4 RESULTS

4.1 Limit Test / Range finding test Performed.

4.1.1 Concentration 0.3, 2.7 and 5.0 mg/kg_{bw}

4.1.2 Number/ percentage of animals showing adverse effects Mortalities were 1/2, 2/2 and 2/2 for the above concentrations (dose levels) respectively.

4.1.3 Nature of adverse effects Mortality.

4.2 Results test substance

4.2.1 Applied concentrations Dose levels (mg/kg_{bw}):

0 (control group dosed with corn oil vehicle only), 0.10, 0.20, 0.25, 0.80, 1.40, 2.00, 2.60, 3.20, 3.80, 4.40, and 5.00.

4.2.2 Effect data (Mortality) See Table A7_5_3_1_1-5 for a summary of the mortalities.

4.2.3 Body weight

Group/ Dose level (mg/kg_{bw})	Mean Bodyweight (g)						
	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Mean Body weight change (Day 0-Day 28)
1/ 0 (control)	1074 (M) 974 (F)	1114 (M) 1027 (F)	1105 (M) 1014 (F)	1082 (M) 1016 (F)	1085 (M) 1020 (F)	1093 (M) 1020 (F)	+19 (M) +46 (F)
11/ 0.10*	1012 (M) 932 (F)	1075 (M) 999 (F)	1050 (M) 993 (F)	1081 (M) 997 (F)	1094 (M) 1007 (F)	1101 (M) 1019 (F)	+89 (M) +87 (F)
2/ 0.20	988 (M) 1001 (F)	1032 (M) 1049 (F)	1015 (M) 1002 (F)	962 (M) 1025 (F)	957 (M) 1063 (F)	967 (M) 1072 (F)	-21 (M) +71 (F)
12/ 0.25*	1051 (M) 1026 (F)	1116 (M) 1055 (F)	1106 (M) 1006 (F)	1097 (M) 981 (F)	1094 (M) 987 (F)	1120 (M) 1030 (F)	+69 (M) +4 (F)
3/ 0.80	1107 (M) 959 (F)	1156 (M) 1014 (F)	1100 (M) 931 (F)	1165 (M) - (F)	1125 (M) - (F)	1155 (M) - (F)	+48 (M) - (F)
4/ 1.40	1095 (M) 1024 (F)	1147 (M) 1068 (F)	1041 (M) 975 (F)	1035 (M) 845 (F)	995 (M) - (F)	- (M) - (F)	- (M) - (F)
5/ 2.00	1127 (M) 1049 (F)	1156 (M) 1082 (F)	1076 (M) 973 (F)	- (M) 755 (F)	- (M) - (F)	- (M) - (F)	- (M) - (F)
6/ 2.60	1079 (M) 1030 (F)	1132 (M) 1044 (F)	1081 (M) 955 (F)	1030 (M) 855 (F)	- (M) - (F)	- (M) - (F)	- (M) - (F)
7/ 3.20	1091 (M) 1017 (F)	1130 (M) 1063 (F)	1006 (M) 943 (F)	- (M) - (F)	- (M) - (F)	- (M) - (F)	- (M) - (F)
8/ 3.80	1050 (M) 1055 (F)	1055 (M) 1036 (F)	932 (M) 835 (F)	- (M) - (F)	- (M) - (F)	- (M) - (F)	- (M) - (F)
9/ 4.40	1123 (M) 1035 (F)	1163 (M) 1062 (F)	1098 (M) 953 (F)	- (M) - (F)	- (M) - (F)	- (M) - (F)	- (M) - (F)
10/ 5.00	1089 (M) 1120 (F)	1064 (M) 1077 (F)	1059 (M) 1035 (F)	- (M) - (F)	- (M) - (F)	- (M) - (F)	- (M) - (F)

NB * Groups 11 and 12 were additional dose levels used to give a better spread of moralities.

4.2.4 Feed consumption

Group	Mean food consumption (g/bird/day) over stated period of Days

	Days -7-0	Days 1-7	Days 8-14	Days 15-21	Days 22-8
1/ 0 (control)	117	120	103	126	123
11/ 0.10*	80	154	103	91	114
2/ 0.20	109	117	83	148	140
12/ 0.25*	120	123	94	100	133
3/ 0.80	120	98	177	514***	1129***
4/ 1.40	111	111	76	180	1500***
5/ 2.00	117	92	89	-	-
6/ 2.60	97	80	50	100	-
7/ 3.20	103	100	120	-	-
8/ 3.80	89	67	400***	-	-
9/ 4.40	100	97	100	-	-
10/ 5.00	81	67	200	-	-

NB * Groups 11 and 12 were additional dose levels used to give a better spread of moralities.

** Primarily food spillage which was impossible to estimate

4.2.5 Concentration / response curve

Given in study report, with the confidence limits from the statistical analysis reported as 0.19-0.50 mg/kg_{bw} (for male and female birds), 0.19-0.67 mg/kg_{bw} (for male birds only), and 0.17-0.53 mg/kg_{bw} (for female birds only.

4.2.6 Other effects

Clinical observations:
The mortality rates in the treatment groups were 0 - 100% with the majority of the mortalities occurring between Days 7 and 10 (see Table A7_5_3_1_2-5 below for a summary of the results). Clinical observations included various degrees of unsteadiness and subdued behaviour.

Necropsy:
With the exception of one bird in Group 2 (0.20 mg/kg_{bw}) there was evidence of haemorrhaging in all birds which died or were sacrificed during the post-dose observation period. No abnormalities were found in birds which were sacrificed at the termination of the study.

4.3 Results of controls

4.3.1 Number/ percentage of animals showing adverse effects

There were no mortalities in the control group and no reported abnormalities at the gross *post-mortem* examination at the end of the study.

4.3.2 Nature of adverse effects

See section 4.3.1 above.

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**	Test substance: brodifacoum; Batch no: Y0052/002/004; Guidelines: USA EPA Proposed Guidelines 163.71-1 Avian single oral LD_{50}, published in the The Federal Register Vol. 43, No. 132 on 10 July 1978 Part II, page 29726; Test species: Mallard duck (*Anas platyrhynchos*). 120 birds were randomly allocated to 12 groups, with each group comprising 10 animals (5 male and 5 females) and were given a single oral dose by gavage in corn oil followed by a 28-day observation period. There was 1 control group and 11 treatment groups at dose levels of 0, 0.10, 0.20, 0.25, 0.80, 1.40, 2.00, 2.60, 3.20, 3.80, 4.40, and 5.00 mg/kg_{bw} brodifacoum. Symptoms of toxicity and mortality were recorded daily throughout the study. Bodyweight and food consumption were measured frequently throughout the study. Gross post-mortem examinations were performed on all birds that died during the test period and on all surviving birds at termination.
5.2	**Results and discussion**	Mortality rates in the treatment groups were 0-100 %, with death generally occurring between Days 7 and 10 of the test period. The clinical observations and the findings at necropsy were generally consistent with those expected following administration of an anticoagulant.
5.2.1	LD_{50}	0.31 mg/kg_{bw} (male and female) 0.36 mg/kg_{bw} (male) 0.30 mg/kg_{bw} (female)
5.3	**Conclusion**	The validity criteria were fulfilled.
5.3.1	Reliability	1
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	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	No 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_2-1: Method of administration of the test substance

Carrier / Vehicle	Details
Water	No
Organic carrier	Yes, corn oil as the dose vehicle.
Concentration of the carrier [% v/v]	The concentration of the test substance in the carrier was 0.05% w/v, with the dose volume (ml/bird) adjusted according to the dose level required and the weight of the bird.
Other vehicle	
Function of the carrier / vehicle	To administer the test substance by gavage.

Table A7_5_3_1_2-2: Test animals

Criteria	**Details**			
Species/strain	Mallard Duck (*Anas platyrhynchos).*			
Source	The County Game Farms, Home Farm, Hothfield, Ashford, Kent.			
Age (in weeks), sex and initial body weight (bw)	**Group**	**Age (weeks)**	**Sex**	**Mean initial bodyweight**
	1	16	5 males and 5 females per treatment group.	1024 g
	11	16		972 g
	2	16		995 g
	12	16		1039 g
	3	16		1033 g
	4	16		1060 g
	5	16		1088 g
	6	16		1055 g
	7	16		1054 g
	8	16		1052 g
	9	16		1079 g
	10	16		1105 g
Breeding population	The County Game Farms, Home Farm, Hothfield, Ashford, Kent.			
Amount of food	Food was available *ad libitum* apart from an overnight starvation period prior to dosing. Water was available at all times. The diet was known to contain no antibiotic or other growth promoter.			
Age at time of first dosing	16 weeks.			
Health condition / medication	Birds used for study assumed to be healthy as no adverse observations noted for pre-treatment period. No medication given (see note above under 'amount of food').			

Table A7_5_3_1_2-3: Test system					
Criteria	**Details**				
Test location	Indoors in holding pens.				
Holding pens	Floor pens measuring 2.0 m x 1.5 m, with wire mesh floors, in a controlled environment poultry building. Each pen contained an automatic drinker and feeder.				
Number of animals	120				
Number of animals per pen [cm²/bird]	10 birds per pen.				
Number of animals per dose	10 per dose level with 1 control group.				
Pre-treatment / acclimation	Birds were maintained under test conditions for 14 days prior to dosing.				
Diet during test	HRC chick diet in meal form.				
Dosage levels (of test substance)	**Group**	**Dose frequency**	**Mean dose volume (ml/bird)**		**Dose level (mg/kg_{bw})**
			Male	**Female**	
	1	Single	10.8	9.8	0
	11	Single	0.2	0.2	0.10
	2	Single	0.4	0.4	0.20
	12	Single	0.5	0.5	0.25
	3	Single	1.8	1.5	0.80
	4	Single	3.1	2.9	1.40
	5	Single	4.5	4.2	2.00
	6	Single	5.6	5.4	2.60
	7	Single	7.0	6.5	3.20
	8	Single	8.0	8.0	3.80
	9	Single	9.9	9.1	4.40
	10	Single	10.9	11.2	5.00
Replicate/dosage level	10 birds per dosage level (5 males and 5 females).				
Dosing method	Gavage				
Dosing volume per application	See above under 'dosage levels (of test substance)'.				
Frequency, duration and method of animal monitoring after dosing	Symptoms of toxicity and mortality were recorded daily throughout the study.				
Time and intervals of body weight determination	Bodyweights were recorded on Days -14, -7, 0, 3, 7, 14, 21 and 28.				

Table A7_5_3_1_2-4: Test conditions (housing)

Criteria	Details
Test temperature	18°C.
Shielding of the animals	The birds were housed indoors by group in purpose built pens.
Ventilation	Ventilation was provided by fans which were adjusted when necessary.
Relative humidity	Recorded daily with a mean value of 77%.
Photoperiod and lighting	Normal daylight pattern was followed.

Table A7_5_3_1_2-5: Mortality data

Test substance dosage level [mg/kg$_{bw}$]	Mortality data	
	Total number per dose level (Day)	Percentage per dose level
0 (controls)	0/10	0
0.10	0/10	0
0.20	4/10 (4,8,13,13)	40
0.25	3/10 (9,16,16)	30
0.80	9/10 (4,7,8,8,8,8,9,10,11)	90
1.40	10/10 (4,7,8,8,8,8,9,10,18,23)	100
2.00	10/10 (4,5,7,7,8,8,8,9,9,15)	100
2.60	10/10 (8,8,8,8,8,9,10,14,14,18)	100
3.20	10/10 (7,8,8,8,8,8,9,9,9,10)	100
3.80	10/10 (3,4,4,7,7,8,8,8,9,10)	100
4.40	10/10 (4,5,5,6,8,8,8,8,9,12)	100
5.00	10/10 (8,8,8,8,9,9,9,9,10,11)	100
LD_{50}	**0.31 mg/kg$_{bw}$**	
Temperature [°C]	18°C.	
Relative humidity	77%	

Table A7_5_3_1_1-7: Validity criteria for avian acute oral toxicity test according to EPA OPPTS 850.2100

	Fulfilled	Not fulfilled
Mortality of control animals <10%	Yes	

Doc IIIA / Section 7.5.3.1.2/01 BPD Data Set IIIA / Annex Point XIII.3.4

Acute/Short term dietary toxicity on birds

5-Day Dietary LC_{50} to Laughing Gulls

Official use only

1 REFERENCE

1.1 Reference

[redacted], (1979). Forty day LC50 - Laughing Gull, Technical Brodifacoum, Final Report. Wildlife International Ltd Report No: 123-125. February 1979 (unpublished)

[redacted]

[redacted]

1.2 Data protection

[redacted]

1.2.1 Data owner

[redacted]

1.2.2 Companies with letters of access

[redacted]

1.2.3 Criteria for data protection

[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

No, study pre-dates guidelines, but study conducted in accordance with the general principles of OECD Guideline for Testing of Chemicals No 205, Avian Dietary Toxicity Test.

2.2 GLP

No. Study pre-dates the requirement for GLP.

2.3 Deviations

Not applicable.

3	**METHOD**		
3.1	**Test material**	Brodifacoum	
3.1.1	Lot/Batch number	Not given in study report.	X
3.1.2	Specification	Please refer to Section 2 of Doc IIIA.	
3.1.3	Purity	Not given in study report.	X
3.1.4	Composition of Product	Not applicable.	
3.1.5	Further relevant properties		
3.1.6	Method of analysis in the diet	The concentrations in the diet were analysed by the sponsoring company, but these were not reported in the study report.	X
3.2	**Administration of the test substance**	Treated and control diets were prepared by the sponsoring company by spiking masticated rodent tissue at the appropriate dietary concentration. Control groups received unspiked masticated rodent tissue. See table A7_5_3_1_2-1 below.	X
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	Laughing Gull (*Larus atricilla*). See table A7_5_3_1_1-2 below.	
3.4.2	Test system	See table A7_5_3_1_1-3 below.	
3.4.3	Diet	Masticated rodent tissue during the 5-day dosing period, followed by 'Southern States cat food' for the 5-week observation period.	
3.4.4	Test conditions	See table A7_5_3_1_1-4 below.	
3.4.5	Duration of the test	40 days (5 day dietary dose followed by 35 day observation period).	
3.4.6	Test parameter	Clinical observations and mortalities.	
3.4.7	Examination / Observation	<u>Clinical examinations and observations</u>: Symptoms of toxicity and mortality were recorded daily throughout the study. <u>Gross macroscopic examinations</u>: Necropsies were performed on the birds which died during the test period.	
3.4.8	Statistics	Not stated in study report.	

4 RESULTS

4.1 Limit Test / Range finding test Not performed.

4.1.1 Concentration

4.1.2 Number/ percentage of animals showing adverse effects

4.1.3 Nature of adverse effects

4.2 Results test substance

4.2.1 Applied concentrations

Dietary concentration (mg/kg_{diet}):

0 (control group), 0.72, 1.62, 3.41, 7.26, 14.02.

4.2.2 Effect data (Mortality)

See Table A7_5_3_1_1-5 for a summary of the mortalities.

4.2.3 Body weight

Mean Bodyweight (g)

Group	Day 0	Day 6	Day 13	Day 20	Day 27	Day 34	Day 40
Control	310	300	300	285	275	270	254
0.72 ppm	305	285	325	300	287	300	275
1.62 ppm	335	315	-	-	-	-	-
3.41 ppm	325	294	-	-	-	-	-
7.26 ppm	300	300	-	-	-	-	-
14.02 ppm	325	275	-	-	-	-	-

4.2.4 Feed consumption

Group	Mean food consumption (g/bird/day) over stated period of Days									
	Day 1*	Day 2*	Day 3*	Day 4*	Day 5*	Wk 1	Wk2	Wk 3	Wk 4	Wk 5
Control	0	29	45	57	89	31	20	28	23	22
0.72 ppm	14	11	19	21	50	24	28	21	30	29
1.62 ppm	4	31	48	62	84	0	0	-	-	-
3.41 ppm	31	31	48	61	68	0	6	-	-	-
7.26 ppm	6	30	45	57	88	0	0	-	-	-
14.02 ppm	9	27	44	55	45	0	0	-	-	-

*** Treatment period**

4.2.5 Concentration /

A no effect level was not established by this study, but the 5-day dietary

	response curve	LC_{50} value was estimated to be <0.72 ppm.
4.2.6	Other effects	Clinical observations: There was a 60% mortality rate at the 0.72 ppm with mortalities occurring on Days 7, 8 and 18. Two of the gulls exhibited symptoms of depression and reduced reaction to external stimuli, and one was found in a prostrate posture prior to death. All surviving gulls remained asymptomatic during the study. A 100% mortality rate occurred in the remaining four higher dose levels, with mortalities occurring between Days 6 and 12 of the study. The gulls were either asymptomatic prior to death or exhibited symptoms of lethargy and depression, reduced reaction to external stimuli, and/or lower limb weakness. Necropsy: The necropsy of the gull that died on Day 7 at the 0.72 ppm dose level revealed no apparent sign of hemorrhagic activity. A white mouldy growth was observed in the dependent portion of the lungs and long aerial mycelia was noted in the lumen of the trachea. It was concluded that death was due to a nodular pulmonary form of aspergillosis. Necropsy results of all remaining gulls which died during the test period were relatively constant, with subcutaneous and/or abdominal extravastians found in most cases. Petechial hemorrhage of the liver and heart were also commonly observed. Several gulls exhibited minute areas of hemorrhaging at the base of the brain and at the apex of the heart. Four cases of external bleeding were observed to have occurred: one gull was found with extravascular blood in the oesophagus and mouth, and three gulls exhibited heavy external bleeding from wounds on the leading edge of their wings.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No mortalities occurred in the negative control group, and all birds were normal in both appearance and behaviour throughout the test period.
4.3.2	Nature of adverse effects	See section 4.3.1 above.
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**	Test substance: brodifacoum; Guidelines: study pre-dates guidelines, but study conducted in accordance with the general principles of OECD Guideline for Testing of Chemicals No 205, Avian Dietary Toxicity Test; Test species: Laughing Gull (*Larus atricilla*). 30 birds were randomly allocated to 6 groups, with each group comprising 5 animals (male and females randomly mixed) and were given the test diets (brodifacoum spiked masticated rodent tissue) for a period of five days followed by a 35-day observation period. There was 1 control group and 5 treatment groups with dietary dose levels of 0.72, 1.62, 3.41, 7.26 and 14.02 ppm brodifacoum. Untreated diet ('Southern States Cat food) was given from Day 5 until termination. Symptoms of toxicity and mortality were recorded daily throughout the study. Bodyweight and food consumption were measure frequently throughout the study. Necropsies were performed on all birds that died during the test period and then on all surviving birds at termination.	
5.2	**Results and discussion**	By Day 12 of the study, a 100% mortality rate had occurred at the 1.62 ppm, 3.41 ppm, 7.62 ppm and 14.02 ppm dose levels. A 50% mortality rate occurred in the 0.72 ppm dose level by Day 18. Necropsies performed on all mortalities revealed signs of hemorrhagic activity in all but one gull.	X
5.2.1	LC_{50}	<0.72 ppm (5-day dietary LC_{50})	
5.3	**Conclusion**	A no effect level was not established by this study, but the LC_{50} value was estimated to be <0.72 ppm. The validity criteria were fulfilled.	
5.3.1	Reliability	2	
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	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
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_2-1: Method of administration of the test substance

Carrier / Vehicle	**Details**
Water	No
Organic carrier	No
Concentration of the carrier [% v/v]	Not given in study report.
Other vehicle	Diet.
Function of the carrier / vehicle	

Table A7_5_3_1_2-2: Test animals

Criteria	**Details**			
Species/strain	Laughing Gull (*Larus atricilla*)			
Source	Birds obtained from Suncoast Seabird Sanctuary, Redington Shores, Florida, USA.			
Age (in weeks), sex and initial body weight (bw)	**Group**	**Mean initial bodyweight**	**Sex**	**Age**
	Control	310 g	Birds randomly assigned to treatment groups without regard to sex.	Age not stated in study report but birds described as 'mature'.
	0.72 ppm	305 g		
	1.62 ppm	335 g		
	3.41 ppm	325 g		
	7.26 ppm	300 g		
	14.02 ppm	325 g		
Breeding population	Birds obtained from Suncoast Seabird Sanctuary, Redington Shores, Florida, USA.			
Amount of food	Both food and water were available *ad libitum* throughout the study.			
Age at time of first dosing	Age not stated in study report but birds described as 'mature'.			
Health condition / medication	Although not stated in study report, birds are presumed to have been in good health at initiation of study, otherwise they would not have been used as subjects. No statement that medication was administered, and therefore again, it is presumed that none was given.			

Table A7_5_3_1_2-3: Test system			
Criteria	**Details**		
Test location	Indoors in specially constructed pens - one for each treatment group.		
Holding pens	The pens were constructed of 2 feet high 20 gauge poultry netting supported by 2 feet high wooden posts. Each of the six pens consisted of a 4 x 6 feet area (*ie* 24 sq ft). The sides of the study pens were covered with black polyethylene to reduce disturbance of the birds during observation. Each of the study pens was provided with a sand floor to prevent injuries to the birds' feet and infra-red lights were utilised as a heat source.		
Number of animals	60		
Number of animals per pen [cm²/bird]	5 birds per pen (*ie* 24/5=4.8 sq ft per bird)		
Number of animals per dose	5 per dose level plus 1 control group.		
Pre-treatment / acclimation	The gulls were kept in a holding pen for a 2 week period prior to the start of the study.		
Diet during test	During the 5-day dosing period, the diet was masticated rodent tissue (spiked with brodifacoum). During the post-exposure observation period, the diet was 'Southern States' cat food, which was supplemented with fish scraps.		
Dosage levels (of test substance)	**Group**	**Dose frequency**	**Nominal dietary concentration (mg/kg_{diet})**
	Control	Not applicable	0
	0.72 ppm	5-day dietary dose	**0.72**
	1.62 ppm	5-day dietary dose	**1.62**
	3.41 ppm	5-day dietary dose	**3.41**
	7.26 ppm	5-day dietary dose	**7.26**
	14.02 ppm	5-day dietary dose	**14.02**
Replicate/dosage level	5 birds per dosage level.		
Dosing method	Dietary.		
Dosing volume per application	Food consumption was recorded by pen during the 5-day exposure period, and then on a weekly basis throughout the 35-day observation period.		
Frequency, duration and method of animal monitoring after dosing	Symptoms of toxicity and mortality were recorded daily throughout the study.		
Time and intervals of body weight determination	Bodyweights were recorded by pen at initiation, and then on Days 6, 13, 20, 27, 34 and at the termination of the study.		

Table A7_5_3_1_2-4: Test conditions (housing)

Criteria	Details
Test temperature	Specific temperature recordings not stated in study report, but it was stated that infra-red lights were utilised as a heat source.
Shielding of the animals	The birds were housed indoors by group in purpose built pens.
Ventilation	Details not given in study report.
Relative humidity	Details not given in study report.
Photoperiod and lighting	9 hours of light per day.

Table A7_5_3_1_2-5: Mortality data

Test substance dosage level (actual) [ppm_{diet} or mg/kg_{diet}]	**Mortality data**	
	Total number per dose level (Day)	**Percentage per dose level**
0 (controls)	0/5	0
0.72	3/5 (7, 8, 18,)	60
1.62	5/5 (7, 7, 8, 8, 9)	100
3.41	5/5 (6, 7, 7, 7, 11)	100
7.26	5/5 (6, 7, 8, 8, 10)	100
14.02	5/5 (6, 6, 6, 8, 8)	100
5-day LC_{50}	<0.72 ppm	
Temperature [°C]	Specific temperature recordings not stated in study report, but it was stated that infra-red lights were utilised as a heat source.	
Relative humidity	Details not given in report.	

Table A7_5_3_1_1-7: Validity criteria for avian acute oral toxicity test according to EPA OPPTS 850.2100

	Fulfilled	**Not fulfilled**
Mortality of control animals <10%	Yes	

Doc IIIA / Section 7.5.3.1.2/02 BPD Data Set IIIA / Annex Point XIII.3.4

Acute/Short term dietary toxicity on birds

5-Day Dietary LC_{50} to Laughing Gulls

Official use only

1 REFERENCE

1.1 Reference [redacted] (1979). Forty day LC50 - Laughing Gull, Technical Brodifacoum, Final Report. Wildlife International Ltd Report No: 123-126. May 1979 (unpublished) [redacted].

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letters of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No, study pre-dates guidelines, but study conducted in accordance with the general principles of OECD Guideline for Testing of Chemicals No 205, Avian Dietary Toxicity Test.

2.2 GLP No. Study pre-dates the requirement for GLP.

2.3 Deviations Not applicable.

3	**METHOD**		
3.1	**Test material**	Brodifacoum	
3.1.1	Lot/Batch number	Not given in study report - brodifacoum containing masticated rodent tissue was supplied already prepared by the sponsoring company (ICI Americas Inc., Goldsboro, North Carolina, USA.	X
3.1.2	Specification	Please refer to Section 2 of Doc IIIA.	
3.1.3	Purity	Not given in study report.	X
3.1.4	Composition of Product	Not applicable.	
3.1.5	Further relevant properties		
3.1.6	Method of analysis in the diet	The concentrations in the diet were analysed by the sponsoring company, but these were not reported in the study report.	X
3.2	**Administration of the test substance**	Treated and control diets were prepared by the sponsoring company by spiking masticated rodent tissue at the appropriate dietary concentration. Control groups received unspiked masticated rodent tissue. See table A7_5_3_1_2-1 below.	X
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	Laughing Gull (*Larus atricilla*). See table A7_5_3_1_1-2 below.	
3.4.2	Test system	See table A7_5_3_1_1-3 below.	X
3.4.3	Diet	Masticated rodent tissue during the 5-day dosing period, followed by 'Southern States cat food' for the 5-week observation period.	
3.4.4	Test conditions	See table A7_5_3_1_1-4 below.	
3.4.5	Duration of the test	40 days (5 day dietary dose followed by 35 day observation period).	
3.4.6	Test parameter	Clinical observations and mortalities.	
3.4.7	Examination / Observation	Clinical examinations and observations: Symptoms of toxicity and mortality were recorded daily throughout the study. Gross macroscopic examinations: Necropsies were performed on the birds which died during the test period.	
3.4.8	Statistics	Not stated in study report.	

4 RESULTS

4.1 Limit Test / Range finding test Not performed.

4.1.1 Concentration

4.1.2 Number/ percentage of animals showing adverse effects

4.1.3 Nature of adverse effects

4.2 Results test substance

4.2.1 Applied concentrations

Dietary concentration (mg/kg_{diet}):

0 (control group), 0.13, 0.34, 0.84, 2.10, 5.26.

4.2.2 Effect data (Mortality)

See Table A7_5_3_1_1-5 for a summary of the mortalities.

4.2.3 Body weight

Mean Bodyweight (g)

Group	Day 0	Day 6	Day 13	Day 20	Day 27	Day 34	Day 40
Control	280	275	290	295	305	315	315
0.13 ppm	285	280	305	255	305	315	320
0.34 ppm	270	240	294	288	294	275	294
0.84 ppm	260	245	280	280	285	275	280
2.10 ppm	275	275	225	250	225	225	250
5.26 ppm	280	275	275	275	275	275	275

4.2.4 Feed consumption

Group	Mean food consumption (g/bird/day) over stated period of Days									
	Day 1*	Day 2*	Day 3*	Day 4*	Day 5*	Wk 1	Wk2	Wk 3	Wk 4	Wk 5
Control	42	42	43	42	41	43	37	38	38	39
0.13 ppm	28	28	28	28	27	43	40	34	34	31
0.34 ppm	14	42	52	38	35	45	37	35	32	28
0.84 ppm	28	29	27	28	26	40	36	31	30	28
2.10 ppm	32	35	33	33	41	31	36	38	38	38
5.26 ppm	27	33	29	30	37	30	28	30	29	29

*** Treatment period**

4.2.5 Concentration / No information given in study report, but the confidence limits from the

	response curve	statistical analysis were reported as 0.8 - 3.3 ppm.
4.2.6	Other effects	Clinical observations: There were no mortalities at the lowest dose level of 0.13 ppm, and all birds in this group remained asymptomatic during the study. At the 0.34 ppm dose level there was 20% mortality with no prior symptoms of toxicity observed. All surviving birds remained symptomatic throughout the study. At the 0.84 ppm dose level there were no mortalities. There was only one clinical observation of some external bleeding from a wing of one bird. At the 2.10 and 5.26 ppm dose levels, there was an 80% mortality rate with mortalities occurring between Days 4 and 13. Clinical observations included lethary and depression, reduced reaction to external stimulie, loss of coordination and lower limb weakness. The surviving birds at these dose levels remained asymptomatic throughout the study. Necropsy: Necropsy results on all birds which died during the study revealed hamorrhages, with subcutaneous and/or abdominal extravasations found in most cases. Hemorrhaging of the mucosa and/or serosa of the intestines was also common. Petechial to ecchymotic hemorrhaging of the liver and heart was found in several birds. There were four cases of extravascular bleeding from wounds on the leading edge of the wings or on the upper mandible of the bill. One gull exhibited extravascular bleeding from the mouth.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	No mortalities occurred in the control group and all birds were normal in both appearance and behaviour throughout the test period.
4.3.2	Nature of adverse effects	See section 4.3.1 above.
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**	Test substance: brodifacoum; Guidelines: study pre-dates guidelines, but study conducted in accordance with the general principles of OECD Guideline for Testing of Chemicals No 205, Avian Dietary Toxicity Test; Test species: Laughing Gull (*Larus atricilla*). 30 birds were randomly allocated to 6 groups, with each group comprising 5 animals (male and females randomly mixed) and were given the test diets (brodifacoum spiked masticated rodent tissue) for a period of five days followed by a 35-day observation period. There was 1 control group and 5 treatment groups with dietary dose levels of 0.13, 0.34, 0.84, 2.10, 5.26 ppm brodifacoum. Untreated diet ('Southern States' cat food) was given from Day 5 until termination. Symptoms of toxicity and mortality were recorded daily throughout the study. Bodyweight and food consumption were measure frequently throughout the study. Necropsies were performed on all birds that died during the test period and then on all surviving birds at termination.
5.2	**Results and discussion**	At the lowest three dose levels (0.13 - 0.84 ppm), there were 0 - 20% mortalities with generally all other birds remaining asymptomatic during the study. At the two highest dose levels (2.10 - 5.26 ppm), there were 80% mortalities in both groups, occurring between Days 4 and 13. The clinical observations and the findings at necropsy were generally consistent with those expected following administration of an anticoagulant.
5.2.1	LC_{50}	1.6 ppm (5-day dietary LC_{50})
5.3	**Conclusion**	The validity criteria were fulfilled.
5.3.1	Reliability	2
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	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
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_2-1: Method of administration of the test substance

Carrier / Vehicle	**Details**
Water	No
Organic carrier	No
Concentration of the carrier [% v/v]	Not given in study report.
Other vehicle	Diet.
Function of the carrier / vehicle	

Table A7_5_3_1_2-2: Test animals

Criteria	**Details**			
Species/strain	Laughing Gull (*Larus atricilla*)			
Source	Birds obtained from Suncoast Seabird Sanctuary, Redington Shores, Florida, USA.			
Age (in weeks), sex and initial body weight (bw)	**Group**	**Mean initial bodyweight**	**Sex**	**Age**
	Control	280 g	Birds randomly assigned to treatment groups without regard to sex.	Age not stated in study report but birds described as 'mature'.
	0.13 ppm	285 g		
	0.34 ppm	270 g		
	0.84 ppm	260 g		
	2.10 ppm	275 g		
	5.26 ppm	280 g		
Breeding population	Birds obtained from Suncoast Seabird Sanctuary, Redington Shores, Florida, USA.			
Amount of food	Both food and water were available *ad libitum* throughout the study.			
Age at time of first dosing	Age not stated in study report but birds described as 'mature'.			
Health condition / medication	Although not stated in study report, birds are presumed to have been in good health at initiation of study, otherwise they would not have been used as subjects. No statement that medication was administered, and therefore again, it is presumed that none was given.			

Table A7_5_3_1_2-3: Test system			
Criteria	**Details**		
Test location	Indoors in specially constructed pens - one for each treatment group.		
Holding pens	The pens were constructed of 2 feet high 20 gauge poultry netting supported by 2 feet high wooden posts. Each of the six pens consisted of a 4 x 6 feet area (*ie* 24 sq ft). The sides of the study pens were covered with black polyethylene to reduce disturbance of the birds during observation. Each of the study pens was provided with a sand floor to prevent injuries to the birds' feet and infra-red lights were utilised as a heat source.		
Number of animals	60		
Number of animals per pen [cm²/bird]	5 birds per pen (*ie* 24/5=4.8 sq ft per bird)		
Number of animals per dose	5 per dose level plus 1 control group.		
Pre-treatment / acclimation	The gulls were kept in a holding pen for a 2 week period prior to the start of the study.		
Diet during test	During the 5-day dosing period, the diet was masticated rodent tissue (spiked with brodifacoum). During the post-exposure observation period, the diet was 'Southern States' cat food, which was supplemented with fish scraps.		
Dosage levels (of test substance)	**Group**	**Dose frequency**	**Nominal dietary concentration (mg/kg_{diet})**
	Control	Not applicable	0
	0.13 ppm	5-day dietary dose	**0.13**
	0.34 ppm	5-day dietary dose	**0.34**
	0.84 ppm	5-day dietary dose	**0.84**
	2.10 ppm	5-day dietary dose	**2.10**
	5.26 ppm	5-day dietary dose	**5.26**
Replicate/dosage level	5 birds per dosage level.		
Dosing method	Dietary.		
Dosing volume per application	Food consumption was recorded by pen during the 5-day exposure period, and then on a weekly basis throughout the 35-day observation period.		
Frequency, duration and method of animal monitoring after dosing	Symptoms of toxicity and mortality were recorded daily throughout the study.		
Time and intervals of body weight determination	Bodyweights were recorded by pen at initiation, and then on Days 6, 13, 20, 27, 34 and at the termination of the study.		

Table A7_5_3_1_2-4: Test conditions (housing)

Criteria	Details
Test temperature	Specific temperature recordings not stated in study report, but it was stated that infra-red lights were utilised as a heat source.
Shielding of the animals	The birds were housed indoors by group in purpose built pens.
Ventilation	Details not given in study report.
Relative humidity	Details not given in study report.
Photoperiod and lighting	17 hours of light per day.

Table A7_5_3_1_2-5: Mortality data

Test substance dosage level (actual) [ppm_{diet} or mg/kg_{diet}]	**Mortality data**	
	Total number per dose level (Day)	**Percentage per dose level**
0 (controls)	0/5	0
0.13	0/5	0
0.34	1/5 (7)	20
0.84	0/5	0
2.10	4/5 (4,6,9,13)	80
5.26	4/5 (5,8,9,10)	80
5-day LC_{50}	1.6 ppm	
Temperature [°C]	Specific temperature recordings not stated in study report, but it was stated that infra-red lights were utilised as a heat source.	
Relative humidity	Details not given in report.	

Table A7_5_3_1_1-7: Validity criteria for avian acute oral toxicity test according to EPA OPPTS 850.2100

	Fulfilled	**Not fulfilled**
Mortality of control animals <10%	Yes	

Doc IIIA / Section 7.5.3.1.2/03 BPD Data Set IIIA / Annex Point XIII.3.4

Acute/Short term dietary toxicity on birds

5-Day Dietary LC_{50} to Bobwhite Quail

Official use only

1 REFERENCE

1.1 Reference

[redacted]). Forty-Day Dietary LC50 - Bobwhite Quail, Technical Brodifacoum, Final Report. Wildlife International Ltd Report No: 123-127, December 1978 (unpublished) [redacted]

[redacted].

1.2 Data protection

[redacted]

1.2.1 Data owner

[redacted]

1.2.2 Companies with letters of access

[redacted]

1.2.3 Criteria for data protection

[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

No, study pre-dates guidelines, but study conducted in accordance with the general principles of OECD Guideline for Testing of Chemicals No 205, Avian Dietary Toxicity Test.

2.2 GLP

No. Study pre-dates the requirement for GLP.

2.3 Deviations

Not applicable.

3 METHOD

3.1	**Test material**	Brodifacoum
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Please refer to Section 2 of Doc IIIA.
3.1.3	Purity	[redacted]
3.1.4	Composition of Product	Not applicable.
3.1.5	Further relevant properties	
3.1.6	Method of analysis in the diet	The concentrations in the diet were nominal, with the test substance assumed to be 100% pure, and therefore, the LC_{50} as reported in the study was of the test substance (technical grade material) as received by the test laboratory.
3.2	**Administration of the test substance**	Treated and control diets were formulated at the testing laboratory prior to dosing by mixing a weighed amount of brodifacoum with a known volume of corn oil, which was then mixed into a weighed amount of diet to give the nominal dietary concentrations. See table A7_5_3_1_2-1 below.
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	Bobwhite quail (*Colinus virginianus).* See table A7_5_3_1_1-2 below.
3.4.2	Test system	See table A7_5_3_1_1-3 below.
3.4.3	Diet	Wildlife International Ltd's game bird starter ration.
3.4.4	Test conditions	See table A7_5_3_1_1-4 below.
3.4.5	Duration of the test	40 days (5 day dietary dose followed by 35 day observation period).
3.4.6	Test parameter	Clinical observations and mortalities.
3.4.7	Examination / Observation	<u>Clinical examinations and observations</u>: Symptoms of toxicity and mortality were recorded daily throughout the study. <u>Gross macroscopic examinations</u>: Necropsies were performed on the birds which died during the test period.
3.4.8	Statistics	Mortality was analysed statistically by probit analysis.

X

4 RESULTS

4.1 Limit Test / Range finding test

Not performed.

4.1.1 Concentration

4.1.2 Number/ percentage of animals showing adverse effects

4.1.3 Nature of adverse effects

4.2 Results test substance

4.2.1 Applied concentrations

Nominal dietary concentration (mg/kg_{diet}):

0 (diet only), 1.0, 1.78, 3.16, 5.62, 10.0, 17.8, 31.6, 56.2, 100

4.2.2 Effect data (Mortality)

See Table A7_5_3_1_1-5 for a summary of the mortalities.

4.2.3 Body weight

Mean Bodyweight (g)

Group	Day 0	Day 6	Day 13	Day 20	Day 27	Day 34	Day 40
Control	20	28	48	65	85	107	124
Control	23	34	57	78	101	123	138
Control	21	27	48	64	81	109	126
Control	20	31	53	73	96	116	132
Control	22	30	47	62	85	106	120
1.0 ppm	24	31	52	73	82	115	136
1.78 ppm	21	29	52	75	99	115	132
3.16 ppm	20	24	48	67	84	100	115
5.62 ppm	25	31	57	77	97	115	137
10.0 ppm	22	28	52	79	102	126	144
17.8 ppm	21	17	-	-	-	-	-
31.6 ppm	23	36	49	69	95	114	133
56.2 ppm	24	31	39	57	81	104	121
100 ppm	24	38	-	-	-	-	-

4.2.4 Feed consumption

Group	Mean food consumption (g/bird/day) over stated period of Days					
	Days 1-5*	Week 1	Week 2	Week 3	Week 4	Week 5
Control	5	7	8	9	13	17
Control	7	9	10	12	13	23
Control	7	10	10	10	12	21
Control	7	9	10	12	13	24
Control	6	6	8	9	13	19

1.0 ppm	7	7	10	10	17	22
1.78 ppm	4	4	10	11	16	27
3.16 ppm	6	6	9	9	13	15
5.62 ppm	4	5	11	15	22	33
10.0 ppm	6	6	9	17	22	35
17.8 ppm	4	-	-	-	-	-
31.6 ppm	10	8	10	14	15	17
56.2 ppm	5	4	9	14	16	24
100 ppm	3	-	-	-	-	-

*** Treatment period**

4.2.5 Concentration / response curve

No information given in study report, but the confidence limits from the statistical analysis were reported as 0.1 - 4.7 ppm.

4.2.6 Other effects

Clinical observations:
The mortality rates in the treatment groups were 20-100% (see Table A7_5_3_1_2-5 below for a summary of the results). There were 2 mortalities at the lowest dose level with no overt symptoms of toxicity. At all other dose levels, the clinical observations of the birds included depression, reduced reaction to stimuli, wing droop, loss of coordination, prostrate posture, ruffled appearance and lower limb weakness. In addition to these symptoms of toxicity, the behavioural observation of toe and nostril picking was observed.

The surviving birds generally appeared normal until termination at the end of the study.

Necropsy:
Many of the birds found dead during the course of the study had undergone autolysis to the extent that no definitive statement could be made about the cause of death. Hematomas in the breast muscles, sings and hocks were a common finding at all dose levels, and epistaxis was also noted. Many birds also showed evidence of toe picking and nostril picking with evident hemorrhage.

Necropsy of the surviving birds at the 56.2 ppm dose level revealed evidence of some old petechial to ecchymotic hemorrhage on the heart of one of the three birds. Aside from a slight mottling of the liver exhibited by one bird at the 3.16 ppm dose level, no other lesions were noted at any dose level.

4.3 Results of controls

4.3.1 Number/ percentage of animals showing adverse effects

There were a total of 6 deaths in the control groups. Toe and nostril picking were concluded to be responsible for these mortalities which occurred during the test period. Aside from this negative social interaction, all other birds were normal in both appearance and behaviour. No gross abnormalities were noted during necropsy of representative birds at the termination of the study.

4.3.2 Nature of adverse effects

See section 4.3.1 above.

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

Test substance: brodifacoum; Batch no: 3/4/5; Guidelines: study pre-dates guidelines, but study conducted in accordance with the general principles of OECD Guideline for Testing of Chemicals No 205, Avian Dietary Toxicity Test; Test species: Bobwhite quail (*Colinus virginianus).*

140 birds were randomly allocated to 14 groups, with each group comprising 10 animals (male and females randomly mixed) and were given the test diets for a period of five days followed by a 35-day observation period. There were 5 control groups and 9 treatment groups with nominal dietary dose levels of 1.0, 1.78, 3.16, 5.62, 10.0, 17.8, 31.6, 56.2 and 100 ppm brodifacoum. Untreated diet was given from Day 5 until termination.

Symptoms of toxicity and mortality were recorded daily throughout the study. Bodyweight and food consumption were measure frequently throughout the study. Necropsies were performed on all birds that died during the test period and on all surviving birds at termination.

5.2 Results and discussion

Mortality rates in the treatment groups were 20-100%, with death occurring between Days 3 and 20 of the test period. The clinical observations and the findings at necropsy were generally consistent with those expected following administration of an anticoagulant. However, it was concluded that the behavioural effect of toe and nostril picking made it difficult to assess the effect of the test substance on mortalities, especially given that this behaviour was concluded to responsible for the mortalities in the control groups.

5.2.1 LC_{50}

0.8 ppm

5.3 Conclusion

Although the clinical observations and findings at necropsy were generally consistent with those expected following administration of an anticoagulant, it was concluded that the mortalities could not be defined as being solely test substance related, given the effects and mortalities in the control groups.

The validity criteria were not fulfilled (>10% mortality in the control groups).

5.3.1 Reliability

2

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	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
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_2-1: Method of administration of the test substance

Carrier / Vehicle	Details
Water	No
Organic carrier	Yes, corn oil used for mixing the test substance into the diet.
Concentration of the carrier [% v/v]	Not given in study report, but the method of administration was in the diet and so the corn oil was used only to enable even distribution of the test substance in the diet.
Other vehicle	Diet.
Function of the carrier / vehicle	To ensure homogenous diet /feed containing the test substance.

Table A7_5_3_1_2-2: Test animals

Criteria	**Details**			
Species/strain	Mallard Duck (*Anas platyrhynchos).*			
Source	Wildlife International Ltd's production flock.			
Age (in weeks), sex and initial body weight (bw)	**Group**	**Age**	**Sex**	**Mean initial bodyweight**
	Control	14 days	Birds randomly assigned to treatment groups without regard to sex.	20 g
	Control	14 days		23g
	Control	14 days		21 g
	Control	14 days		20 g
	Control	14 days		22 g
	1.0 ppm	14 days		24 g
	1.78 ppm	14 days		21 g
	3.16 ppm	14 days		20 g
	5.62 ppm	14 days		25 g
	10.0 ppm	14 days		22 g
	17.8 ppm	14 days		21 g
	31.6 ppm	14 days		23 g
	56.2 ppm	14 days		24 g
	100 ppm	14 days		24 g
Breeding population	Production flock, Wildlife International: blood tested U.S Pullorum-Typhoid Clean.			
Amount of food	Both food and water were available *ad libitum* throughout the study.			
Age at time of first dosing	14 days.			
Health condition / medication	The chicks received no form of antibiotic medication during brooding or throughout the 40 day study.			

Table A7_5_3_1_2-3: Test system			
Criteria	**Details**		
Test location	Indoors in holding pens.		
Holding pens	Beacon (Model B755) battery brooders, with temperature maintained at 100°F throughout the 40 day study.		
Number of animals	140		
Number of animals per pen [cm²/bird]	10 birds per pen.		
Number of animals per dose	10 per dose level with 5 control groups each containing 10 birds.		
Pre-treatment / acclimation	The chicks used in the study were hatched in the same laboratory and therefore had 14 days of acclimation.		
Diet during test	Wildlife International Ltd's game bird starter ration.		
Dosage levels (of test substance)	**Group**	**Dose frequency**	**Nominal dietary concentration (mg/kg_{diet})**
	Control	Not applicable	0
	Control	Not applicable	0
	Control	Not applicable	0
	Control	Not applicable	0
	Control	Not applicable	0
	1.0 ppm	5-day dietary dose	1.0
	1.78 ppm	5-day dietary dose	1.78
	3.16 ppm	5-day dietary dose	3.16
	5.62 ppm	5-day dietary dose	5.62
	10.0 ppm	5-day dietary dose	10.0
	17.8 ppm	5-day dietary dose	17.8
	31.6 ppm	5-day dietary dose	31.6
	56.2 ppm	5-day dietary dose	56.2
	100 ppm	5-day dietary dose	100
Replicate/dosage level	10 birds per dosage level.		
Dosing method	Dietary.		
Dosing volume per application	Food consumption was recorded by pen during the 5-day exposure period, and then on a weekly basis throughout the 28-day observation period.		
Frequency, duration and method of animal monitoring after dosing	Symptoms of toxicity and mortality were recorded daily throughout the study.		
Time and intervals of body weight determination	Bodyweights were recorded by pen at initiation, and then on Days 6, 13, 20, 27, 34 and at the termination of the study.		

Table A7_5_3_1_2-4: Test conditions (housing)

Criteria	Details
Test temperature	100°F.
Shielding of the animals	The birds were housed indoors by group in purpose built pens.
Ventilation	Details not given in study report.
Relative humidity	Details not given in study report.
Photoperiod and lighting	14 hours of light per day.

Table A7_5_3_1_2-5: Mortality data

Test substance dosage level (actual) [ppm_{diet} or mg/kg_{diet}]	**Mortality data**	
	Total number per dose level (Day)	**Percentage per dose level**
0 (controls)	0/10	0
0 (controls)	0/10	0
0 (controls)	2/10	20
0 (controls)	0/10	0
0 (controls)	4/10	40
1.00	2/10 (7,10)	20
1.78	8/10 (5,5,5,6,7,8,10,11)	80
3.16	6/10 (4,5,5,6,6,10)	60
5.62	9/10 (3,4,4,5,5,6,7,7,8)	90
10.0	9/10 (5,5,5,6,7,8,10,15,20)	90
17.8	10/10 (3,3,4,4,4,5,5,10,10,10)	100
31.6	7/10 (6,7,8,8,13,13,14)	70
56.2	7/10 (5,6,7,7,7,7,13)	70
100	10/10 (4,5,5,5,6,6,6,6,6,8)	100
LC_{50}	0.8 ppm (5-day dietary LC_{50})	
Temperature [°C]	100 °F	
Relative humidity	Details not given in report.	

Table A7_5_3_1_1-7: Validity criteria for avian acute oral toxicity test according to EPA OPPTS 850.2100

	Fulfilled	**Not fulfilled**
Mortality of control animals <10%	No	**Yes**

Doc IIIA / Section 7.5.3.1.2/04 BPD Data Set IIIA / Annex Point XIII.3.4

Acute/Short term dietary toxicity on birds

5-Day Dietary LC_{50} to Mallard Ducks

Official use only

1 REFERENCE

1.1 Reference

[redacted], (1978). Dietary LC50 - Mallard Ducks, Technical Brodifacoum, Final Report. Wildlife International Ltd Report No: 123-124, August 1978 (unpublished) [redacted]

1.2 Data protection

1.2.1 Data owner

1.2.2 Companies with letters of access

1.2.3 Criteria for data protection

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

No, study pre-dates guidelines, but study conducted in accordance with the general principles of OECD Guideline for Testing of Chemicals No 205, Avian Dietary Toxicity Test.

2.2 GLP

No. Study pre-dates the requirement for GLP.

2.3 Deviations

Not applicable.

3 METHOD

3.1	**Test material**	Brodifacoum
3.1.1	Lot/Batch number	Batch no: 3/4/5.
3.1.2	Specification	Please refer to Section 2 of Doc IIIA.
3.1.3	Purity	96.5%
3.1.4	Composition of Product	Not applicable.
3.1.5	Further relevant properties	
3.1.6	Method of analysis in the diet	The concentrations in the diet were nominal.
3.2	**Administration of the test substance**	Treated and control diets were formulated at the testing laboratory prior to dosing by mixing a weighed amount of brodifacoum with a known volume of corn oil, which was then mixed into a weighed amount of diet to give the nominal dietary concentrations. See table A7_5_3_1_2-1 below.
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	Mallard Duck (*Anas platyrhynchos).* See table A7_5_3_1_1-2 below.
3.4.2	Test system	See table A7_5_3_1_1-3 below.
3.4.3	Diet	Wildlife International Ltd's game bird starter ration.
3.4.4	Test conditions	See table A7_5_3_1_1-4 below.
3.4.5	Duration of the test	33 days (5 day dietary dose followed by 28 day observation period).
3.4.6	Test parameter	Clinical observations and mortalities.
3.4.7	Examination / Observation	Clinical examinations and observations: Symptoms of toxicity and mortality were recorded daily throughout the study. Gross macroscopic examinations: Necropsies were performed on the birds which died during the test period.
3.4.8	Statistics	Mortality was analysed statistically by probit analysis.

4 RESULTS

4.1	**Limit Test / Range finding test**	Not performed.
4.1.1	Concentration	
4.1.2	Number/ percentage of animals showing adverse effects	
4.1.3	Nature of adverse effects	
4.2	**Results test substance**	
4.2.1	Applied concentrations	Nominal dietary concentration (mg/kg_{diet}) 0 (diet only) 0 (diet only) 0 (diet only) 0 (diet only) 0 (diet only) 100 178 316 562 1000
4.2.2	Effect data (Mortality)	See Table A7_5_3_1_1-5 for a summary of the mortalities.
4.2.3	Body weight	

Mean Bodyweight (g)

Group	Day 0	Day 6	Day 13	Day 20	Day 27	Day 33
Control	202	349	563	819	959	1009
Control	191	363	563	797	927	973
Control	207	246	604	792	896	993
Control	221	270	616	852	991	1021
Control	201	362	594	799	905	982
100 ppm	217	361	617	862	1005	1039
178 ppm	217	320	626	755	904	985
316 ppm	218	266	465	664	839	915
562 ppm	221	309	490	597	806	910
1000 ppm	206	286	-	-	-	-

4.2.4 Feed consumption

Group	Mean food consumption (g/bird/day) over stated period of Days				
	Days 1-5*	Days 6-12	Days 13-19	Days 20-26	Days 27-33
Control	72	86	76	132	122
Control	76	86	82	105	121
Control	86	94	91	114	114
Control	77	90	86	117	129
Control	81	92	86	125	130
100 ppm	84	91	87	109	118
178 ppm	69	93	93	102	122
316 ppm	66	90	86	99	119
562 ppm	64	87	91	100	124
1000 ppm	29	89	-	-	-

*** Treatment period**

4.2.5 Concentration / response curve

A no effect level was not established by this study, and all dose levels demonstrated a mortality greater than 50%.

4.2.6 Other effects

Clinical observations:
Mortality rates in the treatment groups were 60-100% (see Table A7_5_3_1_2-5 below for a summary of the results). Many of the birds which died exhibited no overt symptoms prior to death, apart from blood around the nostrils and bill. Other birds exhibited symptoms ranging from lethargy through depression, reduced reaction to external stimuli, lower limb weakness, prostrate posture and loss of righting reflex to a comatose state. Many birds had bruising and hematomas of the hock region. Some individual bird observations included exhibited convulsions at the 100 ppm dose level and epitaxis prior to death on Day 9, while a bird at the 316 ppm dose level had a hematoma of the upper mandible which appeared as a large nodule near the nostril.

Necropsy:
Necropsies of the birds which died during the test period uniformly demonstrated internal hemorrhage. The myocardial surface showed petechial to ecchymotic hemorrhage and often the abdominal cavity was filled with blood. The liver usually exhibited areas of haemorrhage and was friable.

All surviving birds were necropsied at the termination of the study:

- one of the birds at the 100 ppm dose level showed evidence of hemorrhage near the descending aorta, petechial hemorrhage of the heart and an enlarged and friable liver. The other surviving birds at this dose level showed no overt lesions upon gross necropsy.
- the single surviving bird at the 178 ppm dose level had an extremely friable liver with areas of ecchymotic hemorrhage and showed signs of bruising on the wings.
- at the 316 ppm dose level one bird showed evidence of a massive paracardial hemorrhage which apparently occurred *in extremis*. There was also evidence of ecchymotic and petechial hemorrhage on both the liver and the gizzard. The second bird exhibited a slightly enlarged and extremely friable liver, with areas of hemorrhage.

		- at the 562 ppm dose level one bird exhibited hematomas in the abdominal and thoracic cavities. Friable livers with areas of hemorrhage were a uniform finding.
4.3	**Results of controls**	
4.3.1	Number/ percentage of animals showing adverse effects	There was one mortality in one of the control groups on Day 12. All other birds were normal in both appearance and behaviour throughout the test period.
4.3.2	Nature of adverse effects	See section 4.3.1 above.
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**	Test substance: brodifacoum; Batch no: 3/4/5; Guidelines: study pre-dates guidelines, but study conducted in accordance with the general principles of OECD Guideline for Testing of Chemicals No 205, Avian Dietary Toxicity Test; Test species: Mallard duck (*Anas platyrhynchos*). 100 birds were randomly allocated to 10 groups, with each group comprising 10 animals (male and females randomly mixed) and were given the test diets for a period of five days followed by a 28-day observation period. There were 5 control groups and 5 treatment groups with nominal dietary dose levels of 100, 178, 316, 562 and 1000 ppm brodifacoum. Untreated diet was given from Day 5 until termination. Symptoms of toxicity and mortality were recorded daily throughout the study. Bodyweight and food consumption were measure frequently throughout the study. Necropsies were performed on all birds that died during the test period and then on all surviving birds at termination.
5.2	**Results and discussion**	Mortality rates in the treatment groups were 60-100%, with the death occurring between Days 4 and 25 of the test period. The clinical observations and the findings at necropsy were generally consistent with those expected following administration of an anticoagulant.
5.2.1	LD_{50}	A no effect level was not established by this study, and all dose levels demonstrated a mortality greater than 50%.
5.3	**Conclusion**	The validity criteria were fulfilled, but an LC_{50} value or NOEL could not be determined.
5.3.1	Reliability	3
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	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
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_2-1: Method of administration of the test substance

Carrier / Vehicle	**Details**
Water	No
Organic carrier	Yes, corn oil used for mixing the test substance into the diet.
Concentration of the carrier [% v/v]	Not given in study report, but the method of administration was in the diet and so the corn oil was used only to enable even distribution of the test substance in the diet.
Other vehicle	Diet.
Function of the carrier / vehicle	To ensure homogenous diet /feed containing the test substance.

Table A7_5_3_1_2-2: Test animals

Criteria	**Details**			
Species/strain	Mallard Duck (*Anas platyrhynchos).*			
Source	Wildlife International Ltd's production flock.			
Age (in weeks), sex and initial body weight (bw)	**Group**	**Age**	**Sex**	**Mean initial bodyweight**
	Control	14 days	Birds randomly assigned to treatment groups without regard to sex.	202 g
	Control	14 days		191 g
	Control	14 days		207 g
	Control	14 days		221 g
	Control	14 days		201 g
	100 ppm	14 days		217 g
	178 ppm	14 days		217 g
	316 ppm	14 days		218 g
	562 ppm	14 days		221 g
	1000 ppm	14 days		206 g
Breeding population	Production flock, Wildlife International: blood tested U.S Pullorum-Typhoid Clean.			
Amount of food	Both food and water were available *ad libitum* throughout the study.			
Age at time of first dosing	14 days.			
Health condition / medication	The chicks received no form of antibiotic medication during brooding or throughout the 33 day study.			

Table A7_5_3_1_2-3: Test system			
Criteria	**Details**		
Test location	Indoors in holding pens.		
Holding pens	Beacon (Model B755) battery brooders, with temperature maintained at 72°F throughout the 33 day study..		
Number of animals	100		
Number of animals per pen [cm²/bird]	10 birds per pen.		
Number of animals per dose	10 per dose level with 5 control groups each containing 10 birds.		
Pre-treatment / acclimation	The chicks used in the study were hatched in the same laboratory and therefore had 14 days of acclimation.		
Diet during test	Wildlife International Ltd's game bird starter ration.		
Dosage levels (of test substance)	**Group**	**Dose frequency**	**Nominal dietary concentration (mg/kg_{diet})**
	Control	Not applicable	0
	Control	Not applicable	0
	Control	Not applicable	0
	Control	Not applicable	0
	Control	Not applicable	0
	100 ppm	5-day dietary dose	100
	178 ppm	5-day dietary dose	178
	316 ppm	5-day dietary dose	316
	562 ppm	5-day dietary dose	562
	1000 ppm	5-day dietary dose	1000
Replicate/dosage level	10 birds per dosage level.		
Dosing method	Dietary.		
Dosing volume per application	Food consumption was recorded by pen during the 5-day exposure period, and then on a weekly basis throughout the 28-day observation period.		
Frequency, duration and method of animal monitoring after dosing	Symptoms of toxicity and mortality were recorded daily throughout the study.		
Time and intervals of body weight determination	Bodyweights were recorded by pen at initiation, and then on Days 6, 13, 20, 27 and at the termination of the study.		

Table A7_5_3_1_2-4: Test conditions (housing)

Criteria	Details
Test temperature	72°F.
Shielding of the animals	The birds were housed indoors by group in purpose built pens.
Ventilation	Details not given in study report.
Relative humidity	Details not given in study report.
Photoperiod and lighting	14 hours of light per day.

Table A7_5_3_1_2-5: Mortality data

Test substance dosage level (actual) [ppm_{diet} or mg/kg_{diet}]	**Mortality data**	
	Total number per dose level (Day)	**Percentage per dose level**
0 (controls)	0/10	0
0 (controls)	0/10	0
0 (controls)	1/10	10
0 (controls)	0/10	0
0 (controls)	0/10	0
100	8/10 (7, 9, 12, 13, 14, 15, 16, 17)	80
178	9/10 (4, 8, 8, 8, 9, 9, 11, 13, 13)	90
316	8/10 (9, 9, 11, 12, 13, 17, 22, 23)	80
562	6/10 (6, 13, 14, 19, 20, 25)	60
1000	10/10 (8, 8, 9, 9, 10, 10, 10, 10, 10, 10)	100
LC_{50}	**Could not be determined due to the pattern of mortality.**	
Temperature [°C]	72 °F	
Relative humidity	Details not given in report.	

Table A7_5_3_1_1-7: Validity criteria for avian acute oral toxicity test according to EPA OPPTS 850.2100

	Fulfilled	**Not fulfilled**
Mortality of control animals <10%	Yes	

Doc IIIA / Section 7.5.3.1.3

Effects on reproduction of birds

BPD data set IIIA / Annex Point IIIA XIII 1.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2005) Avian Reproduction Study with Difenacoum in the Japanese Quail (*Coturnix coturnix japonica*). Genesis Laboratories, Inc., Report no. 04012 Unpublished [redacted] [redacted] *In-vivo* experimental work carried out between 31 May 2005 and 18 November 2005.	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. Primary: OECD Test Guideline 206: Avian Reproduction Test, 1984. Secondary: Modified in places to follow OECD Test Guideline "Draft Document 1998": Avian Toxicity Test in the Japanese Quail or Northern Bobwhite, and USEPA Ecological Effects Guideline OPPTS 850.2300: Avian Reproduction Test	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	X
		3 METHOD	

Doc IIIA / Section 7.5.3.1.3

Effects on reproduction of birds

BPD data set IIIA / Annex Point IIIA XIII 1.3

3.1	**Test material**	Difenacoum	
3.1.1	Lot/Batch number	H224750057	
3.1.2	Specification	Please refer to Section 2 of Doc IIIA	
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	Not applicable.	
3.1.5	Further relevant properties	Not applicable.	
3.1.6	Method of analysis	The extraction of difenacoum from avian feed involved homogenisation with acetone followed by evaporation. The extract is then further cleaned up using a hexane:acetonitrile liquid-liquid partition. An aliquot of the acetonitrile phase is taken for quantification by LC-MS/MS using positive ion chemical ionisation. A validated LOQ of 0.01 mg/kg difenacoum was obtained.	
3.2	**Administration of the test substance**	Treated diets prepared and offered *ad libitum*. Refer also to table A7_5_3_1_2-1	X
3.3	**Testing procedure**		
3.3.1	Test organisms	Japanese quail, *Coturnix coturnix japonica.* Refer also to table A7_5_3_1_3-2	X
3.3.2	Test system	Dietary administration offered *ad libitum*. Refer also to table A7_5_3_1_3-3	X
3.3.3	Diet	Mazuri® Exotic Gamebird Breeder was used as the basal feed to prepare all test diets. The test substance was dissolved in HPLC-grade acetone to make a stock solution. For each dietary concentration, an appropriate aliquot of the stock solution was transferred to another container and diluted with additional acetone. The total amount of vehicle added to a batch was set at two percent by weight. The final solution for each dietary level was added to the basal feed in the mixing bowl of a large Hobart mixer. The diet was mixed for 15 minutes after the vehicle was added. The Vehicle Control (VC) diet was always mixed first with neat acetone, followed by the T1, T2, T3 and T4 test diets. Fresh test diets were prepared at least every two weeks. Prepared diets were stored in a walk-in freezer for two weeks, at which time a new batch was mixed.	
3.3.4	Test conditions	Refer to table A7_5_3_1_2-4	X
3.3.5	Duration of the test	Adult Treatment Period: 10 weeks pre-egg laying; 10 weeks egg-laying. Hatchling Observation Period: 14 days post-hatch.	
3.3.6	Test parameter	Adult Parameters: Daily observations, diet consumption, body weight, necropsy including wet weights of the liver, spleen and testes. Reproductive Parameters: Eggs laid, eggshell thickness, defective and	

		cracked eggs, viable embryos, live embryos. Hatchling Parameters: Hatching success/hatchability, hatchling survival, hatchling body weights.
3.3.7	Examination / Observation	The birds were observed daily during the 20 week exposure period. Inspections were made to monitor symptoms that may be indicative of test substance related effects. Birds that died during the treatment period were removed, weighed and necropsied. Feed consumption of each pair of birds was measured weekly during the exposure period. The body weight of each bird was measured at the initiation of the 14-day acclimation period, on day 0, at the end of week 8, and at the end of week 20. At the conclusion of the treatment period, remaining birds were euthanized and necropsied for gross pathological abnormalities. Specific examination was made on the gastro-intestinal tract, liver, kidneys, bile duct, heart, spleen, and reproductive organs. Wet weights of the liver, spleen and testes were measured at the time of necropsy. Other observations were recorded as necessary.
3.3.8	Statistics	Adult endpoints and reproductive parameters were statistically analyzed using TOXSTAT Version 3.4. The experimental unit is each pen (or adult pair), except in the case of adult body weight, in which case the experimental unit is each adult bird. If a data set passed the chi-square test for normal distribution, and Bartlett's test for homogeneity of variance, it was analyzed by ANOVA. If no significant difference was identified by the ANOVA, no additional data was used. If ANOVA identified a difference, then the *post hoc* results generated by TOXSTAT were used. Bonferroni's test was used for pair-wise comparisons of each treatment with the control group. Bonferroni's test is appropriate when the replicates per group were not equal, as was the case in with many of the data sets. Data sets consisting of count data which did not pass the chi-square test and/or Bartlett's test, were transformed and analyzed again. If an appropriate transformation did not succeed in normalizing the distribution, or if the variance was not homogeneous, the original, untransformed data was analyzed by Kruskal-Wallis' non-parametric test (H-statistic). If a post hoc pair-wise comparison was indicated, Dunn's multiple comparison procedure was used. Dunn's procedure compares all possible pairs of means. If no significant difference was identified by the Kruskal-Wallis' test, no additional data was used. Proportional (percentage) data were analyzed with a different procedure. The spread of the data determined the necessary transformation, regardless of the results of the normality and/or homogeneity tests. This procedure is outlined in SOP CO-8.02. Depending upon the results of the transformation, the appropriate analysis of variance procedure was performed. The power of the test is the probability of detecting a difference when

there is a difference. The analysis is a pair-wise comparison of two means. In all cases, the mean values tested were the vehicle control group (VC) and the highest dietary concentration group, treatment level 4 (T4). The rationale for this comparison was that any test substance related effect would be expressed most strongly in the highest dose group. Power analyses were performed using the program XLStatistics. The test parameters were set at:

Significance Level (α):	0.05
Test Hypotheses:	H_0: $\mu_1-\mu_2 = 0$
	H_1: $\mu_1-\mu_2 \neq 0$ (two-tailed test)

Actual standard deviations associated with the means were used since the analyses were performed post hoc. The power statistic is expressed as $(1-\beta)$.

4 RESULTS

Doc IIIA / Section 7.5.3.1.3

Effects on reproduction of birds

BPD data set IIIA / Annex Point IIIA XIII 1.3

4.1	**Limit Test / Range finding test**	Not performed
4.1.1	Concentration	
4.1.2	Number/ percentage of animals showing adverse effects	
4.1.3	Nature of adverse effects	
4.2	**Results test substance**	
4.2.1	Applied concentrations	Nominal Dietary Concentrations: 0 (VC), 0.001 (T1), 0.005 (T2), 0.020 (T3), 0.100 (T4) mg/kg diet, equivelant to: 0 (VC), 0.016 (T1), 0.075 (T2), 0.317 (T3), 1.642 (T4) mg/kg bw at the conclusion of the 20 week treatment period.
4.2.2	Effect data (Mortality and reproductivity)	Adverse effects observed in adult birds durng treatment period:

Doc IIIA / Section 7.5.3.1.3

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Effects on reproduction of birds

Group	Nominal Concentration (mg a.i./kg diet)	Birds Euthanized [a]	Birds Died [b]	Week(s) Found Dead	Initial Number of Birds	Dead (%)	Group Observations
VC	0	9	5	5,7,9,14	40	12.5	Feather loss (head,back), pecking (head), abrasion (head,ear,eye,foot), found dead, and sacrificed.
T1	0.001	5	3	10,11,16	38	7.9	Feather loss (head,back,neck), pecking (head), abrasion (head), hemmorhage (beak). Subdermal hematoma (head), sacrificed, growth on foot, found dead.
T2	0.005	7	2	12,20	40	5.0	Feather loss (head, eye, neck, back), pecking (head), abscess (beak), abrasion (head,foot), sacrificed, subdermal hematoma (head), ataxic, growth on beak, injured (right leg), found dead.
T3	0.020	5	1	7	40	2.5	Feather loss (head,neck), pecking (head), abscess (head), abrasion(foot), hypo-reactivity, abrasion healing, feathers growing, found dead, sacrificed, wing drop, injured (wing), growth on beak, subdermal hematoma (head).
T4	0.100	7	1	16	40	2.5	Feather loss (head, back), pecking (head) abrasion (head,foot), sacrificed, ataxic, fluffed feathers, found dead, growth on beak.

[a] Single birds were euthanized if their pen-mate had died. Both members of a pair were euthanized if the pair was incompatible, described as repeated or routine agonistic behavior which was resulting in severe injury to one or both members of the pair. Both members of some pairs were also euthanized if they met the criterion for excessively low egg production in week 13 of the test. The criterion was egg production in week 13 which was less than or equal to two standard deviations below the mean egg production of the VC group in week 13.
[b] Includes only those birds that were found dead during the 20 week adult observation period. Not included are any birds euthanized during the test.

Reproduction parameters:

Group	Nominal Conc. (mg a.i./ kg diet)	Egg Data							
		Eggs Laid	Mean Eggs /Hen /Week	Mean Hatchlings /Hen /Week	Mean Shell Thickness (mm)	Cracked Eggs (%) [a]	Viable Embryo (%) [b]	Live Embryo (%) [c]	Hatch (%) [d]
VC	0	889	6.5	4.0	0.215	9.8	94.4	95.3	83.5
T1	0.001	973	6.4	3.7	0.207	13.4	94.9	96.2	81.9
T2	0.005	963	6.1	3.2	0.216	10.0	87.2	95.2	77.0
T3	0.020	1056	6.2	4.0	0.215	7.7	95.5	96.2	81.4
T4	0.100	1048	6.4	3.8	0.213	9.9	90.7	97.5	83.0

[a] Percent Cracked Eggs = (cracked eggs/eggs candled) * 100.
[b] Percent Viable embryos = (viable embryos/eggs set) * 100.
[c] Percent Live Embryos = (live embryos/viable embryos) * 100.
[d] Percent Hatch = (hatchlings/viable embryos) * 100.

Refer also to table A7_5_3_1_3-5

NOEC (No Observed Effect Concentration): Greater than the T4 dietary concentration: 0.100 mg/kg diet administered for 20 weeks.

NOEL (No Observed Effect Level): Greater than the T4 ingestion level: 0.01138 mg/kg body weight/day (mean males and females).

4.2.3 Body weight

Adults:

Group	Nominal Concentration (mg a.i./kg diet)	Mean Body Weight (g)		
		Week 0 [a, b]	Week 8 [c]	Week 20 [d]
		Male		
VC	0	211	236	273
T1	0.001	214	251	290
T2	0.005	214	251	284
T3	0.020	205	238	275
T4	0.100	202	234	266
		Female		
VC	0	225	278	304
T1	0.001	224	284	312
T2	0.005	229	279	315
T3	0.020	227	280	302
T4	0.100	231	291	318

[a] Differences in initial body weights (male and female) among groups were not significant when analyzed using ANOVA (F = 2.45, calculated F = 0.176).
[b] Differences in initial male body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 1.974). Differences in initial female body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 0.335).
[c] Differences in week 8 male body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 1.976). Differences in week 8 female body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 0.628).
[d] Differences in week 20 male body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 1.852). Differences in week 20 female body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 0.924).

Hatchlings:

Group	Nominal Concentration (mg a.i./kg diet)	Mean Day 0 Body Weight (g) [a]	Mean Day 14 Body Weight (g) [b]
VC	0	10	68
T1	0.001	10	67
T2	0.005	9	69
T3	0.020	9	59
T4	0.100	10	65

[a] Differences in mean day 0 body weight among groups were not significant when analyzed using Kruskal-Wallis (H = 9.490, calculated H = 2.121).
[b] Differences in mean day 14 body weight among groups were significant when analyzed using Kruskal-Wallis (H = 9.490, calculated H = 29.933), and a *post hoc* test using Dunn's multiple comparison test show T3 being significantly different than all other groups while all other groups, excluding T3, were not significantly different from each other.

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Effects on reproduction of birds

4.2.4 Food consumption

Adults:

Mean Feed Consumption of Adult *Coturnix* During the Avian Reproduction Test With Difenacoum

Group	Nominal Concentration (mg a.i./kg diet)	Feed Consumption (grams/bird/day) Week 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean[a]
VC	0	28	29	26	26	28	26	26	28	30	31	35	36	35	36	36	37	36	37	34	35	32
T1	0.001	28	30	27	28	31	29	29	31	32	34	35	37	37	38	38	38	37	39	36	37	34
T2	0.005	28	30	26	27	30	27	28	29	29	31	33	36	34	36	34	37	35	37	35	36	32
T3	0.020	28	30	27	27	31	27	27	29	30	31	34	36	36	37	37	38	36	36	36	38	33
T4	0.100	26	30	27	27	29	27	28	30	32	34	34	37	36	38	36	38	37	37	37	39	33

[a] Difference in mean feed consumption among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 1.182).

4.2.5 Results of residue analysis

Batch #	Date Prepared	Mean Measured Concentration (mg a.i./kg diet) VC (0.000 mg a.i./kg diet)	T1 (0.001 mg a.i./kg diet)	T2 (0.005 mg a.i./kg diet)	T3 (0.020 mg a.i./kg diet)	T4 (0.100 mg a.i./kg diet)
1	May 9, 2005	-	-	-	0.0177	0.1017
2	June 7, 2005	-	-	-	0.0225	0.1295
11	September 19, 2005	-	-	-	0.0198	0.0845
Mean Measured Concentration		-	-	-	0.0200	0.1052
Standard Deviation		-	-	-	0.0024	0.0227
Percent of Nominal		-	-	-	100.0	105.2

T1 and T2 diets were not analysed as the concentration is below the LOQ of the validated analytical method. However, the lower dietary concentrations are verified indirectly firstly by the careful dilution of the stock solution, secondly by the consistent mixing process used to prepare all levels, and thirdly, by the analytical verification of difenacoum levels in the T3 and T4 diets, which were mixed in the same manner and at the same time.

4.2.6 Other effects

Birds that died during the treatment were necropsied and the significant findings included:

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Group	Nominal Concentration (mg a.i./kg diet)	Birds Euthanized [a]	Birds Died [b]	Week(s) Found Dead	Initial Number of Birds	Dead (%)	Group Observations
VC	0	9	5	5,7,9,14	40	12.5	Feather loss (head,back), pecking (head), abrasion (head,ear,eye,foot), found dead, and sacrificed.
T1	0.001	5	3	10,11,16	38	7.9	Feather loss (head,back,neck), pecking (head), abrasion (head), hemmorhage (beak). Subdermal hematoma (head), sacrificed, growth on foot, found dead.
T2	0.005	7	2	12,20	40	5.0	Feather loss (head, eye, neck, back), pecking (head), abscess (beak), abrasion (head,foot), sacrificed, subdermal hematoma (head), ataxic, growth on beak, injured (right leg), found dead.
T3	0.020	5	1	7	40	2.5	Feather loss (head,neck), pecking (head), abscess (head), abrasion(foot), hypo-reactivity, abrasion healing, feathers growing, found dead, sacrificed, wing drop, injured (wing), growth on beak, subdermal hematoma (head).
T4	0.100	7	1	16	40	2.5	Feather loss (head, back), pecking (head) abrasion (head,foot), sacrificed, ataxic, fluffed feathers, found dead, growth on beak.

[a] Single birds were euthanized if their pen-mate had died. Both members of a pair were euthanized if the pair was incompatible, described as repeated or routine agonistic behavior which was resulting in severe injury to one or both members of the pair. Both members of some pairs were also euthanized if they met the criterion for excessively low egg production in week 13 of the test. The criterion was egg production in week 13 which was less than or equal to two standard deviations below the mean egg production of the VC group in week 13.
[b] Includes only those birds that were found dead during the 20 week adult observation period. Not included are any birds euthanized during the test.

The findings of the terminal necropsies are:

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Gross Necropsy Results of Adult *Coturnix* During the Avian Reproduction Test With Difenacoum
(The number in each column represents the number of birds that displayed the listed findings.)

Observations		Nominal Concentrations (mg a.i./kg diet)				
		VC (0)	T1 (0.001)	T2 (0.005)	T3 (0.020)	T4 (0.100)
Fate:	Found dead	5	2	1	1	1
	Sacrificed	35	36	39	39	39
Total necropsies:		40	38	40	40	40
Feather loss:		20	18	19	17	18
Emaciated:		2	1	2	0	1
Breast muscle atrophy:		2	1	2	0	1
Ventriculus contents:	No feed/grit	2	0	0	0	0
	1/2 full	2	4	2	2	2
	Full	36	34	38	38	38
Enlarged: [a]	Liver	n/a	n/a	n/a	n/a	n/a
	Kidneys	0	2	0	0	0
	Spleen	n/a	n/a	n/a	n/a	n/a
	Bile duct	0	0	0	0	0
Discolored:	Liver	6	3	6	1	5
	Heart	0	0	0	0	0
	Kidneys	0	0	1	0	0
	Spleen	0	1	0	0	0
	Bile duct	0	0	0	0	0
Lesions/Abrasions:	Skin	7	5	3	5	4
Lesions/Growths:	Mouth	0	0	0	0	0
	Esophagus/Crop	1	0	0	0	0
	Proventriculus	0	0	0	0	1
	Ventriculus	0	0	0	0	1
	Intestines	0	0	0	0	0
	Heart	0	0	0	0	0
	Liver	0	0	0	0	0
	Bile Duct	0	0	0	0	0
	Spleen	0	0	0	0	0
	Kidneys	0	0	0	0	0
	Uro-Genital	0	0	0	0	0
Reproductive organs:	Mature follicles	18	19	19	20	20
	Egg in oviduct	14	15	15	17	16
	Immature Testes	0	0	1	0	0

[a] Classification as "enlarged" is subjective for kidneys and bile duct. Livers, spleens, and male testes were weighed and the results presented in Table 21 and in Appendix C3.

Organ weights recorded during the terminal necropsies were:

Group	Nominal Concentration (mg a.i./kg diet)	Organ Body Weight (g)			
		Liver [a]	Spleen [b]	Right Testes [c]	Left Testes [d]
		Male			
VC	0	6.0	0.11	3.8	3.7
T1	0.001	7.5	0.16	3.4	3.6
T2	0.005	7.1	0.16	3.2	3.2
T3	0.020	6.7	0.14	3.4	3.6
T4	0.100	6.4	0.18	3.2	3.4
		Female			
VC	0	9.9	0.20	---	---
T1	0.001	10.9	0.27	---	---
T2	0.005	11.6	0.21	---	---
T3	0.020	9.3	0.22	---	---
T4	0.100	10.8	0.21	---	---

[a] Differences in liver weights among males in the groups were not significant when analyzed using ANOVA Male ($F = 2.53$, calculated $F = 2.299$). Differences in liver weights among females in the groups were significant when analyzed using ANOVA ($F = 2.53$, calculated $F = 4.693$). Although the ANOVA declared significant differences, pair-wise comparisons of each treatment group with the VC group did not find significant differences (Bonferroni's t-test).
[b] Differences in spleen weights among groups were not significant when analyzed using ANOVA Male ($F = 2.53$, calculated $F = 1.693$), Female($F = 2.53$, calculated $F = 0.765$).
[c] Differences in right testes weights among groups were not significant when analyzed using ANOVA Male ($F = 2.53$, calculated $F = 1.387$).
[d] Differences in left testes weights among groups were not significant when analyzed using ANOVA Male ($F = 2.53$, calculated $F = 0.801$).

4.3 Results of controls

4.3.1 Number/ percentage of

All data for the control group is included in the Tables above.

	animals showing adverse effects	
4.3.2	Nature of adverse effects	Although the listed findings are consistent with anticoagulant exposure, the observations do not form a pattern of consistent effects either within groups or across treatment groups. There were eight cases of sub-lethal observations that could be related to anticoagulant exposure. The six cases were distributed among four groups: VC (n=2), T1 (n=2), T2 (n=2), T3 (n=1), and T4 (n=1) treatment groups. Two control group birds were found to have hemorrhaging in the esophagus upon necropsy. This illustrates that the birds were incurring many forms of physical stress and tissue damage that was related to aggressive interactions among pen-mates. While some of the sub-lethal conditions observed may be consistent with anticoagulant exposure, similar observations in the control group suggest that there were other causative factors at work. The lack of any systematic dose response in physical symptoms and in any of the other parameters measured in the test support this conclusion.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Primary Guideline: OECD Test Guideline 206: Avian Reproduction Test, 1984. SecondaryGuidelines: Modified in places to follow OECD Test Guideline "Draft Document 1998": Avian Toxicity Test in the Japanese Quail or Japanese Quail, and USEPA Ecological Effects Guideline OPPTS 850.2300: Avian Reproduction Test. No deviations.

Treated diet was prepared every two weeks and offered *ad libitum* to groups of 10 male and female pairs for 10 weeks pre-egg laying and 10 weeks egg laying. Treated diets contained nominal 0 (VC), 0.001 (T1), 0.005 (T2), 0.020 (T3) and 0.100 (T4) mg/kg diet.

Adults were observed daily and diet consumption, body weight, necropsy including wet weights of the liver, spleen and testes recorded. Eggs were collected daily for 10 weeks. The number of eggs laid, eggshell thickness, defective and cracked eggs, viable embryos, live embryos were recorded. Eggs were incubated and hatching success/hatchability, hatchling survival and hatchling body weight at day 14 were recorded. Parameters were analysed statistically.

5.2 Results and discussion

Of all the parameters measured and analysed in the study, only three were declared to have significant differences, these were: adult female liver weights; the mean body weight of 14-day old hatchlings; and, the mean number of normal hatchlings per hen .

The adult female liver weights were significantly different (lower), according to ANOVA, but no significant differences were identified by pair-wise comparisons of each treatment group mean with the VC group.

The mean body weight of 14-day old hatchlings found the T3 group to be significantly different (lower) from the VC group but this may have been due to behavioural interactions as the hatchling density in the brooders for this group was the highest.

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The mean number of normal hatchlings per hen in the T2 and T4 groups was significantly different (lower) from the VC group. These results do not appear as part of a larger pattern. The T2 group contained two pairs having very low numbers of hatchlings. It is therefore considered to be an artifact of the groupings and the analysis process.

Regarding the adult generation, although the listed symptoms are consistent with anticoagulant exposure, the observations do not form a pattern of consistent effects either within groups or across treatment groups. There were eight cases of sub-lethal observations that could be related to anticoagulant exposure. Two control group birds were found to have haemorrhaging in the oesophagus upon necropsy. This illustrates that the birds were incurring many forms of physical stress and tissue damage that was related to aggressive interactions among pen-mates. While some of the sub-lethal conditions observed may be consistent with anticoagulant exposure, similar observations in the control group suggest that there were other causative factors at work. The lack of any systematic dose response in physical symptoms and in any of the other parameters measured in the study support this conclusion.

Dietary consumption of up to 0.100 mg/kg diet had no observed effect on the body weight, feed consumption, or reproductive performance of adult Japanese quail when administered via the diet for 20 weeks. No effects were attributed to the test substance in egg development, or hatchling observations, hatchling body weights and hatchling feed consumption for 14 days.

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5.2.1	NOEC	**NOEC** (No Observed Effect Concentration): Greater than the T4 dietary concentration: 0.100 mg/kg diet administered for 20 weeks. **NOEL** (No Observed Effect Level): Greater than the T4 ingestion level: 0.01138 mg/kg body weight/day (mean males and females).
5.3	**Conclusion**	The validity criteria can be considered to have been fulfilled. Although the control mortality was very slightly higher than the threshold it is not considered to have affected the integrity of the study. Based on the results of this study with Japanese quail, the NOEC of difenacoum is considered to be greater than 0.100 mg a.i./kg diet. Adult Japanese quail fed difenacoum in the diet for 20 weeks at this level and at three lower levels, did not show any pattern of symptoms consistent with anticoagulant toxicity. There was no suggestion of a dose response at the dietary concentrations listed. All symptoms observed in birds administered difenacoum-treated diets were also observed in the control group. Symptoms observed may have been magnified by the presence of difenacoum in the treated birds, but the degree of interaction cannot be separated and appears to be minor.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

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Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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	

Doc IIIA/Section 7.5.3.1.3 **BPD Data Set IIIA/Annex Point XIII.1.3**	**Effects on birds: Effects on reproduction**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure** [ ]	**Technically not feasible** [✓] **Scientifically unjustified** [✓] **Other justification** [ ]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

Doc IIIA/Section 7.5.3.1.3 **BPD Data Set IIIA/Annex Point XIII.1.3**	**Effects on birds: Effects on reproduction**
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2005

Doc IIIA/Section 7.5.3.1.3 BPD Data Set IIIA/Annex Point XIII.1.3	**Effects on birds: Effects on reproduction**
Evaluation of applicant's justification	The applicant's justification is not sufficient to waive the study on the effects on reproduction of birds. **Mode of action**: RMS observes that effect on birds' reproduction cannot be excluded on the basis of short term or teratogenic effects on mammals. In fact, birds might have a susceptibility to brodifacoum greater than mammals and effects on tissue and organ structure and function and on reproduction cannot be directly extrapolated. Available mammalian data are not considered sufficient to evaluate possible effect on reproduction even in mammalian themselves (fact a two generation study has been requested - see section 'Human health'). In addition, at low concentration various physiological functions may be impaired, and this could adversely affect reproduction and long-term survival. **Exposure** – A typical baiting treatment will last up to 35 day. Consequently, birds might be exposed continuously to the active substance for an extended period, leading to progressive accumulation during the critical period of reproduction. - Some species of predatory bird may be exposed to rodenticides through primary and secondary poisoning. - Effects on reproduction endpoints might well occur at concentration lower than lethal ones. - No reference is given for the mentioned monitoring study (which compounds?) showing lack of effect in predatory population. In this respect, it should be considered that prior exposure to sub-lethal concentrations may result in increased susceptibility of birds to the acutely toxic effects of rodenticides when they will be afterwards exposed to the substance. **Technical feasibility** - The technical difficulties are recognized but are not such to make a study impossible. It should be possible to design the test choosing appropriately low concentrations of active substance (administered via food or drinking water), such as not significant additional mortality compared to the control occur. - Based on PEC/PNEC rations risk to birds via primary poisoning is greater than the risk via secondary poisoning. - If there is evidence of marked differences in sensitivity among species (Which study? Which species?), this could be used for deriving an assessment factor to apply in order to take this uncertainty into account.

Doc IIIA/Section 7.5.3.1.3 **BPD Data Set IIIA/Annex Point XIII.1.3**	**Effects on birds: Effects on reproduction**
Conclusion	In conclusion the request for waivingf the reproduction study, based on considerations on mode of action, exposure and technical feasibility, is not considered sufficiently justified. The submission of a study is required to assess possible risk at population level.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Doc IIIA/Section 7.5.3.1.3 **BPD Data Set IIIA/Annex Point XIII.1.3**	**Effects on birds: Effects on reproduction**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure** [ ]	**Technically not feasible** [✓] **Scientifically unjustified** [✓] **Other justification** [ ]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [ ]		
Evaluation by Competent Authorities		
Use separate "evaluation boxes" to provide transparency as to the		

Doc IIIA/Section 7.5.3.1.3 **BPD Data Set IIIA/Annex Point XIII.1.3**	**Effects on birds: Effects on reproduction**
	comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2005

Doc IIIA/Section 7.5.3.1.3 BPD Data Set IIIA/Annex Point XIII.1.3	**Effects on birds: Effects on reproduction**
Evaluation of applicant's justification	The applicant's justification is not sufficient to waive the study on the effects on reproduction of birds. **Mode of action**: RMS observes that effect on birds' reproduction cannot be excluded on the basis of short term or teratogenic effects on mammals. In fact, birds might have a susceptibility to brodifacoum greater than mammals and effects on tissue and organ structure and function and on reproduction cannot be directly extrapolated. Available mammalian data are not considered sufficient to evaluate possible effect on reproduction even in mammalian themselves (fact a two generation study has been requested - see section 'Human health'). In addition, at low concentration various physiological functions may be impaired, and this could adversely affect reproduction and long-term survival. **Exposure** – A typical baiting treatment will last up to 35 day. Consequently, birds might be exposed continuously to the active substance for an extended period, leading to progressive accumulation during the critical period of reproduction. - Some species of predatory bird may be exposed to rodenticides through primary and secondary poisoning. - Effects on reproduction endpoints might well occur at concentration lower than lethal ones. - No reference is given for the mentioned monitoring study (which compounds?) showing lack of effect in predatory population. In this respect, it should be considered that prior exposure to sub-lethal concentrations may result in increased susceptibility of birds to the acutely toxic effects of rodenticides when they will be afterwards exposed to the substance. **Technical feasibility** - The technical difficulties are recognized but are not such to make a study impossible. It should be possible to design the test choosing appropriately low concentrations of active substance (administered via food or drinking water), such as not significant additional mortality compared to the control occur. - Based on PEC/PNEC rations risk to birds via primary poisoning is greater than the risk via secondary poisoning. - If there is evidence of marked differences in sensitivity among species (Which study? Which species?), this could be used for deriving an assessment factor to apply in order to take this uncertainty into account.

Doc IIIA/Section 7.5.3.1.3 **BPD Data Set IIIA/Annex Point XIII.1.3**	**Effects on birds: Effects on reproduction**
Conclusion	In conclusion the request for waivingf the reproduction study, based on considerations on mode of action, exposure and technical feasibility, is not considered sufficiently justified. The submission of a study is required to assess possible risk at population level.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

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Effects on reproduction of birds

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			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2005) Avian Reproduction Study with Difenacoum in the Japanese Quail (*Coturnix coturnix japonica*). Genesis Laboratories, Inc., Report no. 04012 Unpublished [redacted] [redacted] *In-vivo* experimental work carried out between 31 May 2005 and 18 November 2005.	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. Primary: OECD Test Guideline 206: Avian Reproduction Test, 1984. Secondary: Modified in places to follow OECD Test Guideline "Draft Document 1998": Avian Toxicity Test in the Japanese Quail or Northern Bobwhite, and USEPA Ecological Effects Guideline OPPTS 850.2300: Avian Reproduction Test	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	X
		3 METHOD	

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3.1	**Test material**	Difenacoum	
3.1.1	Lot/Batch number	H224750057	
3.1.2	Specification	Please refer to Section 2 of Doc IIIA	
3.1.3	Purity	96.7% w/w	
3.1.4	Composition of Product	Not applicable.	
3.1.5	Further relevant properties	Not applicable.	
3.1.6	Method of analysis	The extraction of difenacoum from avian feed involved homogenisation with acetone followed by evaporation. The extract is then further cleaned up using a hexane:acetonitrile liquid-liquid partition. An aliquot of the acetonitrile phase is taken for quantification by LC-MS/MS using positive ion chemical ionisation. A validated LOQ of 0.01 mg/kg difenacoum was obtained.	
3.2	**Administration of the test substance**	Treated diets prepared and offered *ad libitum*. Refer also to table A7_5_3_1_2-1	X
3.3	**Testing procedure**		
3.3.1	Test organisms	Japanese quail, *Coturnix coturnix japonica.* Refer also to table A7_5_3_1_3-2	X
3.3.2	Test system	Dietary administration offered *ad libitum*. Refer also to table A7_5_3_1_3-3	X
3.3.3	Diet	Mazuri® Exotic Gamebird Breeder was used as the basal feed to prepare all test diets. The test substance was dissolved in HPLC-grade acetone to make a stock solution. For each dietary concentration, an appropriate aliquot of the stock solution was transferred to another container and diluted with additional acetone. The total amount of vehicle added to a batch was set at two percent by weight. The final solution for each dietary level was added to the basal feed in the mixing bowl of a large Hobart mixer. The diet was mixed for 15 minutes after the vehicle was added. The Vehicle Control (VC) diet was always mixed first with neat acetone, followed by the T1, T2, T3 and T4 test diets. Fresh test diets were prepared at least every two weeks. Prepared diets were stored in a walk-in freezer for two weeks, at which time a new batch was mixed.	
3.3.4	Test conditions	Refer to table A7_5_3_1_2-4	X
3.3.5	Duration of the test	Adult Treatment Period: 10 weeks pre-egg laying; 10 weeks egg-laying. Hatchling Observation Period: 14 days post-hatch.	
3.3.6	Test parameter	Adult Parameters: Daily observations, diet consumption, body weight, necropsy including wet weights of the liver, spleen and testes. Reproductive Parameters: Eggs laid, eggshell thickness, defective and	

		cracked eggs, viable embryos, live embryos. Hatchling Parameters: Hatching success/hatchability, hatchling survival, hatchling body weights.
3.3.7	Examination / Observation	The birds were observed daily during the 20 week exposure period. Inspections were made to monitor symptoms that may be indicative of test substance related effects. Birds that died during the treatment period were removed, weighed and necropsied. Feed consumption of each pair of birds was measured weekly during the exposure period. The body weight of each bird was measured at the initiation of the 14-day acclimation period, on day 0, at the end of week 8, and at the end of week 20. At the conclusion of the treatment period, remaining birds were euthanized and necropsied for gross pathological abnormalities. Specific examination was made on the gastro-intestinal tract, liver, kidneys, bile duct, heart, spleen, and reproductive organs. Wet weights of the liver, spleen and testes were measured at the time of necropsy. Other observations were recorded as necessary.
3.3.8	Statistics	Adult endpoints and reproductive parameters were statistically analyzed using TOXSTAT Version 3.4. The experimental unit is each pen (or adult pair), except in the case of adult body weight, in which case the experimental unit is each adult bird. If a data set passed the chi-square test for normal distribution, and Bartlett's test for homogeneity of variance, it was analyzed by ANOVA. If no significant difference was identified by the ANOVA, no additional data was used. If ANOVA identified a difference, then the *post hoc* results generated by TOXSTAT were used. Bonferroni's test was used for pair-wise comparisons of each treatment with the control group. Bonferroni's test is appropriate when the replicates per group were not equal, as was the case in with many of the data sets. Data sets consisting of count data which did not pass the chi-square test and/or Bartlett's test, were transformed and analyzed again. If an appropriate transformation did not succeed in normalizing the distribution, or if the variance was not homogeneous, the original, untransformed data was analyzed by Kruskal-Wallis' non-parametric test (H-statistic). If a post hoc pair-wise comparison was indicated, Dunn's multiple comparison procedure was used. Dunn's procedure compares all possible pairs of means. If no significant difference was identified by the Kruskal-Wallis' test, no additional data was used. Proportional (percentage) data were analyzed with a different procedure. The spread of the data determined the necessary transformation, regardless of the results of the normality and/or homogeneity tests. This procedure is outlined in SOP CO-8.02. Depending upon the results of the transformation, the appropriate analysis of variance procedure was performed. The power of the test is the probability of detecting a difference when

there is a difference. The analysis is a pair-wise comparison of two means. In all cases, the mean values tested were the vehicle control group (VC) and the highest dietary concentration group, treatment level 4 (T4). The rationale for this comparison was that any test substance related effect would be expressed most strongly in the highest dose group. Power analyses were performed using the program XLStatistics. The test parameters were set at:

Significance Level (α): 0.05

Test Hypotheses: H_0: $\mu_1-\mu_2 = 0$

H_1: $\mu_1-\mu_2 \neq 0$ (two-tailed test)

Actual standard deviations associated with the means were used since the analyses were performed post hoc. The power statistic is expressed as $(1-\beta)$.

4 RESULTS

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4.1	**Limit Test / Range finding test**	Not performed
4.1.1	Concentration	
4.1.2	Number/ percentage of animals showing adverse effects	
4.1.3	Nature of adverse effects	
4.2	**Results test substance**	
4.2.1	Applied concentrations	Nominal Dietary Concentrations: 0 (VC), 0.001 (T1), 0.005 (T2), 0.020 (T3), 0.100 (T4) mg/kg diet, equivelant to: 0 (VC), 0.016 (T1), 0.075 (T2), 0.317 (T3), 1.642 (T4) mg/kg bw at the conclusion of the 20 week treatment period.
4.2.2	Effect data (Mortality and reproductivity)	Adverse effects observed in adult birds durng treatment period:

Group	Nominal Concentration (mg a.i./kg diet)	Birds Euthanized [a]	Birds Died [b]	Week(s) Found Dead	Initial Number of Birds	Dead (%)	Group Observations
VC	0	9	5	5,7,9,14	40	12.5	Feather loss (head,back), pecking (head), abrasion (head,ear,eye,foot), found dead, and sacrificed.
T1	0.001	5	3	10,11,16	38	7.9	Feather loss (head,back,neck), pecking (head), abrasion (head), hemmorhage (beak). Subdermal hematoma (head), sacrificed, growth on foot, found dead.
T2	0.005	7	2	12,20	40	5.0	Feather loss (head, eye, neck, back), pecking (head), abscess (beak), abrasion (head,foot), sacrificed, subdermal hematoma (head), ataxic, growth on beak, injured (right leg), found dead.
T3	0.020	5	1	7	40	2.5	Feather loss (head,neck), pecking (head), abscess (head), abrasion(foot), hypo-reactivity, abrasion healing, feathers growing, found dead, sacrificed, wing drop, injured (wing), growth on beak, subdermal hematoma (head).
T4	0.100	7	1	16	40	2.5	Feather loss (head, back), pecking (head) abrasion (head,foot), sacrificed, ataxic, fluffed feathers, found dead, growth on beak.

[a] Single birds were euthanized if their pen-mate had died. Both members of a pair were euthanized if the pair was incompatible, described as repeated or routine agonistic behavior which was resulting in severe injury to one or both members of the pair. Both members of some pairs were also euthanized if they met the criterion for excessively low egg production in week 13 of the test. The criterion was egg production in week 13 which was less than or equal to two standard deviations below the mean egg production of the VC group in week 13.
[b] Includes only those birds that were found dead during the 20 week adult observation period. Not included are any birds euthanized during the test.

Reproduction parameters:

Group	Nominal Conc. (mg a.i./ kg diet)	Egg Data							
		Eggs Laid	Mean Eggs /Hen /Week	Mean Hatchlings /Hen /Week	Mean Shell Thickness (mm)	Cracked Eggs (%) [a]	Viable Embryo (%) [b]	Live Embryo (%) [c]	Hatch (%) [d]
VC	0	889	6.5	4.0	0.215	9.8	94.4	95.3	83.5
T1	0.001	973	6.4	3.7	0.207	13.4	94.9	96.2	81.9
T2	0.005	963	6.1	3.2	0.216	10.0	87.2	95.2	77.0
T3	0.020	1056	6.2	4.0	0.215	7.7	95.5	96.2	81.4
T4	0.100	1048	6.4	3.8	0.213	9.9	90.7	97.5	83.0

[a] Percent Cracked Eggs = (cracked eggs/eggs candled) * 100.
[b] Percent Viable embryos = (viable embryos/eggs set) * 100.
[c] Percent Live Embryos = (live embryos/viable embryos) * 100.
[d] Percent Hatch = (hatchlings/viable embryos) * 100.

Refer also to table A7_5_3_1_3-5

NOEC (No Observed Effect Concentration): Greater than the T4 dietary concentration: 0.100 mg/kg diet administered for 20 weeks.

NOEL (No Observed Effect Level): Greater than the T4 ingestion level: 0.01138 mg/kg body weight/day (mean males and females).

4.2.3 Body weight

Adults:

Group	Nominal Concentration (mg a.i./kg diet)	Mean Body Weight (g)		
		Week 0 [a, b]	Week 8 [c]	Week 20 [d]
		Male		
VC	0	211	236	273
T1	0.001	214	251	290
T2	0.005	214	251	284
T3	0.020	205	238	275
T4	0.100	202	234	266
		Female		
VC	0	225	278	304
T1	0.001	224	284	312
T2	0.005	229	279	315
T3	0.020	227	280	302
T4	0.100	231	291	318

[a] Differences in initial body weights (male and female) among groups were not significant when analyzed using ANOVA (F = 2.45, calculated F = 0.176).
[b] Differences in initial male body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 1.974). Differences in initial female body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 0.335).
[c] Differences in week 8 male body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 1.976). Differences in week 8 female body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 0.628).
[d] Differences in week 20 male body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 1.852). Differences in week 20 female body weights among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 0.924).

Hatchlings:

Group	Nominal Concentration (mg a.i./kg diet)	Mean Day 0 Body Weight (g) [a]	Mean Day 14 Body Weight (g) [b]
VC	0	10	68
T1	0.001	10	67
T2	0.005	9	69
T3	0.020	9	59
T4	0.100	10	65

[a] Differences in mean day 0 body weight among groups were not significant when analyzed using Kruskal-Wallis (H = 9.490, calculated H = 2.121).
[b] Differences in mean day 14 body weight among groups were significant when analyzed using Kruskal-Wallis (H = 9.490, calculated H = 29.933), and a *post hoc* test using Dunn's multiple comparison test show T3 being significantly different than all other groups while all other groups, excluding T3, were not significantly different from each other.

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Effects on reproduction of birds

4.2.4 Food consumption

Adults:

Mean Feed Consumption of Adult *Coturnix*
During the Avian Reproduction Test With Difenacoum

Group	Nominal Concentration (mg a.i./kg diet)	Feed Consumption (grams/bird/day) Week 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean[a]
VC	0	28	29	26	26	28	26	26	28	30	31	35	36	35	36	36	37	36	37	34	35	32
T1	0.001	28	30	27	28	31	29	29	31	32	34	35	37	37	38	38	38	37	39	36	37	34
T2	0.005	28	30	26	27	30	27	28	29	29	31	33	36	34	36	34	37	35	37	35	36	32
T3	0.020	28	30	27	27	31	27	27	29	30	31	34	36	36	37	37	38	36	36	36	38	33
T4	0.100	26	30	27	27	29	27	28	30	32	34	34	37	36	38	36	38	37	37	37	39	33

[a] Difference in mean feed consumption among groups were not significant when analyzed using ANOVA (F = 2.53, calculated F = 1.182).

4.2.5 Results of residue analysis

Batch #	Date Prepared	Mean Measured Concentration (mg a.i./kg diet) VC (0.000 mg a.i./kg diet)	T1 (0.001 mg a.i./kg diet)	T2 (0.005 mg a.i./kg diet)	T3 (0.020 mg a.i./kg diet)	T4 (0.100 mg a.i./kg diet)
1	May 9, 2005	-	-	-	0.0177	0.1017
2	June 7, 2005	-	-	-	0.0225	0.1295
11	September 19, 2005	-	-	-	0.0198	0.0845
Mean Measured Concentration		-	-	-	0.0200	0.1052
Standard Deviation		-	-	-	0.0024	0.0227
Percent of Nominal		-	-	-	100.0	105.2

T1 and T2 diets were not analysed as the concentration is below the LOQ of the validated analytical method. However, the lower dietary concentrations are verified indirectly firstly by the careful dilution of the stock solution, secondly by the consistent mixing process used to prepare all levels, and thirdly, by the analytical verification of difenacoum levels in the T3 and T4 diets, which were mixed in the same manner and at the same time.

X

4.2.6 Other effects

Birds that died during the treatment were necropsied and the significant findings included:

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Effects on reproduction of birds

Group	Nominal Concentration (mg a.i./kg diet)	Birds Euthanized [a]	Birds Died [b]	Week(s) Found Dead	Initial Number of Birds	Dead (%)	Group Observations
VC	0	9	5	5,7,9,14	40	12.5	Feather loss (head,back), pecking (head), abrasion (head,ear,eye,foot), found dead, and sacrificed.
T1	0.001	5	3	10,11,16	38	7.9	Feather loss (head,back,neck), pecking (head), abrasion (head), hemmorhage (beak). Subdermal hematoma (head), sacrificed, growth on foot, found dead.
T2	0.005	7	2	12,20	40	5.0	Feather loss (head, eye, neck, back), pecking (head), abscess (beak), abrasion (head,foot), sacrificed, subdermal hematoma (head), ataxic, growth on beak, injured (right leg), found dead.
T3	0.020	5	1	7	40	2.5	Feather loss (head,neck), pecking (head), abscess (head), abrasion(foot), hypo-reactivity, abrasion healing, feathers growing, found dead, sacrificed, wing drop, injured (wing), growth on beak, subdermal hematoma (head).
T4	0.100	7	1	16	40	2.5	Feather loss (head, back), pecking (head) abrasion (head,foot), sacrificed, ataxic, fluffed feathers, found dead, growth on beak.

[a] Single birds were euthanized if their pen-mate had died. Both members of a pair were euthanized if the pair was incompatible, described as repeated or routine agonistic behavior which was resulting in severe injury to one or both members of the pair. Both members of some pairs were also euthanized if they met the criterion for excessively low egg production in week 13 of the test. The criterion was egg production in week 13 which was less than or equal to two standard deviations below the mean egg production of the VC group in week 13.
[b] Includes only those birds that were found dead during the 20 week adult observation period. Not included are any birds euthanized during the test.

The findings of the terminal necropsies are:

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Effects on reproduction of birds

Gross Necropsy Results of Adult *Coturnix* During the Avian Reproduction Test With Difenacoum
(The number in each column represents the number of birds that displayed the listed findings.)

Observations		Nominal Concentrations (mg a.i./kg diet)				
		VC (0)	T1 (0.001)	T2 (0.005)	T3 (0.020)	T4 (0.100)
Fate:	Found dead	5	2	1	1	1
	Sacrificed	35	36	39	39	39
Total necropsies:		40	38	40	40	40
Feather loss:		20	18	19	17	18
Emaciated:		2	1	2	0	1
Breast muscle atrophy:		2	1	2	0	1
Ventriculus contents:	No feed/grit	2	0	0	0	0
	1/2 full	2	4	2	2	2
	Full	36	34	38	38	38
Enlarged: [a]	Liver	n/a	n/a	n/a	n/a	n/a
	Kidneys	0	2	0	0	0
	Spleen	n/a	n/a	n/a	n/a	n/a
	Bile duct	0	0	0	0	0
Discolored:	Liver	6	3	6	1	5
	Heart	0	0	0	0	0
	Kidneys	0	0	1	0	0
	Spleen	0	1	0	0	0
	Bile duct	0	0	0	0	0
Lesions/Abrasions:	Skin	7	5	3	5	4
Lesions/Growths:	Mouth	0	0	0	0	0
	Esophagus/Crop	1	0	0	0	0
	Proventriculus	0	0	0	0	1
	Ventriculus	0	0	0	0	1
	Intestines	0	0	0	0	0
	Heart	0	0	0	0	0
	Liver	0	0	0	0	0
	Bile Duct	0	0	0	0	0
	Spleen	0	0	0	0	0
	Kidneys	0	0	0	0	0
	Uro-Genital	0	0	0	0	0
Reproductive organs:	Mature follicles	18	19	19	20	20
	Egg in oviduct	14	15	15	17	16
	Immature Testes	0	0	1	0	0

[a] Classification as "enlarged" is subjective for kidneys and bile duct. Livers, spleens, and male testes were weighed and the results presented in Table 21 and in Appendix C3.

Organ weights recorded during the terminal necropsies were:

Group	Nominal Concentration (mg a.i./kg diet)	Organ Body Weight (g)			
		Liver [a]	Spleen [b]	Right Testes [c]	Left Testes [d]
		Male			
VC	0	6.0	0.11	3.8	3.7
T1	0.001	7.5	0.16	3.4	3.6
T2	0.005	7.1	0.16	3.2	3.2
T3	0.020	6.7	0.14	3.4	3.6
T4	0.100	6.4	0.18	3.2	3.4
		Female			
VC	0	9.9	0.20	---	---
T1	0.001	10.9	0.27	---	---
T2	0.005	11.6	0.21	---	---
T3	0.020	9.3	0.22	---	---
T4	0.100	10.8	0.21	---	---

[a] Differences in liver weights among males in the groups were not significant when analyzed using ANOVA Male ($F = 2.53$, calculated $F = 2.299$). Differences in liver weights among females in the groups were significant when analyzed using ANOVA ($F = 2.53$, calculated $F = 4.693$). Although the ANOVA declared significant differences, pair-wise comparisons of each treatment group with the VC group did not find significant differences (Bonferroni's t-test).
[b] Differences in spleen weights among groups were not significant when analyzed using ANOVA Male ($F = 2.53$, calculated $F = 1.693$), Female($F = 2.53$, calculated $F = 0.765$).
[c] Differences in right testes weights among groups were not significant when analyzed using ANOVA Male ($F = 2.53$, calculated $F = 1.387$).
[d] Differences in left testes weights among groups were not significant when analyzed using ANOVA Male ($F = 2.53$, calculated $F = 0.801$).

4.3 Results of controls

4.3.1 Number/ percentage of

All data for the control group is included in the Tables above.

	animals showing adverse effects	
4.3.2	Nature of adverse effects	Although the listed findings are consistent with anticoagulant exposure, the observations do not form a pattern of consistent effects either within groups or across treatment groups. There were eight cases of sub-lethal observations that could be related to anticoagulant exposure. The six cases were distributed among four groups: VC (n=2), T1 (n=2), T2 (n=2), T3 (n=1), and T4 (n=1) treatment groups. Two control group birds were found to have hemorrhaging in the esophagus upon necropsy. This illustrates that the birds were incurring many forms of physical stress and tissue damage that was related to aggressive interactions among pen-mates. While some of the sub-lethal conditions observed may be consistent with anticoagulant exposure, similar observations in the control group suggest that there were other causative factors at work. The lack of any systematic dose response in physical symptoms and in any of the other parameters measured in the test support this conclusion.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Primary Guideline: OECD Test Guideline 206: Avian Reproduction Test, 1984. SecondaryGuidelines: Modified in places to follow OECD Test Guideline "Draft Document 1998": Avian Toxicity Test in the Japanese Quail or Japanese Quail, and USEPA Ecological Effects Guideline OPPTS 850.2300: Avian Reproduction Test. No deviations.

Treated diet was prepared every two weeks and offered *ad libitum* to groups of 10 male and female pairs for 10 weeks pre-egg laying and 10 weeks egg laying. Treated diets contained nominal 0 (VC), 0.001 (T1), 0.005 (T2), 0.020 (T3) and 0.100 (T4) mg/kg diet.

Adults were observed daily and diet consumption, body weight, necropsy including wet weights of the liver, spleen and testes recorded. Eggs were collected daily for 10 weeks. The number of eggs laid, eggshell thickness, defective and cracked eggs, viable embryos, live embryos were recorded. Eggs were incubated and hatching success/hatchability, hatchling survival and hatchling body weight at day 14 were recorded. Parameters were analysed statistically.

5.2 Results and discussion

Of all the parameters measured and analysed in the study, only three were declared to have significant differences, these were: adult female liver weights; the mean body weight of 14-day old hatchlings; and, the mean number of normal hatchlings per hen .

The adult female liver weights were significantly different (lower), according to ANOVA, but no significant differences were identified by pair-wise comparisons of each treatment group mean with the VC group.

The mean body weight of 14-day old hatchlings found the T3 group to be significantly different (lower) from the VC group but this may have been due to behavioural interactions as the hatchling density in the brooders for this group was the highest.

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The mean number of normal hatchlings per hen in the T2 and T4 groups was significantly different (lower) from the VC group. These results do not appear as part of a larger pattern. The T2 group contained two pairs having very low numbers of hatchlings. It is therefore considered to be an artifact of the groupings and the analysis process.

Regarding the adult generation, although the listed symptoms are consistent with anticoagulant exposure, the observations do not form a pattern of consistent effects either within groups or across treatment groups. There were eight cases of sub-lethal observations that could be related to anticoagulant exposure. Two control group birds were found to have haemorrhaging in the oesophagus upon necropsy. This illustrates that the birds were incurring many forms of physical stress and tissue damage that was related to aggressive interactions among pen-mates. While some of the sub-lethal conditions observed may be consistent with anticoagulant exposure, similar observations in the control group suggest that there were other causative factors at work. The lack of any systematic dose response in physical symptoms and in any of the other parameters measured in the study support this conclusion.

Dietary consumption of up to 0.100 mg/kg diet had no observed effect on the body weight, feed consumption, or reproductive performance of adult Japanese quail when administered via the diet for 20 weeks. No effects were attributed to the test substance in egg development, or hatchling observations, hatchling body weights and hatchling feed consumption for 14 days.

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5.2.1	NOEC	**NOEC** (No Observed Effect Concentration): Greater than the T4 dietary concentration: 0.100 mg/kg diet administered for 20 weeks.
		NOEL (No Observed Effect Level): Greater than the T4 ingestion level: 0.01138 mg/kg body weight/day (mean males and females).
5.3	**Conclusion**	The validity criteria can be considered to have been fulfilled. Although the control mortality was very slightly higher than the threshold it is not considered to have affected the integrity of the study. Based on the results of this study with Japanese quail, the NOEC of difenacoum is considered to be greater than 0.100 mg a.i./kg diet. Adult Japanese quail fed difenacoum in the diet for 20 weeks at this level and at three lower levels, did not show any pattern of symptoms consistent with anticoagulant toxicity. There was no suggestion of a dose response at the dietary concentrations listed. All symptoms observed in birds administered difenacoum-treated diets were also observed in the control group. Symptoms observed may have been magnified by the presence of difenacoum in the treated birds, but the degree of interaction cannot be separated and appears to be minor.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Doc IIIA / Section 7.5.3.1.3 **Effects on reproduction of birds**

BPD data set IIIA / Annex Point IIIA XIII 1.3

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	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	

Doc IIIA/Section 7. BPD Data Set IIIA/Annex Point 7.5.5	**Bioconcentration, terrestrial**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [✓] **Limited exposure** []	**Technically not feasible** [] **Scientifically unjustified** [] **Other justification** [✓]	
Undertaking of intended data submission [[ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	[redacted]	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIA/Section 7. BPD Data Set IIIA/Annex Point 7.5.5	**Bioconcentration, terrestrial**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [✓] **Limited exposure** []	**Technically not feasible** [] **Scientifically unjustified** [] **Other justification** [✓]	
Detailed justification:	[redacted]	
Undertaking of intended data submission [[ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	[redacted]	
Evaluation of applicant's justification	[redacted]	
Conclusion	[redacted]	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIA/Section 7.5.6/01

BPD Data Set IIIA/Annex Point XIII.3

Effects on other terrestrial non-target organisms

Secondary toxicity of brodifacoum to American Kestrels.

Official use only

1 REFERENCE

1.1 Reference

[redacted]. Secondary LC50 study of the toxicity of the anticoagulant brodifacoum to American Kestrels (*Falco sparvarius*). ICI Americas Inc. Report number: TMUE0017/B (unpublished), [redacted]

1.2 Data protection

[redacted]

1.2.1 Data owner

[redacted]

1.2.2 Companies with letter of access

[redacted]

1.2.3 Criteria for data protection

[redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

No guideline was stated in the report. The study was conducted in accordance with accepted scientific principles of the time and the protocol was similar to that described by Heath *et al*, 1972. Comparative dietary toxicities of pesticides to birds. *Bur. Sport Fish. and Wildl. Spec. Sci. Rep. Wildl.* 152, pg 57.

2.2 GLP

No, the study pre-dates the requirements of GLP.

2.3 Deviations

No. However, there were two notable exceptions to the procedures described in the protocol described by Heath *et al*, 1972. All the birds were individually caged and each bird was offered 30g of vole tissue per day (on a metal tray) rather than food supplied *ad libitum*.

3 MATERIALS AND METHODS

3.1	**Test material**	The vole bait was prepared containing 100ppm brodifacoum and a bait base of lightly rolled oat-groat bait prepared using brodifacoum 0.25% liquid concentrate (JF 05074) as supplied by ICI America. Voles were fed this bait and on the 5th day all surviving voles were culled and were ground whole to form a pooled sample, which were immediately frozen. Portions of this were then offered to the kestrels.	
3.1.1	Lot/Batch number	Not stated	X
3.1.2	Specification	Not stated	X
3.1.3	Purity	Not stated	X
3.1.4	Further relevant properties	None	
3.1.5	Radiolabelling	The test material was not radiolabelled.	
3.1.6	Method of analysis	Five aliquots of 30g of the ground tissue were taken for analysis by HPLC to determine the brodifacoum residue.	
3.2	**Reference substance**	No	
3.2.1	Method of analysis for reference substance		
3.3	**Testing/estimation procedure**		
3.3.1	Test system/ performance	This secondary toxicity study using American Kestrels was carried out to investigate the potential for brodifacoum to cause secondary poisoning to predators or scavengers from eating primary/target animals. The kestrels, 28 males and 20 females, were trapped in Colorado and California. Five groups of eight mature kestrels were each fed on one of five concentrations of brodifacoum in vole tissue for 5 consecutive days. Treated tissue was diluted with untreated vole tissue to provide the five nominal test concentrations. These concentrations were 0.3, 0.8, 1.6, 3.2 and 6.0ppm. The birds were individually caged and was offered 30g of vole tissue per day. A control group of eight kestrels was offered 30g per day of untreated vole tissue for 5 consecutive days. All birds were observed for 90 days after treatment period. All birds that died during the observation period were subjected to a *post-mortem*. The carcasses were then frozen and sent to ICI for analysis. All surviving birds were released.	
3.3.2	Estimation of bioconcentration	Bioconcentration was not measured. The study was performed to investigate the potential of brodifacoum to cause secondary poisoning to American Kestrels after consumption of voles fed on a brodifacoum-containing bait.	

4 RESULTS

4.1 Experimental data

4.1.1 Bait consumption

A total of 125 voles were offered the treated bait (100ppm brodifacoum) and the average vole weighed 48g. The average bait consumption during the 4 days was 10.5g (range 0.4 to 21.8g). 64 of the voles died on or before the fifth day. It was estimated that approximately 70% of the ingested brodifacoum was excreted or metabolised, based on the pooled vole tissue containing 6.7ppm brodifacoum and the average vole consuming 10.5g of bait equivalent to 21.9mg/kg brodifacoum.

4.1.2 Bodyweight

Initially the bodyweights were 101 and 118g for males and females respectively. Immediately prior to being offered the treated vole tissue virtually all of the birds had gained bodyweight. Average male was 113g and average female was 131g. The bodyweights between treatment groups were uniform. After the 90 day observation period, the birds had gained weight, males averaged 134.3g and females averaged 152.5g.

4.1.3 Tissue consumption

The bird bodyweight values were used to calculate the theoretical mean maximum consumption of brodifacoum in mg/kg for each treatment group.

Treatment group, ppm					
	0.3	**0.8**	**1.6**	**3.2**	**6.0**
mg/kg	0.4	1.0	2.1	3.8	7.3

These values provide relative comparisons between groups as some spillage of vole tissue during consumption was unavoidable. It was assumed that dehydration of vole tissue prior to consumption did not result in loss of brodifacoum.

4.1.4 Mortality

Kestrel mortality at various levels of treatment.	
Treatment level, ppm of brodifacoum in vole tissue	**Mortality**
0	0/8
0.3	0/8
0.8	1/8
1.6	0/8
3.2	0/8
6.0	4/8 (1 mortality not treatment related)

Three of the mortalities in the 6.0ppm group were due to haemorrhagic lesions in the pericardial and/or peritoneal cavities and one bird had a ruptured cloaca causing severe peritonitis. The other bird mortality in this group and also the bird mortality in the 0.8ppm group, occurred during the first post-treatment week but had no significant lesions.

No other mortality or signs of toxicity were observed in individuals from the 3.2, 1.6, 0.8 and 0.3ppm concentration groups.

4.1.5 Other Observations

One to five magpies were observed scavenging daily under the kestrel cages. Four days after the last treated vole tissue was fed to the kestrels, a magpie was found dead. Haemorrhages were reported in the body cavities and pectoral muscle.

4.2 Estimation of bioconcentration

Not the purpose of the study.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

This secondary toxicity study using American Kestrels was carried out to investigate the potential for brodifacoum to cause secondary poisoning to predators or scavengers from eating primary target animals.

The vole bait was prepared containing 100ppm brodifacoum and a bait base of lightly rolled oat-groat bait prepared using brodifacoum 0.25% liquid concentrate (JF 05074) as supplied by ICI America. Voles were fed this bait and on the 5th day all surviving voles were culled and were ground whole to form a pooled sample, which were immediately frozen. Treated tissue was diluted with untreated vole tissue to provide the five nominal test concentrations, 0.3, 0.8, 1.6, 3.2 and 6.0ppm. No guideline was stated in the report, but the study was conducted in accordance with accepted scientific principles of the time and the protocol was similar to that described by Heath *et al*, 1972.

Five groups of eight mature kestrels were each fed on one of five concentrations of brodifacoum in vole tissue for 5 consecutive days. The birds were individually caged and were offered 30g of vole tissue per day.

A control group of eight kestrels was offered 30g per day of untreated vole tissue for 5 consecutive days.

All birds were observed for 90 days after treatment period. All birds that died during the observation period were subjected to a *post-mortem*. The carcasses were then frozen and sent to ICI for analysis. All surviving birds were released.

5.2 Results and discussion

A total of 125 voles were offered the treated bait (100ppm brodifacoum). The average bait consumption during the 4 days was 10.5g (range 0.4 to 21.8g). 64 of the voles died on or before the fifth day. It was estimated that approximately 70% of the ingested brodifacoum was excreted or metabolised, based on the pooled vole tissue containing 6.7ppm brodifacoum and the average vole consuming 10.5g of bait equivalent to 21.9mg/kg brodifacoum.

The kestrel bodyweights were uniformly spread between the treatment groups. The birds continued to gain bodyweight throughout the treatment period and during the 90 day observation period. The bird bodyweight values were used to calculate the mean maximum consumption of brodifacoum in mg/kg for each treatment group.

Summary of Kestrel Mortality

Bird No.	Sex	Treatment, ppm	Whole body residue, ppm	Gross pathology
70	M	0.8	0.23	No significant lesions
59	F	6.0	0.24	No significant lesions
47	M	6.0	0.20	Haemorrhagic
60	M	6.0	0.12	Slightly haemorrhagic
56	M	6.0	0.10	Ruptured cloaca, non-haemorrhagic

Magpie	-	-	0.17	Haemorrhagic

No mortality or signs of toxicity were observed in individuals from the 3.2, 1.6 and 0.3ppm concentration groups.

One to five magpies were observed scavenging daily under the kestrel cages. Four days after the last treated vole tissue was fed to the kestrels, a magpie was found dead. Haemorrhages were noted in the body cavities and pectoral muscle.

5.3 Conclusion

The objective of the study was to estimate a vole-kestrel brodifacoum secondary LC50. This was not achieved and the cause of failure was the selection of concentrations was too low; a starting point of 3.8mg/kg brodifacoum in the vole tissue and a longer treatment period may have been more realistic.

The degree of kestrel secondary toxicity that may occur in field operations cannot be predicted from this laboratory study. Under field conditions the degree of exposure to non-target animals would depend on dose and treatment levels, methods of use, local ecological situations and the behaviour of the target and non-target species.

5.3.1 Reliability 2

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 2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	
	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*
Reliability	*Discuss if deviating from view of rapporteur member state*
Findings	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	*Discuss if deviating from view of rapporteur member state*

Doc IIIA/Section 7.5.6 Effects on other terrestrial non-target organisms

BPD Data Set IIIA/Annex Point XIII.3 Primary and secondary toxicity of brodifacoum to Carrion Crows.

Official use only

1 REFERENCE

1.1 Reference [redacted], 1986. Safety evaluation of Agrochemicals: R170431 and PP581; Sub-acute oral toxicity to Carrion Crows. Institute of Terrestrial Ecology (Natural Environmental Research Council), Report number: ICI/NERC F6/95/129 (unpublished), [redacted]

1.2 Data protection [redacted]

1.2.1 Data owner [redacted]

1.2.2 Companies with letter of access [redacted]

1.2.3 Criteria for data protection [redacted]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No guideline was stated in the report. The study was conducted in accordance with accepted scientific principles of the time and the protocol contained in the original report.

2.2 GLP No, the study pre-dates the requirements of GLP.

2.3 Deviations No.

3 MATERIALS AND METHODS

3.1	**Test material**	Brodifacoum. Primary exposure – test material incorporated into maize meal and normal diet (dog food) of the crows at 10, 100 or 1000ppm. Secondary exposure – the crows are fed on voles, *Microtus pennsylvanicus* which had been intoxicated with a known amount of test material and minced to give a homogenous distribution of any residues present, 'vole burger'. Nominal concentration in vole burger was 4.5mg/kg.
3.1.1	Lot/Batch number	Primary exposure – [redacted] Secondary exposure – mixture of [redacted] and [redacted]
3.1.2	Specification	Not stated
3.1.3	Purity	Primary exposure – [redacted] Secondary exposure – mixture of [redacted]% w/w and [redacted]% w/w.
3.1.4	Further relevant properties	None
3.1.5	Radiolabelling	The test material was not radiolabelled.
3.1.6	Method of analysis	
3.2	**Reference substance**	No
3.2.1	Method of analysis for reference substance	
3.3	**Testing/estimation procedure**	
3.3.1	Test system/ performance	The study was designed to compare the sub-acute dietary toxicity of 2 anticoagulant rodenticides, brodifacoum and [redacted], to Carrion crows, *Corvus corone corone*. Only the results for brodifacoum have been summarised here. Twenty-six wild caught carrion crows, already acclimatised to outdoor flight cages were supplied by the sponsor. Each bird was ringed for identification purposes. Birds were housed in groups of 4 in covered outside flights and subject to normal weather conditions. The flights were of wood and wire-mesh construction with plastic roofs and concrete floors. All contained perches. Each bird was individually caged for the purposes of dosing only. The birds were acclimatised for a period of 47 days. Two birds died during the acclimatisation period, but no specific cause of death could be identified. Birds were allocated to groups at random. All were >12 months old at dosing (no juvenile plumage present). Sex was not determined as plumage in the sexes is very similar.

Treatment groups:

Group No.	Test material	Dietary exposure regime	No. of birds	Bird No.
1	Brodifacoum	Primary	4	11, 12, 13, 14
2	Brodifacoum	Secondary	4	15, 16, 17, 18
5	Control	-	4	6, 8, 9, 10
6	Uncaged control	-	4	(2), 3, 4, 5

Groups 3 and 4 are not included here as these groups were using the compound R170431 which is not being summarised here. Bird 2 was placed into group 4 as a mortality occurred in this group.

Eight sequentially increasing doses were given to the birds at 14 day intervals. Doses were nominally 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 mg of brodifacoum per kg and birds were exposed by primary and secondary exposure. Primary dosing was brodifacoum on maize meal and mixed with dog food, the normal diet and fed to the birds. Secondary dosing was brodifacoum-fed voles that were minced into a 'vole burger' and fed to the birds, normal diet was not provided.

Birds were weighed at 14 day intervals. For measurement of general condition and early indication of treatment related effects, general observations of behaviour and pelage were made.

Blood samples were taken during the acclimatisation period to provide a plasma blank for use in clotting factor assays and to identify any problems with techniques. Blood samples were taken on days -6, -3 and 0 to establish pre-treatment levels and variation between the birds. After the first and each subsequent dose blood samples were taken after 3, 7, 10 and 14 days. Blood samples were taken, mixed with tri-sodium citrate (1 part blood: 1 part citrate) and centrifuged. 200µl aliquots of the plasma were frozen and transported to the sponsor for analysis of prothrombin time and Factor X.

3.3.2 Estimation of bioconcentration

Bioconcentration was not measured. The study was designed to compare the sub-acute dietary toxicity of 2 anticoagulant rodenticides, brodifacoum and R170431, to Carrion crows, *Corvus corone corone,* when exposed by the primary and secondary route.

4 RESULTS

4.1 Experimental data

4.1.1 Consumption/ acceptability

The nominal dose and amount consumed by each individual bird was determined. In almost all cases where the dose was presented by the primary route the entire dose appeared to have been consumed. Where the test material was presented by the secondary route, in the form of a vole burger, after the second dose uneaten vole burger remained. These amounts were small and it was considered that the actual dose consumed was near to that intended.

Percent of dose estimated to have been consumed by each bird at each dose level.

	% consumed Nominal dose, mg/kg							
Group/Bird No.	**0.1**	**0.2**	**0.4**	**0.8**	**1.6**	**3.2**	**6.4**	**12.8**
Control/6	98	99	99	100	100	98	100	98
Control/8	98	99	96	98	91	63	96	99
Control/9	97	98	98	99	99	98	98	100
Control/10	99	92	96	98	98	81	99	98
1/11	100	89	-	-	-	-	-	-
1/12	87	95	95	95	95	100	-	-
1/13	99	99	100	99	99	98	-	-
1/14	97	86	98	98	86	-	-	-
2/15	99	97	89	94	68	80	-	-
2/16	95	95	96	84	-	-	-	-
2/17	99	71	81	77	91	-	-	-
2/18	90	92	57	58	-	-	-	-

- indicates that the bird had died or had been sacrificed.

4.1.2 Effects of anticoagulation

Graphical presentation of the response of Factor X to the brodifacoum dose is contained in the original report. Factor X reduced in all birds dosed with brodifacoum; this however returned to normal after a time. The magnitude and duration of the response was dose related.

Graphical presentation of the prothrombin times is also contained in the original report. Birds fed brodifacoum by the primary route showed the most marked increase in prothrombin times, however, the birds fed by the secondary route also showed a marked increase but to a slightly lesser extent.

4.1.3 Mortality

All birds that were exposed to brodifacoum either by the primary or secondary route died or were sacrificed. 6/8 deaths resulted from haemorrhage or because of some form of anaemia. There were no deaths in either the control or uncaged control groups and the *post-mortem* results were normal.

4.1.4	Other observations	All birds that either died or were sacrificed lost plumage condition, they often had a heavy mite or feather louse infestation and stopped perching, spending most of their time on the concrete floor. Body weights were effectively constant throughout the study, except when a bird was close to death. Control birds were in good general condition throughout the whole study.
4.2	**Estimation of bioconcentration**	Not the purpose of the study.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The test material was brodifacoum presented *via* primary and secondary exposure routes. For primary exposure, brodifacoum (FAJ7266, 99.3% w/w) was incorporated into maize meal and normal diet (dog food) of the crows at 10, 100 or 1000ppm. For secondary exposure, brodifacoum (mixture of 159/4503 and 159/45-4, 994.4% w/w and 95.7% w/w) was fed to voles, *Microtus pennsylvanicus*, these were then minced to give a homogenous distribution of any residues present, 'vole burger'. These vole burgers where then fed to the crows. The study was designed to compare the sub-acute dietary toxicity of 2 anticoagulant rodenticides, brodifacoum and R170431, to Carrion crows, *Corvus corone corone*. Only the results for brodifacoum have been summarised here. Twenty-six wild caught carrion crows, already acclimatised to outdoor flight cages were supplied by the sponsor. Each bird was ringed for identification purposes. Birds were allocated to groups of 4 at random. All were >12 months old at dosing (no juvenile plumage present). Sex was not determined as plumage in the sexes is very similar. Group 1 were exposed to brodifacoum *via* primary exposure using the maize meal and dog food mix. Group 2 were exposed to brodifacoum *via* the secondary route using the vole burgers. Group 5 and 6 were controls. Groups 3 and 4 are not included in this summary as they involved using a different compound. Eight sequentially increasing doses were given to the birds at 14 day intervals. Doses were nominally 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 mg of brodifacoum per kg. Birds were weighed at 14 day intervals. For measurement of general condition and early indication of treatment related effects, general observations of behaviour and pelage were made. Blood samples were taken during the acclimatisation period to provide a plasma blank for use in clotting factor assays and to identify any problems with techniques. Blood samples were taken on days -6, -3 and 0 to establish pre-treatment levels and variation between the birds. After the first and each subsequent dose, blood samples were taken after 3, 7, 10 and 14 days. Blood samples were taken, mixed with tri-sodium citrate (1 part blood: 1 part citrate) and centrifuged. 200µl aliquots of the plasma were frozen and transported to the sponsor for analysis of prothrombin time and Factor X.
5.2	**Results and discussion**	In almost all cases where the dose was presented by the primary route the entire dose appeared to have been consumed. Where the test material was presented by the secondary route, in the form of a vole burger, after the second dose uneaten vole burger remained; these amounts were small

and it was considered that the actual dose consumed was near to that intended.

Factor X reduced in all birds dosed with brodifacoum. This however returned to normal after a time. The magnitude and duration of the response was dose related. Birds fed brodifacoum by the primary route showed the most marked increase in prothrombin times, however, the birds fed by the secondary route also showed a marked increase but to a slightly lesser extent.

All birds that were exposed to brodifacoum either by the primary or secondary route died or were sacrificed. 6/8 deaths resulted from haemorrhage or because of some form of anaemia. There were no deaths in either the control or uncaged control groups and the *post-mortem* results were normal.

All birds that either died or were sacrificed lost plumage condition, they often had a heavy mite or feather louse infestation and stopped perching, spending most of their time on the concrete floor. Body weights were effectively constant throughout the study, except when a bird was close to death. Control birds were in good general condition throughout the whole study.

5.3 Conclusion

There was little difference in the effects of brodifacoum when presented by either the primary or secondary routes of exposure.

However, it is not possible with the data available from this study to state unequivocally that when used in the field these results would be reflected. There are so many factors that this study has not examined which can influence the quantity of a substance that a bird/animal may be exposed to.

5.3.1 Reliability 2

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	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

	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*
Reliability	*Discuss if deviating from view of rapporteur member state*
Findings	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Doc IIIA/Section 7.5.6/02

BPD Data Set IIIA/Annex Point XIII.3

Field trial used to assess the risks to non-target organisms.

			Official use only
1	**REFERENCE**		
1.1	**Reference**	[redacted], 1981. Hazards to barn owls associated with the use of Talon (brodifacoum bait) for controlling rats and house mice. US Fish and Wildlife Service, Denver Wildlife Research Centre. Report number RIC0619 (unpublished) [redacted]. [redacted]1980. Talon: Rodent baiting sites of the Barn Owl Secondary hazard study. ICI Americas Inc. Report Series TMUD3335/B (unpublished) [redacted].	
1.2	**Data protection**	[redacted]	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
1.2.3	Criteria for data protection	[redacted]	
1.3	**Guideline study**	No guidelines were stated in the reports. The study was carried out in accordance with the accepted scientific principles of the time.	
1.4	**Deviations**	No	
2	**METHOD**		
2.1	**Test Substance (Biocidal Product)**		
2.1.1	Trade name/ proposed trade name	Talon Pellets	X
2.1.2	Composition of Product tested	The product contains 0.005% w/w brodifacoum as the active substance. 0.15% declomycin (demethylchlorotetracycline or DMCT) was added. This fluoresces under UV light in the lower jaw of any rodents which have consumed the bait. By collecting and examining owl pellets under UV light, any owls that had consumed rodents that had consumed the bait could be determined.	X
2.1.3	Physical state and nature	RB: Ready-for-use pellet bait (GCPF code).	
2.1.4	Monitoring of active substance concentration	No	
2.1.5	Method of analysis	Not applicable	
2.2	**Reference substance**	No	
2.2.1	Method of analysis for reference substance	Not applicable	
2.3	**Testing procedure**		

Doc IIIA/Section 7.5.6/02 **BPD Data Set IIIA/Annex Point XIII.3**		**Field trial used to assess the risks to non-target organisms.**
2.3.1	Test population / inoculum / test organism	Refer Table 1.2
2.3.2	Test system	Refer Table 1.3
2.3.3	Application of TS	Refer Table 1.4
2.3.4	Test conditions	Refer Table 1.5
2.3.5	Duration of the test / Exposure time	A total of 25 Talon Pellet field treatments were carried out during the study and the duration of these treatments ranged between 17 and 53 days. Collection of owl pellets and radiotelemetry of the barn owls was carried out pre- and post-Talon Pellet treatments at some or all of the sites.
2.3.6	Number of replicates performed	None
2.3.7	Controls	No
2.4	**Examination**	
2.4.1	Effect investigated	The secondary hazard of the anticoagulant rodenticide bait, Talon Pellets, when used for controlling rats and mice on farms.
2.4.2	Method for recording / scoring of the effect	Talon Pellet Field Treatment. Visual assessment, pre- and post-census bait consumption and tracking patches were employed to determine the effectiveness of the Talon Pellet treatment. Visual assessment was recorded using the following scale: 0 = no rodent activity 1 = light rodent activity 2 = medium rodent activity 3 = heavy rodent activity 4 = very heavy rodent activity. An approximation of bait consumption was calculated using the following scale:

Visual score	Approx. consumption per rat bait station, g	Approx. consumption per mouse bait station, g
0	0	0
1	25	12
2	50	24
3	75	36
4	100	48

Tracking patches were scored using the following scale:

0 = 0 tracks (no activity)
1 = 5 tracks (light activity)
2 = 10 tracks (medium activity)

		3 = 15 tracks (heavy activity) 4 = 20 tracks (very heavy activity) <u>Owl monitoring.</u> Owl pellets were collected pre- and post-Talon Pellet treatment to determine if any rodents had been consumed that had eaten the bait which contained DMCT, the fluorescing agent. Also, an indication of the common prey items taken by the birds could be evident. Radio-telemetry was employed to track and identify individual birds. During the early phases of the study tracking of general owl movements were made every 2 to 3 days. After the Talon Pellet treatment efforts were made to locate most owls daily during roosting and feeding periods.
2.4.3	Intervals of examination	All sites employed a 3 day pre-treatment census period and a 3 day post-treatment census. The details of visits during the treatment period are not contained in the report. Owl pellets were collected in conjunction with other trial activities, intervals were not stated in the report. Radio-tracking of owls was carried out every 2 to 3 days initially, but then daily, especially after the Talon Pellet treatments were completed.
2.4.4	Statistics	None
2.4.5	Post monitoring of the test organism	Yes. A 3 day post-treatment census was carried out, this was used to determine the effectiveness of the Talon Pellet treatment. Owl pellets were collected to determine if any owls had taken rodents that had consumed Talon Pellets as a prey item. Radio-tracking of owls was carried out to determine if there was an impact on the surrounding owl populations due to the use of the rodenticide, Talon Pellets.

Doc IIIA/Section 7.5.6 **Field trial used to assess the risks to non-target organisms.**

BPD Data Set IIIA/Annex Point XIII.3

3 RESULTS

3.1 Efficacy

The data contained in the reports does not detail the efficacy of the Talon Pellet treatments as the intention of the study was to expose owls to farms which were being treated with Talon Pellets, not to monitor the efficacy at each of the treated sites.

However, a total of 346 bait stations were placed using a 33.3kg of Talon Pellets. Approximately, 19.3kg of bait was consumed during the trials. Treatment periods ranged from 17 to 53 days. Generally, control of rats and mice was considered to have been very good.

3.2 Effects against organisms or objects to be protected

Analysis of the regurgitated pellets showed that meadow voles were the most common prey item (75.7%) for all sites. Some Norway rats (3.9%) and house mice (2.2%) occurred in some pellet collections, but they made up only a small part of the diet of barn owls in the study area.

Refer IIIB7_7_7_1 – 1 Chart of Owl Pellet Analysis.

Examination of fluorescence under UV light of pellets collected after the Talon Pellet treatment, indicated that no rodents that consumed the Talon Pellets bait were consumed by barn owls that then regurgitated pellets.

Summary of regurgitated barn owl pellet analysis of pellets collected on treated sites post-treatment.
(All jaws were negative when examined under UV light for fluorescence.)

		Prey Species*					
Site No.	**No. days post-treatment**	**MP**	**RN**	**MM**	**BB**	**OP**	**Other**
2	20	-	1	-	-	-	-
14	20-21	9	-	-	2	-	-
18	12	22	-	2	-	-	-
37	10	-	-	1	-	-	-
38	12	8	-	-	-	-	-
	52-54	42	-	-	1	-	-
	59	11	-	-	-	-	1
	65	4	-	-	-	-	-
	89	54	-	-	2	2	-
		9	-	-	1	-	2
53	4	26	-	-	-	-	-
	27	14	-	-	-	1	-
	33	41	-	1	-	-	3
TOTAL	-	**240**	**1**	**4**	**6**	**2**	**6**

* MP = *Microtus pennsylvanicus*, RN = *Rattus norvegicus*, MM = *Mus musculus*, BB = *Blarina brevicauda*, OP = *Oryzomys palustris*, Other =

unknown and *Condylura cristata*.

34 barn owls were captured and radio-equipped, 26 adults (17 females and 9 males) and 8 newly fledged young of the year.

Initially (early July) 13 adult birds were actively being radio-tracked. Contact was lost with a number of the owls and some moulted their tail feathers and the transmitter. Further owls were added and 13 owls were continually tracked until early August. Contact was lost with the majority of young birds that were tracked as it was presumed that they had left the area.

3.3 Other effects

Several reasons for owl mortality were recorded. 2 electrocutions, 2 highway kills, 2 predator kills, 1 found dead (cause unknown) and one reported shooting.

Summary of results from brodifacoum residue analysis of available barn owl carcasses.

Species	Description	Site No.	Brodifacoum residue
Barn owl	Female, car kill, pre-treatment	-	Not detected
Barn owl	Newly hatched chick, pre-treatment	51	Not detected
Barn owl	Juvenile killed by another owl, pre-treatment	-	Not detected
Barn owl	Juvenile, found dead 1 day post-treatment	65	Not detected
Barn owl	Juvenile, electrocuted 6 days post-treatment	2	Trace (<0.05ppm)
Barn owl	Juvenile, probable car kill, 26 days post-treatment	2	Not detected

3.4 Efficacy of the reference substance

Not applicable.

3.5 Efficacy limiting factors

3.5.1 Occurrences of resistances

Resistance to brodifacoum was not discussed in the study report.

3.5.2 Other limiting factors

None

4 RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS

4.1 Reasons for laboratory testing

Not applicable, this is a field study to assess risks to non-target organisms.

4.2 Intended actual scale of biocide application

Not applicable

Doc IIIA/Section 7.5.6 **Field trial used to assess the risks to non-target organisms.**

BPD Data Set IIIA/Annex Point XIII.3

4.3	**Relevance compared to field conditions**	
4.3.1	Application method	Not applicable
4.3.2	Test organism	Not applicable
4.3.3	Observed effect	Not applicable
4.4	**Relevance for read-across**	Not applicable

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The test material, Talon pellets (containing 0.005% brodifacoum, plus addition of 0.15% demethylchlorotetracycline (DMCT). The DMCT fluoresces under UV light in the lower jaw of rodents after consumption of the bait) were used for the control of rats and mice on farms. Radiotelemetry on barn owls was carried out to determine the effect of the rodenticide treatment on the surrounding barn owl populations. No guideline method was used, but the study was conducted in accordance with the accepted scientific principles of the time.

Owls were captured, using a variety of methods including nets. No mortalities or injuries were recorded during the capture phase. The owls were banded (i.e. ringed), radio-equipped (transmitter attached to two central tail feathers by a tail clip) and released. The radio transmitters used in the study were provided by Bioelectronic Unit, Denver Wildlife Research Unit. All were in the range 164MHz band, they weighed approximately 10g, however, twenty transmitters had mortality circuits and weighed approximately 14g. Attempts to locate the owls were made daily during roosting and feeding periods.

Appropriate sites were chosen, that had barn owls present, either nesting or roosting sites. Regurgitated pellets were collected from most sites prior to treatment. Some of the initial collections had pellets which were over 1 year old. After initial collections, pellets were collected during the treatment and also the post-treatment period.

Rodent activity was evaluated using visual assessment of sites and tracking patches. After rodent presence was established, tracking boards and bait stations were laid. Track scores were recorded for three days pre-treatment and three days post-treatment as a means of population census. At 15/25 sites the bait was weighed at placement and conclusion of the treatment period; on the remaining 10 sites, visual assessments on bait consumption were carried out.

One of the sites was set up as a 'worst case'. An abandoned barn was used that had an owl box set up. A female barn owl took up residence in the box. 39 rats were released into the barn and provided with an excess supply of water and food (in the form of dog food). Following, this acclimatisation period, tracking patches were laid to determine a pre-treatment census. 100g of Talon Pellets bait was laid at 10 bait stations, throughout the barn on 24th July. The barn was left

		undisturbed, apart from to re-fill water supplies, to encourage the owl to stay. The bait stations were removed on 19th August, all of the bait had been consumed. The owl had laid a clutch of eggs, therefore, to reduce disturbance, the post-treatment census was not carried out. The barn was only entered again on 11th September to release 100 house mice. 15 bait stations containing Talon Pellets were placed in the barn. On 27th September all of the bait was removed, bait consumption and track scores were recorded. Post-treatment tracking was carried out over two days, before all equipment was removed.
5.2	**Reliability**	Efficacy evaluation was not the purpose of the study.
5.3	**Assessment of efficacy, data analysis and interpretation**	35 active barn owl nests were located in the entire study area. Of these nests, 23 were located in trees, 4 in barns (one nest box), 3 in silos (two nest boxes), 3 inside chimneys, 1 in a warehouse and 1 in a water tank. 34 barn owls in total were captured, radio-equipped and released. 26 were adults (9 males and 17 females) and 8 were newly fledged young. Some of the radio-transmitters were lost due to the moulting of the tail feathers and mortalities. A total of 346 bait placements were made with 33.3kg of Talon Pellets. Approximately, 19.3kg of bait was consumed during the trial. Treatment periods ranged from 17 to 53 days. Generally, control of rats and/or mice was considered to have been very good. Even though some farms had substantial populations of *Rattus norvegicus* and *Mus musculus*, adult barn owls did not appear to spend much time hunting around the farm. The radiotelemetry data also indicated that adult barn owls spent most time hunting away from farms, in fields and marshes with high populations of voles. Analysis of the collected regurgitated pellets showed that meadow voles (*Microtus pennsylvanicus*) were the most common prey item (75.7%). While some *Rattus norvegicus* and *Mus musculus* occurred in some pellets they made up only a small part of the diet in the study area. Examination of the fluorescence under UV light of the pellets collected during the post-treatment period, indicated that no rodents that consumed the Talon Pellets bait were eaten by barn owls that regurgitated the pellets. 7 barn owl mortalities were recorded, 2 electrocutions, 2 road kills, 2 predator kills and 1 unknown cause. A reported shooting was also documented during the study. Analysis was carried out on these carcasses but only one presented a brodifacoum residue of 0.05ppm, however, death was attributed to electrocution.
5.4	**Conclusion**	This study shows that *Rattus norvegicus* and *Mus musculus* are consumed by barn owls to some extent but generally the percentage consumption of commensal rodents is low. The study also showed that barn owls did not hunt in and around farms. The results of the study indicate that the potential for barn owl mortality as a result of Talon Pellet rodenticide baiting around farms appears to be low.

Doc IIIA/Section 7.5.6 Field trial used to assess the risks to non-target organisms.

BPD Data Set IIIA/Annex Point XIII.3

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Comments	[redacted]
Summary and conclusion	[redacted]
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Comments	*Discuss if deviating from view of rapporteur member state*
Summary and conclusion	*Discuss if deviating from view of rapporteur member state*

Doc IIIA/Section 7.5.6 Field trial used to assess the risks to non-target organisms.

BPD Data Set IIIA/Annex Point XIII.3

Tables for Method

1.1 (mixed) Population / Inoculum (*if necessary; include separate table for different samples*)

1.2 Test organism (*if applicable*)

Criteria	Details
Species	Norway rat, *Rattus norvegicus* House mouse, *Mus musculus* Barn owl, *Tyto alba*
Strain	Wild
Source	Not applicable
Laboratory culture	Not applicable
Stage of life cycle and stage of stadia	Not applicable
Mixed age population	Yes
Other specification	None
Number of organisms tested	Not reported.
Method of cultivation	None
Pre-treatment of test organisms before exposure	Rodent activity was evaluated using visual assessment of sites and tracking patches. Census bait consumption and tracking patches were employed to determine the presence of populations of rats and house mice. Differentiation between rats and mice was made by observing the size of the prints on the tracking patches. Owls were captured using a variety of techniques. The owls were banded *i. e.* ringed, radio-equipped and released.
Initial density/number of test organisms in the test system	Not reported.

Doc IIIA/Section 7.5.6 Field trial used to assess the risks to non-target organisms.

BPD Data Set IIIA/Annex Point XIII.3

1.3 Test system

Criteria	Details
Culturing apparatus / test chamber	35 active barn owl nests were located in the entire area (1100km^2 in Southern western New Jersey). Two nests were located in unoccupied farms, 8 were in residential and industrial areas, 2 in trees within 100m of occupied farms and the rest (23) were on occupied farms. A total of 25 sites were treated with Talon Pellets to determine if baiting on and around farms would present a secondary hazard to barn owls. The area baited was mainly agricultural, with the majority of farms having at least one large building on site. The sites were selected on the basis that they were within 1 to 1½ miles of a known owl roosting or nesting site. This led to a clustering of sites, but gave the owls a higher potential level of exposure.
Number of vessels / concentration	None
Test culture media and/or carrier material	Talon Pellets with the addition of fluorescing agent, DMCT, were used as supplied by the manufacturer.
Nutrient supply	None
Measuring equipment	Census baiting and tracking patches were employed. Regurgitated pellets were collected from most sites prior to treatment. Some of the initial collections had pellets which were over 1 year old. After initial collections, pellets were collected during the treatment and also post-treatment period, to determine if barn owls had consumed rodents that had consumed the bait. Radio-transmitters provided by Bioelectronic Unit, Denver Wildlife Research Unit. All were in the range 164MHz band, and weighed approximately 10g, however 20 transmitters had mortality circuits and weighed approximately 14g.