Section 7.5.3.1.3 Effects on reproduction of birds

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Official use only

1 REFERENCE

1.1	**Reference**	[redacted]; Cypermethrin: Reproduction study with the northern bobwhite quail; [redacted] Dates of experimental work: 9 October 2002 – 14 April 2003
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, FIFRA guidleline 71-4 OECD guideline 206
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, study was performed on the technical grade active substance
3.1.5	Further relevant properties	
3.1.6	Method of analysis	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. The individual procedural recoveries were in the range 88-107%. 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 mixing cypermethrin (dissolved in acetone) into a premix which was then used for weekly preparation of the final diet.

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3.3	**Testing procedure**	
3.3.1	Test organisms	See table A7_5_3_1_3-1
3.3.2	Test system	See table A7_5_3_1_3-2
3.3.3	Diet	See table A7_5_3_1_3-2
3.3.4	Test conditions	See table A7_5_3_1_3-3
3.3.5	Duration of the test	21 days
3.3.6	Test parameter	Adult bodyweight, adult feed consumption, eggs laid, eggs cracked, viable embryos, live embryos, hatchlings, 14 day old survivors, eggshell thickness and offspring bodyweight.
3.3.7	Examination / Observation	All birds were observed daily. Body weights of the adults were recorded before dosing and at two week intervals up to eight weeks after dosing, and then at the end of the study. Food consumption per pen was recorded weekly. During egg laying, eggshell thickness was measured on one egg from odd numbered pens during odd numbered weeks and from even numbered pens during even numbered weeks, when available. Eggs were examined for embryo viability, on day 11-12 of incubation, and survival, on day 21 of incubation The group body weight (by parental pen) of hatchlings was determined after hatching and after an additional 14 days. Macroscopic examinations were recorded at post-mortem on birds which died during the study and on all surviving birds at the end of the exposure period.
3.3.8	Statistics	Not applicable, the NOEC was determined based on no-effects at the highest dose tested.
		4 RESULTS
4.1	**Limit Test / Range finding test**	Performed (38 day pilot study)
4.1.1	Concentration	Three treatment groups (5 pairs/group) were fed diets containing 160, 400 and 1000 ppm cypermethrin respectively. A non-treated diet was also fed to a fourth control group.
4.1.2	Number/ percentage of animals showing adverse effects	No treatment related mortalities occurred in the control, 400 or 1000 ppm dose groups. One incidental mortality did occur in the 160 ppm group, however following observations at necropsy this single mortality was considered to be unrelated to treatment. Additionally there were no signs of toxicity nor any treatment-related effects on feed consumption, bodyweight or egg production at any of the concentrations tested.
4.1.3	Nature of adverse effects	No treatment-related effects were observed in the pilot study.

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4.2 Results test substance

4.2.1 Applied concentrations

Dietary analysis: The absence of test substance or co-eluting substance was confirmed for control samples. Homogeneity testing was carried out for the 160 and 1000 ppm a.i. test concentrations (six samples at each concentration, from top, middle and bottom of left and right sections of the mixing vessel). Means and standard deviations were 178±10.2 and 972±12.9 ppm a.i. respectively, with coefficients of variation of 5.73 and 1.33% respectively.

Measured concentrations of cypermethrin in the freshly prepared then frozen diet ranged from 93 to 104% of the nominal values. Measured concentrations in diet samples collected from feeders after seven days at ambient also ranged from 93 to 104% of the Day 0 values.

4.2.2 Effect data (Mortality and reproductivity)

Mortality: No treatment-related mortalities occurred during the study. Two incidental mortalities occurred, one in the 160 ppm a.i. treatment group and the other in the 400 ppm a.i. treatment group.

The mortality in the 160 ppm a.i. treatment group was a female found dead on Day 2 of Week 12. Prior to death, the female was noted to have extensive head and neck lesions and appeared weak. At necropsy the bird was well muscled, with a body weight of 210 g. Yolk remnants were found in the abdominal cavity. The spleen was pale and slightly enlarged, the kidneys were pale, the cecal contents were pasty, and the ovary was developing. At necropsy, the female's pen mate was noted to have a slightly enlarged spleen, but was otherwise unremarkable.

The mortality in the 400 ppm a.i. treatment group was a female that was euthanized on Day 2 of Week 19 due to her debilitated condition. Prior to death the female was noted as thin and exhibited depression, a ruffled appearance, lethargy, foot lesions and associated lameness. At necropsy, the bird was emaciated, with a body weight of 136 g. There was a loss of muscle mass with the keel prominent. The liver and kidneys were pale and the ovary was regressed. At necropsy, the female's pen mate was noted to have areas of hyperemia in the small intestine, but was otherwise unremarkable.

Reproductive effects: There were no treatment-related effects upon reproductive performance at any of the concentrations tested. When compared to the control group, there were no statistically significant differences in any of the reproductive parameters measured in the 160, 400, or 1000 ppm a.i. treatment groups.

The NOEC was determined to be 1000 ppm (1000 mg a.s./kg feed) based on the highest dose level tested.

See Table A7_5_3_1_3-4

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4.2.3	Body weight	There was a slight, but statistically significant ($p < 0.05$) increase in the mean body weight of the males in the 400 ppm a.i. treatment group at termination of the adult portion of the study. Since the difference observed was small, not concentration dependant, and represented an increase in body weight, it was not considered to be related to treatment. Thus it was concluded that there were no treatment-related effects on the body weight of adult birds during the study. See Table A7_5_3_1_3-4
4.2.4	Food consumption	There were inconsistent, but statistically significant, increases in food consumption for different dose groups at different times during the study. In all cases the differences from the control were very small and were not considered to be treatment-related. See Table A7_5_3_1_3-4
4.2.5	Results of residue analysis	Not performed
4.2.6	Other effects	Clinical signs: There were no treatment-related effects on behaviour or appearance of adult birds during the study. No overt signs of toxicity were observed at any of the concentrations tested. Incidental clinical observations noted during the test included those normally associated with injuries and penwear. Autopsy: No treatment-related macroscopic abnormalities were observed in any birds examined.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No adverse effects shown by any animals in the control group
4.3.2	Nature of adverse effects	None found

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Cypermethrin technical active substance (96.5% purity) was administered in the diet to twenty-nine week old northern bobwhite quail (*Colinus virginianus*) at nominal doses of 0, 160, 400 and 1,000 ppm a.i. for 21 weeks. There were two birds housed in each pen (one male and one female) and 16 pens for each dose level of cypermethrin and the untreated control.

Body weights of the adults were recorded before dosing and at two week intervals up to eight weeks after dosing, and then at the end of the study. Food consumption per pen was recorded weekly.

Any eggs were collected daily and examined for cracking and eggshell thickness measured. Eggs were incubated and examined for embryo viability and survival and allowed to hatch.

Macroscopic examinations were recorded at *post-mortem* on birds which died during the study and on all surviving birds at the end of the exposure period.

5.2 Results and discussion

No treatment-related mortalities occurred during the study. There were no treatment-related effects on behaviour or appearance of adult birds and no overt signs of toxicity were observed at any of the concentrations tested. It was concluded that there were no treatment-related effects on the body weight of adult birds and any small differences in feed consumption were not considered to be treatment-related.

There were no treatment-related effects upon reproductive performance at any of the concentrations tested and no treatment-related macroscopic abnormalities were observed in any birds examined at autopsy.

5.2.1 NOEC

The NOEC (21 weeks) = 1000 mg a.s./kg feed or 92.0 mg a.s./kg bw/d

5.3 Conclusion

Validity criteria can be considered as fulfilled.

See table A7_5_3_1_3-5.

5.3.1 Reliability

1

5.3.2 Deficiencies

No

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

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 versionis adopted.
Conclusion	Applicant's versionis adopted.
Reliability	1

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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_3-1: Test animals

Criteria	Details
Species/strain	Northern bobwhite quail (Colinus virginianus)
Source	[redacted]
Sex	Males an females (determined by visual examination of plumage)
Initial body weight (bw)	187-249 g at initiation of the test
Breeding population	All birds were from the same hatch, approaching their first breeding season and had not been used in any previous testing.
Amount of food	Ad libitum during acclimitisation and during the test. Average feed consumption was determined per pen per week by weighing the freshly filled feeder on day 0 and recording the amount of additional diet added during the week before re-weighing the feeder on day 7 of each week.
Age at time of first dosing	29 weeks
Health condition / medication	Birds were examined prior to test initiation for physical injuries and appeared to be in good health. Neither the adults nor offspring were given any medication during the study.

Table A7_5_3_1_3-2: Test system

Criteria	Details
Test location	Indoors in holding pens
Holding pens	Georgia Quail Farm Manufacturing model no. 0330 with a floor area of 1377 cm^2, measuring approximately 27 x 51 cm. Pens had sloping roves with a ceiling height of 20-25 cm and were constructed of galvanised wire mesh and sheeting.
Number of animals (male/female)	64 pairs (1 male, 1 female per pen, 16 pens per treatment group)
Number of animals per pen [cm^2/bird]	1 male and 1 female per pen [688.5 cm^2/ bird]
Number of animals per dose	16 pens (16 males, 16 females) per treatment group
Pre-treatment / acclimation	All birds were acclimitised to the caging facilities for 16 weeks prior to study initiation and were observed daily during this period. Throughout acclimitisation birds were given water and feed *ad libitum*.
Diet during test	The basal diet was formulated to Wildlife International's specifications by Agway Inc. and contained at least 27% protein, 2.5% fat and no more than 5% fibre. Although the basal diet conatained approximately 1.1% calcium, an additional 5% of limestone (38.5% calciul) was added to the basal diets of adults for the purpose of shell formation. Offspring received basal diet without test substance and without the limestone supplement.
Dosage levels (of test substance)	Untreated control, 160, 400, 1000 mg a.s./kg feed (adjusted for purity of test substance). Dose levels were based on results of a range-finder study. Control diet and each treated diet were prepared weekly and presented to the birds once per week.
Replicate/dosage level	16 pens (1 pair of birds) per dose level
Dosing method	Ad libitum
Dosing volume per application	As required
Frequency, duration and method of animal monitoring after dosing	All birds were observed daily throughout the study for signs of toxicity or abnormal effects. Additionally all offspring were observed daily from hatching until 14 days of age.
Time and intervals of body weight determination	Measured at test initiation, at the end of test weeks 2, 4, 6, 8 and at adult termination. Bodyweights were not measured during egg laying so as to avoid any adverse effects of handling on egg production.

Incubation, storing and hatching	Eggs were collected daily and stored in a cold room until incubation, with all eggs laid in a weekly interval being considered as one lot. Prior to incubation the eggs were weighed and examined for cracking. During egg laying, eggshell thickness was measured on one egg from odd numbered pens during odd numbered weeks and from even numbered pens during even numbered weeks, when available. Eggs were incubated at 37.4°C and examined for embryo viability, on day 11-12 of incubation, and survival, on day 21 of incubation, before allowing them to hatch. The temperature and humidity of the incubator were kept constant by forced air currents and the eggs were rotated through an arc of 90° each hour.
Test period after egg-laying	The egg laying phase of the study lasted approximately 11 weks and was followed by a post-adult termination phase (incubation, hatching and 14 day offspring rearing period) lasting 6 weeks.
Turning of eggs	Yes, the incubator was equipped with an autiomatic egg rotation device
Collection period for eggs	The egg laying phase of the study lasted approximately 11 weks.

Table A7_5_3_1_3-3: Test conditions (housing)

Criteria	**Details**
Test temperature	The study room was maintained at 20.6±1.6°C(SD)
Shielding of the animals	Yes, only birds associated with this study were kept in the study room to avoid excessive disturbance
Ventilation	15 room air volumes every hour were vented and replaced with fresh air
Relative humidity	relative humidity 34 ±13%(SD)
Photoperiod and lighting	8:16 hour light/dark regime for the first seven weeks followed by a 17:7 light/dark regime until termination.
Storing, incubation and hatching conditions for eggs	Eggs were incubated at 37.4°C. The temperature and humidity of the incubator were kept constant by forced air currents and the eggs were rotated through an arc of 90° each hour.
Environmental conditions for young birds	On day 21 of incubation eggs were transferred to a Petersime Hatcher. Wire mesh baskets were used to keep hatchlings separated by parental pen of origin. Eggs were not rotated in the hatcher. The average temperature in the hatching compartment was 37.2 ±0.0ºC (SD) and a relative humidity of approximately 77% (recorded daily)

Table A7_5_3_1_3-4: Values of reproduction ability

Reproductive parameter	Test Group (ppm)			
	Control	**160**	**400**	**1000**
No. of Replicates	16	15	15	16
Mean adult food consumption (g/bird/day)	18.57	19.29	18.67	20.05
Mean adult body weight at term, males (g)	216	219	229*	212
Mean adult body weight at term, females (g)	244	236	234	246
No. eggs laid[1]	812	755	613	808
Eggs laid (% of maximum laid[4])	72	72	58	72
Eggs cracked (% of total laid)	2	3	2	1
No. eggs set	715	663	539	723
Viable embryos (% of eggs set)	86	92	96	97
Live three-week embryos (% of viable embryos)	99	100	100	100
Hatchlings (% of live three-week embryos)	96	96	96	95
No. of hatchlings	596	586	502	661
No. of 14 day survivors	574	556	489	622
14-day old survivors (% of hatchlings)	96	94	96	93
Mean body weight of hatchlings ± SD (g) [3]	6 ± 1	6 ± 0	6 ± 1	6 ± 1
Mean body weight of 14-day old survivors ± SD (g)	28 ± 3	26 ± 3	27 ± 2	26 ± 3
Hatchlings (% of eggs set)	82	87	92	91
14 day old survivors (% of eggs set)	79	83	88	85
Mean egg shell thickness ± SD (mm)	0.230 ± 0.013	0.228 ± 0.013	0.228 ±0.017	0.228 ± 0.014
Eggs laid/hen	51	50	41	51
Eggs laid/hen/day[2]	0.51	0.51	0.41	0.51
14 day old survivors/hen	36	37	33	39
Hatchlings (% of maximum set)	57	60	52	64
14 day old survivors (% of maximum set)	55	57	50	60

[1] Represents the total number of eggs laid in each group.

[2] Based on 99 days of egg production.

[3] Hatchlings found dead were not weighed

[4] Maximum laid is defined as the largest number of eggs laid by any one hen

Differences between the control and each treatment groups were not significant ($p > 0.05$) except for *.

% values represent pen means for each experimental group.

Table A7_5_3_1_3-5: Validity criteria for bird reproduction test according to OECD 206

	Fulfilled	Not fulfilled
Mortality of control animals <10%	**yes**	
Average number of 14-day-old survivors per hen in controls ≥ 14, 12 and 24 for mallard duck, bobwhite quail and Japanese quail	**yes**	
Average eggshell thickness for the control group ≥ 0.34, 0.19 and 0.19 mm for mallard duck, bobwhite quail and Japanese quail	**yes**	
Concentration of the test substance in the diet ≥ 80 % of the nominal concentration throughout the test period	**yes**	