

**Data Evaluation Report on the Acute Dietary Toxicity of Difethialone Technical to Black-billed Magpies (*Pica pica*) and European Ferrets (*Mustela putoris furo*)**


PMRA Submission Number {.....}

EPA MRID Number 466565-01

<b>Data Requirement:</b>	PMRA Data Code	{.....}
	EPA DP Barcode	D323274
	OECD Data Point	{.....}
	EPA MRID	466565-01
	EPA Guideline	Non-guideline

<b>Test material:</b>	Difethialone Technical Difethialone Pellets	<b>Purity:</b> Technical-grade (not further specified) 23.94 mg ai/kg (approx. 2.4% ai)
Common name	Difethialone	
Chemical name:	IUPAC: 3-[(1 <i>RS</i> ,3 <i>RS</i> ;1 <i>RS</i> ,3 <i>SR</i> )-3-(4'-Bromobiphenyl-4-yl)-1,2,3,4-tetrahydro-1-naphthyl]-4-hydroxy-1-benzothi-in-2-one CAS name: 3-[3-(4'-Bromo[1,1'-biphenyl]-4-yl)-1,2,3,4-tetrahydro-1-naphthalenyl]-4-hydroxy-2 <i>H</i> -1-benzothiopyran-2-one CAS No.: 104653-34-1 Synonyms: LM2219	

**Primary Reviewer:** Christie E. Padova  
Staff Scientist, Dynamac Corporation

**Signature:**   
**Date:** 2/03/06

**Secondary Reviewer:** Teri S. Myers  
Senior Scientist, Cambridge Environmental Inc.

**Signature:**   
**Date:** 2/09/06

**Primary Reviewer:** Shannon Borges, Biologist  
EPA/OPP/EFED/ERB – II

**Date:** 01/08/07



**Secondary Reviewer(s):** {.....}  
{EPA/OECD/PMRA}

**Date:** {.....}

**Reference/Submission No.:** {.....}

<b>Company Code</b>	{.....}	[For PMRA]
<b>Active Code</b>	{.....}	[For PMRA]
<b>Use Site Category:</b>	{.....}	[For PMRA]
<b>EPA PC Code</b>	128967	

**Date Evaluation Completed:** 10-02-2006

**CITATION:** Savarie, P.J. 2005. Secondary toxicity hazard assessment of difethialone in black-billed magpies (*Pica pica*) and European ferrets (*Mustela putoris furo*). Unpublished study performed by National Wildlife Research Center, USDA/APHIS/Wildlife Services, Fort Collins, CO. Laboratory Project ID: QA-385. Study submitted by LiphaTech, Inc., Milwaukee, WI. Studies were conducted from the 1997 to 1999, and the final report was submitted September 8, 2005.

**DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the secondary dietary toxicity of a pesticide to predator avian and mammalian species. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.



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## EXECUTIVE SUMMARY:

An assessment of the potential secondary hazard of difethialone was made by comparing accumulated residues in rats (primary exposed animal) to the dietary levels that cause mortality in magpies and ferrets (secondary exposed animals).

The dietary toxicity of technical-grade difethialone to adult black-billed magpies (*Pica pica*) was assessed in a 30-day acute range-finding study conducted with two birds per level at measured concentrations of 0, 0.31, 0.95, 8.5, and 91.6 mg ai/kg diet, and in a 33-day acute definitive study conducted with ten birds per level at mean-measured concentrations of 0, 1.17, 2.31, 8.14, and 17.9 mg ai/kg diet. The dietary toxicity of technical-grade difethialone to 8-11 week old European ferrets (*Mustela putoris furo*) was assessed in a 33-day acute range-finding study conducted with 1 animal/sex/level at measured concentrations of 0, 0.089, 1.02, 8.37, and 112 mg ai/kg diet and in a 35-day acute range-finding study conducted with 1 animal/sex/level at measured concentrations of 0, 212, 422, 780, and 1585 mg ai/kg diet. For both secondary species, there was usually a 3-day pre-treatment period, a 5 day treatment period, followed by a 25-/27-day post-treatment period.

In magpies, the reviewer calculated the LC<sub>50</sub> to be 4.48 mg ai/kg diet (no reliable confidence intervals). Signs of toxicity were observed in birds consuming diets at the ≥8.5 mg ai/kg levels and included hemorrhaging (as indicated by blood around and under the cage, the food dish, and the perch), dark feces, lethargy, fluffed feathers, sternal recumbency, and/or wing droop. The NOAEC for mortality and clinical signs of toxicity (only endpoints assessed) was 0.95 mg ai/kg diet. The primary reviewer for EPA notes that NOAEC values are based on gross external observations. Although necropsy was not included in the protocol, lack of observations of external hemorrhaging does not preclude the existence of internal hemorrhage that would also indicate toxicosis. According to the US EPA classification, difethialone would be classified as very highly toxic to black-billed magpies on an acute dietary basis.

In ferrets, the reviewer calculated the LC<sub>50</sub> to be 97.7 mg ai/kg (no reliable confidence intervals). There was no mortality during the first range-finding study, where the LC<sub>50</sub> was >112 mg ai/kg diet. In the second study, mortality data were variable and the sample size was low; these factors precluded the ability to derive a reliable toxicity estimate. Red urine (presumably from blood), blood under cage, lethargy, ataxia, and/or labored breathing were observed in animals exposed at ≥1.02 mg ai/kg diet levels. The NOAEC for mortality and clinical signs of toxicity (only endpoints assessed) was 0.089 mg ai/kg diet. Additional data may be requested to further define the dietary LC<sub>50</sub> to ferrets. Due to small sample sizes and lack of reliable confidence limits, caution should be taken in utilizing the result as a definitive LC<sub>50</sub> point estimate for ferrets.

The accumulation of formulated difethialone pellets (containing 2.4% ai) in male albino rat (*Rattus norvegicus*) carcasses was also assessed using two different feeding scenarios, one in which animals were offered bait feed for 3 days then euthanized, and one in which animals were offered bait feed until poisoning and death occurred. Rats that were euthanized on day 3 consumed a mean of 4.3 mg ai/kg bw and the mean difethialone body residue was 2.8 ppm. Rats that were fed continuously with the same diet had a mean time of death of 5.2 days (range of 4-7 days) and consumed an average of 4.5 mg ai/kg bw. The mean difethialone body residue was 3.1 ppm. No statistical difference (2-sample t-test) was observed between residue levels of rats fed for 3 days and euthanized, and those fed until death occurred.

This study is scientifically sound, but was not designed to fulfill any current U.S. EPA data requirement and is therefore classified as SUPPLEMENTAL. Data obtained from this study indicate that poisoning symptoms could be manifested in magpies and ferrets by feeding on rodents consuming difethialone-treated bait diet. In addition, it was observed that magpies are much more sensitive to difethialone poisoning than ferrets.

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## Results Synopsis

Test Organism Size/Age(Mean Weight): Magpies: Adult or 1<sup>st</sup> year (118.9-193.0 g)  
Ferrets: 8-11 weeks old (398.5-763.0 g)  
Rats: Approximately 2 months old (277.3-314.0 g)

### Magpie (secondary species)

LC<sub>50</sub>: 4.48 mg ai/kg diet 95% C.I.: Not reliably determined  
NOAEC: 0.95 mg ai/kg diet  
Endpoint(s) affected: Mortality, clinical signs of toxicity (only endpoints assessed)

### Ferret (secondary species)

LC<sub>50</sub>: 97.7 mg ai/kg diet (observed) 95% C.I.: Not reliably determined  
NOAEC: 0.089 mg ai/kg diet  
Endpoint(s) affected: Mortality, clinical signs of toxicity (only endpoints assessed)  
**Note:** Observed LC<sub>50</sub> is based on test with only 2 animals per treatment level.

### Rat (primary species)

Carcass accumulation after 3 days: 2.8 ppm  
Carcass accumulation under actual use conditions: 3.1 ppm  
t-test p-value=0.28

## I. MATERIALS AND METHODS

**GUIDELINE FOLLOWED:** This study was not submitted to fulfill any current Subdivision E guideline requirement. The protocol from this study was derived from communication between the National Wildlife Research Center and the U.S. EPA during 1994-1995. Deviations from sound scientific practice and/or typical acute dietary toxicity studies include:

1. Pre-test health (including mortality rates) of the animals was not described.
2. The concentration of corn oil in the treated diets was not specified. It was reported that the concentration of corn oil in the control diet (also the base diet used in preparation for the treated diets) was 1.52%; however, additional corn oil was used in the preparation of the treated diets.
3. In the protocol deviations table, it was reported that plastic (instead of ceramic) bowls were used as food bowls for the magpies and ferrets. The tendency for substances to adsorb to plastic is much greater than for ceramic products, and therefore plastic is not recommended for contact with treated feed unless it is demonstrated that the potential for adsorption is low.
4. A definitive LC<sub>50</sub> ferret study was not conducted.
5. The composition of the magpie and ferret diet could not be discerned from the photocopy of the label provided. Vitamin K is an antidote for anticoagulants, and may reduce the toxicity up to an order of magnitude.

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6. Analytical data reported for the range-finding magpie study (observed concentrations and liver residue), the 33-day range-finding ferret study (observed concentrations and liver residues), and the 35-day ferret range-finding study (observed concentrations only) could not be verified by the reviewer (refer to Reviewer's Comments section).
7. Effects of difethialone on body weight and food consumption of the secondary species were not assessed. These are typical endpoints assessed during acute dietary studies.
8. Based on information provided in the study, difethialone appears to be susceptible to photodegradation. As new diets were offered daily to mapies and ferrets, the stability of difethialone in Pedigree dog food should have been verified for 1 day of ambient storage. The stability of difethialone during frozen storage in all applicable matrices was verified during the study.
9. The homogeneity of difethialone was not assessed in treated magpie and ferret feed (not applicable for the rat bait, as this is a formulated product and the homogeneity is assumed). Spot-checking of the raw data by the reviewer indicated that homogeneity may not have been achieved during preparation of the diets.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. It was reported that the study was performed in accordance with GLP 40 CFR Part 160 with the following exceptions: amendments and deviations from the study protocol were documented but not signed by the Study Director or filed with QA in a timely manner, and technical difethialone was not tracked.

**A. MATERIALS:**

- 1. Test Material**
- a. Difethialone Technical (magpie and ferret studies)
  - b. Difethialone Pellets (rat study)

**Description:**

- a. Powder
- b. Pellets, solid

**Lot No./Batch No. :**

- a. M008 and 8010
- b. 21798

**Purity:**

- a. 96.9 and 100.45%, respectively
- b. Mean of two assays: 23.94 mg/kg (2.4% ai)

**Stability of Compound Under Test Conditions:** The ambient stability of (technical-grade) difethialone in the basal dog food diet was not assessed. Frozen storage stability studies indicated that difethialone was stable in all applicable matrices (i.e., basal dog food, magpie and ferret liver, and whole rat carcass) when stored frozen for approximately 4-6 weeks. As the rat diet was a formulated difethialone bait, its stability was assumed under normal use conditions.

**Storage Conditions of Test Chemicals:** Not reported

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**Stability of Compound**

**Under Storage Conditions:** Stable in container when protected from light and moisture (ai). Average shelf life is 4 years.

**Physicochemical properties of difethialone.**

Parameter	Values	Comments
Water solubility at 20EC	Not reported	Practically Insoluble in water.
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

**2. Test organism:**

**Species (common and scientific names):** Black-billed magpies (*Pica pica*)  
 European ferrets (*Mustela putoris furo*)  
 Sprague-Dawley derived albino rat (*Rattus norvegicus*)

**Age at study initiation:** Magpies: Adult or 1<sup>st</sup> year  
 Ferrets: 8-11 weeks old  
 Rats: Approximately 2 months old

**Weight at study initiation:** Magpies: 118.9-193.0 g  
 Ferrets: 398.5-763.0 g  
 Rats: 277.3-314.0 g

**Source:** Magpies: Captured from wild populations near Fort Collins and Denver, CO and Millville, UT.  
 Ferrets: Purchased from Marshall Farms, North Rose, NY and vaccinated against canine distemper.  
 Rats: Purchased from Simonsen Laboratories, Inc., Gilroy, CA.

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**B. STUDY DESIGN:**

**1. Experimental Conditions**

**Table 1: Experimental Parameters**

Parameter	Details	Remarks
<p><u>Acclimation</u>                      Period:                      Conditions: (same as test or not)                      Feeding:                      Health: (any mortality observed)</p>	<p><u>Magpies</u> were quarantined for 14 days under the same conditions as used for testing. The length of <u>ferret</u> acclimation, if any, was not reported. Magpies and ferrets were fed Pedigree® dog food with chunky beef, and provided water <i>ad libitum</i>. <u>Rats</u> were quarantined for 7 days under laboratory conditions and maintained on a diet of pelleted Purina Rodent Laboratory Chow® and water, <i>ad libitum</i>.</p>	<p>Pre-test health (including mortality rates) of the animals was not described.</p> <p>Ferrets were provided a bowl with corn cob bedding that served as a “nest” box.</p>
<p>Pen size and construction materials</p>	<p><u>Magpies</u>: galvanized steel cages, 21 x 20 x 15 in.  <u>Ferrets</u>: stainless steel cages, 25 x 9.5 x 7 in. (first range-finding study) or 24 x 18 x 14 in. (second range-finding study)  <u>Rats</u>: stainless steel cages, 13.5 x 7 x 7 in.</p>	<p>All animals were housed individually upon arrival.</p>

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Parameter	Details	Remarks
Test duration	<p><u>Magpies:</u> 30-day (5 days treated + 25 days untreated) range-finding study 33-day [3 day untreated (prebait) + 5 days treated + 25 days untreated] definitive study</p> <p><u>Ferrets:</u> 33-day [3 day untreated (prebait) + 5 days treated + 25 days untreated] range-finding study 35-day [3 day untreated (prebait) + 5 days treated + 27 days untreated] range-finding study</p> <p><u>Rats:</u> 3- or 13-day definitive study</p>	<p>Rats fed for 3 days were euthanized; rats fed for 13 days were allowed to die from the difethialone.</p> <p>The 3-day pretreatment periods were conducted to acclimate magpies and ferrets to the test diets.</p> <p>The second ferret range-finding study was extended 2 days because day 25 post-treatment fell on a Sunday, and Monday was an official holiday.</p>
<p><u>Test concentrations:</u> Nominal: Actual:</p>	<p><u>Magpies:</u> <u>Range-finding study:</u> Target: 0, 0.1, 1, 10, and 100 mg ai/kg diet Actual: 0, 0.31, 0.95, 8.5, and 91.6 mg ai/kg diet <u>Definitive study:</u> Target: 0, 2, 4, 8, and 16 mg ai/kg diet Actual: 0, 1.17, 2.31, 8.14, and 17.9 mg ai/kg</p> <p><u>Ferrets:</u> <u>33-day Range-finding study:</u> Target: 0, 0.1, 1, 10, and 100 mg ai/kg diet Actual: 0, 0.089, 1.02, 8.37, and 112 mg ai/kg diet <u>35-day Range-finding study:</u> Target: 0, 200, 400, 800, and 1600 mg ai/kg diet Actual: 0, 212, 422, 780, and 1585 mg ai/kg</p> <p><u>Rats:</u> <u>Single-choice feeding:</u> Target: 0.0025% Observed: 0.002394%</p>	<p>Actual concentrations were corrected for the reference standard brodifacoum as well as for corresponding QC recoveries. However, concentration data from the range-finding magpie study, and both ferret studies could not be verified by the reviewer (refer to Reviewer's Comments section).</p> <p>To account for diet moisture loss, three bowls each containing about 90 g of control diet (for magpies) or 250 g of control diet (for ferrets) were placed in the animal room and the average loss of moisture was subtracted from the amount of diet consumed.</p>

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Parameter	Details	Remarks
<p><u>Solvent/vehicle, if used</u> type: amount:</p>	<p><u>Magpie and ferret:</u> Corn oil 1.52% (control/base diets)  <u>Rat:</u> N/A</p>	<p>The concentration of corn oil in the treated diets was not specified.</p>
<p>Diet preparation and feeding</p>	<p><u>Magpie and ferret:</u> A control/base diet was prepared by combining blended (Waring blender) cans of Pedigree dog food with corn oil in a Kitchen Aid mixer. This mixture was purged with nitrogen and sealed with parafilm.</p> <p>For the highest-level treated diets for each experiment, difethialone powder was quantitatively transferred with corn oil to a bowl containing a portion of base diet and the mixture was blended with a Kitchen Aid mixer. The lower diets were serially diluted from this mixture.</p> <p>All diets were offered on the day of preparation, or stored frozen for an unspecified length of time until use.</p> <p><u>Rats:</u> Difethialone-treated pelleted diet or placebo pelleted diet were offered neat (only source of food).</p>	<p>For the magpie definitive study, the base diet was prepared as described, except the dog food was passed through a meat grinder prior to use.</p>
<p>Feed withholding period</p>	<p>None</p>	
<p>Stability and homogeneity of test material in the diet determined (Yes/No)</p>	<p>Stability – frozen conditions only Homogeneity – not assessed</p>	
<p><u>Number of animals per replicate/groups</u></p>	<p>Each replicate consisted of one animal.</p>	



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Parameter	Details	Remarks
<u>Number of replicates/group</u>	<p><u>Magpies:</u>  <u>Range-finding study:</u>                      2 per treatment and control levels  <u>Definitive study:</u>                      10 per treatment and control levels</p> <p><u>Ferrets:</u>  <u>Both Range-finding studies:</u>                      2 (1/sex) per treatment and control levels</p> <p><u>Rats:</u>                      5 control and 10 per treatment level</p>	
<u>Test conditions</u> temperature: relative humidity(%): photoperiod:	18.49-25.453°C 0.09-55.64% 12 hours light/12 hours dark	
Reference chemical, if used	None reported	

**2. Observations:**

**Table 2: Observations**

Parameters	Details	Remarks
Parameters measured (mortality/body weight/ mean feed consumption/ others)	<p><u>Magpie and ferret:</u>                      Mortality                      Food consumption (during treatment period)                      Body weight                      Liver residue</p> <p><u>Rat:</u>                      Mortality (13-day feeding period only)                      Food consumption                      Body residue</p>	<p>For body residue determination in rats, the head, feet, tail, and pelt were removed and the remaining carcass was ground before assay.</p> <p>Pre-treatment body weights and corrected diet concentrations were used to calculate the quantity of difethialone consumed (mg ai/kg bw).</p>

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Parameters	Details	Remarks
Indicate the stability and homogeneity of test chemical in the diet	Difethialone was stable in basal dog food, magpie and ferret liver, and whole rat carcass when these samples were stored frozen for approximately 4-6 weeks.  The homogeneity of difethialone in dog food diet was not assessed.	For details regarding stability results, refer to Reviewer's Comments section.
Indicate if the test material was regurgitated	None reported	
Treatments on which necropsies were performed	None	
Observation intervals	Daily	
Were raw data included?	Yes, sufficient	

**II. RESULTS AND DISCUSSION:**

**A. MAGPIE:**

30-Day range-finding study: Both birds from each of the 8.5 and 91.6 mg ai/kg diet levels died between 1 and 5 days post-treatment; calculated consumption was 6.2-138.7 mg ai/kg bw. Signs of toxicity were observed at these levels and included spots of blood under cage, lethargy, fluffed feathers, sternal recumbency, and/or wing-drop. No mortality or signs of toxicity were observed in control birds, or in birds offered 0.31, or 0.95 mg ai/kg diets (calculated consumption of 0.29-0.88 mg ai/kg bw). The NOAEC was 0.95 mg ai/kg diet. Although livers of all test birds were analyzed for difethialone, control samples were contaminated during sample handling and/or preparation and results are considered invalid.

33-Day definitive study: Cumulative mortality was 0, 30, 50, 70, and 50% following 5 days of exposure to treated diet at 0 (control), 1.17, 2.31, 8.14, and 17.9 mg ai/kg, respectively, and occurred from the third day of treatment through 3 days post-treatment. Calculated consumption was 0, 1.5, 3.5, 10.4, and 22.4 mg ai/kg bw in the treatment groups, respectively. As mortality was not progressively linear, the study author-calculated LC<sub>50</sub> (by probit analysis) was 7.34 mg ai/kg diet, with no reliable confidence intervals. The predominant sign of toxicity was hemorrhaging, as indicated by blood observed under the cage, under the tray and in food dish, on the perch, and dark feces in several birds from all test levels. Lethargy was also observed in two birds at the 17.9 mg ai/kg level. Food consumption (recorded during the exposure phase) and body weight changes (pre- and post-treatment recordings) were not assessed in relation to treatment by the study authors. Difethialone levels in the liver averaged 1.52, 1.57, 1.80, and 2.69 ppm in decedent birds and 0.64, 0.72, 0.69, and 0.82 ppm in surviving birds from the 1.17, 2.31, 8.14, and 17.9 mg ai/kg levels, respectively. The livers of control animals were not analyzed.

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**Table 3: Effect of Difethialone on Mortality of Magpies (*Pica pica*).**

Treatment, mg ai/kg diet Measured (and Nominal) <sup>1</sup>	Mean product consumed, mg ai/kg bw <sup>2</sup>	No. of birds per treatment	Cumulative mortality					Mean Liver Residue, ppm Decedents / Survivors
			Day 1 PT	Day 3 PT	Day 4 PT	Day 5 PT	Day 25 PT	
<b>30-Day Range-Finding</b> (5 days treatment + 25 days post-treatment)								
Solvent control	0	2 (1 M, 1 F)	0	0	0	0	0	--- / 8.15 <sup>3</sup>
0.31 (0.1)	0.34	2 (2 F)	0	0	0	0	0	--- / 0.18 <sup>3</sup>
0.95 (1)	0.88	2 (2 F)	0	0	0	0	0	--- / 0.75 <sup>3</sup>
8.5 (10)	6.8	2 (2 F)	1	1	1	2	2	1.61 / --- <sup>3</sup>
91.6 (100)	105	2 (1 M, 1 F)	1	2	2	2	2	2.04 / --- <sup>3</sup>
NOAEC		0.95 mg ai/kg diet						
LC <sub>50</sub>		Not estimated						
<b>33-Day Definitive</b> (3 days pre-treatment + 5 days treatment + 25 days post-treatment)								
Solvent control	0	10 (7 M, 3 F)	0	0	0	0	0	Not analyzed <sup>4</sup>
1.17 (2)	1.5	10 (5 M, 5 F)	3	3	3	3	3	1.52 / 0.64
2.31 (4)	3.5	10 (7 M, 3 F)	4	5	5	5	5	1.57 / 0.72
8.14 (8)	10.4	10 (4 M, 6 F)	3	7	7	7	7	1.80 / 0.69
17.9 (16)	22.4	10 (6 M, 4 F)	4	5	5	5	5	2.69 / 0.82
NOAEC		<1.17 mg ai/kg diet						
LC <sub>50</sub>		7.4 mg ai/kg diet (no reliable confidence intervals)						

<sup>1</sup> Measured concentrations were corrected for a surrogate (reference) standard (brodifacoum), and QC recoveries.

<sup>2</sup> Pre-treatment body weight was used to calculate mg/kg difethialone consumed using the measured concentrations and the following formula: mg/kg = mg/kg in diet x diet consumed (g) ÷ pre-treatment bw (g).

<sup>3</sup> Controls were contaminated and data are considered invalid.

<sup>4</sup> All control liver samples were homogenized and combined to provide QC control samples.

**B. FERRET:**

33-Day range-finding study: No mortality was observed in ferrets treated for 5 days at measured dietary concentrations of 0.089-112 mg ai/kg diet; calculated consumption was 0.14-166.5 mg ai/kg bw. The observed LC<sub>50</sub> was >112 mg ai/kg diet. The only clinical sign of toxicity was red urine (presumably from blood) observed in all females at the ≥1.02 mg ai/kg diet levels and in the male at the 112 mg ai/kg diet level. The NOAEC was 0.089 mg ai/kg diet. Liver residues were not detected (<0.091 ppm) in control samples, averaged <0.20 ppm at the 0.089 mg ai/kg diet level, and averaged 0.60-0.76 ppm at the 1.02-112 mg ai/kg diet levels.

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35-Day range-finding study: Mortalities were observed with the higher dietary concentrations; however, they were not dose-responsive. Cumulative mortality was 2/2, 1/2, 0/2, and 2/2 at the 212, 422, 780, and 1585 mg ai/kg diet, respectively, and occurred between 6 and 11 days post-treatment. Calculated consumption averaged 473, 563, 1748, and 2850 mg ai/kg bw for the treatment levels, respectively. An LC<sub>50</sub> value was not reported. The two ferrets at the 212 mg ai/kg diet level died without having exhibited prior clinical effects. Effects observed in ferrets from the higher levels included blood under cage, lethargy, ataxia, and/or labored breathing. The NOAEC was <212 mg ai/kg diet. Liver residues were not detected (<0.091 ppm) in control samples, ranged from 1.0-1.2 ppm in decedent ferrets, and 0.51-0.62 in surviving ferrets.

**Table 4: Effect of Difethialone on Mortality of Ferrets (*Mustela putoris furo*).**

Treatment, mg ai/kg diet Measured (and Nominal) <sup>1</sup>	Mean product consumed, mg ai/kg bw <sup>2</sup>	No. of ferrets per treatment	Cumulative mortality					Mean Liver Residue, ppm Decedents / Survivors
			Day 5 PT	Day 7 PT	Day 9 PT	Day 11 PT	Day 25 (or 27) PT	
<b>33-Day Range-Finding</b> (3 days pre-treatment + 5 days treatment + 25 days post-treatment)								
Solvent control	0	2 (1M, 1F)	0	0	0	0	0	--- / <LOD <sup>3</sup>
0.089 (0.1)	0.14	2 (1M, 1F)	0	0	0	0	0	--- / <0.20 <sup>4</sup>
1.02 (1)	1.3	2 (1M, 1F)	0	0	0	0	0	--- / 0.62
8.37 (10)	11.2	2 (1M, 1F)	0	0	0	0	0	--- / 0.76
112 (100)	166.5	2 (1M, 1F)	0	0	0	0	0	--- / 0.60
NOAEC	0.089 mg ai/kg diet							
LC <sub>50</sub>	>112 mg ai/kg diet (observed)							
<b>35-Day Definitive</b> (3 days pre-treatment + 5 days treatment + 27 days post-treatment)								
Solvent control	0	2 (1M, 1F)	0	0	0	0	0	--- / <LOD <sup>3</sup>
212 (200)	473	2 (1M, 1F)	0	0	1	2	2	1.2 / ---
422 (400)	563	2 (1M, 1F)	0	0	0	1	1	1.1 / 0.62
780 (800)	1748	2 (1M, 1F)	0	0	0	0	0	--- / 0.54
1585 (1600)	2850	2 (1M, 1F)	0	1	1	2	2	1.1 / ---
NOAEC	<212 mg ai/kg diet							
LC <sub>50</sub>	Not determined							

<sup>1</sup> Measured concentrations were corrected for a surrogate (reference) standard (brodifacoum), and QC recoveries.

<sup>2</sup> Pre-treatment body weight was used to calculate mg/kg difethialone consumed using the measured concentrations and the following formula: mg/kg = mg/kg in diet x diet consumed (g) ÷ pre-treatment bw (g).

<sup>3</sup> LOD = 0.091 ppm.

<sup>4</sup> Difethialone detected in one of two samples at 0.20 ppm.



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Rat (primary species)

Carcass accumulation after 3 days: 2.8 ppm

Carcass accumulation under actual use conditions: 3.1 ppm

t-test p-value=0.28

**F. STUDY DEFICIENCIES:**

There were no deviations from sound scientific practice that affected the validity of this study. This study, however, was not designed to fulfill any current Subdivision E guideline requirement.

**G. REVIEWER'S COMMENTS:**

Results of the reviewer's statistical analyses differed slightly from those of the study author. The reviewer similarly could not generate reliable LC<sub>50</sub> estimates (i.e., with sound 95% confidence intervals), but those that were derived were lower than those reported by the study author. The reviewer's more conservative toxicity estimates are reported in the Executive Summary and Conclusions sections.

The study author reported in the protocol deviations table that a definitive LC<sub>50</sub> test was not conducted for ferrets because the observed ferret LC<sub>50</sub> from the range-finding studies was >100 ppm, and that animals would not be exposed to concentrations higher than this.

Difethialone concentrations in dog food diet were determined using HPLC with UV (262 nm) detection (methods described in detail in Appendix 5). The LOD was reported as 0.085 mg/kg. Results (raw data) from sample analyses were provided in Appendix 8. Measured concentrations in the diet were adjusted for recoveries of a co-analyzed brodifacoum reference standard. In addition, QC samples were prepared and analyzed concurrently with the test samples; however, data reported in Appendix 8 were apparently not adjusted for the QC recoveries obtained. Corrected concentration data provided in the summary tables (Tables 2a – 2e) for the definitive magpie study were verified by the reviewer when the raw data were corrected for QC recoveries (for both treated feed and liver residue data). In addition, data provided for the liver residues in the 35-day ferret study and body residues in rat results could also be verified by the reviewer when QC adjustments were made. However, data provided in the appendix for the 30-day magpie range-finding study (corrected concentrations and liver residues), the 33-day ferret range-finding study (corrected concentrations and liver residues), and 35-day ferret range-finding study (corrected concentrations only) could not be verified by the reviewer, with or without QC adjustments.

The storage stability of difethialone in each sample matrix was investigated; results were provided in the analytical report (Appendix 8) and are summarized below. A two-sided t-test was used to compare results following storage with those obtained following extraction of freshly-prepared QC samples. In all cases, there were no statistical differences. Ambient stability of difethialone in Pedigree dog food was not verified.

Matrix	Fortification Range (mg ai/kg)	Length of Storage (days)	Temperature of Storage (°C)	Recovery (% of Target)
Pedigree dog food	1990-2010	56	-33	92.8 ± 2.3
Magpie liver	2.02-2.06	61	Not reported	91.5 ± 2.4
Ferret liver	2.04-2.12	56	Frozen (not further specified)	101 ± 1.2
Rat carcass	9.95-10.0	42	-33	90.6 ± 4.1

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The moisture loss from the dog food was also determined for each experiment, and the average loss of moisture was subtracted from the amount of diet consumed by the magpies/ferrets.

Although a photocopy of the label of the basal dog food used in the magpie and ferret studies (Pedigree with Chunky Beef, Soy Free) was provided, the quality of the copy was poor, and the ingredient list could not be clearly read. Since the dog food used for the studies is no longer manufactured (at least under the same name), only a similar diet could be found at [www.Pedigree.com](http://www.Pedigree.com). In the similar diet (Pedigree with Beef), Vitamin K was not a constituent.

One female magpie that was used in the range-finding study (0.95 mg ai/kg diet level) was classified as 1<sup>st</sup> year. It was reported that young magpies do not molt their wing feathers until the summer after they are hatched and the amount of black on the primary feathers is much greater than in older birds. The age of the remainder of birds used for testing was adult.

Due to typically delayed mortality for anticoagulant pesticides, EPA requires an observation period of at least 15 days (longer if death occurs in the last 3 days). For all secondary species experiments, the study duration was sufficient to accurately assess treatment-related mortality.

Samples of untreated dog food containing 1.52% corn oil from the magpie definitive study and untreated pelleted rodent diet from the rat dietary study were each submitted for organochlorine (LOD = 0.01 ppm) and organophosphorus (LOD = 0.05 ppm) pesticides screening analyses. These pesticides were not detected.

It was reported in the protocol deviations table that not enough magpies were collected in Colorado, and therefore additional ones were obtained from Utah. In addition, not enough birds in total could be collected for a 1:1 male:female ratio during testing. The study author reported that this deviation is probably minimal because some male and female magpies died on each of the difethialone treated diets.

## H. CONCLUSIONS:

This study is scientifically sound, but was not designed to fulfill any current U.S. EPA data requirement and is therefore classified as Supplemental. Based on investigations pertaining to acute dietary toxicity in two secondary species, and dietary accumulation in one primary species, the following conclusions were made:

### Magpie (secondary species)

LC<sub>50</sub>: 4.48 mg ai/kg diet                      95% C.I.: Not reliably determined  
NOAEC: 0.95 mg ai/kg diet  
Endpoint(s) affected: Mortality, clinical signs of toxicity (only endpoints assessed)

### Ferret (secondary species)

LC<sub>50</sub>: 97.7 mg ai/kg diet (observed)                      95% C.I.: Not reliably determined  
NOAEC: 0.089 mg ai/kg diet  
Endpoint(s) affected: Mortality, clinical signs of toxicity (only endpoints assessed)  
**Note:** Observed LC<sub>50</sub> is based on test with only 2 animals per treatment level.

### Rat (primary species)

Carcass accumulation after 3 days: 2.8 ppm  
Carcass accumulation under actual use conditions: 3.1 ppm  
t-test p-value=0.28

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Poisoning symptoms could be manifested in magpies and ferrets by feeding on rodents consuming difethialone-treated diet.



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**III. REFERENCES:**

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**APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:**

**MAGPIES**

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 2.31

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	3.277296	3.631629	0	+INFINITY

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	2.898512	1	.3823664

SLOPE = .5012421

95 PERCENT CONFIDENCE LIMITS = -.3521234 AND 1.354608

**LC50 = 4.478493**

**95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY**

LC10 = 1.310467E-02

95 PERCENT CONFIDENCE LIMITS = 0 AND .6082736

**FERRETS**

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
1585	2	2	100	25
780	2	0	0	25
422	2	1	50	75
212	2	2	100	25

THE BINOMIAL TEST SHOWS THAT 0 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

**AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 97.69071**

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

**RATS**

ethanized rats            fed until death            paired t-test result (2-tailed)

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3.3	3.4	<b>0.276640316</b>
2.1	3.3	
3.2	3.2	
3.2	2.7	
2.8	2.5	
2.5	3.5	
3	1.9	
2.7	4.3	
3	3.2	
2.2	3.1	
<b>2.8</b>	<b>3.11 average</b>	