouse traps no.2 (Grahnb, Sweden1) specially designed for small mammals and in the meadow by DeuFa1 single capture traps which avoid intrusion of predators due to a different closing device.

The traps were built up every second week. On each plot a trapping session lasted for two nights. The traps were checked at sunrise and sunset, at least every 12 hours. After the trapping sessions the traps were collected from the plots.

Animals captured the first time with a body weight ≥ 10 g were anaesthetized with ether and individually marked with a passive integrated transponder (PIT, 12 x 2.12 mm, 0.11 g, Torvan2/Alvic3), which was subcutaneously injected with an aseptic syringe. For each trapped individual, the date, PIT number, trap location, species, sex, weight, and reproduction status was recorded. Captured shrews were recorded but not marked or examined due to requirements of the authorities.

Vegetation height of the herb layer was estimated by using a metering rule at every trap check.

II. RESULTS AND DISCUSSION

The most abundant species in modern pome orchards was found to be the common vole. Apart from the common vole, the yellow-necked mouse was also captured within modern pome orchards but to a far smaller extent. All other species were captured only occasionally. The common vole's colonisation of traditional meadows and modern pome orchards did not seem to depend primarily on the different habitat type but rather on ground vegetation height.

Populations decreased when the ground vegetation was mowed, mulched or pastured in both habitat types. In undisturbed traditional meadow habitats recapture rates and the rate of non-adult individuals were in general higher which indicates a stable population and reproduction. High ground vegetation provides better protection against predators and shelter. Therefore, traditional meadows with high ground vegetation were probably preferred when spring reproduction started.

Regular mulched modern pome orchards provide less protection against avian predators and consequently less shelter. Therefore, they were probably not very attractive in spring and only used on a small scale. In summer when primary habitats like traditional meadows were well occupied the modern pome orchards were used as secondary habitat to a larger extent – including reproduction.

Common vole populations seem to depend on the presence of primary habitats like e.g. meadows.

Species characteristics

Meadows, pastures and every type of set aside grassland are the preferred habitat of the common vole and therefore it is the most common species in these types of habitats. The common vole is a common and widespread microtine species in central Europe with seasonal and multi-annual variation in population density. The primary habitat is grassland and the secondary habitat is cultivated land. The population density reaches its peak in autumn, during winter the population decreases and reaches its minimum in spring. In case of low population densities the common vole lives solitary in territories of its own. In case of high population densities and scarce space, communal nest sides of mother and daughters can occur.

Female common voles born in spring and summer start to reproduce the same year and generally die after the breeding season during the winter. Females born at the end of the breeding season in autumn overwinter as subadults (sexually immature) and get sexually mature in spring. Populations in typical common vole environments have a 3 (2 - 4)-year multi-annual cycle. The density during the first year is very low, increases over the second year. Two preconditions may then lead to vole mass outbreaks: the high reproduction potential of the common vole and optimal environmental conditions like space, food, vegetation cover, and good climate conditions. After the mass outbreak, the population crashes.

III. CONCLUSIONS

The most abundant species in modern pome orchards was found to be the common vole. Apart from the common vole, the yellow-necked mouse was also captured within modern pome orchards but to a far smaller extent. All other species were captured only occasionally.

(2006)

RMS comment: Study acceptable. Agree with the choice of focal species. It can be noted that the common vole is the species used for screening TER calculation for Orchard scenario.

In this study PT and PD values for common vole in orchards were not determined. Therefore, the refined risk assessment was based on default values given in EFSA 2009 guidance document.

However, a study for determining the residue level in plants and vertebrates contaminated by a **CA2735** application according to the GAP was conducted in orchard in Germany (KIIIA 10.1.2.1/03, Knäbe, S. 2009 and KIIIA 10.1.2.1/04, Mende, P. 2009).

According to the guidance document from EFSA 2009, the long-term risk to mammals can be assessed on the basis of maximum residue concentrations in food items. The previous evaluation was therefore refined taking into consideration the maximum residue concentrations determined in a study conducted under realistic conditions. The different scenarios considered are presented in table 9.3.2.1.9.

Table 9.3.2.1.9: different scenarios considered in orchards.

Diet Composition & Portion of Diet	Residue _{MAX}			Vole
	Scenario 1	Scenario 2	Scenario 3	PD
Animal matter (RUD 1)	0.61 ^a	3.09 ^b	0.67 ^c	0.00 (PD 1)
Plant matter (RUD 2)	0.08 ^d	0.08 ^d	0.08 ^d	1.00 (PD 2)

^a Soil Dwelling Arthropods (Vortis sampling) (Knäbe, S. 2009)

^b Crop & Soil dwelling Arthropods (Earwig traps) (Knäbe, S. 2009)

^c Soil dwelling Arthropods (Pitfall traps) (Knäbe, S. 2009). Here the highest mean residue was chosen as a realistic estimate.

^d Short vegetation (Knäbe, S. 2009)

Vole is an herbivorous mammal feeding exclusively on grass so only the residue obtained on plants will be considered.

The refined chronic risk assessment is presented in tables 9.3.2.1.10, 9.3.2.1.11 and 9.3.2.1.12. It is based on the FIRfield/b.w., the PT and PD values experimentally determined as well as two mean residue and maximum residue concentrations.

Table 9.3.2.1.10 : Refined long-term mammal Risk Assessment for Amitrole in orchards (scenario 1: soil-dwelling arthropods and short vegetation) based on the maximum dose rate, 13 L/ha corresponding to 0.993 kg a.s./ha (application under the row).

Species	Size & Diet Guild	FIR/BW	AV	PT	RUD ₁	PD1	RUD ₂	PD2	ftwa	DDD*	TER (5)
Vole (focal species)	small herbivorous mammal	1.33	1	1	0.61	-	0.08	1	0.53	0.0564	16.0
Lagomorph (generic focal species)	large herbivorous mammal	0.5	1	1	0.61	-	0.08	1	0.53	0.0212	42.5
Wood mouse (generic focal species)	Omnivorous mammal	0.27	1	1	0.61	0.25	0.08	0.75	0.53	0.0304	29.6
Shrew (generic focal species)	Small insectivorous mammal	0.55	1	1	0.61	1	0.08	-	0.53	0.178	5.1

*DDD = FIRfield/bw x PT x ((RUD₁ x PD 1) + (RUD₂ x PD 2)) x ftwa

Table 9.3.2.1.11: Refined long-term mammal Risk Assessment for Amitrole in orchards (scenario 2: crop- and soil-dwelling arthropods [earwig traps] + short vegetation) based on the maximum dose rate, 13 L/ha corresponding to 0.993 kg a.s./ha (application under the row).

Species	Size & Diet Guild	FIR/BW	AV	PT	RUD 1	PD1	RUD 2	PD2	ftwa	DDD*	TER (5)
Vole (focal species)	small herbivorous mammal	1.33	1	1	3.09	-	0.08	1	0.53	0.0564	16.0
Lagomorph (generic focal species)	large herbivorous mammal	0.5	1	1	3.09	-	0.08	1	0.53	0.0212	42.5
Wood mouse (generic focal species)	Omnivorous mammal	0.27	1	1	3.09	0.25	0.08	0.75	0.53	0.119	7.6
Shrew (generic focal species)	Small insectivorous mammal	0.55	1	1	3.09	1	0.08	-	0.53	0.9007	1.0

*DDD = FIRfield/bw x PT x ((RUD 1 x PD 1) + (RUD 2 x PD 2)) x ftwa

Table 9.3.2.1.12: Refined long-term mammal Risk Assessment for Amitrole in orchards (scenario 3: soil-dwelling arthropods [pitfall traps] + short vegetation) based on the maximum dose rate, 13 L/ha corresponding to 0.993 kg a.s./ha (application under the row).

Species	Size & Diet Guild	FIR/BW	AV	PT	RUD 1	PD1	RUD 2	PD2	ftwa	DDD	TER (5)
Vole (focal species)	small herbivorous mammal	1.33	1	1	0.67	-	0.08	1	0.53	0.0564	16.0
Lagomorph (generic focal species)	large herbivorous mammal	0.5	1	1	0.67	-	0.08	1	0.53	0.0212	42.5
Wood mouse (generic focal species)	Omnivorous mammal	0.27	1	1	0.67	0.25	0.08	0.75	0.53	0.0326	27.6
Shrew (generic focal species)	Small insectivorous mammal	0.55	1	1	0.67	1	0.08	-	0.53	0.195	4.6

*DDD = FIRfield/bw x PT x ((RUD 1 x PD 1) + (RUD 2 x PD 2)) x ftwa

Based on the lowest NOEL (0.91 mg/kg b.w.) in rats, the TER range from 9 to 58. Therefore, when CA2735 is used according to the good Agricultural Practice, it is not expected to present any significant long-term risk to mammals foraging in orchards.

RMS comment: Based on the lowest NOEL (0.91 mg/kg b.w.) in rats, the TER is equal to 16 for the focal species (vole) when the default value from the EFSA guidance document for FIR/bw, PT and PD, and max residue concentration determined in the field are considered. Therefore, risk is acceptable for the focal species determined experimentally (vole).

For the additional generic focal species (Lagomorph, woodmouse and shrew listed in the EFSA guidance document) the risk is acceptable for lagomorphs and woodmouse for all scenarios but not acceptable for shrew for scenario 2 and 3. However for these species all the other parameters (PT and PD) used in the calculation are default parameters.

Therefore, when CA2735 is used according to the good Agricultural Practice, it is not expected to present any significant long-term risk to herbivorous and omnivorous mammals foraging in Orchards. However, long term risk assessment for insectivorous mammals should be refined at national level (level 4)

B.9.3.2.2. Secondary poisoning risk assessment

According to the EC Guidance Document on Risk Assessment for Birds and Mammals, substances with a log P_{OW} greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains. Bioaccumulation of Amitrole is not expected since the log P_{OW} (-0.969) is below 3. Thus, secondary poisoning through CA2735 via the food chain can be excluded.

B.9.3.2.1. Risk from drinking water

Exposure via drinking water

Most of the mammals can in principle satisfy (at least parts of) their daily water demand via food uptake. However, this potential depends on the water content of the diet items, which is lowest for seeds. Therefore, the assessment methodology for the risk to mammals of pesticides in drinking water as provided below uses a small granivorous mammal as focal species at Tier 1.

In the EFSA Journal (2009)⁷, the drinking water rate (DWR) for the focal mammal species with a body weight of 21.7 g is given as 5.1 mL/d (equivalent to **0.24 L/kg b.w./day**). This DWR is used in the following risk assessment.

- Uptake from contaminated water collected in pools on plants:

According to EFSA (2009) the leaf scenario clearly reflects a worst-case situation with regard to the risk to mammals. It is relevant for spray application only. Formation of pools that would serve as drinking water supply for mammals requires a certain plant morphology. Leaves must point upwards and at the same time must be closely pressed against other leaves or the stem at their basis to form cavities that could hold water over a considerable amount of time. Also, these structures must be accessible to mammals, i.e. must be able to sit on the plant for drinking.

No uptake from contaminated water collected in pools on plants is expected to occur as CA2735 is not applied on the green parts of vine or grape but under the row at the foot of trees and/or vine. Any weeds present are not considered to be of any relevance for this form of exposure either in spatial terms or in time.

- Uptake from contaminated water collected in puddles on soil:

European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. [139 pp.].

Small mammals may be exposed to residues of **CA2735** by drinking water in puddles on soil within or close to the target area. According to the EFSA Journal (2009), the concentration in puddles (PEC_{puddle}) is calculated by the following equation:

$$PEC_{\text{puddle}} = (AR / 10) / [1000 \times (w + Koc \times s)]$$

where: AR = application rate [g/ha]; divided by 10 to achieve rate in mg/m²,
w = 0.02 (pore water term: volume),
s = 0.0015 (soil term: volume, density, organic carbon content)

The TERa and TERlt are calculated as follows:

$$TERa = LC_{50} / (PEC_{\text{puddle}} \times DWR) \text{ and } TERlt = NOEC / (PEC_{\text{puddle}} \times DWR) \text{ with } DWR = 0.24.$$

The TER calculation results are presented in the **Table 10.3.2.3-1**.

Table 10.3.2.3-1: TER calculation resulting from a water intake from a contaminated puddle.

Dose (g/ha)	PEC (mg/L)	Endpoint (mg/kg b.w.)	TERa / TERlt
890	0.57*	LC ₅₀ : 5000	> 1000
		NOEC: 0.9	7

* based on a Koc of 89 (see Vol 3 B.8).

The water intake from contaminated puddles by small mammals does not present any significant risk under the recommended conditions of use of **CA2735**.

B.9.3.3. RMS conclusion

The risk assessments for mammals were conducted in accordance to EFSA guidance (European Food Safety Authority; Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438).

The relevant endpoints for the risk assessment for mammals are LD50 = 5000 mg/kg b.w. and NOEL of 0.9 mg/kg b.w./d.

In a first screening step risk assessment, the acute TER value is higher than the trigger value of 10 for small herbivorous mammals for orchards and vineyard.

In a first screening step and Tier 1 risk assessment, the long-term TER values are lower than the trigger value of 5 for small herbivorous, large herbivorous, small insectivorous and omnivorous mammals.

Therefore a higher tier risk assessment is conducted. The following refinement parameters are taken into account:

The focal species for the relevant feeding guilds, proportion of time spent in the treated field (PT factor) and proportions of food items obtained from the treated area (PD factor):

In vineyard, the focal species was the woodmouse and this species was therefore considered in the higher tier risk with a refined PT value of 0.41. PT and PD were not refined for other species.

In orchard, the focal species was the vole and this species was therefore considered in the higher tier risk. This species is the same that the generic focal species for screening and Tier 1 calculation. PT and PD were not refined.

Moreover, the evaluation was refined taking into consideration the maximum residue concentrations determined in a study conducted under realistic conditions. The different scenarios considered are presented in the following table:

Diet Composition & Portion of Diet	Maximum measured Residue		
	Scenario 1	Scenario 2	Scenario 3

Animal matter	0.61 ^a	3.09 ^b	0.67 ^c
Plant matter	0.08 ^d	0.08 ^d	0.08 ^d

^a Soil Dwelling Arthropods (Vortis sampling) R_{max}^b Crop & Soil dwelling Arthropods (Earwig traps) R_{max}^c Soil dwelling Arthropods (Pitfall traps) Mean R_{max}^d Short vegetation R_{max}

Thus, based on the combined application of the two main refinement parameters (PT and realistic RUD values), all TERs are above the trigger of 5 for herbivorous and omnivorous mammals. TER for small insectivorous are lower than the trigger of 5. However for this species all other parameters (PT and PD) used in the calculation are default parameters. Risk assessment for insectivorous mammals should be further refined at national level.

TER values for Vineyard are:

Species	Wood mouse (focal species)	Vole (generic focal species)	Lagomorph (generic focal species)	Shrew (generic focal species)
Diet Guild	Omnivorous	Small herbivorous	Large herbivorous	Small insectivorous
TER	18.4 to 72.2	16	54.4	1 to 5.1

TER values for Orchards are:

Species	Vole (focal species)	Wood mouse (generic focal species)	Lagomorph (generic focal species)	Shrew (generic focal species)
Diet Guild	Small herbivorous	Omnivorous	Large herbivorous	Small insectivorous
TER	16	7.6 to 29.6	42.5	1 to 5.1

Concerning risk from drinking water, the TER_{acute} and TER_{repro} are above the trigger of 5 indicating an acceptable risk.

Due to the low log K_{OW} (< 3) of amitrole, the risk to birds through secondary poisoning had not to be assessed. The application of CA2735, therefore, does not raise concerns regarding an accumulation in the food chain or secondary poisoning.

The use of CA2735 in accordance to the recommended uses is acceptable to mammals.

B.9.4. EFFECT ON BEES (ANNEX IIA 8.3.1 ; ANNEX IIIA 10.4)

This point was addressed in the dossier submitted and reviewed for the inclusion of amitrole in the annex I. Therefore, there is no need for summarizing again the studies. The main endpoints are shown in table 9.4.7.1.1. Please also refer to review report SANCO/ 6839/VI/97-final, 22/03/2001.

B.9.4.1. Contact and oral acute toxicity (annex IIA 8.3.1.1 ; annex IIIA 10.4.1)

B.9.4.1.1. Acute toxicity of amitrole on bees

Please refer to initial point 9.4.

B.9.4.1.2. Acute toxicity of CA2735 on bees

Please refer to initial point 9.4.

B.9.4.2. Bee brood feeding testing (annex IIA 8.3.1.2)

No data required.

B.9.4.3. Residue tests (annex IIIA 10.4.2)

No data required.

B.9.4.4. Cage tests (annex IIIA 10.4.3)

No data required.

B.9.4.5. Field tests (annex IIIA 10.4.4)

No data required.

B.9.4.6. Tunnel tests (annex IIIA 10.4.5)

No data required.

B.9.4.7. Risk assessment**B.9.4.7.1. Toxicity data**

Toxicity data are summarised in the table below.

Table 9.4.7.1.1 toxicity data for bees

Test substance	Type of test	Toxicity value ($\mu\text{g a.s./bee}$)	Reference
Amitrole	Contact (48h)	$\text{LD}_{50} > 100 \mu\text{g a.s./bee}$	EU review report 6839/VI/97-final, 22/03/2001
	Oral (48h)	$\text{LD}_{50} > 152 \mu\text{g a.s./bee}$	EU review report 6839/VI/97-final, 22/03/2001
CA2210	Contact (72h)	$\text{LD}_{50} > 200 \mu\text{g preparation/bee}$ ($> 42 \mu\text{g a.s./bee}$)	EU (DAR)
	Oral (72h)	$\text{LD}_{50} = 200 \mu\text{g preparation/bee}$ ($> 42 \mu\text{g a.s./bee}$)	EU (DAR)

B.9.4.7.2. HQ calculation**Table 9.4.7.2.1: HQ for honeybees**

Test substance	Type of test	Toxicity endpoint ($\mu\text{g a.s./bee}$)	Exposure (g/ha)	HQ
Amitrole	Contact	> 100	2977 (a.s.)	< 30
	Oral	> 152		< 20
CA2210	Contact	> 200	14522 (preparation)	< 73
	Oral	$= 200$		< 73

When considering the a.s., the hazard quotients for oral (Q_{HO}) and contact exposure (Q_{HC}) are below the trigger of 50, indicating that the active substance doesn't present any unacceptable risk to bees.

When considering the formulated product, both the Q_{HO} and the Q_{HC} are lower than 73. However, CA2735 is applied under the row and the spray is directed to the foot of trees and vine plants. Therefore, the actual dose rate applied represents only 1/3 of the nominal rate of 13 L/ha.

On this basis, the Q_{HO} and the Q_{HC} are therefore lower than 24 indicating a low risk to bees of CA2735.

Therefore, no significant risk to bees is expected from the application of CA2735.

B.9.4.8. RMS conclusion

The risk assessment for bees was conducted in accordance with SANCO/10329/2002 rev. 2 final (Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (2002)).

Hazard Quotients for both acute oral and acute contact exposure were calculated to be ≤ 20 and < 30 , respectively. These values are below the relevant trigger of 50 as defined in Commission Regulation (EU) No 546/2011 indicating an acceptable risk to bees. When considering the formulated product, the Hazard Quotients for both acute oral and acute contact exposure are lower than 73. However, CA2735 is applied under the row and the spray is directed to the foot of trees and vine plants. Therefore, the actual dose rate applied represents only 1/3 of the nominal rate of 13 L/ha. On this basis, the Hazard Quotients are therefore lower than 24 indicating a low risk to bees of CA2735.

The use of CA2735 in accordance to the recommended uses is acceptable to bees.

B.9.5. EFFECTS ON OTHER ARTHROPOD SPECIES (ANNEX IIA 8.3.2 ; ANNEX IIIA 10.5)

B.9.5.1. Laboratory, extended laboratory and semi-field tests (annex IIA 8.3.2 ; annex IIIA 10.5.1)

The effects of amitrole on beneficials were investigated in a series of studies performed under laboratory conditions. The results are summarized in table 9.5.4.1.1. All the laboratory studies were reviewed at the E-U level, please refer to review report SANCO/ 6839/VI/97-final 22/03/2001. New extended laboratory studies were summarized.

B.9.5.2.1. Toxicity of CA2735 on *Aphidius colemani* (laboratory test)

Please refer to B.9.5.1

B.9.5.2.2. Toxicity of CA2735 on *Typhlodromus pyri* (laboratory test)

Please refer to B.9.5.1

B.9.5.2.3. Toxicity of CA2735 on *Orius insidiosus* (laboratory test)

Please refer to B.9.5.1

B.9.5.2.4. Toxicity of CA2735 on *Poecilus cupreus* (laboratory test)

Please refer to B.9.5.1

B.9.5.2.5. Toxicity of CA2735 on *Pardosa armentata* (laboratory test)

Please refer to B.9.5.1

B.9.5.2.6. Toxicity of CA2735 on *Aleochara bilineata* (laboratory test)

Please refer to B.9.5.1

B.9.5.2.7. Toxicity of Weedazol TL on *Aphidius rhopalosiphii* (extended laboratory test)

□ Reference: KIIA 8.8.2/01

□ Author(s); year: Candolfi M. P. 1996

❑ **Title:** EXTENDED LABORATORY TEST TO EVALUATE THE SIDE EFFECTS OF WEEDAZOL TL APPLIED TO PLANTS ON ADULT *APHIDIUS RHOPALOSIPHI* (HYMENOPTERA: BRACONIDAE) BASED ON MEAD-BRIGGS (1994).

❑ **Report No:** 95-034-1002

❑ **Guidelines:** Mead-Briggs, 1994

❑ **Deviations:**

❑ **GLP:** yes

❑ **Executive summary**

In this two-phase laboratory study, 36 adult *Aphidius rhopalosiphi* (6 replicates of 6 wasps) survival and behaviour were determined during a period of 48 hours of exposure to Weedazol TL sprayed on plant material. Subsequently, the 18 wasps (6 replicates of 3 wasps) were allowed to parasitize aphids over 24 hours, and after 7 days their fecundity (or parasitization efficiency) was assessed. Mortality and parasitization rates were then used to calculate the E-value (beneficial capacity) of the test substance to *Aphidius rhopalosiphi* under extended laboratory conditions.

Behaviour: There were differences in the observed behavioural patterns of *Aphidius rhopalosiphi* 0.5 hours after treatment application. In the control, the wasps were distributed equally on the plant surface and on the (untreated) cylinder wall, whereas in the 20 L/ha Weedazol TL treatment, the majority of the wasps were located on the cylinder. This behaviour was also observed after 2 hours of exposure. It is concluded that a 20 L/ha Weedazol TL treatment seems to cause an avoidance behaviour of the wasps of the sprayed plant surfaces. This repellent effect of Weedazol was substantiated by the observations on *Aphidius rhopalosiphi* activity. After 0.5 and 2 hours, almost all control wasps were resting, but in the Weedazol TL treatment, a high percentage of wasps were walking.

Mortality: After 48 hours of exposure to Weedazol TL significantly more wasps had died (47.2%) when compared to the control (2.8%). 100% mortality was observed in the toxic standard treatment after 24 hours of exposure. Female mortality during the 24-hour parasitization period was also significantly higher in the Weedazol TL treatment (66.7%) when compared to the control treatment (11.1 %).

Fecundity: The wasps of the control parasitized 6.8 mummies per surviving female, while the wasps exposed to Weedazol TL parasitized 1.0 mummie per surviving female. This difference was statistically significant.

Based on the effects of exposure on survival and reproduction, Weedazol TL, according to the IOBC scheme, is classified as "moderately harmful" to *Aphidius rhopalosiphi*, under extended laboratory conditions, at the application rate of 20 L/ha.

❑ **Materials and methods**

A. Materials

- | | |
|-----------------------------|---|
| 1. Test material: | Weedazol TL |
| Description: | deep brown liquid |
| Lot/Batch no.: | J4-1077 |
| Active substance content: | amitrole: 236 g/L |
| Stability of test compound: | date of expiry: 10/1997 |
| 2. Control: | purified water |
| Solvent/Vehicle: | none |
| Toxic reference: | Perfekthion (a.s. content: 340 g/L dimethoate) |
| 3. Test organisms – | |
| Species: | <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae) |
| Life stage/age: | adult females, ≤ 48 hours |
| Source: | PK NOTzlingszuchten, Welzheim, Germany |
| No. of wasps per group: | 36 (6 replicates/group, 6 wasps/replicate) for mortality, 18 (6 replicates/group, 3 wasps/replicate) for reproduction |

4. Environmental conditions –

Temperature:	18.5 – 21.5°C
Relative humidity:	63 - 90%
Photoperiod:	16 h light
Light intensity:	2647 - 3120 lux
5. Test substrate: barley seedlings (*Hordeum vulgare*) infested with *Rhopalosiphum padi* (Linnaeus) (Homoptera: Aphididae) (10 to 20 per pot)
6. Test duration:

phase 1:	48 hours
phase 2:	11 days

B. Study design and method

1. In life dates: 22/08 – 01/09/1995
2. Test phases:

phase 1:	mortality and behavioural changes of adults
phase 2:	reproduction test with surviving females from phase 1
3. Description of test procedures:

In the first phase of the test (exposure phase), adult wasps (not older than 48 hours) were exposed to treated plants for 48 hours. All treatments (control, test substance and toxic standard) were replicated 6 times, with each replicate consisting of 6 female wasps (a total of 36 wasps per treatment). On the morning of the test, the plants to be sprayed were pre-treated with a fructose solution (25% w/v in water). A light coating of the solution was applied using a hand sprayer, 1 to 2 hours prior to treatment application. The plants were left to dry on the laboratory bench. The sugar provided both food and a foraging stimulus for the wasps. The soil in the pots was then covered with a layer of dry sand to create a uniform surface. Once the treatments had been applied with the calibrated sprayer and the test substance had dried on the plants, they were enclosed in the untreated acrylic cylinders. Thereafter, the test units were connected to the air pump system in the environmental chamber. The wasps were taken from the holding vessels in batches of 2 to 5, using a small aspirator and gently blown from the aspirator into the test units.

In the second phase of the test (fecundity phase), the effects of Weedazol TL on the fecundity of the wasps (parasitization capacity) were assessed using females, randomly selected from each treatment. After the 48 hours of the exposure phase, surviving wasps were transferred to the untreated aphid-infested plants, using an aspirator. The plants were enclosed under a clear acrylic cylinder. Three wasps were placed in each arena and depending on the survival rate, a maximum of 6 replicates were set up (requiring 18 wasps, randomly chosen). The pots of aphid-infested plants and parasitoids were maintained under the same environmental conditions. The wasps were removed after 24 hours. Any aphid mummies that subsequently developed were counted 7 days later.

4. Observations:

Phase 1: Observations on the position (plant, cylinder, sand) of the individual wasps were made at 30-minute intervals during the initial 3 h after introduction of the wasps to determine whether fresh residues of the test product were repellent. The condition of the wasps (live, affected, moribund, dead) was recorded at approximately 2, 24 and 48 hours after their introduction.

Phase 2: Mortality of the adult wasps was recorded at removal of the test areas. The number of mummies that developed on each plant was recorded 10 days after removal of the adults.

5. Statistics:

Percent mortality and percent affected wasps data were tested for significance using the Fisher Exact test. Fecundity data was checked for normality using the Shapiro-Wilk's test. If the assumption of normality was met, the effect of treatment with Weedazol TL was tested for significance against the control using the t-test. If

assumption of normality was not met, treatments were compared using the nonparametric Mann-Whitney U-test. The computer program used to run the statistical analysis was "Statistica for Windows".

Behavioural data were analysed as follows: the data recorded on the position of the wasps in each replicate during the 10 observations performed at 0.5 and 2 hours after treatment application were pooled as follows: the total number of insects on the plant (on the cylinder or on the sand) equals the number of insects on the plant at observation 1 + number of insects on the plant at observation 2 + ... + number of insects on the plant at observation 10. These calculated total numbers of insects on the plants, on the cylinder or on the sand, were then used to determine a mean value for each treatment. Data recorded at 0.5 and 2 hours after treatment applications was analysed separately.

In order to determine the effect of the treatment with Weedazol TL on test organism mortality (corrected mortality, M-value), the mortality observed among organisms exposed to the test substance treatment after 48 hours of exposure was corrected for control mortality according to Schneider-Orelli.

□ Findings:

II. Results and discussion

Behavioural Observations

Aphidius rhopalosiphi position: At 0.5 hours after treatment application, the majority of the wasps were located on the cylinder in the Weedazol TL treatment, while in the control, this distribution of the wasps was approximately 50% on the plant and 50% on the cylinder. In the toxic standard treatment, a higher number of wasps were on the sand in comparison to the control and Weedazol TL treatments. Two hours after treatment application, the position of the wasps in the control and Weedazol TL treatment was similar to the observations made at 0.5 hours after treatment application. This behaviour (more wasps on the cylinder when compared to the control) led to the conclusion that Weedazol TL may have a repellent effect on *Aphidius rhopalosiphi*. In the toxic standard, almost all the wasps were on the sand, as they were affected by the treatment.

Aphidius rhopalosiphi activity: At 0.5 hours after treatment application, most of the *Aphidius rhopalosiphi* in the control were resting while in the Weedazol TL treatment, approximately 50% of the wasps were walking. As expected in the toxic standard treatment, significantly more wasps were affected when compared to the other two treatments, where the wasps did not show any abnormal behaviour.

Two hours after treatment application, the behaviour of the wasps exposed to Weedazol TL and to the control was similar to the observations made at 0.5 hours after treatment application. In the Weedazol TL treatment, most of the wasps were walking (approximately 50%), whereas in the control treatment, approximately 10% of the wasps were walking. This observation led to the conclusion that the wasps tried to walk away from the Weedazol TL-treated areas. All the wasps in the toxic standard treatment were affected (dead or abnormal behaviour) 2 hours after treatment application.

Mortality and Behaviour

After 48 hours of exposure (termination of the exposure phase of the study), 2.8%, 47.2% and 100% mortality was observed among wasps exposed to the control, the Weedazol TL and the toxic standard treatment, respectively. The percent mortality in the Weedazol TL treatment was significantly higher when compared to the control after 48 hours of exposure.

The mortality of the control and toxic standard were within the range expected for these treatments. After 24 and 48 hours of exposure, 19.4% and 22.2% of the wasps exposed to Weedazol TL treatment showed coordination problems, respectively. The control wasps did not show any co-ordination problems. During the second phase of the test (fecundity assessment), significantly more female wasps died during the 24-hour parasitization period in the Weedazol TL treatment (average of 66.7%) when compared to the control (11.1 %).

Fecundity

The mean number of parasitized aphids per female (No. of mummies) of the control was 6.8, whereas the wasps which survived the Weedazol TL exposure parasitized an average of 1.0 aphid per female. This difference was statistically significant. The female wasps exposed to the toxic standard did not survive the 48-hour exposure period (first phase of the test) and therefore, could not be set up for the second phase.

Based on the effects of exposure on survival and reproduction, Weedazol TL is classified, according to the IOBC scheme, as "moderately harmful" to *Aphidius rhopalosiphi* at the application rate of 20 L/ha under extended laboratory conditions.

Environmental conditions

The air temperature in the environmental chamber ranged from 18.5 to 21.5°C. Relative humidity ranged from 63 to 90%. The light intensity ranged from 2647 to 3120 lux during the entire study.

❑ Conclusions:

The relevant endpoints are summarised in table 8.8.2.1-1.

Table 8.8.2.1-1: main study results

Test system:	<i>Aphidius rhopalosiphi</i> , life stage at test start: adult Extended laboratory test on barley seedlings Spray application to whole plants Introduction of test organisms: post application to freshly dried residues		
Test method:	Mead-Briggs et al. (1994)		
Test substance:	Weedazol TL		
Mortality and reproduction:	Application rate [L prod./ha]	Mean mortality [%]	Mean number of mummies / female
	Control	11.1 ± 17.2	6.8 ± 4.4
	20	66.7 ± 21.1	1.0 ± 2.0
Corrected mortality	45.71%		
Reproduction ability	0.1469		
E-value (Overmeer & van Zon)	92%		

Based on the effects of exposure on survival and reproduction, Weedazol TL is classified, according to the IOBC scheme, as "moderately harmful" to *Aphidius rhopalosiphi* at the application rate of 20 L/ha under extended laboratory conditions.

Comment (RMS): Study acceptable. Weedazol TL could be tested at more than one dose rate in order to determine a dose response effects from more data. Another study was conducted more recently with CA2210 with 5 dose rates. This study is summarized below.

B.9.5.2.8. Toxicity of CA2210 on *Aphidius rhopalosiphi* (extended laboratory test)

- ❑ **Reference:** KIIA 8.8/02
- ❑ **Author(s); year:** Moll M., 2006
- ❑ **Title:** Effect of CA2210 on the parasitoid *Aphidius rhopalosiphi*, extended laboratory study – dose-response test
- ❑ **Report No:** 24842002
- ❑ **Guidelines:** Mead-Briggs et al., 2000 & improvements by the ring-test group (Mead-Briggs et al., 2002)
- ❑ **Deviations:**
- ❑ **GLP:** yes
- ❑ **Executive summary**

The effect of CA2210 (active ingredient: 229 g/L Amitrole) applied at 5 dose rates (556, 1667, 5000, 15000 and 45000 mL product/ha) on the parasitoid *Aphidius rhopalosiphi* was measured in the laboratory via contact on treated plant surfaces (exposure period), compared to a water treated control and to a toxic standard.

Additionally, an assessment for sub lethal effects on parasitisation activity of the female survivors (post-exposure period) was performed in test item treatment groups with < 50 % corrected mortality.

Under extended laboratory conditions the LR₅₀ of CA2210 was estimated to be greater than 45000 mL CA2210/ha. The reproductive capacity of *A. rhopalosiphi* was statistically significantly reduced by 15000 mL CA2210/ha compared to the control, but the effect was below the trigger value of 50% for reproduction. Therefore there was no effect on reproduction up to and including 45000 mL CA2210/ha.

□ Materials and methods:

A. Materials

1. Test material: CA2210
Description: brown liquid
Lot/Batch no.: RD-816-166B
Active substance content: amitrole: 229 g/L (analysed)
Stability of test compound: date of expiry: 11/2005
2. Control: purified water
Solvent/Vehicle: none
Toxic reference: Perfekthion (a.s. content: 400 g/L dimethoate)
3. Test organisms –
Species: *Aphidius rhopalosiphi* (Hymenoptera: Braconidae)
Life stage/age: adult females, ≤ 51 hours
Source: Katz Biotech A.G., Germany
No. of wasps per group: 36 (6 replicates/group, 5 wasps/replicate) for mortality, 15-20 (15-20 replicates/group, 1 wasp/replicate) for reproduction
4. Environmental conditions –
Temperature: 18 - 22°C
Relative humidity: 61 - 90%
Photoperiod: 16 h light
Light intensity: 900 - 3000 lux
5. Test substrate: Barley seedlings (*Hordeum vulgare*) infested with *Rhopalosiphum padi*
6. Test duration: phase 1: 48 hours
phase 2: 11-12 days

B. Study design and method

1. In life dates: 27/06 – 09/08/2005
2. Test phases: phase 1: mortality and behavioural changes of adults
phase 2: reproduction test with surviving females from phase 1
3. Description of test procedures:

In the first phase of the test (exposure phase), adult wasps were exposed to treated plants for 48 hours. All treatments (control, test substance and toxic standard) were replicated 6 times, with each replicate consisting of 5 female wasps (a total of 30 wasps per treatment). On the morning of the test, the plants to be sprayed were pre-treated with a fructose solution (10% w/v in water). A light coating of the solution was applied using a hand sprayer, 30 minutes to 2 hours and 20 minutes prior to treatment application. The plants were left to dry on the laboratory bench. Once the treatments had been applied with the calibrated sprayer and the test substance had dried on the plants, they were enclosed in the untreated acrylic cylinders. The treatments consisted in one control group (water alone), CA2210 at dose rates of 556, 1667, 5000, 15000 and 45000 mL/ha. The reference product was used at 10 mL/ha. Thereafter, the test units were connected to the air pump system in the environmental

chamber. The wasps were taken from the holding vessels in batches of 2 to 5, using a small aspirator and gently blown from the aspirator into the test units.

In the second phase of the test (fecundity phase), the effects of Weedazol TL on the fecundity of the wasps (parasitization capacity) were assessed using females, randomly selected from each treatment. After the 48 hours of the exposure phase, surviving wasps were transferred to the untreated aphid-infested plants, using an aspirator. The plants were enclosed under a clear acrylic cylinder. One wasps were placed in each arena and depending on the survival rate, 15-20 replicates were set up. The pots of aphid-infested plants and parasitoids were maintained under the same environmental conditions. The wasps were removed after 24 hours. Any aphid mummies that subsequently developed were counted 11-12 days later.

4. Observations:

Phase 1: Observations on the position (plant, cylinder, sand) of the individual wasps were made at 30-minute intervals during the initial 3 h after introduction of the wasps to determine whether fresh residues of the test product were repellent. The condition of the wasps (live, affected, moribund, dead) was recorded at approximately 2, 24 and 48 hours after their introduction.

Phase 2: Mortality of the adult wasps was recorded at removal of the test areas. The number of mummies that developed on each plant was recorded 11-12 days after removal of the adults.

5. Statistics:

The LR_{50} of the mortality was determined directly from the raw data. Mortality data were analysed for significance using the Fisher Exact Test, which is a distribution-free test and does not require testing for normality or homogeneity prior analysis. Settling data were tested for normal distribution and homogeneity of variance using the Kolmogoroff-Smirnov-Test ($\alpha = 0.05$) and the Cochran-Test ($\alpha = 0.05$).

Test item: Because the data were normally distributed and homogenous the Dunnett-Test (multiple comparison, two-sided, $\alpha = 0.05$) was used.

Toxic standard: Because the data were normally distributed and homogenous the Student-T-Test for homogenous variances (pair wise comparison, two-sided, $\alpha = 0.05$) was used.

Reproduction was tested for normal distribution and homogeneity of variance using the Kolmogoroff-Smirnov-Test ($\alpha = 0.05$) and the Cochran-Test ($\alpha = 0.05$). Because reproduction data were normally distributed and homogenous the Dunnett-Test (multiple comparison, two-sided, $\alpha = 0.05$) was used. The software used to perform the statistical analysis was ToxRat Professional, Version 2.09, ToxRat® Solutions GmbH, © 2001-2005.

□ Findings:

The main results are presented in table 8.8.2.1-2.

Test system:	<i>Aphidius rhopalosiphi</i> , life stage at test start: adult Extended laboratory test on barley seedlings Spray application to whole plants Introduction of test organisms: post application to freshly dried residues				
Test method:	Mead-Briggs et al. (2000)				
Test substance:	CA2210				
Mortality and reproduction:	Application rate [mL prod./ha]	Mortality [%]		Reproduction	
		Mean	Corrected	Rate (mummies/female)	Reduction [%]
	Control	0 ± 0	-	39.1 ± 20.4	-
	556	3.3 ± 8.2	3.3	33.1 ± 12	15.3
	1167	0 ± 0	0	33.9 ± 15.7	13.2
	5000	0 ± 0	0	34.5 ± 21.8	11.7

	15000	36.7 ± 15.1 (sign)	36.7	22.3 ± 14.3 (sign)	42.9
	45000	46.7 ± 20.7 (sign)	46.7	26.8 ± 15.3	31.4
LR ₅₀	> 45000 mL prod./ha				
ER ₅₀	> 45000 mL prod./ha				

❑ **Conclusion:**

Based on the effects of exposure on survival and reproduction under extended laboratory conditions, the LR₅₀ and ER₅₀ (reproduction) were shown to be greater than 45000 mL/ha.

- ❑ **Comment (RMS): Study acceptable. This study is more reliable than the previous one as further dose rates have been tested and allow to observe a dose response effect. The results of this study will be used in risk assessment for *Aphidius rhopalosiphi***

B.9.5.2.9. Toxicity of CA2210 on *Typhlodromus pyri* (extended laboratory test)

- ❑ **Reference:** KIIA 10.5.2.2/01
- ❑ **Author(s); year:** Rosenkranz B., 2006
- ❑ **Title:** Effect of CA2210 on the predatory mite *Typhlodromus pyri*, extended laboratory study – dose-response test
- ❑ **Report No:** 24843062
- ❑ **Guidelines:** Blümel et al., 2000 & Oomen et al., 1988
- ❑ **Deviations:**
- ❑ **GLP:** yes
- ❑ **Executive summary**

The effect of CA2210 (active ingredient: 229 g/L Amitrole) applied at 5 dose rates (0.938, 1.875, 3.75, 7.5 and 15 mL product/ha) on the predatory mite *Typhlodromus pyri* was measured in the laboratory via contact on treated plant surfaces (exposure period), compared to a water treated control and to a toxic standard. Additionally, an assessment for sub lethal effects (reproduction) was performed.

The results of this study do not indicate statistically significant lethal effects on the predatory mite *Typhlodromus pyri* exposed to 1.875 mL/ha CA2210 in 200 L water/ha on natural leaf surface. Significant acute lethal effects were observed at dosages of 3.75 mL/ha CA2210 and higher (Bonferroni t-test, $\alpha = 0.05$). The LR₅₀ value was determined to be 3.6 mL CA2210/ha (Probit analysis).

The reproduction was not affected up to and including a rate of 3.75 mL/ha CA2210 in 200 L water/ha, the highest rate tested in the reproduction assessment (Dunnnett-test, $\alpha = 0.05$).

❑ **Materials and methods:**

A. Materials

- Test material: CA2210
Description: brown liquid
Lot/Batch no.: RD-816-166B
Active substance content: amitrole: 229 g/L (analysed)
Stability of test compound: date of expiry: 11/2005
- Control: purified water
Solvent/Vehicle: none
Toxic reference: Perfekthion (a.s. content: 400 g/L dimethoate)
- Test organisms –

Species:	<i>Typhlodromus pyri</i> (Scheuten)
Life stage/age:	protonymphs, not older than 24 h
Source:	Katz Biotech A.G., Germany
No. of mites per group:	60 (6 replicates/group, 10 mites/replicate)

4. Environmental conditions –

Temperature:	24 - 26°C
Relative humidity:	60 - 90%
Photoperiod:	16 h light
Light intensity:	330 - 780 lux

5. Test substrate: vine plants (*Vitis vinifera*)**B. Study design and method**

1. In life dates: 27/06 – 05/09/2005
2. Test phases: phase 1: mortality
phase 2: reproduction test

3. Description of test procedures:

Mites were exposed to treated plants including one control group, 5 test substance groups and 1 toxic standard group). Each consisted of 6 replicate with 10 mites/group (a total of 60 mites per treatment). Once the treatments had been applied with the calibrated sprayer and the test substance had dried on the plants, they were enclosed in the untreated acrylic cylinders and the mites introduced. The treatments consisted in one control group (water alone), CA2210 at dose rates of 0.938, 1.875, 3.75, 7.5 and 15 mL product/ha.

4. Observations:

Number of living, dead and escaped mites counted at day 2 and day 7 after test initiation. Further 3 assessments were carried out with a maximum interval of 3 days up to day 14 (inclusive).

The sex-ratio for reproduction testing at day 7 was 1 male per 5 females at a minimum. If at day 7 the sex-ratio was less than 1 male per 5 females, males originating from another replicate from the same treatment were added until an appropriate sex-ratio was reached.

Number of eggs laid and number of live and dead juvenile stages per female counted at day 7, day 9, day 11 and day 14 after test initiation in the control and all treatment groups with a 7 day corrected mortality < 50 %.

5. Statistics:

Mortality data were tested for nominal distribution and homogeneity of variance using Kolmogoroff Smirnov-test ($\alpha = 0.05$) and Cochran-Test ($\alpha = 0.05$). Because mortality data were normally distributed and homogeneous Dunnett-Test (multiple comparison, two-sided, $\alpha = 0.05$) was used.

Mortality data of the control and the toxic standard were also tested for normal distribution and homogeneity of variance using Kolmogoroff Smirnov-test ($\alpha = 0.05$) and Cochran Test ($\alpha = 0.05$). Because the data for not homogeneous Welch t-test (single comparison, two-sided, $\alpha = 0.05$) was used.

Reproduction data were tested for normal distribution and homogeneity of variance using Rls-test ($\alpha = 0.05$) and Cochran-Test ($\alpha = 0.05$). Because reproduction data were normally distributed and homogeneous Dunnett Test (multiple comparison, two-sided, $\alpha = 0.05$) was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 2.09, ToxRat® Solutions GmbH, Alsdorf, Germany.

The LR₅₀ value based on the mortality after 7 days of exposure to the test item on the test organisms, its 95 % confidence limits and the X2 goodness of fit were estimated by applying Probit-Analysis, based on the corrected mortality data recorded after the first week of exposure. The software used to perform the statistical analysis was ToxRat Professional, Version 2.09, ToxRat® Solutions GmbH, Alsdorf, Germany.

WARNING: This document forms part of an EC evaluation data package and should not be read in isolation. Registration must not be granted on the basis of this document.

❑ **Findings:**

The main results are presented in table 10.5.2.2-1.

Test system: <i>Typhlodromus pyri</i>						
Extended laboratory test on vine (Blümel et al., 2000 & Oomen et al., 1988)						
Test substance: CA2210						
Application	Control	0.938 mL/ha	1.875 mL/ha	3.75 mL/ha	7.5 mL/ha	15 mL/ha
Mortality [%]	18.3	21.7	26.7	56.7	100	100
Significance	-	n.s.	n.s.	p<0.05	p<0.05	p<0.05
Corrected mortality	-	4.2	10.3	47	100	100
LR₅₀	3.6 mL/ha (3.2 – 4.1 mL/ha)					
Reproduction rate (egg/female)	5.6	4.8	3.2	8	Not evaluated	Not evaluated
Significance	-	n.s.	p<0.05	n.s.	-	-
ER₅₀	3.75 mL/ha					
Effect on reproduction	-	14.3	42.9	42.9	-	-

A mortality of 100% was observed in the toxic reference group.

❑ **Conclusion:**

The results of this study do not indicate some statistically significant lethal effects on the predatory mite *Typhlodromus pyri* exposed to 1.875 mL/ha CA2210 in 200 L water/ha on natural leaf surface.

Significant acute lethal effects were observed at dosages of 3.75 mL/ha and higher (Bonferroni t-test, $\alpha = 0.05$). The LR₅₀ value was determined to be 3.6 mL/ha (Probit analysis).

The reproduction was not affected up to and including a rate of 3.75 mL/ha, the highest rate tested in the reproduction assessment (Dunnnett-test, $\alpha = 0.05$). Only at the middle rate of 1.875 mL/ha, statistically significant differences to the control were observed. However, this effect is considered to be not test item related, because no effects occurred in the higher treatment rates. The reduction in this treatment group could be explained with biological variability.

❑ **Comment (RMS): Study acceptable**

B.9.5.2.10. Toxicity of CA2210 on *Pardosa* sp (extended laboratory test)

- ❑ **Reference:** KIIA 10.5.2.3/01
- ❑ **Author(s); year:** Schmitzer S., 2006
- ❑ **Title:** Effect of CA2210 on the wolf spider *Pardosa* sp. In the laboratory, extended laboratory study
- ❑ **Report No:** 24841066
- ❑ **Guidelines:** BBA draft guideline 1994 and IOBC / OILB Heimbach et al., 2000
- ❑ **Deviations:**
- ❑ **GLP:** yes
- ❑ **Executive summary**

The effect of CA2210 (active ingredient: 229 g/L Amitrole) applied at 3 dose rates (0.22, 7.5 and 15 L product/ha) on the spiders *Pardosa* sp. was measured in the laboratory via contact on natural soil, compared

to a water treated control and to a toxic standard. Additionally, an assessment for sub lethal effects on feeding rate was performed in test item treatment groups.

After 14 days of exposure, no mortality occurred after treatment with 0.22 L/ha. Three percent mortality was found in the 7.5 L/ha dose group (corresponding to 0.1% corrected mortality). There was no statistic significance when compared to the control group. In the 15.0 L/ha dose group, 23.5% mortality was found (corresponding to 21.2% corrected mortality), which was statistically significant compared to the control.

No reduction of food consumption of the spiders was found after treatment with 0.22 L/ha compared to the control spiders. Food consumption was slightly reduced in the 7.5 L/ha and 15.0 L/ha dose groups compared to the control group. However, this decrease was not statistically significant when compared to the controls.

CA2210 related behavioural abnormalities were observed during the first 4 days of the experiment in the 15.0 L/ha treatment group but were no longer seen afterwards. No behavioural abnormalities occurred in the other treatment groups.

The reference item resulted in a mortality of 88.2 % (87.9 % corrected mortality).

□ Materials and methods:

A. Materials

1. Test material: CA2210
Description: brown liquid
Lot/Batch no.: RD-816-166B
Active substance content: amitrole: 229 g/L (analysed)
Stability of test compound: date of expiry: 11/2005
2. Control: purified water
Solvent/Vehicle: none
Toxic reference: Perfekthion (a.s. content: 400 g/L dimethoate)
3. Test organisms –
Species: Wolf spider (Araneae: Lycosidae). All individuals of the experiment were determined and the following species were found: *Pardosa armentata*: 78 spiders; *Pardosa agrestis*: 35 spiders; *Pardosa pratrivaga*: 26 spiders; *Pardosa palustris*: 16 spiders; *Pardosa proxima*: 3 spiders; *Pardosa pullata*: 1 spider and subadults: 10 spiders (one spider from the 7.5 L/ha dose group could not be determined, since this spider escaped during the experimental trial).
Life stage/age: adults and subadults
Source: outdoor collected spiders close to Munster (Westfalen) and Rossdorf (Hessen) Germany
No. of spiders per group: 34 replicates per variant with 17 replicates containing 1 adult male and 17 replicates per variant containing 1 adult female
4. Environmental conditions –
Temperature: 18 - 22°C
Relative humidity: 61 - 88%
Photoperiod: 16 h light
Light intensity: 710 - 1360 lux
5. Test substrate: soil Lufa 2.1 with *Drosophila spec.* as food
6. Test duration: 14 days

B. Study design and method

1. In life dates: 16/05 – 30/05/2005

2. Description of test procedures:

Wolf spider *Pardosa armentata* (46.2 %), *Pardosa agrestis* (20.7 %), *Pardosa prativaga* (15.4 %), *Pardosa palustris* (9.5 %), *Pardosa proxima* (1.8 %), *Pardosa pullata* (0.6 %), and subadults (5.9 %) were exposed to CA2210 at three dose rate, 0.22, 7.5 and 15 L /ha. A concurrent control group (water) and a toxic reference were also assayed. Spiders were distributed into 34 replicates per variant with 17 replicates containing 1 adult male and 17 replicates per variant containing 1 adult female. The spiders were exposed to direct application on natural soil (LUF A 2.1).

3. Observations:

After 2 hours and on day 1, 2, 3, 4, 7, 8, 10, 11 and 14, the number of dead or damaged individuals were assessed. The food consumption was assessed on days 1, 2, 3, 4, 8 and 11.

4. Statistics:

Mortality data were analysed for significance using Fisher's Exact Test, which is a distribution-free test and does not require testing for normality or homogeneity prior analysis.

Food consumption data were tested for normal distribution and homogeneity of variance using Kolmogoroff-Smirnov Test ($\alpha = 0.05$) and Cochran's-Test ($\alpha = 0.05$). Because these data were normally distributed and variance homogenous, Student-t test (pair wise comparison, one-sided smaller, $\alpha = 0.05$), was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 2.09, ® ToxRat Solutions GmbH, © 2005.

❑ Findings:

The main results are presented in table 8.8.2.1-4.

Application rate	Mortality [%]	Corrected mortality (%)	Flies consumed per spider per day	Reduction feeding capacity (%)
0.22	0 (n.s.)	0	3.4 (n.s.)	0
7.5	3 (n.s.)	0.1	2.9 (n.s.)	15
15	23.5 (sign.)	21.2	3.0 (n.s.)	12
Control	2.9	-	3.4	-

❑ Conclusion:

The dose rate of 15 L/ha caused a significant increase in the spider mortality. Neither feeding capacity reduction nor behavioural abnormality were seen whichever the dose rate.

❑ Comment (RMS): Study acceptable

B.9.5.2.11 Toxicity of Weedazol TL on Orius insidiosus (extended laboratory test)

❑ **Reference:** KIIA 10.5.2.4/01

❑ **Author(s); year:** Candolfi M. P. 1996

❑ **Title:** EXTENDED LABORATORY TEST TO EVALUATE THE SIDE EFFECTS OF WEEDAZOL TL APPLIED TO PLANTS ON THE PREDATOR *ORIU* *INSIDIOSUS* (HETEROPTERA: ANTHOCORIDAE)

❑ **Report No:** 95-036-1002

❑ **Guidelines:** IOBC, EPPO (BEART group)

- ☐ **Deviations:**
- ☐ **GLP:** yes
- ☐ **Executive summary**

In this two-phase laboratory study, 100 bugs *Orius insidiosus* (10 replicates of 10 insects) survival and reproduction were determined during a period of 8 days of exposure to Weedazol TL sprayed on plant material at the dose rate of 20 L/ha. Mortality was recorded on test days 1, 5 and 8 of the exposure phase of the study. No reproduction data were collected during this study due to 100% mortality of the *Orius insidiosus* exposed to the Weedazol TL treatment at the end of the exposure phase.

A significant effect of treatment with Weedazol TL on *Orius insidiosus* mortality was observed. At the termination of the exposure phase of the test (Day 8), mean cumulative mortality of 9.1 %, 100% and 97% was observed in the control, the Weedazol TL and the toxic standard treatment, respectively.

Based on the E-value (E = 100%), Weedazol TL is classified according to the IOBC scheme as "Harmful" to *Orius insidiosus*, under extended laboratory conditions.

- ☐ **Materials and methods:**

A. Materials

1. Test material: Weedazol TL
Description: deep brown liquid
Lot/Batch no.: J4-1077
Active substance content: amitrole: 236 g/L
Stability of test compound: date of expiry: 10/1997
2. Control: purified water
Solvent/Vehicle: none
Toxic reference: Parathion Flussig (a.s. content: 186 g/L parathion)
3. Test organisms –
Species: *Orius insidiosus* (Heteroptera: anthocoridae)
Life stage/age: nymphs in the second nymphal stage
Source: Koppert B.V., The Netherlands
No. of wasps per group: 100 (10 replicates/group, 10 bugs/replicate)
4. Environmental conditions –
Temperature: 23 – 27°C
Relative humidity: 60 - 90%
Photoperiod: 16 h light
Light intensity: 2500 - 3000 lux
5. Test substrate: corn (*Zea mais*)

B. Study design and method

1. In life dates: 18/10 – 26/10/1995
2. Description of test procedures:

In the first phase of the test (exposure phase), bugs (nymphs in the second nymphal stage) were exposed to treated plants (corn) for 8 days. All treatments (control, test substance and toxic standard) were replicated 10 times, with each replicate consisting of 10 bugs (a total of 100 bugs per treatment).

Treatment concentration for the test material (Weedazol TL) was determined from the proposed maximum labelled field application rate of 20 L/ha diluted to the equivalent of 200 L/ha. The toxic standard Parathion flussig was applied at a concentration of 0.1%. The control was deionised water.

At test initiation, the plants were sprayed with the appropriate solution. After the plants had dried, the test units were assembled. Thereafter, 10 *Orius insidiosus* second-stage nymphs were impartially selected from the holding vessels and added to each test unit, using a soft, fine brush. In an attempt to eliminate bias in assigning the insects to specific test units, the insects were impartially transferred to the test units by random selection from the holding vessels and introduced one at a time to the first replicate of the control, followed by the first replicate of the test substance and then the first replicate of the toxic reference standard treatment until each test unit had 1 *Orius insidiosus*.

The same procedure was repeated for the second replicate of each treatment and so on, until all 10 replicates had 10 insects. Insects that were damaged during transfer were replaced. The food supply, *Ephestia* sp. eggs, was also added to each test unit. The test units were then placed within the environmental chamber and connected to the air circulation system. Throughout the exposure phase of the study, additional food was added to the test units any time biological assessments were performed. The exposure phase of the study terminated when the insects reached the last nymphal stage (Test Day 8).

3. Observations:

On Days 1, 5 and 8 $\pm$ 1 (fifth nymphal stage), the test units were examined individually and the number of dead, surviving and missing (probably cannibalised) nymphs was recorded.

4. Statistics:

Mortality and missing *Orius insidiosus* data were checked for normality using the Shapiro-Wilk's test (Weber *et al.*, 1989). Since the assumptions of normality were not met, the effects of treatment with Weedazol TL were tested for significance against the control using the nonparametric Mann-Whitney U test (Zar, 1984). The computer program used to run the statistical analysis was "Statistica for Windows", by StatSoft, Inc., Tulsa, OK, U.S.A.

□ Findings:

A significant effect of treatment with Weedazol TL on *Orius insidiosus* mortality was observed. At the termination of the exposure phase of the test (Day 8), mean cumulative mortality of 9.1%, 100% and 97% was observed in the control, the Weedazol TL and the toxic standard treatment, respectively. The mortality observed among the *Orius insidiosus* exposed to the control and the toxic standard was within the range expected for these treatments.

If anthocorids do not have an escape route, as was the case in the test system, they cannot protect themselves from being attacked and predated. The percentage of missing insects at the end of the exposure phase represents effectively the percentage of *Orius insidiosus* predated, because, at that time, the test system was opened and thoroughly searched for any insects that were alive or dead. At the end of the exposure phase, no differences in predation rate (percent missing *Orius insidiosus*) were observed. Percent missing *Orius insidiosus* was 6%, 6% and 5% in the control, the Weedazol TL and the toxic standard treatment, respectively. The predation rates observed were within the range usually seen in *Orius insidiosus* studies.

No reproduction data were collected during this study due to 100% mortality of the *Orius insidiosus* exposed to the Weedazol TL treatment at the end of the exposure phase.

The air temperature in the environmental chamber ranged from 23.5 to 26.0°C. Relative humidity ranged from 69 to 81%. The light intensity at the test unit level was 2507 lux.

□ Conclusion:

Based on the E-value (E = 100%), Weedazol TL is classified according to the IOBC scheme as "Harmful" to *Orius insidiosus*, under extended laboratory conditions.

□ **Comment (RMS): Study acceptable. Lower doses rate could have been tested as there was 100 % effect observed at the only one tested dose.**

B.9.5.2.12. Toxicity of CA2210 on *Chrysoperla carnea* (extended laboratory test)

- ❑ **Reference:** KIIA 10.5.2.4/02
- ❑ **Author(s); year:** Rosenkranz B., 2006
- ❑ **Title:** Effect of CA2210 on the lacewing *Chrysoperla carnea*, extended laboratory study – dose-response test
- ❑ **Report No:** 24844047
- ❑ **Guidelines:** Vogt et al., 2000
- ❑ **Deviations:**
- ❑ **GLP:** yes
- ❑ **Executive summary**

The effect of CA2210 (active ingredient: 229 g/L Amitrole) applied at 5 dose rates (850, 1543, 2778, 5000 and 9000 mL product/ha) on the lacewing *Chrysoperla carnea* was measured in the laboratory via contact on treated plant surfaces (exposure period), compared to a water treated control and to a toxic standard. Additionally, an assessment for sub lethal effects (reproduction) was performed.

In the different test item treatments, mortalities between 22.0% - 44.0% were observed. This resulted in corrected mortalities (Schneider-Orelli) between 4.9 and 31.7%. According to the effects on mortality in the different treatment groups the LR₅₀ was determined to be higher than 9000 mL/ha CA2210, the highest rate tested in this study.

Up to and including the 2778 mL/ha treatment group the number of eggs per female per day was ≥ 15 and the hatching rate was $\geq 70\%$. In the two highest test item treatment groups, fertility of *Chrysoperla carnea* was affected after the first reproduction assessment. After a second reproduction assessment the number of eggs per female per day in those treatment groups did recover: ≥ 15 .

Under extended laboratory conditions the LR₅₀ of CA2210 was higher than 9000 mL/ha in 200 L deionised water/ha, the highest rate tested in this study. Reproduction of adult *C. carnea* was affected in the first assessment week at dose rates of 5000 mL/ha and above. No adverse effect on reproduction was observed in an additional reproduction assessment for these test rates that has been conducted one week after the first reproduction assessment.

- ❑ **Materials and methods:**

A. Materials

- | | |
|-------------------------------|--|
| 1. Test material: | CA2210 |
| Description: | brown liquid |
| Lot/Batch no.: | RD-816-166B |
| Active substance content: | amitrole: 229 g/L (analysed) |
| Stability of test compound: | date of expiry: 11/2005 |
| 2. Control: | purified water |
| Solvent/Vehicle: | none |
| Toxic reference: | Perfekthion (a.s. content: 400 g/L dimethoate) |
| 3. Test organisms – | |
| Species: | lacewing <i>Chrysoperla carnea</i> |
| Life stage/age: | larvae, pupae and adults (males and females). 2-3 day old larvae at test start |
| Source: | Ibacon, Germany |
| No. of mites per group: | between 28 and 41 lacewings per replicate |
| 4. Environmental conditions – | |
| Temperature: | 24 - 26°C |

Relative humidity:	60 - 90%
Photoperiod:	16 h light
Light intensity:	1360 - 1660 lux

5. Test substrate: vine plants (*Vitis vinifera*)

B. Study design and method

1. In life dates: 29/06 – 04/10/2005
2. Test phases: phase 1: mortality
phase 2: reproduction test

3. Description of test procedures:

Lacewings were exposed to treated plants including one control group, 6 test substance groups and 1 toxic standard group). Each consisted of 50 replicate with 1 – 2 units per replicate (between 28 and 41 lacewings per replicate). Once the treatments had been applied with the calibrated sprayer and the test substance had dried on the plants, they were enclosed in the untreated acrylic cylinders and the lacewings introduced. The treatments consisted in one control group (water alone), CA2210 at dose rates of 850, 1543, 2778, 5000 and 9000 mL product/ha. Eleven to 18 days until cocoons were transferred (using forceps) to the oviposition cages. Pupae were carefully cleaned from *Sitotroga* eggs using a fine brush. After hatching the adults were sexed by investigating the abdomen for characteristic marks.

For assessment of fertility the numbers of eggs were counted after 24 hour egg-laying periods ('checks'). Two checks were done within one week. Eggs of each sample were incubated in separate plastic boxes. For assessment of fertility, hatched larvae were removed daily. *Sitotroga* eggs were added to avoid cannibalism. At 5 - 8 days after hatching of larvae from the eggs, any remaining eggs where no live larva had hatched were determined as unhatched. The number of hatched larvae was determined after hatching of all larvae. Then the hatching rate was calculated by counting the unhatched eggs.

The experiment was prolonged for 2 further checks in the control and the test item treatment groups of 5000 mL/ha and 9000 mL/ha, because fecundity and fertility was affected in the 1st week of assessment in these test item treatment groups and did not reached 15 eggs per female per day. Therefore in agreement with the sponsor a second week of assessment were carried out in order to investigate the reproduction behaviour more in details.

4. Observations:

The number of living and dead larvae and number of pupae developed were determined at least 3 times a week after test start and number of adults hatched was checked regularly.

The reproductive performance of the lacewings was assessed for the control group and all test item groups. No toxic standard reproduction testing was performed. The number of eggs were counted after egg laying periods (checks of 24 hours), 2 checks were done within one week. The number of larvae was determined after hatching of all larvae and the hatching rate was calculated.

5. Statistics:

Mortality data were analysed for significance using the Fisher Exact Test, which is a distribution-free test and does not require testing for normality or homogeneity prior analysis. The software used to perform the Probit-Analysis was ToxRat Professional, Version 2.09, ToxRat® Solutions GmbH, Alsdorf, Germany.

❑ Findings:

The main results are presented in table 10.5.2.4-1.

Test system: <i>Chrysoperla carnea</i>						
Extended laboratory test on vine (Vogt et al., 2000)						
Test substance: CA2210						
Application	Control	850 mL/ha	1543 mL/ha	2778 mL/ha	5000 mL/ha	9000 mL/ha
Mortality [%]	18	30	22	22	28	44
Significance	-	n.s.	n.s.	n.s.	n.s.	p<0.05
Corrected mortality	-	14.6	4.9	4.9	12.2	31.7
LR₅₀	> 9000 mL/ha					
Reproduction rate 1st (egg/female) assessment	27.8	38.4	40.4	26.2	10.1	11.6
Hatching rate 1st assessment	92.1	91.5	91.7	93	94	94.4
Reproduction rate 2nd (egg/female) assessment	26.1	Not evaluated	Not evaluated	Not evaluated	17.7	24.4
Hatching rate 2nd assessment	93.3				91.3	92.5

A mortality of 81.3% was observed in the toxic reference group.

❑ Conclusion:

Under extended laboratory conditions the LR₅₀ of CA2210 was higher than 9000 mL/ha, the highest rate tested in this study.

Reproduction of adult *C. carnea* was affected in the first assessment week at dose rates of 5000 mL/ha and above. No adverse effect on reproduction was observed in an additional reproduction assessment for these test rates that has been conducted one week after the first reproduction assessment.

❑ Comment (RMS): Study acceptable

B.9.5.3. Field tests (annex IIIA 10.5.2)

A field study to evaluate the effects of CA2735 on predatory mite population development in a vineyard in southern France is on-going. The study plan has been submitted and the draft final report should be available in December 2012. This study should be submitted at national level to conclude on the in-field risk.

B.9.5.4. Risk assessment for other non-target arthropod species**B.9.5.4.1. Toxicity data for non-target arthropods in laboratory studies and field studies****Table 9.5.4.1.1: Effect of CA2735 on non-target arthropods**

Species	Results	Reference
Studies using artificial substrates		
<i>Aphidius colemani</i> (adults; lab. test)	Effect (mortality): 80% (4.72 kg as/ha; test substance: SL 236 g a.s./L)	EU review report 6839/VI/97-final, 22/03/2001
<i>Typhlodromus pyri</i> (protonymphs; lab. test)	Effect (mortality): 100% (4.72 kg as/ha; test substance SL 236 g a.s./L)	
<i>Orius insidiosus</i> (nymphs, 2nd stage; lab. test)	Effect (mortality): 100% (4.72 kg as/ha; test substance SL 236 g a.s./L)	
<i>Poecilus cupreus</i> (adults; lab. test)	Effect (mortality): 3.7% (4.72 kg as/ha; test substance SL 236 g a.s./L)	
<i>Poecilus cupreus</i> (adults; lab. test)	Effect (mortality): 0% Effect (food consumption) = 1.18 (ratio treated/control) (14.6 kg a.s./ha; test substance WP 50% w/w a.s.)	
<i>Pardosa armentata</i> (adults)	Effect (mortality): 100% (4.9 kg a.s./ha; test substance WP 50% w/w a.s.)	
<i>Aleochara bilineata</i> (adults; lab. test)	Effect (mortality): 0% Effect (reproduction) = 0.01 (ratio treated/control) (5.0 kg a.s./ha; test substance WP 50% w/w a.s.)	
<i>Aleochara bilineata</i> (adults; lab. test)	Effect (mortality): 11.7% Effect (fecundity) = 1.0 (ratio treated/control) (5.0 kg a.s./ha; test substance WP 50% w/w a.s.)	
Studies under extended laboratory conditions		
<i>Aphidius rhopalosiphii</i> KIIA 8.8.2/01	Corrected mortality: 45.71% Reproduction ability: 0.1469 E-value (Overmeer & van Zon): 92% (20 L/ha - WEEDAZOL TL: SL, 236 g/L)	Candolfi M. P. 1996
<i>Aphidius rhopalosiphii</i> KIIA 8.8.2/02	LR ₅₀ : > 45000 mL prod./ha ER ₅₀ : > 45000 mL prod./ha (CA2210: SL, 229 g/L)	Moll M., 2006
<i>Typhlodromus pyri</i> KIIA 8.8.2/03	LR ₅₀ : 3.6 mL prod./ha ER ₅₀ : > 3.75 mL prod./ha (highest tested dose in the reproduction phase) (CA2210: SL, 229 g/L)	Rosenkranz B., 2006
<i>Pardosa</i> sp.	Effect (mortality): 21.2% at 15000 mL/ha Effect (feeding capacity): none up to	Schmitzer S., 2006

KIIA 8.8.2/04	15000 mL/ha (CA2210: SL, 229 g/L)	
<i>Orius insidiosus</i>	Effect (mortality): 100% (20 L/ha - WEEDAZOL TL: SL, 236 g/L)	Candolfi M. P. 1996
KIIA 8.8.2/05		
<i>Chrysoperla carnea</i>	LR ₅₀ : > 9000 mL prod./ha ER ₅₀ : > 9000 mL prod./ha (2nd assessment)	Rosenkranz B., 2006
KIIA 8.8.2/06	(CA2210: SL, 229 g/L)	

B.9.5.4.2. Exposure in-field and off-field of non-target arthropods

The maximum single application rate is 13 L preparation/ha. No Multiple Application Factor (MAF) needs to be considered.

Off-field exposure = Application rate × (drift factor d ÷ vegetation distribution factor e)

^d Drift factor = drift rate/100

^e vegetation distribution factor of 10 was considered to be appropriate for 2D extended lab studies with *T.pyri* and of 1 for 3D extended lab study with *Aphidius rhopalosiph*.

B.9.5.4.3. Risk assessment for CA2735

For assessing the risk to *Typhlodromus pyri* and *Aphidius rhopalosiph* of CA2735 according to the GAP table, the exposure and corresponding hazard quotient (HQ) approach were based on the recommendations of the ESCORT 2 workshop (March 2000)⁸ and the Working Document SANCO/10329/2002⁹. The following equation was used to calculate the HQ values for in-field and off-field exposure scenarios:

$$\text{In-field HQ} = \frac{\text{Application rate}^a \times \text{MAF}^b}{\text{LR}_{50}^c}$$

^a Single application rate in mL product/ha

^b Multiple Application Factor [ratio between the half-life of the product and the spray interval together with the number of applications]. CA2735 is applied only once per season and therefore, the MAF value is 1.

^c Lethal Rate 50, application rate causing 50% mortality under worst case conditions in mL product/ha

The HQ values for off-crop exposure were calculated according to the following equation:

$$\text{Off-field HQ} = \frac{\text{Application rate} \times \text{MAF} \times \left(\frac{\text{drift factor d}}{\text{vegetation distribution factor e}} \right)}{\text{LR}_{50}} \times \text{correction factor f}$$

^d Drift factor = % drift rate/100

^e vegetation distribution factor of 10 was considered to be appropriate for 2D extended lab studies with *T.pyri* and of 1 for 3D extended lab study with *Aphidius rhopalosiph*.

^f safety factor of 5, covers differences in sensitivity between different species

It should be noted that CA2735 is applied under the row. Spraying is directed to the foot of trees and vine plants. For such applications, farmers use equipment with hydraulic nozzles and the drift can be compared to that determined in industrial crops. Therefore, the drift values given in Escort 2 for field crops were selected in the off-field HQ calculation.

The resulting HQ values for CA2735 are presented in Table 9.5.4.3.1.

⁸ Escort 2 Workshop: Guidance Document on Regulatory Testing and Risk Assessment Procedures for Plant Protection Product with Non Target Arthropods". Candolfi et al, 21-23 March 2000.

⁹ SANCO/10329/2002 rev. 2 final (October 2002). "Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC".

Table 9.5.4.3.1 In-field and off-field risk hazard quotient for terrestrial arthropods, from the proposed use of CA2735

Test species	LR ₅₀	Application rate x MAF	Exposure scenario	Estimated exposure	HQ [Trigger = 1*]
<i>Typhlodromus pyri</i>	3.6 mL/ha (mortality & reproduction)	13000 mL/ha ⁴	In-field	13000 mL/ha	3611
			Off-field_1m ¹	36 mL/ha	50
			Off-field_50 m ²	0.78 mL/ha	1.08
			Off-field_75 m ³	0.52 mL/ha	0.72
<i>Aphidius rhopalosiphi</i>	> 45000 mL/ha (mortality & reproduction)		In-field	13000 mL/ha	0.3
			Off-field_1m ¹	360 mL/ha	0.04

* Trigger of 1 is equivalent to 50% effect (from extended laboratory studies) at the estimated exposure

¹ Assuming spray drift of 2.77%, for 1 application to field crops, in line with ESCORT 2.

² Assuming spray drift of 0.06%, for 1 application to field crops, in line with ESCORT 2.

³ Assuming spray drift of 0.04%, for 1 application to field crops, in line with ESCORT 2.

⁴ Maximum dose rate used in orchard and vineyard.

Notifier

For *Aphidius rhopalosiphi*, the in-field and off-field hazard quotients are below the trigger value of 1 indicating that there is no significant risk to this species after the application of **CA2735** in orchard and vineyards.

For the most sensitive species to CA2210, *Typhlodromus pyri*, the HQ in field indicates a significant risk. The off-field risk is acceptable when a buffer zone of 75 meters is applied (HQ = 0.72). However, the relevance of such a value is questionable. *Typhlodromus pyri* is a foliage-dwelling species. It is located on the lower face of vine and tree leaves. Since the application of **CA2735** is not crop-directed, the exposure of this species will not be likely significant even though it is difficult to quantify the exposure of the foliage-dwelling species. Typhlodromes usually live on the leaves and their exposure will be limited.

Because **CA2735** will be applied from January to April as well as from mid-October to mid-December in vineyards and orchards, the overwintering period should be considered.

Zacharda¹⁰ (1989) investigated the location of *Typhlodromus pyri* in an apple orchard according to season. Diapausing females of *T. pyri* overwintered hidden in crevices in the bark of the stem and main branches of apple trees. Direct observations of overwintering females showed that they were aggregated in clusters. Males were never found overwintering. In the last week of April, some of the females left their winter shelters and moved to the canopy. First they occupied the spurs on the main branches, with only a few on the 1-year-old shoots on the periphery of the canopy. In October most mites were found on current-year shoots and no mites were found on spurs. At the end of the growing season, there were only a few mites on trees and adult females gradually entered diapause and moved to winter shelters. In the apple orchard studied by Zacharda, the life-cycle of *T. pyri* is completed on the apple tree where all the developmental stages occur. Only diapausing females overwinter in crevices in bark; males die at the end of growing season within the canopy of the apple tree. In spring, the field population of *T. pyri* becomes mobile, multiplying only gradually within the first few weeks. Only active females, not those in winter shelters, lay eggs. The first current-year generation appears in mid-May. Two current-year generations develop during the growing season. The reproduction of females changes during the growing season, decreasing strikingly in summer; this can be observed at the population level. Females enter diapause gradually from the last week of August until the end of September.

¹⁰ Zacharda, M. 1989. Seasonal history of *Typhlodromus pyri* (Acari: Mesostigmata: Phytoseiidae) in a commercial apple orchard in Czechoslovakia. Exp. Appl. Acarol. 6: 307- 325

Both *Typhlodromus occidentalis* and *Typhlodromus pyri* overwinter as adult females (Lee and Davis¹¹, 1968 and Herbert¹², 1962), respectively. In Australia, *Typhlodromus* spp. reportedly overwinter as adult females under loose bark and bud scales (Readshaw¹³, 1975).

This is in broad agreement with reports from other countries of *Typhlodromus pyri* overwintering in the splintered ends of dying twigs and deep crevices surrounding old scars (Chant¹⁴, 1959) and in crevices in the bark (Solomon¹⁵, 1975).

It also agrees with the finding of *Typhlodromus occidentalis* overwintering in bark (Lee and Davis¹³, 1968), in peduncles (fruit stalks) (Caltagirone¹⁶, 1970) and underneath old scale coverings (McMurtry and Flaherty¹⁷, 1977; McMurtry¹⁸, 1981).

In vineyards, typhlodromes also overwinter as adult females on the bark and wood of vines (James¹⁹, 2000). It was also shown that the tydeid, *Homeopronematus anconai* (Baker) (formerly identified as *Pronematus anconai*) was a food source for *Metaseiulus* (*Typhlodromus*) *occidentalis* (Nesbitt). Both species also overwinters in grape buds, and *H. anconai* may continued to serve as food for the predator (Fike Knop and Hoy²⁰, 1983).

In early winter - late fall, typhlodromes use bark and wood of vine as a shelter and therefore, they are not significantly exposed to amitrole. In spring and summer, when females move to the canopy, due to the mode of application, non-crop directed spraying on the foot of trees and vine plants, the mite exposure is also very low and only negligible effects are expected.

For the other foliage-dwelling arthropods, depending on the species, amitrole has a strong impact (*Orius insidiosus*) with a 100% mortality at 20 L/ha of CA2210 or only acceptable effects (*Chrysoperla carnea*) with a LR50 > 9000 mL/ha of CA2210 resulting in an in-field HQ of <14.

When considering the soil-dwelling arthropods, the most exposed species, the effects of amitrole were investigated on several species. Except *Pardosa armentata* for which a 100% mortality was observed at 4.9 kg a.s./ha, the other species are quite less sensitive. However, it should be noted that in a more recent study under extended-laboratory conditions, a mortality of 21.2% was recorded at a dose rate of 15 L/ha (CA2210).

In addition, no or few effects were seen on *Aleochara bilineata* (mortality of 0 and 11.7% at 5 kg a.s./ha). The same slight impact was noted on *Poecilus cupreus* with mortalities ranging from 0 to 3.7% with dose rates of 14.6 kg a.s./ha (WP 50%) and 4.72 kg a.s./ha (SE 236 g/L), respectively.

In addition, Pollini (2010) has investigated the effects of early spring – late winter application of amitrole in orchards and vineyards. He concluded that taking into consideration the spontaneous flora present when amitrole is applied as well as during its "persistence" on weeds, presence of beneficials may be considered as negligible or even unexisting. Therefore, adverse effects on predator populations (ladybirds, mites, Chrysopidae and/or syrphs) following a late winter or an early spring application can be considered as negligible.

¹¹ Lee, M.S. and Davis, D.W. 1968. Life history and behaviour of the predatory mite *Typhlodromus occidentalis* in Utah. Ann. Entomol. Soc. Am. 61 : 251-255.

¹² Herbert, H.J. 1962. Overwintering females and the number of generations of *Typhlodromus* (*T.*) *pyri* Scheuten (Acarina: Phytoseiidae) in Nova Scotia. Can. Entomol. 94: 233- 242.

¹³ Readshaw, J.L. 1975. The ecology of tetranychid mites in Australian orchards. J. Appl. Ecol. 12: 473-479.

¹⁴ Chant, D.A. 1959. Phytoseiid mites (Acarina: Phytoseiidae). Part I. Bionomics of seven species in southeastern England. Can. Entomol. Suppl. 12: 1-44.

¹⁵ Solomon, M.G. 1975. The colonisation of an apple orchard by predators of the fruit tree red spider mite. Ann. Appl. Biol. 80: 119- 122.

¹⁶ Caltagirone, L.E. 1970. Overwintering sites for *Melaseiulus occidentalis* in peach orchards. 1. Econ. Entomol. 63: 340-341.

¹⁷ McMurtry, J.A. and Flaherty, D.L. 1977. An ecological study of phytoseiid and tetranychid mites on walnut in Tulare County, California. Environ. Entomol. 6: 287- 292.

¹⁸ McMurtry, J.A. 1981. The use of phytoseiids for biological control: progress and future prospects. In Recent advances in knowledge of the Phytoseiidae. Proceedings of Formal Conference of the Acarology Society of America, MA Hoy (ed.), pp. 23-48.

¹⁹ James D. G. 2000. Reproductive diapause in *Typhlodromus doreenae* Schicha (Acari: Phytoseiidae) **International Journal of Acarology** 26 (1), 101-103.

²⁰ Fike Knop N. and Hoy M. A. 1983. Tydeid mites in vineyards. California Agriculture, November-December 1983, 16-18.

In conclusion, the most sensitive species to amitrole are foliage-dwelling species. However, due to the mode of application of CA2735 (spraying under the row with the jet directed to the foot of trees or vine plants), such species are not likely significantly exposed to amitrole. On the other hand, the most exposed species (soil-dwelling species) are far less sensitive and no significant was observed on several species such as *Pardosa* sp., *Aleochara bilineata* and *Poecilus cupreus*

Risk mitigation measurements: no spray buffer zone of 75 meters

RMS comment:

A potential risk has been identified in-field and off-field for *T.pyri*, the representative species for mites. According to ESCORT 2, a refined in-field risk assessment has to be provided, based on a higher tier study (aged residue study or field study for example). The two standard species (*Aphidius rhopalosiphi* and *Typhlodromus pyri*) are used in risk assessment (HQ calculation) as they cover risk assessment for other non target arthropods. Therefore, even if *Typhlodromus pyri* might be considered not relevant in field in relation with the GAPs, the endpoint from the test conducted with this species has to be used in risk assessment in-field and off-field in order to cover all other non target arthropods. Off field risk is acceptable with respect to a no spray buffer zone of 75 meters. A field study to evaluate the effects of CA2735 on predatory mite population development in a vineyard in southern France was performed and submitted in December 2012. It is reported by the notifier that amitrole application has no impact on the population of *Typhlodromus pyri*.

RMS comment: Considering the late submission of this study (December 2012), it was not possible for RMS to assess it. This study should be submitted at national level (level 4).

B.9.5.5. RMS conclusion

The risk assessment for non-target arthropods other than bees was conducted in accordance to ESCORT 2 (Candolfi et al. 2000: Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods) and SANCO/10329/2002 rev. 2 final (Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (2002).

Based on the results in the Tier 2 extended laboratory studies with the standard species *A. rhopalosiphi* and *T. pyri*, only the in-field HQ for *Typhlodromus pyri* is above the trigger of 1 (corresponding to $\geq 50\%$ effect at the predicted in-field exposure) and needs to be refined. The off-field HQ is below the trigger of 1 (corresponding to $< 50\%$ effect at the predicted off-field exposure) indicating an acceptable off-field risk with respect to a no spray buffer zone of 75 meters.

A potential risk has been identified in-field and off-field for *T.pyri*, the representative species for mites. According to ESCORT 2, a refined in-field risk assessment has to be provided, based on a higher tier study (aged residue study or field study for example). The two standard species (*Aphidius rhopalosiphi* and *Typhlodromus pyri*) are used in risk assessment (HQ calculation) as they cover risk assessment for other non target arthropods. Therefore, even if *Typhlodromus pyri* might be considered not relevant in field in relation with the GAPs, the endpoint from the test conducted with this species has to be used in risk assessment in-field and off-field in order to cover all other non target arthropods. Off field risk is acceptable with respect to a no spray buffer zone of 75 meters. A field study to evaluate the effects of CA2735 on predatory mite population development in a vineyard in southern France was performed and submitted in December 2012. It is reported by the notifier that amitrole application has no impact on the population of *Typhlodromus pyri*. Considering the late submission of this study (December 2012), it was not possible for RMS to assess it. This study should be submitted at national level (level 4).

On the other hand, the most exposed species (soil-dwelling species) are far less sensitive and no significant was observed on several species such as *Pardosa* sp., *Aleochara bilineata* and *Poecilus cupreus* at higher doses than recommended application rate. No in-field and therefore off-field risk is indicated *Pardosa* sp., *Aleochara bilineata* and *Poecilus cupreus* with effect levels of below 50%.

B.9.6. EFFECTS ON EARTHWORMS (ANNEX IIA 8.4 ; ANNEX IIIA 10.6.1)

This point was addressed in the dossier submitted and reviewed for the inclusion of amitrole in the annex I. Therefore, there is no need for summarizing again the studies. The main endpoints are shown in table 9.6.4.1.1. Please also refer to review report SANCO/ 6839/VI/97-final, 22/03/2001.

B.9.6.1. Acute toxicity (annex IIA 8.4.1 ; annex IIIA 10.6.1.1)**B.9.6.1.1. Acute toxicity of amitrole for earthworms**

Please refer to B.9.6.

B.9.6.1.2. Acute toxicity of 1,2,4-triazole for earthworms

Please refer to B.9.6. For 1,2,4-triazole, see the DAR of tebuconazole.

B.9.6.1.3. Acute toxicity of Weedazol TL for earthworms

Please refer to B.9.6.

B.9.6.2. Sublethal effects (annex IIA 8.4.2 ; annex IIIA 10.6.1.2)

Not relevant, please refer to review report SANCO/6839/VI/97-final, 22/03/2001.

B.9.6.3. Field studies (annex IIIA 10.6.1.3)

Based on the risk assessment presented in b.9.6.4, no field testing with earthworms is regarded necessary.

B.9.6.4. Risk assessment for earthworms**B.9.6.4.1. Toxicity data****Table 9.6.4.1.1: Toxicity of amitrole, 1,2,4-triazole and WEEDAZOL TL on earthworm**

Substance	Test species	Test system	Toxicity (mg a.s./kg soil dry weight)		Reference
			LC ₅₀	NOEC	
amitrole	<i>Eisenia fetida</i>	acute	>448	-	EU review report 6839/VI/97-final, 22/03/2001
1,2,4-triazole	<i>Eisenia foetida</i>	acute	>1000	-	DAR of tebuconazole
	<i>Eisenia foetida</i>	chronic	-	1	DAR of tebuconazole
WEEDAZOL TL	<i>Eisenia foetida</i>	acute	>1000 mg formulation/kg soil dry weight (equivalent to >221.5 mg a.s./kg soil dry weight)		EU (DAR)

B.9.6.4.2. Exposure

Amitrole can be used for agricultural uses and non crop uses (on permeable and impermeable surfaces). PECsoil are calculated in the same way for agricultural uses and non crop uses on permeable surfaces. For non crop uses on impermeable surfaces, PECsoil calculation is not considered relevant.

Amitrole is an herbicide and therefore is not directed to the crop but to the ground. The applicant noted that amitrole is not applied on bare soil but on well developed weeds (growth stage 40-60), and therefore used an interception value of 90%.

RMS does not agree with this value since the cover by weeds may be very different from a case to another. In addition, the interception by weeds is usually not taken into account in PEC calculations. The standard practice for these kinds of uses is to use a worst-case interception value of 0%. This value is used in the RMS calculations. The calculated PEC soil for amitrole was 3.967 mg/kg.

PEC_{soil} were calculated assuming a soil depth of 5 cm and a soil bulk density of 1.5 g/cm³. Amitrole is not considered as persistent, and therefore no plateau concentration was calculated.

The metabolite 1,2,4-triazole (formed by photodegradation with a maximum occurrence in soil of 9.9%) was included in the risk assessment for soil organisms by the applicant.

For the metabolite 1,2,4-triazole, the calculated initial PEC in soil is 0.323 mg/kg.

1,2,4-triazole can be considered as persistent (DT₉₀ of 717.6 days). A plateau concentration was calculated in 5 cm (background value in 5 cm + PEC_{soil,ini} in 5 cm). The calculated PEC plateau is 0.494 mg/kg (after 8 years). The PEC plateau is considered in the following risk assessment.

RMS notes that according to the sustained GAP for agricultural uses (application period), photodegradation is not expected to be a major route of degradation of amitrole in soil and therefore 1,2,4-triazole is not expected to be formed in significant amounts. Therefore, to the RMS opinion, a risk assessment was not strictly required. However, RMS considers that if further agricultural uses occurring when photodegradation may be important would be sustained, it should be discussed whether 1,2,4-triazole may need to be included in the risk assessment or not. Therefore, risk assessment for 1,2,4-triazole are presented for information for sustained GAP for agricultural uses. It is highlighted that photodegradation and microbial degradation will be concomitant and concurrent processes. Therefore, if photodegradation occurs in field, the maximum occurrence reached is expected to be lower than 9.9%.

B.9.6.4.3. Risk assessment

Table 9.6.4.3.1: TER of amitrole and 1,2,4-triazole on earthworm

Substance	Test species	Test system	Toxicity endpoint (mg a.s./kg soil dry weight)	PEC _{soil} (mg a.s./kg)	TER	Trigger
amitrole	<i>Eisenia fetida</i>	acute	>448	3.967	>113	10
1,2,4-triazole	<i>Eisenia fetida</i>	acute	>1000	0.494	>2024	10
	<i>Eisenia fetida</i>	chronic	1	0.494	2	5

For amitrole, the acute TER following a single application in vineyard or orchards was calculated to be >10 (TER > 113). Used according to the manufacturer recommendations, CA2735 does not present any significant risk to earthworms. Given the DT₅₀ and DT₉₀ of amitrole in soil, as well as the low toxicity of amitrole in earthworms, no long-term risk assessment is needed.

For 1,2,4-triazole, the acute TER following a single application in vineyard or orchards was calculated to be >2024. No unacceptable acute risk to earthworms is anticipated with 1,2,4-triazole following the use of CA2735 according to the GAPs. The long term TER for 1,2,4-triazole following a single application in vineyard or orchards was calculated to be 2. Refined long term risk should be submitted at national level when necessary. However, RMS notes that according to the sustained GAP, photodegradation is not expected to be a major route of degradation of amitrole in soil and therefore 1,2,4-triazole is not expected to be formed in significant amounts (<9.9%) and does not need to be assessed (see volume 3 B.8.).

No unacceptable long term risk to earthworms is anticipated with 1,2,4-triazole following the use of CA2735 according to the GAPs

B.9.6.5. RMS conclusion

The risk assessment for earthworms was conducted in accordance to SANCO/10329/2002 rev. 2 final (Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (2002)).

Under the conservative assumptions of a Tier 1 assessment and underlying the most conservative PEC_{soil}, the acute TER for earthworms exposed to amitrole is above the trigger of 10, as defined in Commission Regulation (EU) No 546/2011 indicating acceptable risk to earthworms. As amitrole is not persistent in soil and no high risk is indicated for acute risk, reproductive testing is not necessary.

The metabolite 1,2,4-triazole (photodegradation metabolite with a maximum occurrence in soil of 9.9%) was included in the risk assessment for soil organisms by the applicant. The acute TER for earthworms exposed to 1,2,4-triazole is above the trigger of 10 indicating acceptable acute risk to earthworms. The long term TER for earthworms exposed to 1,2,4-triazole is below the trigger of 5 indicating necessary for refinement. However, RMS notes that according to the sustained GAP, photodegradation is not expected to be a major route of degradation of amitrole in soil and therefore 1,2,4-triazole is not expected to be formed in significant amounts (<9.9%). RMS considers that if further uses occurring when photodegradation may be important would be sustained, it should be discussed whether 1,2,4-triazole may need to be included in the risk assessment or not.

The use of CA2735 in accordance to the recommended uses is acceptable to earthworms.

B.9.7. EFFECTS ON OTHER SOIL NON-TARGET MACRO-ORGANISMS (ANNEX IIIA 10.6.2)

This point was addressed in the dossier submitted and reviewed for the inclusion of amitrole in the annex I. The results reported above indicate no persistence in soil. Moreover, there is a low toxicity to soil-dwelling arthropods and earthworms. Therefore, no study on other non-target organisms was considered to be necessary. For 1,2,4-triazole, see the DAR of tebuconazole. The main endpoint is shown in table 9.7.2.1

B.9.7.1. Toxicity of 1,2,4-triazole for *Folsomia candida*

Please refer to B.9.7. For 1,2,4-triazole, see the DAR of tebuconazole.

B.9.7.2. Risk assessment for other soil non-target macro-organisms

B.9.7.2.1. Toxicity data of 1,2,4-triazole

Table 9.7.2.1: Toxicity of 1,2,4-triazole on other soil non-target macro-organisms

Substance	Test species	Test system	Toxicity (mg a.s./kg soil dry weight)		Reference
			LC ₅₀	NOEC	
1,2,4-triazole	<i>Folsomia candida</i>	chronic	-	1.8	DAR of tebuconazole

B.9.7.2.2. Risk assessment

Table 9.7.2.2.1: TER of 1,2,4-triazole on other soil non-target macro-organisms

Substance	Test species	Test system	Toxicity endpoint (mg a.s./kg soil dry weight)	PEC _{soil} (mg a.s./kg)	TER	Trigger
1,2,4-triazole	<i>Folsomia candida</i>	chronic	1.8	0.494	3.64	5

B.9.7.3. RMS conclusion

The risk assessments for other soil macro-organisms were conducted in accordance to SANCO/10329/2002 rev. 2 final (Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (2002)).

For amitrole, the results indicate no persistence in soil (see vol 3 B.8.). Moreover, there is a low toxicity to soil-dwelling arthropods and earthworms. Therefore, no study on other non-target organisms was considered to be necessary.

The long term TER for soil macro-organisms *Folsomia candida* exposed to 1,2,4-triazole is below the trigger of 5 indicating necessary for refinement. However, RMS notes that according to the sustained GAP, photodegradation is not expected to be a major route of degradation of amitrole in soil and therefore 1,2,4-triazole is not expected to be formed in significant amounts (<9.9%). RMS considers that if further uses occurring when photodegradation may be important would be sustained, it should be discussed whether 1,2,4-triazole may need to be included in the risk assessment or not.

The use of CA2735 in accordance to the recommended uses is acceptable to soil macro-organisms other than earthworms.

B.9.8. EFFECTS ON SOIL NON-TARGET MICRO-ORGANISMS (ANNEX IIA 8.5 ; ANNEX IIIA 10.7)

This point was addressed in the dossier submitted and reviewed for the inclusion of amitrole in the annex I. Therefore, there is no need for summarizing again the studies. The main endpoints are shown in paragraphe B.9.8.3. Please also refer to review report SANCO/ 6839/VI/97-final, 22/03/2001. For 1,2,4-triazole, see the DAR of tebuconazole. The main endpoint is shown in paragraph B.9.8.3.

B.9.8.1. Laboratory testing (annex IIA 8.5 ; annex IIIA 10.7.1)

B.9.8.1.1. Toxicity of amitrole on soil respiration and nitrification

Please refer to B.9.8.

B.9.8.1.2. Toxicity of 1,2,4-triazole on soil respiration and nitrification

Please refer to B.9.8. For 1,2,4-triazole, see the DAR of tebuconazole.

B.9.8.2. Additional testing (annex IIIA 10.7.2)

Deviation of the measured activity is lower than 25%. Therefore, further information is not required.

B.9.8.3. Risk assessment for soil micro-organisms

For amitrole, transient retardation of nitrification processes, recovery after 42 days (up to 20 mg/kg soil). No effect on carbon mineralisation up to 20 mg/kg soil. On the basis of the recommended GAP, one single application of 13 L/ha, the initial PEC soil was calculated: 3.97 mg/kg. This results in a margin of safety of 5.

For 1,2,4-triazole, no effects on nitrogen and carbon mineralisation after 28 days at up to 0.353 mg/kg soil. On the basis of the recommended GAP, one single application of 13 L/ha, the PEC plateau was calculated: 0.494 mg/kg. However, RMS notes that according to the sustained GAP, photodegradation is not expected to be a major route of degradation of amitrole in soil and therefore 1,2,4-triazole is not expected to be formed in significant amounts (<9.9%). Moreover, there are no effects in the test at the maximum tested dose of 0.353 mg/kg soil.

Therefore, the use of CA2735 according to the GAP does not present any significant risk to soil microorganisms.

B.9.8.4. RMS conclusion

The risk assessment for soil micro-organisms was conducted in accordance to SANCO/10329/2002 rev. 2 final (Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (2002)).

Amitrole, even when tested at higher soil concentrations than the maximum PEC_{soil} did not result in effects above $\pm 25\%$ on the nitrogen transformation and respiration of soil microorganisms as defined in Commission Regulation (EU) No 546/2011.

For 1,2,4-triazole, no effects on nitrogen and carbon mineralisation after 28 days at up to 0.353 mg/kg soil. On the basis of the recommended GAP, one single application of 13 L/ha, the PEC plateau was calculated: 0.494 mg/kg. However, RMS notes that according to the sustained GAP, photodegradation is not expected to be a major route of degradation of amitrole in soil and therefore 1,2,4-triazole is not expected to be formed in significant amounts ($<9.9\%$). Moreover, there are no effects in the test at the maximum tested dose of 0.353 mg/kg soil. RMS considers that if further uses occurring when photodegradation may be important would be sustained, it should be discussed whether 1,2,4-triazole may need to be included in the risk assessment or not.

The use of CA2735 in accordance to the recommended uses is acceptable to soil microorganisms and their functions.

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

B.9.9.1. Effects of amitrole on non-target plants

As a part of the studies conducted for investigating the effects on seed germination and vegetative vigour of terrestrial plants (US-EPA series 122-1 and 123-1), several terrestrial plants were exposed to either 5 or 9 concentrations of technical amitrole, respectively. The study is summarized below.

B.9.9.1.1. Effects on seedling emergence and vegetative vigour of 12 species

- ☐ **Reference:** KIIA 8.16./01
- ☐ **Author(s); year:** Cross J. W. 1993
- ☐ **Title:** Non-Target Phytotoxicity of Amitrole in Terrestrial Use – Part B – seed germination and vegetative vigour
- ☐ **Report No:** 198501 Part B
- ☐ **Dates of experimental work:**
- ☐ **Guidelines:** US-EPA - Pesticide Assessment Guidelines (PAG) Subdivision J - Hazard Evaluation, Non-target Plants - Series 122-1 and Series 123-1 - Tiers I and 2 Combined
- ☐ **Deviations:**
- ☐ **GLP:** yes
- ☐ **Executive summary**

Seed Germination

The test substance was dissolved and diluted in 0.2 mM calcium sulphate to concentrations calculated to correspond to the maximum field application level (24.0 ppm, equivalent to 9.92 kg/ha) and two-fold dilutions thereof (to the equivalent of 0.31 kg/ha). These solutions were used to moisten plastic seed germination pouches, each pouch pre-loaded with ten seeds of a single plant species. The species tested were: leek, maize, oats, rye, wheat (Monocots) and bush bean, cucumber, lettuce, radish, soybean and sunflower (Dicots). The pouches were placed in a sealed plastic container and incubated in a constant temperature incubator at 25°C. The test duration was seven days. At the end of this period, the seeds were scored for germination. The results were subjected to analysis of variance (ANOVA) and linear regression analysis of $\log(\text{dosage})$ versus number of seeds germinated.

The results indicated no significant effect (no $p < 0.05$, where p is the calculated probability) of Amitrole on seed germination of leek, maize, oats, rye, wheat, bush bean, cucumber, radish, soybean, or sunflower. With lettuce, there was an inhibitory effect at the 0.005 confidence level and a significant negative relationship between

log(dosage) and number of seeds germinated ($R^2 = 0.362$, $p=0.008$, where R^2 is the square of the regression coefficient). The calculated EC₂₅ value for lettuce was 0.35 kg/ha equivalent.

Vegetative Vigour

Seeds of each test species were planted in pots, at least five plants per pot. After the plants had reached the two to three true leaf stage, the plants were sprayed with solutions of Amitrole dissolved in water. The species tested were leek, maize, oats, rye, wheat (Monocots) and bush bean, cucumber, lettuce, pepper, radish, soybean and sunflower (Dicots). Amitrole was dissolved in water only and diluted to concentrations calculated to provide a field rate of 9.92 kg/ha and two-fold dilutions thereof. Each of these solutions was sprayed on three replicate pots of each species. The plants were maintained in a temperature-controlled greenhouse under conditions of natural light. The plants were observed at one week (7 or 8 days) and at 18 or 19 days after treatment. Observations were made of the stature and general condition of each plant. Since chlorosis and/or necrosis of the new growth zone were identified as the major observable effect, this effect was consistently scored on a 0 to 10 scale. Re-treatment at lower application levels was made when the initial spray treatments exceeded the NOEL (no observable effects level).

Chlorosis: Amitrole caused chlorosis of the zone of new growth (buds and new leaf blades in dicots, leaf bases in monocots). At higher treatment levels, this chlorosis progressed to necrosis and, ultimately, plant death. At lower levels, only new growth was affected, with older parts of the plant remaining undamaged. At marginal levels of phytotoxicity, the portion of the plant that had undergone new growth at the time of treatment remained chlorotic but later growth appeared normal. The species varied in their sensitivity to Amitrole.

Stature: Amitrole also caused a reduction in plant stature. This effect was less dramatic than the effect of chlorosis of new growth, but was consistently observed.

Dry weight: After the last observation, the plants were harvested at the base of the stem and the tops were dried and weighed. There was a consistent and significant reduction of the harvested dry weight of all species with increasing treatment levels.

EC₅₀ and EC₂₅ levels: Since the chlorosis score was consistently observed in all species, this was used for calculation of these effect levels, except in radish where natural senescence of older leaves caused inconsistent scoring. The EC₅₀ estimates by linear regression in monocots ranged from approximately 0.431 kg/ha in rye and wheat [0.112 kg/ha] to 1.86 kg/ha in leek. In dicots, EC₅₀ values ranged from approximately 0.15 kg/ha in sunflower to as much as 7.7 kg/ha in radish (radish based on dry weight). The EC₅₀ for peppers (which grew slowly under these conditions) at the second observation was 0.744 kg/ha but declined at the third observation to 0.037 kg/ha. The calculated EC₂₅ levels varied. The values for monocots ranged from approximately 0.16 kg/ha (wheat) to 1.364 kg/ha (leek). In dicots the EC₂₅ values were generally lower, ranging from 0.0372 kg/ha (sunflower) to 0.4 kg/ha (soybean).

NOEL Levels: No observable effect levels were determined by tests of significance for each treatment level versus, the control group for that species. The NOEL levels for monocots ranged from 0.0372 kg/ha in rye and wheat to 1.24 kg/ha in leek. In dicots NOEL ranged from 0.2 kg/ha in sunflower to approximately 2.48 kg/ha for radish.

Determination of Amitrole in Treatment Solutions via Liquid Chromatographic Analysis. The concentration of Amitrole, 3-amino-1,2,4-triazole, in treatment solutions was determined directly by liquid chromatography. Actual concentrations for the seed germination treatments were found to be within 91% to 101 % (avg 93.9% $\pm$ 2.4% std dev) of the target concentration. Actual concentrations for the vegetative vigour treatments were within 93% to 100% (avg 96.7% $\pm$ 2.7% std dev) of the target concentration.

☐ **Materials and methods:**

A. MATERIALS

- | | |
|-------------------------------------|---|
| 1. Test Material: | aminotriazole |
| Description: | not mentioned |
| Lot/batch #: | L1-4420 |
| Purity of active ingredient: | 98.22% |
| Stability of test compound: | not mentioned, assumed to be stable during the experimental phase. |
| 2. Vehicle and/or control: | water |
| 3. Test plants - | |
| Species: | leek, maize, oats, rye, wheat (Monocots) and bush bean, cucumber, lettuce, radish, soybean and sunflower (Dicots) |
| Environmental conditions: | |
| Temperature: | 24 ± 2°C |
| pH: | not applicable |
| Light intensity: | not stated |

B. STUDY DESIGN AND METHODS:

- 1. In life dates:** 18/09/1992-12/02/1993

2. Experimental treatments and Observations**Seed Germination**

The test substance was dissolved and diluted in 0.2 mM calcium sulphate to concentrations calculated to correspond to the maximum field application level (24.0 ppm, equivalent to 9.92 kg/ha) and two-fold dilutions thereof (to the equivalent of 0.31 kg/ha). These solutions were used to moisten plastic seed germination pouches, each pouch pre-loaded with ten seeds of a single plant species. The species tested were: leek, maize, oats, rye, wheat (Monocots) and bush bean, cucumber, lettuce, radish, soybean and sunflower (Dicots). The pouches were placed in a sealed plastic container and incubated in a constant temperature incubator at 25°C. The test duration was seven days rather than five days due to slow germination of the control seeds. Lettuce seeds received light during the incubation period, but the remaining crops were incubated in the dark. At the end of this period, the seeds were scored for germination. Seeds were deemed germinated if the radicle had emerged > 5 mm from the seed coat. Seeds were scored as emergent if both radicle and shoot / hypocotyl had emerged from the seed or caryopsis. Other notes were taken of the appearance of the seeds, where appropriate, and each germination pouch was photocopied.

Vegetative Vigour

Seeds of each test species were planted in pots, at least five plants per pot. After the plants had reached the two to three true leaf stage, the plants were sprayed with solutions of Amitrole dissolved in water. The species tested were leek, maize, oats, rye, wheat (Monocots) and bush bean, cucumber, lettuce, pepper, radish, soybean and sunflower (Dicots).

An electrically-driven sprayer was used for all applications. Sprayer output was calibrated by spraying a dye solution onto a series of 20x20 cm glass plates and determining the volume of spray deposited on each plate by colorimetric techniques. Amitrole was dissolved in water only and diluted to concentrations calculated to provide a field rate of 9.92 kg/ha and two-fold dilutions thereof. Each of these solutions was sprayed on three replicate pots of each species. The plants were maintained in a temperature-controlled greenhouse under conditions of natural light. Records were maintained of watering, fertilization and other cultural practices. Readings of ambient light were made in the greenhouse on the date of each treatment and on the day of each observation. The plants were observed at one week (7 or 8 days) and at 18 or 19 days after treatment. The second observation was made at 18 or 19 days, rather than two weeks, at the request of the sponsor, since it is known that Amitrole is a slow-acting herbicide. Pepper plants were also scored at 26 days after treatment, since these plants grew more slowly than the others. Observations were made of the stature and general condition of each plant. Since chlorosis and/or necrosis of the new growth zone were identified as the major observable effect, this effect was

consistently scored on a 0 to 10 scale (0 = no effect, 10 = maximum effect on the character being scored). Photographs were taken of the plants at each scoring. Re-treatment at lower application levels was made when the initial spray treatments exceeded the NOEL (no observable effects level).

3. Statistics

The results were subjected to analysis of variance (ANOVA) and linear regression analysis of log(dosage) versus number of seeds germinated.

□ Findings:

Seed Germination

The results indicated no significant effect (no $p < 0.05$, where p is the calculated probability) of Amitrole on seed germination of leek, maize, oats, rye, wheat, bush bean, cucumber, radish, soybean, or sunflower. With lettuce, there was an inhibitory effect at the 0.005 confidence level and a significant negative relationship between log(dosage) and number of seeds germinated ($R^2 = 0.362$, $p = 0.008$, where R^2 is the square of the regression coefficient). The calculated EC₂₅ value for lettuce was 0.35 kg/ha equivalent.

Vegetative Vigour

Chlorosis

Amitrole caused chlorosis of the zone of new growth (buds and new leaf blades in dicots, leaf bases in monocots). At higher treatment levels, this chlorosis progressed to necrosis and, ultimately, plant death. At lower levels, only new growth was affected, with older parts of the plant remaining undamaged. At marginal levels of phytotoxicity, the portion of the plant that had undergone new growth at the time of treatment remained chlorotic but later growth appeared normal. The species varied in their sensitivity to Amitrole.

Stature

Amitrole also caused a reduction in plant stature. This effect was less dramatic than the effect of chlorosis of new growth, but was consistently observed.

Dry weight

After the last observation, the plants were harvested at the base of the stem and the tops were dried and weighed. There was a consistent and significant reduction of the harvested dry weight of all species with increasing treatment levels.

EC₅₀ and EC₂₅ levels

Since the chlorosis score was consistently observed in all species, this was used for calculation of these effect levels, except in radish where natural senescence of older leaves caused inconsistent scoring. EC₅₀ and EC₂₅ levels were estimated by linear regression analysis of normally-transformed chlorosis scores and (for comparison) the EC₅₀ levels were also estimated by graphical interpolation from plots of chlorosis score vs. Treatment Level. The EC₅₀ estimates by linear regression [visually interpolated values in brackets] in monocots ranged from approximately 0.431 kg/ha in rye and wheat [0.112 kg/ha] to 1.86 kg/ha in leek. In dicots, EC₅₀ values ranged from approximately 0.15 kg/ha in sunflower to as much as 7.7 kg/ha in radish (radish based on dry weight). The EC₅₀ for peppers (which grew slowly under these conditions) at the second observation was 0.744 kg/ha but declined at the third observation to 0.037 kg/ha. The calculated EC₂₅ levels varied. The values for monocots ranged from approximately 0.16 kg/ha (wheat) to 1.364 kg/ha (leek). In dicots the EC₂₅ values were generally lower, ranging from 0.0372 kg/ha (sunflower) to 0.4 kg/ha (soybean).

NOEL Levels

No observable effect levels were determined by tests of significance for each treatment level versus, the control group for that species. The NOEL levels for monocots ranged from 0.0372 kg/ha in rye and wheat to 1.24 kg/ha in leek. In dicots NOEL ranged from 0.2 kg/ha in sunflower to approximately 2.48 kg/ha for radish.

Determination of Amitrole in Treatment Solutions via Liquid Chromatographic Analysis. The concentration of Amitrole, 3-amino-1,2,4-triazole, in treatment solutions was determined directly by liquid chromatography. Actual concentrations for the seed germination treatments were found to be within 91% to 101 % (avg 93.9% ± 2.4% std

dev) of the target concentration Actual concentrations for the vegetative vigour treatments were within 93% to 100% (avg 96.7% $\pm$ 2.7% std dev) of the target concentration.

❑ Conclusion

There was no observed effect of Amitrole over the mandated concentration range (up to the maximum field treatment of 9.92 kg/ha) on the germination of the species tested, with the exception of lettuce. With lettuce there was a significant and dose-dependant inhibitory effect on germination, but the maximum inhibition observed was less than 50%, relative to the control.

Chlorosis of the new growth zone was the primary observable effect of Amitrole in each species tested. This chlorosis became evident only after the new growth emerged from the growing points of the plant. The degree of chlorosis was dosage-dependent. At higher treatment levels, this chlorosis developed to tissue necrosis and (at the highest levels) plant death. The development of chlorosis was paralleled by inhibition of growth, evidenced by a reduction in plant stature and resulting in a reduction of dry weight of the treated plants in a dose-dependent manner.

The sensitivity of the different plant species to Amitrole varied, as indicated by the calculated EC₅₀ levels for the species tested. The calculated EC₅₀ estimates for monocots ranged from approximately 0.41 kg/ha to 1.86 kg/ha in leek. In dicots, EC₅₀ ranged from approximately 0.15 kg/ha in sunflower to as much as 0.66 kg/ha in cucumbers (7.07 kg/ha in radish, based on dry weight).

The no observable effects level also varied among the different species, ranging from 0.038 kg/ha in wheat and rye and 0.02 kg/ha in lettuce and sunflower to 1.24 kg/ha in leek. Radish gave an NOEL (based on dry weight) of 2.48 kg/ha. Leek and radish were the least sensitive species tested. Lettuce and sunflower were the most sensitive.

- ❑ **Comment (RMS): Study acceptable. In the study report, doses are given in lb/acre and have been converted in kg/ha in the summary. However, this conversion is not well understood as in this case the conversion factor of 1.12085 should be used and it seems that it's not always the case. Concerning the worst case endpoint reported as an EC₅₀ of 0.15 kg/ha in the summary, the corresponding endpoint in the study report was EC₅₀=0.12 lb/acre. Therefore, applying the conversion factor, this results in EC₅₀=0.1345 kg/ha. This value is used in the risk assessment.**

Seedling emergence was tested more recently with technical amitrole. As a part of the studies conducted for investigating the effects on seed germination and vegetative vigour of terrestrial plants (US-EPA series 122-1 and 123-1), several terrestrial plants were exposed to either 5 or 9 concentrations of technical amitrole, respectively. The study is summarized below.

B.9.9.1.2. Effects on seedling emergence of 10 species

- ❑ **Reference:** KIIA 8.16/02
- ❑ **Author(s); year:** Porch J. R. 1998
- ❑ **Title:** AMITROLE: A TOXICITY TEST TO DETERMINE THE EFFECTS OF THE TEST SUBSTANCE ON SEEDLING EMERGENCE OF TEN SPECIES OF PLANTS
- ❑ **Report No:** 461-109
- ❑ **Guidelines:** OPPTS Number 850.4225
- ❑ **Deviations:**
- ❑ **GLP:** yes
- ❑ **Executive summary**

The effects of a single spray application of Amitrole on seedling emergence, growth, and condition of ten species of plants were determined after spraying different dose rates of the active substance. The experimental design for this study consisted of five treatment groups and a control group. Each treatment group had four replicate pots with ten seeds planted in each pot. The control group had eight replicate pots with ten seeds planted in each pot. A single application of Amitrole was made to each treatment group. Spray mixtures were applied at a spray

volume of 93.5 mL/m², which is equivalent to the minimum proposed spray volume for a foliar application. Nominal concentrations of the spray mixtures applied to each treatment group were 120, 300, 751, 1870, and 4700 ppm of active substance, which corresponded to application rates of 0.124, 0.31, 0.78, 1.98 and 4.849 kg a.s./ha, respectively. Seeds were impartially assigned to prelabelled growth pots on the day of test initiation. The replicate pots were placed in a randomised block design on a greenhouse table after spray mixtures were applied. Observations of emergence were made on Day 10 and the day of test termination, while observations of height, condition, and fresh weight were made only on the day of test termination. Dry weights were determined from seedling shoots collected at test termination.

Treatment-related effects resulting from a single spray application of Amitrole to planted seeds were observed in all ten test species. The most sensitive species tested, as determined by the highest test concentration at which no treatment-related effects were observed (NOEC), were cabbage and lettuce, which had NOECs of 0.124 kg/ha, while the NOECs for carrot, onion, rye grass, and tomato seedlings were each 0.31 kg/ha. The least sensitive species were bean, corn, soybean, and wheat, which had NOECs of 0.78 kg/ha.

□ Materials and methods:

A. MATERIALS

1. **Test Material:** aminotriazole
Description: white powder
Lot/batch #: 17051718
Purity of active ingredient: 91.7%
Stability of test compound: not mentioned, assumed to be stable during the experimental phase.
2. **Vehicle and/or control:** water
3. **Test plants - Species:** see table below.

Test Species		Seed Source	Planting Depth
Bean	<i>Phaseolus vulgaris</i>	Meyer Seed Co., Baltimore, MD	2cm
Cabbage	<i>Brassica oleracea</i>	Southern States Coop., Richmond, VA	6mm
Carrot	<i>Daucus carota</i>	Territorial Seed Co., Cottage Grove, OR	6mm
Corn	<i>Zea mays</i>	Asgrow Seed Co., Galena, MD	2cm
Lettuce	<i>Lactuca sativa</i>	Territorial Seed Co., Cottage Grove, OR	6mm
Onion	<i>Allium cepa</i>	Territorial Seed Co., Cottage Grove, OR	6mm
Rye	<i>Lolium perenne</i>	Meyer Seed Co., Baltimore, MD	6mm
Soybean	<i>Glycine max</i>	Asgrow Seed Co., Galena, MD	2cm
Tomato	<i>Lycopersicon esculentum</i>	Meyer Seed Co., Baltimore, MD	6mm
Wheat	<i>Triticum aestivum</i>	Arrowhead Mills, Inc., Hereford, TX	2cm

3. Test soil -

The soil used for the test represented a sandy loam soil, and was composed of kaolinite clay, industrial quartz sand and peat mixed in a 4:50:5 ratio (w:w:w). Crushed limestone was added to buffer the pH of the soil, and a slow-release fertilizer was added to provide nutrients essential for plant growth. The particle size distribution was 81% sand, 7% silt, and 12% clay, and the organic matter content was 2.8%.

Environmental conditions:

- Temperature:** 24 ± 2°C
pH: not applicable
Light intensity: 0.001 kW/m²

B. STUDY DESIGN AND METHODS:

- 1. In life dates:** Cabbage, Carrot, Onion, Rye Grass, Tomato: September 15-29, 1997
Corn, Lettuce, Soybean, Wheat: September 16-30, 1997
Bean: September 16-October 1, 1997

2. Experimental treatments and Observations

Seeds were planted in plastic pots (approximately 16 cm in diameter and 12 cm deep) on the day of test substance application. A template was used to gently compact the soil and leave ten uniform holes for planting. One indiscriminately selected seed was then planted in each hole, for a total of ten seeds in each pot. Holes were then closed by slightly depressing the soil surface.

Spray mixtures were applied at a spray volume of 93.5 mL/m², which is equivalent to the minimum proposed spray volume for a foliar application. Nominal concentrations of the spray mixtures applied to each treatment group were 120, 300, 751, 1870, and 4700 ppm of active substance, which corresponded to application rates of 0.124, 0.31, 0.78, 1.98 and 4.84 kg a.s./ha, respectively.

Observations on Day 10 were made to document seedling emergence. Observations on Day 14 were made to document seedling emergence and growth, and to determine changes in the general condition of seedlings following the application of the test substance. Observations consisted of noting whether emergence had or had not occurred and assessing the condition of each seedling. Emergence was defined as the presence of visible plant tissue above the surface of the soil. Seedling condition was described by noting the presence or absence of possible signs of phytotoxicity such as colour changes, necrosis, leaf curling, chlorosis, plant lodging or plant stunting. Each emerged seedling was then assigned a numerical score that described the plant condition. The growth of emerged seedlings was evaluated by assessing the height, fresh shoot weight, and dry shoot weight of seedlings living at test termination.

Seedling height was measured with a ruler to the nearest whole centimetre from the surface of the soil to either the apical meristem (bean, soybean, and tomato) or the tip of the tallest leaf of the seedling (cabbage, carrot, corn, lettuce, onion, rye grass, and wheat). At test termination, the stems of all living seedlings were clipped at soil level. The above-ground (shoot) portions of all living seedlings within a replicate were then weighed as a group. The resulting weight was divided by the number of seedlings included in the measurement in order to determine the mean shoot fresh weight. After fresh weights were determined, the collected shoots were placed in labelled bags and dried to a constant weight in a drying oven. When dry, the shoots were again weighed as a group, and the mean shoot dry weight for each replicate was calculated.

3. Statistics

Statistical analyses were used to aid in the evaluation of effects of application of the test substance on seedling emergence, survivorship, mean shoot weight (fresh and dry), and seedling height. Statistical analyses included the determination of effect concentrations (EC estimates) using the linear interpolation and resampling methods of Norberg-King in the ICPIN computer program when possible. EC₂₅ and EC₅₀ values were defined as the concentrations of test substance that caused 25 and 50% changes, respectively, in the variable of interest (e.g. seedling emergence, weight, or height) relative to the control group. Data were also evaluated to determine the lowest-observed-effect-concentration (LOEC) and the no-observed-effect-concentration (NOEC) for emergence and growth. The LOEC was defined as the lowest concentration of test substance used in the study that showed an effect on the variable of interest. The NOEC was defined as the concentration of test substance used in the study that was immediately below the LOEC. Dunnett's test was used to aid in establishing the LOEC and NOEC by determining which treatment groups differed significantly from the control group. Analyses were performed using the two-tailed DUNNETT option of the GLM (general linear model) procedure of SAS 6.11. Alpha was set at 0.05.

□ Findings:

Samples of spray mixtures were collected and analysed. Recoveries were shown to range from 96 to 100% of the nominal concentrations.

Bean. There were no treatment related effects observed upon the emergence or survivorship of bean seedlings at any test concentration. At test termination, the mean number of emerged, living seedlings per replicate in the treatment groups ranged from 94 to 101% of the control mean. There were apparent treatment-related effects

upon the growth of seedlings in the 4.84 kg/ha group. The mean height and dry weight of the 4.84 kg/ha group were reduced (67 and 81%, respectively) and were significantly different from the control means.

There were treatment-related signs of phytotoxicity observed in the 1.98 and 4.84 kg/ha groups. By Day 10, chlorosis attributed to the treatment was observed in both the 1.98 and 4.84 kg/ha groups. At test termination, slight to moderate chlorosis was intermittently observed in two of the four 1.98 kg/ha replicates and frequently observed in all four 4.84 kg/ha replicates. On Day 14, seedlings in 11 of the 28 replicates in these groups were moderately wilted. The wilt was attributed to a water deficit, and appeared to be temporary. Therefore, all of the replicates were watered, and test termination was delayed until the next day (Day 15) to allow recovery. On Day 15 the seedlings had regained their turgor, but slight necrosis remained on many of the previously wilted seedlings in both treatment and control groups. The wilt and resulting necrosis did not appear to adversely impact the overall health of the seedlings. Because of the treatment-related chlorosis observed in seedlings of the 1.98 kg/ha group, the NOEC for bean seedlings was determined to be 0.78 kg/ha. The most sensitive quantitative endpoint was seedling height, with a calculated EC_{25} of 3.84 kg/ha.

Cabbage. There was no apparent treatment-related effect upon the emergence of cabbage seedlings. Mean emergence at test termination for the 0.124, 0.31, 0.78, 1.98 and 4.84 kg a.s./ha groups were 97, 103, 94, 78, and 92% of the control mean emergence, respectively. There were clear effects upon the growth of cabbage seedlings at all test concentrations from 0.31 kg a.s./ha and above. Seedling growth in the 0.31, 0.78, 1.98 and 4.84 kg a.s./ha groups was reduced relative to the control group, and appeared to be dose responsive. Mean dry weight and mean height of the 0.78, 1.98 and 4.84 kg a.s./ha groups were significantly reduced, while mean fresh weight of 0.31, 0.78, 1.98 and 4.84 kg a.s./ha groups were significantly reduced. Treatment-related chlorosis and necrosis were present in all replicates of the 0.31, 0.78, 1.98 and 4.84 kg a.s./ha groups, and the severity of the conditions appeared to form a clear dose response pattern. The number of seedlings with condition scores of 80 or above (indicating severe damage to seedling health) was 9, 23, and 31 in the 0.78, 1.98 and 4.84 kg a.s./ha groups, respectively. Four, two, and six seedlings died shortly after emerging in the 0.78, 1.98 and 4.84 kg a.s./ha treatment groups, respectively. Although incidental seedling mortality is not unexpected, the high number of severely damaged and dead seedlings in the 0.78, 1.98 and 4.84 kg a.s./ha groups in this study was considered treatment-related. Because of the apparent treatment-related effects upon growth and condition at 0.31 kg a.s./ha, the NOEC for cabbage seedlings was determined to be 0.124 kg a.s./ha. The most sensitive quantitative endpoint for cabbage in this test was fresh weight, which resulted in a calculated EC_{25} of 0.26 kg a.s./ha.

Carrot. There were no apparent treatment related reductions in the emergence of carrot seedlings. Mean emergence in the 0.124, 0.31, 0.78, 1.98 and 4.84 kg a.s./ha groups was 100, 91, 89, 91, and 94% of the control mean, respectively. However, since many seedlings in the higher concentration groups did not survive until test termination, survivorship was evaluated in addition to emergence. The mean number of surviving seedlings in the control, 0.124, 0.31, 0.78, 1.98 and 4.84 kg a.s./ha groups were 8.00, 8.25, 7.75, 7.50, 4.50, and 5.00, respectively. Mean survivorship in the 1.98 and 4.84 kg a.s./ha treatment groups was significantly ($p < 0.05$) different from the control group mean.

Due to the small size and great variability of the seedlings in all treatment groups, the seedling growth data were difficult to interpret. Although the mean fresh weights of the treatment groups ranged from 66 to 76% of the control mean fresh weight, their distribution did not appear to be dose responsive, and none was significantly different from the control mean. The mean dry weight of the treatment groups ranged from 73 to 91 % of the control mean. Although reductions in dry weight were not as great as those observed in the fresh weight, the 1.98 and 4.84 kg a.s./ha group means were 77 and 73% of control, respectively, and both were significantly different from the control mean ($p < 0.05$). Mean heights of the 0.124, 0.31, 0.78, 1.98 and 4.84 kg a.s./ha treatment groups were 95, 89, 88, 95, and 77% of the control mean, respectively. Only the 4.84 kg a.s./ha group mean was significantly ($p < 0.05$) different from the mean of the control group. Apparent treatment-related necrosis and chlorosis were observed in the 0.78, 1.98 and 4.84 kg a.s./ha treatment groups. Although incidental necrosis was observed in all groups, the number of affected individuals was much greater in the three highest concentration test groups (4 to 9 seedlings per group) than in the control group (one seedling). Because of the apparent treatment-related effects upon condition 0.78 kg a.s./ha, the NOEC for carrot seedlings was determined to be 0.31 kg a.s./ha. The most sensitive quantitative endpoint for carrot seedlings in this test was fresh weight, which resulted in a calculated EC_{25} of 0.112 kg a.s./ha. However, due to the distribution of the data as described above, this EC estimate may not be reliable. The EC_{25} estimates calculated for survival, height, and dry weight were 1.36, > 4.83 and 4.22 kg a.s./ha, respectively.

Corn. There were no apparent treatment-related effects upon the emergence or growth of corn seedlings at any of the concentrations tested. Mean emergence in the treatment groups ranged from 98 to 100% of the control

mean. One of the emerged corn seedlings died during the test, but the cause of the mortality could not be attributed to treatment. Mean fresh and dry weights of the treatment groups ranged from 108 to 117% and 104 to 116% of the control means, respectively, while mean heights of the treatment groups ranged from 104 to 107% of the control mean. Slight chlorosis was observed on seedlings in the 1.98 and 4.84 kg a.s./ha groups and was attributed to treatment. Therefore, the NOEC for corn was determined to be 0.78 kg a.s./ha. The calculated EC₂₅ for all other test endpoints was > 4.84 kg a.s./ha, the highest test concentration.

Lettuce. There were no apparent treatment related effects upon the emergence of lettuce seedlings. Mean emergence at test termination in the 0.124, 0.31, 0.78, 1.98 and 4.84 kg a.s./ha groups was 105, 85, 85, 115 and 82% of the control mean. Apparent treatment-related effects were observed on seedling growth (fresh and dry weights and height) in the 0.31, 0.78, 1.98 and 4.84 kg a.s./ha groups. Weight reductions in these treatment groups were dose-responsive, and were similar in both fresh and dry weights. Treatment group mean fresh weights for the 0.31, 0.78, 1.98 and 4.84 kg a.s./ha groups were 71, 74, 53 and 16% of the control mean, respectively, while mean dry weights for the treatment groups were 81, 77, 65, and 40% of the control mean, respectively. The mean fresh weights of the 1.98 and 4.84 kg a.s./ha groups and the mean dry weight of 4.84 kg a.s./ha group were significantly different ($p < 0.05$) from the respective control means. Although the 0.31 and 0.78 kg a.s./ha group means were not significantly different ($p > 0.05$) from the control means for fresh or dry weight, the consistency of the reductions and the clear dose-response pattern indicate that the reductions are treatment-related. Additionally, treatment-related reductions in mean seedling height were observed in the 0.31, 0.78, 1.98 and 4.84 kg a.s./ha groups, whose mean heights were 82, 82, 62, and 37% of the control mean, respectively. Treatment-related chlorosis of seedlings was observed in 0.78, 1.98 and 4.84 kg a.s./ha treatment groups. Although incidental chlorosis and necrosis of seedlings occurred throughout the treatment groups, both the number of affected individuals and the severity of the chlorosis increased with the test concentration. There were two apparently treatment-related seedling mortalities in the 0.78 kg a.s./ha group and seven in the 4.84 kg a.s./ha group. Because of the observed effects upon seedling growth, the test concentration of 0.124 kg a.s./ha was determined to be the NOEC for lettuce seedlings. The most sensitive endpoint was fresh weight, which had a calculated EC₂₅ of 0.29 kg a.s./ha.

Onion. There was no treatment-related effect upon the emergence of onion seedlings in the 0.124, 0.31, 0.78, 1.98 kg a.s./ha treatment groups. Mean emergence in 4.84 kg a.s./ha group was reduced (73% of control), but the means were not significantly different. However, the reduction was considered treatment-related since it was accompanied by reductions in both weight (fresh and dry) and height, as well as apparent signs of phytotoxicity, as described below. Seedlings in the 0.78 kg a.s./ha group showed apparent treatment-related reductions in fresh weight and height. Mean fresh weights of the 0.78, 1.98 and 4.84 kg a.s./ha groups were 74, 54 and 28% of the control mean, respectively, while mean seedling heights in the same groups were 78, 74 and 43% of the control mean, respectively. Treatment-related effects on dry weight were apparent in the 1.98 and 4.84 kg a.s./ha groups, which had group means that were 64 and 54% of the control mean, respectively. Effects on the condition of seedlings, characterized by chlorosis, were apparent at 0.78 kg a.s./ha. The severity of the chlorosis was much greater in the 1.98 and 4.84 kg a.s./ha groups. One seedling mortality was observed and attributed to treatment in the 4.84 kg a.s./ha group. One seedling in the 0.31 kg a.s./ha group also died prior to test termination, but since no other seedlings in that group exhibited any signs of phytotoxicity, the mortality was considered incidental. Because of the apparent treatment-related effects upon condition at 0.78 kg a.s./ha, the NOEC for onion seedlings was determined to be 0.31 kg a.s./ha. The most sensitive endpoints for onion seedlings in this test were height and fresh weight. The EC₂₅ and EC₅₀ for height were calculated to be 0.72 and 3.72 kg a.s./ha, respectively, while the EC₂₅ and EC₅₀ for fresh weight were calculated to be 0.73 and 2.37 kg a.s./ha, respectively.

Rye Grass. There was no apparent treatment-related effect upon the emergence of rye grass seedlings at any test concentration. Mean emergence at test termination in the 0.124, 0.31, 0.78, 1.98 and 4.84 kg a.s./ha groups was 100, 100, 97, 95, and 103% of the control mean, respectively. Treatment-related effects on seedling growth appeared restricted to the 1.98 and 4.84 kg a.s./ha groups. Mean seedling height and dry weight in these two groups were reduced and significantly different ($p < 0.05$) than their respective control group means.

Mean fresh weight in the 1.98 and 4.84 kg a.s./ha groups were 65 and 28% of the control mean respectively, although only the 4.84 kg a.s./ha group mean was significantly different ($p < 0.05$) than the mean fresh weight of the control seedlings. Treatment-related effects upon seedling condition were observed in the 0.78, 1.98 and 4.84 kg a.s./ha groups. Chlorosis and necrosis were frequently observed in these treatment groups, and the incidence and severity of the conditions appeared to be dose-responsive. Three seedlings in the 0.78 kg a.s./ha group and four seedlings in the 4.84 kg a.s./ha group died prior to test termination. The seedling deaths were attributed to

treatment; Because of the apparent treatment-related effects upon condition at 0.78 kg a.s./ha, the NOEC for rye grass seedlings was determined to be 0.31 kg a.s./ha. The most sensitive endpoints for rye grass seedlings in this test were fresh and dry weight. The EC₂₅ and EC₅₀ for fresh weight were calculated to be 1.49 and 3.1 kg a.s./ha, respectively, while the EC₂₅ and EC₅₀ for dry weight were calculated to be 1.49 and 3.6 kg a.s./ha, respectively.

Soybean. There was no apparent treatment-related effect on the emergence of soybean seedlings at test concentrations up to 4.84 kg a.s./ha, the highest concentration tested. At test termination, mean emergence of seedlings in the treatment groups ranged from 98 to 100% of the control mean. Treatment-related effects on seedling growth and condition were apparent in 1.98 and 4.84 kg a.s./ha groups. Mean seedling fresh weight, dry weight, and height in these groups were reduced and significantly different from their respective control group means. Observations of chlorosis and necrosis on seedlings were made at test termination, and were attributed to treatment. Because of the effects on growth and condition of seedlings in the 1.98 kg a.s./ha, the NOEC for soybeans in this study was determined to be 0.78 kg a.s./ha. The most sensitive test endpoint was seedling height, which resulted in a calculated EC₂₅ estimate of 2.97 kg a.s./ha.

Tomato. Treatment-related effects on the emergence of tomato seedlings were observed in, and restricted to the 4.84 kg a.s./ha group. Mean seedling emergence in that treatment group was 43% of the control mean. Mean emergence in 0.124, 0.31, 0.78, and 1.98 kg a.s./ha treatment groups were 103, 100, 76, and 100% of the control mean. Treatment-related reductions in seedling growth were observed in the 1.98 and 4.84 kg a.s./ha groups. Mean fresh weights of seedlings in the 1.98 and 4.84 kg a.s./ha groups were 68 and 14% of the control mean, respectively, while mean seedling dry weights of the same two treatment groups were 76 and 46% of the control mean, respectively. Mean heights of seedlings in the 1.98 and 4.84 kg a.s./ha treatment groups were 80 and 43% of the mean height of control seedlings, respectively. For each parameter tested, the mean of the 4.84 kg a.s./ha group was significantly different from the control mean ($p < 0.05$), while the mean of the 1.98 kg a.s./ha group was not significantly different ($p > 0.05$) from the control group mean. Treatment-related effects upon seedling condition were observed in the 0.78, 1.98 and 4.84 kg a.s./ha groups. Chlorosis and necrosis were observed in the three highest concentration treatment groups, and the incidence and severity of the conditions appeared to be dose-responsive. In addition, treatment-related mortalities were observed in the groups. Four, three, and nine seedlings died prior to test termination in the 0.78, 1.98 and 4.84 kg a.s./ha groups respectively. Several seedlings throughout all test groups (except 4.84 kg a.s./ha) failed to shed their seed coats upon emergence, which resulted in poor seedling condition at test termination. However, the failure to shed seed coats was not attributed to treatment. Because of the treatment-related mortality, chlorosis, and necrosis observed in the 0.78 kg a.s./ha group, the NOEC for tomato seedlings was determined to be 0.31 kg a.s./ha. The survivorship, height, fresh weight, and dry weight of tomato seedlings in this test resulted in calculated EC₂₅ estimates of 2.11, 2.23, 1.49 and 1.86 kg a.s./ha, respectively.

Wheat. There was no observed treatment-related effect on the emergence of wheat seedlings at any test concentration. Mean seedling emergence in the treatment groups ranged from 100 to 105% of the control mean. Apparent treatment-related reductions in growth were observed in the 4.84 kg a.s./ha group. Mean fresh weight, dry weight, and height of seedlings in this group were 70, 77, and 83% of the control group means, respectively, and were significantly ($p < 0.05$) different from the control means. Apparent treatment-related effects upon seedling condition were observed in the 1.98 and 4.84 kg a.s./ha groups. There was a slight, but apparently treatment-related increase in the number of necrotic plants in 1.98 kg a.s./ha group, and a noticeable increase in both the occurrence and the severity of necrosis in the 4.84 kg a.s./ha group. Two seedlings in the 1.98 kg a.s./ha group died during the test as a result of the treatment. Incidental leaf curl, necrosis and chlorosis were observed on seedlings in the control, 0.31 and 0.78 kg a.s./ha groups, but was not attributed to treatment. Because of the apparent treatment-related necrosis and mortality observed in the 1.98 kg a.s./ha group, the NOEC for wheat seedlings was determined to be 0.78 kg a.s./ha. The most sensitive test endpoint for wheat was fresh weight, which resulted in a calculated EC₂₅ of 4.09 kg a.s./ha.

❑ **Conclusion:**

Treatment-related effects resulting from a single spray application of Amitrole to planted seeds were observed in all ten test species. The most sensitive species tested, as determined by the highest test concentration at which no treatment-related effects were observed (NOEC), were cabbage and lettuce, which had NOECs of 0.124 kg a.s./ha, while the NOECs for carrot, onion, rye grass, and tomato seedlings were each 0.31 kg a.s./ha. The least sensitive species were bean, corn, soybean, and wheat, which had NOECs of 0.78 kg a.s./ha.

- ❑ **Comment (RMS): Study acceptable. Some EC₅₀ values have not been reported in the summary whereas they are available in the study report. Therefore, the worst case EC₅₀ value reported in**

the study report is 0.55 lb/acre (cabbage). In the study report, doses are given in lb/acre and have been converted in kg/ha in the summary. However, this conversion is not always well understood as in this case the conversion factor of 1.12085 should be used and seems that it is not always the case. The worst case endpoint reported in the study report is an EC₅₀ of 0.55 lb/acre (cabbage). Applying the conversion factor, this results in EC₅₀=0.616 kg/ha. This value is used in the risk assessment.

B.9.9.1.3. Risk assessment for non-target plants

B.9.9.1.3.1. Toxicity data of amitrole

Table 9.9.2.3.1.1: Endpoints from plant studies with amitrole

Test Type	Test species	Ecotoxicological endpoint lowest EC ₅₀ (kg a.s./ha)	Reference
seedling emergence and growth test	Bean, Cabbage, Carrot, Corn, Lettuce, Onion, Rye, Soybean, Tomato, Wheat	Lowest EC ₅₀ (Cabbage) 0.616 kg a.s./ha	Porch J. R. 1998
Vegetative vigour	leek, maize, oats, rye, wheat (Monocots) and bush bean, cucumber, lettuce, pepper, radish, soybean and sunflower (Dicots)	Lowest EC ₅₀ (sunflower): 0.1345 kg a.s./ha	Cross J. W. 1993

B.9.9.1.3.2. Exposure

Table 9.9.2.3.2.1: Off-crop exposure for non-target terrestrial plants

Crop	Application rate (kg a.s./ha)	Distance (m)	Drift (%)	Interception (%)	PEC (kg a.s./ha)
Vine, orchards	2.977	1	2.77	0	0.0825
		5	0.57	0	0.0170

B.9.9.1.3.3. Risk assessment

Table 9.9.2.3.3.1: TER calculations

Test type	EC ₅₀ (kg a.s./ha)	Distance (m)	Drift (%)	PEC (kg a.s./ha)	TER	Trigger
Vegetative vigour (worst case)	0.1345	1	2.77	0.0825	1.63	5
		5	0.57	0.0170	7.9	5

The Working Document SANCO/10329/2002²¹ indicates that spray drift is considered the key exposure route for terrestrial plants located in the vicinity of the treated area.

It should be noted that CA2735 is applied under the row. Spraying is directed to the foot of trees and vine plants. For such applications, farmers use equipment with hydraulic nozzles and the drift can be compared to that determined in industrial crops. Therefore, the drift values given in Escort 2 for field crops were selected in the off-field HQ calculation.

Based on the maximum dose rate, 2.977 kg a.s./ha and the lowest ED50 determined on sunflower (seedling emergence) at 0.15 kg a.s./ha, the TER is lower than 5. When considering a buffer zone of 5 m, the spray drift is 0.57% and therefore, the exposure of adjacent plants is 0.017 kg a.s./ha. Under these conditions, the TER is 7.9. Because in both the vegetative vigour test and the seedling emergence study were carried out on more than 6 species, the trigger value is 5.

²¹ SANCO/10329/2002 rev. 2 final (October 2002). "Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC".

Therefore, when a buffer zone of 5 m between the treated areas and the adjacent plants is respected, CA2735 used according the manufacturer recommendations does not present any significant risk to non-target terrestrial plants.

Overall conclusion for risk management

The result of the risk assessment for terrestrial plants on vegetative vigour and seedling emergence shows a TER values above the trigger of 5, when a buffer zone of 5 m is considered. Thus, it can be concluded that there will be no risk to terrestrial non-target plants located outside of the treated area and exposed to CA2735 when a buffer zone of 5 m is considered.

B.9.9.2. RMS conclusion

The risk assessment for terrestrial non-target plants was conducted in accordance to SANCO/10329/2002 rev. 2 final (Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (2002)).

Underlying the most conservative effect endpoints obtained from seedling emergence and vegetative vigour tests for plants treated with amitrole, TERs exceed the trigger value of 5 at distances of 5 m.

The use of CA2735 in accordance to the recommended uses is acceptable to non-target plants with mitigation measure (5 m buffer zone).

B.9.10. EFFECTS ON BIOLOGICAL METHODS OF SEWAGE TREATMENT (ANNEX IIA 8.7)

This point was assessed at EU level (see addendum to amitrole monograph). A study was submitted to address this point (Armstrong, 1999) and was accepted. Therefore, there is no need for summarizing again the studies. The main endpoints are shown in 9.10.2.

B.9.10.1. Effects to activate sludge

Please refer to B.9.10.

B.9.10.2. Risk assessment

The effect of amitrole on the respiration rate of micro-organisms contained in activated sludge after a contact time of 3h was assessed. Amitrole was tested at 10, 31,6, 100, 316 and 1000 mg/L with 3,5 dichlorophenol as a reference. No decrease in oxygen consumption was observed at any tested level. The EC50 > 1000 mg/L.

B.9.10.3. RMS conclusion

The effect of amitrole on the respiration rate of micro-organisms contained in activated sludge after a contact time of 3h was assessed. Amitrole was tested at 10, 31,6, 100, 316 and 1000 mg/L with 3,5 dichlorophenol as a reference. No decrease in oxygen consumption was observed at any tested level. The EC50 > 1000 mg/L.

B.9.11. REFERENCES RELIED ON

Section B9 ecotoxicology (Annex IIA, point 8, Annex IIIA, point 10)
New Annex II Data and information

Annex Point	Author (s)	Year	Title Source (were different from company) Company, report No GLP status Published or not	Data protection claimed Y/N	Owner
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Annex point	Author (s)	Year	Title Source (were different from company) Company, report No GLP status Published or not	Data protection claimed Y/N	Owner
IIA 8.2.1.3/01	[REDACTED]	1985	Acute toxicity of aminotriazole to bluegill sunfish (<i>Lepomis macrochirus</i>) [REDACTED] GLP :N		NUFARM
IIA 8.2.1.3/02	[REDACTED]	1993	Amitrole technical: acute toxicity to sheepshead minnow (<i>Cyprinodon variegatus</i>) under static renewal conditions. [REDACTED] not published GLP :Y		NUFARM
IIA 8.3.1.3/01	Collins, K.	1993	Amitrole technical: acute toxicity to mysids under static conditions. SLI report n° 93-4-4736 Not published GLP :Y	Y	NUFARM
IIA 8.3.1.4/01	Dionne, E.	1993	Amitrole technical: acute toxicity to Estaren Oyster (<i>Sstostrea virginica</i>) under flow through conditions. SLI report n° 93-6-4816 Not published GLP :Y	Y	NUFARM
IIA 8.4/01	Palmer, J.S. and Krueger, H.O.	1998	Amitrole: a 5-d toxicity test with the freshwater alga (<i>Selenastrum capricornatum</i>). Wildlife International Ltd (USA) report n° 461A-101 Not published GLP :Y	Y	NUFARM
IIA 8-4/02	Cross, J.	1993	Non target phytotoxicity of amitrole in terrestrial use. Growth & reproduction of <i>Selenastrum capricornatum</i> . EPL BAS report n° 198S01 part A. GLP :Y	Y	NUFARM
IIA 8.4/03	Palmer, J.S. and Krueger, H.O.	1998	Amitrole: a 5-d toxicity test with the freshwater diatom (<i>Navicula pelliculosa</i>). Wildlife International Ltd (USA) report n° 461A-103 Not published GLP :Y	Y	NUFARM
IIA 8.4/04	Palmer, J.S. and Krueger, H.O.	1998	Amitrole: a 5-d toxicity test with the marine diatom (<i>Skeletonema costatum</i>). Wildlife International Ltd (USA) report n° 461A-102 Not published GLP :Y	Y	NUFARM
IIA 8.8.2./01	Candolfi, M.P.	1996	. Extended laboratory test to evaluate the side effects of Weedazol TL applied to plants on adult, <i>Aphidius rhopalosiphii</i> (Hymenoptera: Braconidae) based on Meadbriggs (1994). Springborn Lab. Europe (CH) report n° 95-034-1002 Not published GLP :Y	Y	NUFARM
IIA 8.8.2/02	Moll, M.	2006	Effects of CA2210 on the Parasitoid, <i>Aphidius rhopalosiphii</i> (Hymenoptera, Aphidiidae)-Extended Lab study. Dose response test. Ibacon (D) report n° 24842002. Not published GLP :Y	Y	NUFARM
IIA	Rosenkranz,	2006	Effects of CA2210 on the Predatory mite,	Y	NUFARM

Annex point	Author (s)	Year	Title Source (were different from company) Company, report No GLP status Published or not	Data protection claimed Y/N	Owner
8.8.2/03	B.		Typhlodromus pyri-Extended Lab study. Dose response test. Ibacon (D) report n° 24843062 Not published. GLP :Y		
IIA 8.8.2./04	Schmitzer, S.	2006	Effect of CA 2210 on the wolf spider, pardosa spec. In the laboratory-Extended Laboratory Study. Ibacon report n° 24841066 Not published GLP :Y		NUFARM
IIA 8.8.2./05	Candolfi, M.P.	1996	. Extended laboratory test to evaluate the side effects of Weedazol TL applied to plants on the predator, Orius insidiosus (Heteroptera: Anthocoridae). Springborn Lab. Europe (CH) report n° 95-036-1002 Not published GLP :Y	Y	NUFARM
IIA 8.8.2./06	Rosenkranz, B.	2006	Effects of CA2210 on the Lacewing Chrysoperla carnea-Extended Lab study. Dose response test. Ibacon (D) report n° 24844047 Not published GLP :Y	Y	NUFARM
IIA 8.16/01	Cross, J.	1993	Non target phytotoxicity of amitrole in terrestrial use. Seed germination and vegetative vigor. EPL BAS report n° 198S01 part B. GLP :Y	Y	NUFARM
IIA 8.16/02	Porch, J & al.	1998	Amitrole: a toxicity test to determine the effects of the test substance on seedling emergence of ten species of plants. WIL report n° 461109 GLP :Y	Y	NUFARM
IIA 8.16/03		2012	CA 2735: A two generations reproduction study with the mallard using pulsed exposure. not published GLP :Y	Y	NUFARM
IIA 8.16/04		2012	Fish two generations life cycle test with the Medaka (Oryzias latipes) GLP : Y Not published	Y	NUFARM

Annex III Data and information

Annex point	Author (s)	Year	Title Source (were different from company) Company, report No GLP status Published or not	Data protection claimed Y/N	Owner
IIIA 10.1.2.1/01		2006	Bird species in vineyards in France and Italy: Field data for the determination of focal species	Y	Birds and mammals TF

Annex point	Author (s)	Year	Title Source (were different from company) Company, report No GLP status Published or not	Data protection claimed Y/N	Owner
			Report N°: RA05-005 Unpublished GLP : Y		
IIIA 10.1.2.1/02		2007	Generic field monitoring of birds in vineyards in France Report N°: RA05223-2 Unpublished GLP : Y	Y	Birds and mammals TF
IIIA 10.1.2.1/03	Knäbe, S.	2009	Residues of Amitrole in Arthropods as Food Sources for Wild Birds and Mammals after Application of Weedazol (Northern Germany 2008) GAB eurofins, Germany Nufram SAS Report N°: S08-00383 Unpublished GLP : Y	Y	NUFARM
IIIA 10.1.2.1/04	Mende, P.	2009	Analytical Phase Report. Residues of Amitrole in Arthropods as Food Sources for Wild Birds and Mammals after Application of Weedazol (Northern Germany 2008) GAB eurofins, Germany Nufram SAS Report N°: S08-00383 Unpublished GLP : Y	Y	NUFARM
IIIA 10.1.2.2/01,		2006	Bird species in pome fruit orchards in Poland and Italy: Field data for the determination of focal species Report N°: RA05-002 Unpublished GLP : Y	Y	Birds and mammals TF
IIIA 10.1.2.2/02		2007	Generic field monitoring of selected bird species in orchards in southern Germany Unpublished GLP : Y	Y	Birds and mammals TF
IIIA 10.3.1.4/01		2007	Generic field monitoring of mammals in vineyards in France Report N°: RA05223-1 Unpublished GLP : Y	Y	Birds and mammals TF
IIIA 10.3.1.5/01		2006	Small mammal monitoring in modern pome orchards of Baden-Württemberg and Thuringia	Y	Birds and mammals TF

Annex point	Author (s)	Year	Title Source (were different from company) Company, report No GLP status Published or not	Data protection claimed Y/N	Owner
			Report N°: RC05-021 Unpublished GLP : Y		
IIIA 10.5	Pollini.	2010	Position paper NTA: amitrole: study on possible adverse effects versus polliniser and other beneficial insects. Not published GLP : N	Y	NUFARM
IIIA 10.5.2.1/01	Candolfi M. P.	1996	Extended laboratory test to evaluate the side effects of weedazol TL applied to plants on adult <i>Aphidius Rhopalosiphii</i> (Hymenoptera: braconidae) based on mead-briggs (1994) Springborn Laboratories, USA Nufarm Report: 95-034-1002 Unpublished GLP : Y ⇒ please refer to KIA 8.8.2/01	Y	NUFARM
IIIA 10.5.2.1/02	Moll M.	2006	Effect of CA2210 on the parasitoid <i>Aphidius rhopalosiphii</i> , extended laboratory study – dose-response test Ibacon, Germany Nufarm Report: 24842002 Unpublished GLP : Y ⇒ please refer to KIA 8.8.2/02	Y	NUFARM
IIIA 10.5.2.2/01	Rosenkranz B.	2006	Effects of CA2210 on the predatory mite <i>Typhlodromus pyri</i> , extended laboratory study, dose-response test Ibacon, Germany Nufarm Report: 24843062 Unpublished GLP : Y ⇒ please refer to KIA 8.8.2/03	Y	NUFARM
IIIA 10.5.2.3/01	Schmitzer S.	2006	Effect of CA2210 on the wolf spider <i>Pardosa sp.</i> In the laboratory, extended laboratory study Ibacon, Germany Nufarm Report: 24841066 Unpublished GLP : Y ⇒ please refer to KIA 8.8.2/04	Y	NUFARM
IIIA 10.5.2.4/01	Candolfi M. P.	1996	Extended laboratory test to evaluate the side effects of weedazol TL applied to plants on the predator <i>Orius insidiosus</i> (Heteroptera: Anthocoridae)	Y	NUFARM

Annex point	Author (s)	Year	Title Source (were different from company) Company, report No GLP status Published or not	Data protection claimed Y/N	Owner
			Springborn Laboratories, USA Nufarm Report: 95-036-1002 Unpublished GLP : Y ⇒ please refer to KIIA 8.8.2/05		
IIIA 10.5.2.4/02	Rosenkranz B.	2006	Effect of CA2210 on the lacewing <i>Chrysoperla carnea</i> , extended laboratory study – dose-response test Ibacon, Germany Nufarm Report: 24844047 Unpublished GLP : Y ⇒ please refer to KIIA 8.8.2/06	Y	NUFARM