Section 7.5.3.1.2 Short term (dietary) toxicity to birds
Annex Point IIIA XIII 1.2 **Avian dietry toxicity test**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]; Cypermethrin: A dietary LC50 study with the Northern bobwhite quail ; [redacted] Dates of experimental work: 25 July 2002 – 2 August 2002	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Chimac Agriphar s.a.	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, US EPA OPPTS number 850.2200, FIFRA subdivision E - section 71-2. OECD Guideline 205.	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	2001060167	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	96.5%w/w	
3.1.4	Composition of Product	Not applicable, test carried out on active substance	
3.1.5	Further relevant properties	Due to the low water solubility of cypermethrin, the test substance was dissolved in acetone before being mixed into the diet.	
3.1.6	Method of analysis in the diet	The method of analysis was developed by Wildlife International Ltd (study report available). Samples were extracted with ethyl acetate and the concentration of cypermethrin in the extracts determined by Gas Chromatography with Electron Capture Detection (GC-ECD). Calibration samples were run alongside each sample set. The LOD was set at 0.10 ng on-column and the LOQ set at 100 ppm a.s. based on the lowest matrix fortification level. Procedural recovery of the analytical method were 106% and 104% for samples taken on days 0 and 5, respectively. The measured concentrations of the dietary samples were not corrected for procedural recovery.	
3.2	**Administration of the test substance**	Test substance was administered in the diet. Test diets were prepared by suspending the test article in solvent (acetone) prior to mixing with feed.	

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3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Test organisms	See table A7_5_3_1_1-1
3.4.2	Test system	see table A7_5_3_1_1-2
3.4.3	Diet	All birds were fed a game bird ration formulated to in-house specifications (full diet preparation is provided in the study report). An amount of diet sufficient to last the 5 day exposure period was prepared for each treatment and control group and presented to the birds at test initiation. Test diets were prepared by suspending the test article in solvent (acetone) prior to mixing with feed using a Hobart mixer. Samples of diet were collected at preparation (day 0) to verify the test concentration and to confirm the stability and homogeneity in the diets. Samples were also collected from feed troughs of the control, low and high dose groups on day 5 of the test to assess stability of the test substance under test conditions.
3.4.4	Test conditions	See table A7_5_3_1_1-3
3.4.5	Duration of the test	10 days acclimation + 5 days exposure + 3 days observation
3.4.6	Test parameter	Mortality, signs of toxic effects, food consumption, bodyweight.
3.4.7	Examination / Observation	Twice daily, with the exception of day 8 when birds were observed once before euthanasia.
3.4.8	Statistics	Not required as no mortality occured during the study. Therefore the LC50 was based on the highest test concentration. No statistical analysis was applied to separate mean responses among treatment groups for the endpoints of food consumption and bodyweights.

4 RESULTS

4.1	**Limit Test / Range finding test**	Not performed
4.2	**Results test substance**	
4.2.1	Applied concentrations	Test diets from the 562 and 5620 ppm a.i. test concentrations were used to evaluate homogeneity. Means and standard deviations for the two test concentrations were 599±13.5 ppm a.i. and 5730± 183 ppm a.i., representing 107% and 102% of nominal respectively. The coefficients of variation for these concentrations were 2.25% and 3.19% respectively. Samples collected during the test to verify test substance concentrations for the 1000, 1780 and 3160 ppm a.i. diets had means of 1020, 1900 and 3260 ppm a.i. respectively representing 102%, 107% and 103% of nominal. Stability of the test compound was verified by analysis of diet samples from feeders after being held at ambient for 5 days. Values averaged

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		104% and 108% of the Day 0 values for the 562 and 5620 ppm a.i. test concentrations respectively.
4.2.2	Effect data (Mortality)	No mortalities occurred during the test in any of the test groups or the controls
4.2.3	Body weight	There was a decrease in mean body weight gain during the treatment period in birds receiving cypermethrin at 3160 and 5620 ppm a.i. test concentrations compared with untreated controls (see table A7_5_3_1_1-4).
4.2.4	Feed consumption	There were no apparent treatment-related effects on feed consumption at any of the test concentrations during the exposure period (see table A7_5_3_1_1-4).
4.2.5	Concentration / response curve	Not applicable
4.2.6	Other effects	There were no clinical signs of toxicity in the control group or in the treatment groups at 562 and 1000 ppm a.i. concentrations. All birds in these groups remained in good health throughout study. In the 1780 ppm a.i. treatment group signs of toxicity were noted on the morning of Day 1. These were wing droop, ruffled appearance and lethargy. These signs of toxicity were observed through to Day 7 for all except two birds which remained normal in appearance and behaviour. One bird continued to display a ruffled appearance until test termination but was nevertheless gaining weight and improving in condition. Lesions from toe picking were also noted in two birds during the course of the test. In the 3160 ppm a.i. treatment group signs of toxicity were first noted on the morning of Day 1. These were hyperexcitability, exhibited by all five birds in the first pen, and wing droop or ruffled appearance exhibited by two birds in the second pen. By the afternoon of Day 7 all birds were normal in appearance and behaviour. In the 5620 ppm a.i. treatment group signs of toxicity were first noted on the morning of Day 1. These were wing droop and ruffled appearance and were exhibited by up to seven birds. These signs of toxicity were observed only up to and through Day 7 for all except two birds which continued to display a ruffled appearance at test termination. These birds were nevertheless gaining weight and improving in condition.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	None, all control birds remained in good health throughout the study.
4.3.2	Nature of adverse effects	Not applicable
4.4	**Test with reference substance**	Not performed

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Cypermethrin technical active substance (purity 96.5%) was administered in the diet to 10-day old northern bobwhite quail (*Colinus virginianus*) at nominal doses of 0, 562, 1000, 1780, 3160 and 5620 ppm a.i. for five days according to OECD guideline 205 (10 birds of undetermined sex/treatment group) .

Mortalities, health and clinical observations were recorded twice daily for seven days after the beginning of dosing, and once prior to euthanasia, on Day 8 of the test. Individual body weights were measured at Days 0 (test initiation), 5 and 8. Average food consumption was determined by measuring the change in weight of feed presented to the birds during the exposure period and the post-exposure period.

5.2 Results and discussion

No mortality observed in any treatment group. No clinical signs of toxicity nor modification of appearance and behaviour at 562 and 1000 g/kg food. Signs of toxicity (wing droop, ruffled appearance, lethargy, hyper excitability) were observed at 1780, 3160 and 5620 mg/kg food.

Treatment-related effect in bodyweight was observed during the exposure period at 3160 and 5620 mg/kg food.

Clinical signs/feed consumption: no apparent treatment-related effect during exposure period.

LC50 (5d) > 5620 mg a.s./kg feed or > 1376 mg a.s./kg bw/d, based on the mean body weight of 24.5 g and the food consumption of 6 g /day reported in the 5620 mg/kg treatment group.

The no observed effect concentration (NOEC) was 1000 ppm a.i. (1000 mg a.s./kg feed) based upon signs of toxicity in birds receiving the 1780 ppm a.i. test concentration.

5.2.1 LD_{50}

> LC_{50} (5 days) = 5620 mg a.s./kg feed based on no mortality at the highest dose level

5.3 Conclusion

Validity criteria were fulfilled; no mortality was recorded in any of the three control groups.

LC50 (5d) > 5620 mg a.s./kg feed

NOEC 1000 mg a.s./kg feed

5.3.1 Reliability

1

Study was evaluated and accepted under directive 91/414/EC

5.3.2 Deficiencies

No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2008
Materials and Methods	Applicant’s version is acceptable.
Results and discussion	Applicant's version is a dopted.

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Conclusion	Applicant's version is a dopted.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_5_3_1_1-1: Test animals

Criteria	**Details**
Species/strain	Northern bobwhite quail (Colinus virginianus)
Source	[redacted]
Sex	Undetermined as all birds were immature
Initial body weight (bw)	16-25 g at initiation of the test
Breeding population	Birds obtained from production flock. All birds were from the same hatch.
Amount of food	Ad libitum during acclimitisation and during the test. Average feed consumption was determined by measuring the change in weight of feed presented to the birds during the exposure period and the post-exposure period. The accuracy of food consumption could be affected by wastage.
Age at time of first dosing	10 days
Health condition / medication	Birds appeared to be in good health at test initiation and received no form of antibiotic medication during acclimitisation or test period.

Table A7_5_3_1_1-2: Test system

Criteria	**Details**
Test location	Indoor in thermostatically controlled brooding pens
Holding pens	Each pen had a floor space of approximately 72 x 90 cm and a ceiling height of 23 cm. External walls, ceilings and floors were made of galvanized steel wire and sheeting.
Number of animals	Total number of tested animals = 80
Number of animals per pen [cm²/bird]	5 birds/pen [1.3 cm²/bird]
Number of animals per dose	10 chicks per dose group (5 treatment groups plus 3 untreated control groups).
Pre-treatment / acclimation	All birds were acclimitised to the caging facilities from the day of hatch until initiation of the test. Throughout acclimitisation birds were fed the game bird ration and received a water soluble vitamin mix via their water. Water and feed were supplied *ad libitum.*
Diet during test	All birds were fed a game bird ration formulated to Wildlife International Ltd's specifications. This comprosed a minimum of 27% protein and 2.5% crude fat with a maximum of 5% crude fibre. The diet also contained vitamin and mineral pre-mix.
Dosage levels (of test substance)	Nominal dietary test concentrations were 0, 562, 1000, 1780, 3160, 5620 mg/kg feed. Sufficient quantity of test diet to last the 5 day exposure period were prepared at test initiation and administered at day 0.
Replicate/dosage level	Not applicable, only one preparation/dose.
Feed dosing method	Birds were allowed access to the feed *ad libitum*
Dosing volume per application	Not applicable
Frequency, duration and method of animal monitoring after dosing	Mortalities, health and clinical observations were recorded twice daily for seven days after the beginning of dosing, and once prior to euthanasia, on Day 8 of the test.
Time and intervals of body weight determination	Individual body weights were measured at Days 0 (test initiation), 5 and 8.

Table A7_5_3_1_1-3: Test conditions (housing)

Criteria	**Details**
Test temperature	Average temperature in the brooding compartment of the pens was 39±2ºC(SD) with the average room temperature for the study being 29.8±1.2ºC(SD).
Shielding of the animals	Not specified
Ventilation	The air handling system was designed to vent up to 15 room air volumes per hour and replace them with fresh air.
Relative humidity	Average relative humidity of 56±4ºC(SD).
Photoperiod and lighting	16 hours of light per day (maintained by clock), with an average of 173 lux of illumination

Table A7_5_3_1_1-4: Effect Data

Treatment Group ppm a.i.	**Mean body weight (g)**			**Mean food consumption (g/bird/day)**	
	Day 0	**Day 5**	**Day 8**	**During dosing**	**Post-dosing**
0	21	32 (11)	39 (7)	7	8
562	20	31 (11)	39 (7)	6	9
1000	20	32 (12)	40 (7)	6	8
1780	21	31 (10)	38 (7)	6	8
3160	21	31 (9)	38 (8)	7	11
5620	21	28 (7)	36 (8)	6	10

Bracketed figures are the mean change, calculated using individual body weights

Reported figures have been rounded to the nearest whole number