Section A7.5.3.1.2 Short-term toxicity on birds (1)

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			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted], 1986, Subacute dietary LC50 of Preventol A4-S to Bobwhite Quail, [redacted], Toxicology Report No. [redacted], 1986-09-04.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer Chemicals AG	
1.2.2	Companies with letter of access	-	
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/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; according to US-EPA, FIFRA Guideline, Section 163, 71-2 (1984) as well as the US-EPA Toxic Substances Control Act (TSCA) and the ASTM Standard Practice (E857-81) "Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species"	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Dichlofluanid	
3.1.1	Lot/Batch number	Batch No. [redacted]; Source: Mobay Corp., Organic and Rubber Chemicals Division	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	[redacted]	X
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	low water solubility: 1.3 mg/l	
3.1.6	Method of analysis in the diet	Liquid Chromatography with UV-VIS Detector, Waters Z-Module, Radial Compression Column Unit (10 cm x 8 mm, Novapack ODS, 5 µm), repeatability, reliability and recovery of a.i. were confirmed; Results of feed analysis conducted by Hazleton Lab., Inc., USA (Study No. 86-175-04). Analysis of protein, moisture, fat, ash, crude fiber, carbohydrates, calories, several heavy metals, several aflatoxins, several organophosphates/organochlorine insecticides and PCB.	
3.2	**Administration of the test substance**	See table A7_5_3_1_2-1	
3.3	**Reference substance**	No	

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3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Test organisms	See table A7_5_3_1_2-2	
3.4.2	Test system	See table A7_5_3_1_2-3	
3.4.3	Diet	See table A7_5_3_1_2-4	
3.4.4	Test conditions	See table A7_5_3_1_2-4	
3.4.5	Duration of the test	8 days: 5 treatment days + 3 days post-exposure observation period	
3.4.6	Test parameter	Mortality, toxic signs, body weight changes, feed consumption, necropsy examinations	
3.4.7	Examination/ Observation	See table A7_5_3_1_2-3	
3.4.8	Statistics	Body weight and feed consumption: The control group mean data was compared using t-test with $P \leq 0.05$ (Sokal, R.R. & F.J. Rohlf (1969): Biometry. Freeman & Co, San Fransisco, USA) and all body weight gain data of treatment group was subjected to analysis of variance (ANOVA) with $P \leq 0.05$ (Sokal, R.R. & F.J. Rohlf (1969)). If ANOVA indicated significant treatment effects, the means of the treatment levels were compared to that of controls using the Williams test (Williams, D.A.: A test for differences between treatment means when several dose levels are compared with a zero dose control. Biometrics, 27, 103-117. Williams, D.A.: The comparison of several dose levels with a zero dose control. Biometrics, 28, 519-531). When a treatment mean was significantly different from the control means, that treatment was considered a toxicant effect level. All statistical analysis were conducted using software supplied by SAS Institute Inc., Cary, North Carolina, USA.	
		4 RESULTS	
4.1	**Limit Test / Range finding test**	Limit test was performed	
4.1.1	Concentration	See data given below	
4.1.2	Number/ percentage of animals showing adverse effects	See data given below	
4.1.3	Nature of adverse effects	See data given below	
4.2	**Results test substance**		
4.2.1	Applied concentrations	Nominal concentration in diet: 5000 ppm; measured concentrations: see table A7_5_3_1_2-3	X
4.2.2	Effect data (Mortality)	One mortality was noted in the treatment group on day 5. Gross clinical observations (i.e. face was pecked) and postmortem examination of this	

		bird suggest that death was due to cage mate aggression. No deaths which could be directly attributed to test substance occurred.
4.2.3	Body weight	See table A7_5_3_1_2-5
4.2.4	Feed consumption	See table A7_5_3_1_2-5
4.2.5	Concentration / response curve	Not applicable
4.2.6	Other effects	No clinically observable signs of toxicity were noted in treated birds. No compound-related gross lesions were noted in postmortem examination of birds sacrificed at study termination. Not test substance-related effects: hock and nares scabs (1 bird), prominent keel bone (1 bird), empty crop (1 bird), postmortem autolysis (1 bird)
4.3	**Results of controls**	See table A7_5_3_1_2-5
4.3.1	Number/ percentage of animals showing adverse effects	1 bird showed red renal zone
4.3.2	Nature of adverse effects	1 bird showed red renal zone
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	-
4.4.2	Results	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	A subacute avian dietary toxicity test was conducted to estimate the toxicity of Preventol A4-S to Bobwhite quail (*Colinus virginianus*) when exposed to the diet for a period of 5 days. The test complies with US-EPA FIFRA Guideline, Section 163.71-2 (1984) as well as those of the US-EPA Toxic Substances Control Act (TSCA) and the ASTM Standard Practice (E857-81) "Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species". One group of 10 birds was fed a diet containing 5000 ppm Preventol A4-S for a period of 5 days. Two additional non-treated groups of 10 birds each were maintained as concomitant controls. All groups were maintained on Preventol A4-S free feed for a three-day observation period following the five-day exposure period.
5.2	**Results and discussion**	One death was observed in the Preventol A4-S exposure group; however, clinically observable signs and necropsy findings indicate that death was due to wounds received as a result of cage mate aggression. No grossly observable signs of toxicity were noted. Statistically significant decreases in body weight occurred in Preventol A4-S treated birds when compared with controls; however, no differences in feed consumption were apparent. No compound-related gross lesions were noted at necropsy of quails sacrificed at study termination.

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5.2.1	LC_{50}	> 5000 ppm test substance
5.2.2	NOEC	< 5000 ppm test substance
5.3	**Conclusion**	Two of the three validity criteria for short-term avian toxicity test according to OECD Guideline 205 are fulfilled: 1. The mortality rate in the control was below 10%, 2. Test substance concentration is > 80% of nominal concentration throughout the dosing period. One criterion is not fulfilled: the lowest treatment level causing no compound-related mortality or other observable toxic effects.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	13/12/04
Materials and Methods	Accept applicant's version with the comment that: **3.1.3** The purity of dichlofluanid was [redacted] but the percentage of active substance in Preventol A4-S was compensated for in the calculation of the dietary concentration. * The feed was prepared simultaneously for the 2 short-term toxicity on birds studies and analysis done on the same batch.
Results and discussion	Accept applicant's version with the comment that: **4.2.1** The measured concentrations were 4892 on day 0 and 5570 on day 5.
Conclusion	Accept applicant's version.
Reliability	Reliability = 1
Acceptability	Acceptable The UK CA considers that the test is acceptable. The fulfilment criteria of showing the lowest concentration to show no effects has not been met, as only a limit test was performed. The UK CA considers it was acceptable to do a limit test as this is the approach suggested by SETAC.
Remarks	All endpoints and data presented in the summary and tables have been checked against the original summary and are correct.
	COMMENTS FROM ...
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_2-1: Method of administration of the test substance

Carrier/Vehicle	Details
Water	No
Organic carrier	Yes, corn oil and ethanol
Concentration of the carrier [% v/v]	Diet preparation: Appropriate amounts of Preventol A4-S, corn oil and ethanol were combined in a 250 ml Erlenmeyer flask and added to the feed while mixing in a Hobart mixer. Feed for control groups: 0 ppm Preventol A4-S, 160 g corn oil, 150 ml ethanol, 15.84 kg feed. Feed for treatment group: Control: 5000 ppm Preventol A4-S (= 39.548 mg), 70 g corn oil, 150 ml ethanol, 6.89 kg feed.
Other vehicle	Yes, feed (Purina Gamebird Startena)
Function of the carrier / vehicle	Facilitation of uptake

Table A7_5_3_1_2-*2*: Test animals

Criteria	Details
Species/Strain	Bobwhite quail (*Colinus virginianus*)
Source	[redacted]
Age (in weeks), sex and initial body weight (bw)	At an age of 3 days the quails were obtained; Sex: unknown; Body weights at age of 10 days: 18-21 g
Breeding population	No data
Amount of food	Food and water were available ad libitum, prior to and throughout the study.
Age at time of first dosing	Age: 10 days, body weights 18-21 g
Health condition / medication	No prophylactic medication.

Table A7_5_3_1_2-3: Test system

Criteria	Details
Test location	Indoor, in steel brooders
Holding pens	galvanized steel brooders (90 x 70 x 23 cm), pelletized wood was used as cage bedding und was changed once during the study.
Number of animals	30 (unknown sex)
Number of animals per pen [cm²/bird]	10 birds of unknown sex (630 cm²/bird)
Number of animals per dose	Two control groups, each 10 birds of unkown sex, One dose group with 10 birds of unkown sex
Pre-treatment / acclimatisation	Acclimatisation period 7 days, birds were examined upon receipt and daily throughout the acclimatisation period. Less than 5% mortality was noted prior test initiation and all unsuitable birds (injured, deformed etc.) were eliminated from inclusion in the test. Food (Purina Gamebird Startena) and water were available ad libitum, prior to and throughout the study.
Diet during test	Food (Purina Gamebird Startena) and water were available ad libitum throughout the study.
Dosage levels (of test substance)	Nominal concentration in diet: 5000 ppm; the birds were fed for 5 days; measured concentrations: 4892 ± 73 ppm (day 0, three samples taken for homogeneity analysis), 5570 ppm (sample taken on day 5 from initial feed mix for stability determination).
Replicate/dosage level	One dose group with 10 birds; gang housed in a breeder
Feed dosing method	Orally by feed
Dosing volume per application	The group of birds was fed a diet containing 5000 ppm a.i. for a period of 5 days, ad libitum.
Frequency, duration and method of animal monitoring after dosing	After 5 treatment days, birds were given control feed for 3 days (post-exposure observation period). Observations for mortality and toxic signs were made twice daily except on weekends when only one observation per day was made; feed consumption for each group was recorded daily. At the end of the study, all surviving birds were sacrificed by CO_2 asphyxiation. Necropsy examinations were conducted on all birds at study termination, as well as on all birds that died during the in-life phase of the study.
Time and intervals of body weight determination	Body weights were recorded on day 0, 5 and 8

Table A7_5_3_1_2-4: Test conditions (housing)

Criteria	Details
Test temperature	37.8 ± 0.5 °C.
Shielding of the animals	No data
Ventilation	No data
Relative huminity	No data
Photoperiod and lighting	8/16 hour light/dark cycle

Table A7_5_3_1_2-5: Average body weight change and feed consumption of animals during study

			CONTROL 1	CONTROL 2	DOSE GROUP
Mean body weight [g]	Treatment days	Day 0	17.5	16.9	18.3
		Day 5	30.2	29.6	25.6
	Observation day	Day 8	38.6	38.6	33.9
Daily food consumption [g/bird/day]	Treatment days	Day 1	5.5		4.0
		Day 2	4.9		3.7
		Day 3	3.4		3.3
		Day 4	5.2		6.8
		Day 5	6.5		6.8
	Observation days	Day 6	6.0		6.5
		Day 7	5.3		4.7
		Day 8	4.2		3.6
		Mean	5.1		4.9

Table A7_5_3_1_2-6: Validity criteria for short-term avian toxicity test according to OECD Guideline 205

	fulfilled	Not fulfilled
Mortality of control animals < 10%	**X**	
Test substance concentration > 80% of nominal concentration throughout the dosing period	**X**	
Lowest treatment level causing no compound-related mortality or other observable toxic effects		**X**