UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Data Review

SUBJECT: Chlorophacinone (067707): secondary hazard studies

FROM: William Erickson, Biologist
ERB3/EFED
W. Erickson 4/18/01

THRU: Arnet Jones, Chief
ERB3/EFED
Arnet W. Jones 04/18/2001

TO: Susan Lewis, Chief
SRRD

Attached are the DERs for the secondary hazard studies for mammals (MRID No. 446314-01) and birds (446314-02) submitted to support 0.005% ai chlorophacinone bait. The studies are classified as Supplemental. Because nontarget risks of anticoagulant and other rodenticides are currently under review, the need for any additional testing will not be addressed until that assessment is completed. Secondary-hazard studies to support 0.01% ai chlorophacinone bait have not been submitted.

Some problems exist with the chlorophacinone studies. Mortality should not be the only measurement endpoint for assessing adverse secondary effects. Sublethal effects, such as prolonged blood-coagulation time, should be measured in both treated and untreated animals. External signs of toxicosis are useful, but all test animals also should undergo a complete necropsy when the study is terminated. In the ferret study, only animals that died were reported to have undergone a necropsy. All magpies were said to have been necropsied, but in neither study were the necropsy raw data provided. EFED requests that the testing laboratory and the registrant discuss test protocols before any additional tests are conducted with chlorophacinone or any other rodenticide.

Additionally, a study previously submitted to evaluate secondary hazard to raptors feeding on rodents poisoned with 0.005% ai bait has been upgraded from "Invalid" to "Supplemental". The citation for that study is provided below, and a copy of the DER is attached.

> Askham, L. 1988. Chlorophacinone - secondary hazard evaluation of chlorophacinone in raptors. Submitted by LiphaTech, Inc., Milwaukee, WI. Study conducted by Washington State University, Pullman, WA. EPA MRID No. 407514-02.

Although there are deficiencies in that study relating to sample size and residue analysis of partially decayed vole carcasses, the study provides some useful information that can be used in assessing secondary toxicity of chlorophacinone.

Contact Bill Erickson at 305-6212 if you have any questions.

Chlorophacinone: Mammalian Secondary Hazard and Target Species Residue Study With 0.005% ai Bait

Study Title: Secondary hazard study using chlorophacinone-killed laboratory rats fed to domestic Ferrets (*Mustela putorius furo*)

Date: October 22, 1996

Authors: Ahmed, M. S., J. Baroch, L. Carlet, and D. Whaley

Laboratory: Genesis Laboratories, Inc.
10122 N. E. Frontage Road
Wellington, CO 80549

Sponsor: LiphaTech, Inc.
3101 W. Custer Avenue
Milwaukee, WI 53209

Lab. Study No.: 96004

MRID No.: 446314-01

Reviewer: William Erickson, Biologist, ERB3/EFED W. Erickson 4/18/01

Approved by: Henry D. Craven 4/18/01

Results Synopsis: Eleven (55%) of 20 domestic ferrets died after feeding for five consecutive days on laboratory rats poisoned with 0.005% ai pelleted chlorophacinone bait. Whole-body residue in 4 rats ranged from 0.18 to 0.80 ppm.

Study Classification: Supplemental for 70-A-SS (Secondary poisoning, mammal) for 0.005% ai bait
Supplemental for 70-C-SS (Residue in target species) for 0.005% ai bait

Materials and Methods	
Test species:	Domestic ferret (*Mustela putorius furo*); 8-11 weeks old
Source:	Marshall Farms USA, Inc. North Rose, NY

Materials and Methods	
Test material fed to ferrets:	Laboratory rats (Sprague Dawley strain) poisoned with 0.005% ai Rozol Paraffinized Pellets (no-choice) for 5 consecutive days
Housing conditions and pre- and post-test food adequate?	Ferrets were housed individually in plastic-coated wire pens (61 x 76 x 46 cm) suspended over metal collection pans; Ferret lab. diet was provided ad libitum during pre- and post-treatment periods
Pre-test acclimation period	13 days
Ferrets healthy pre-test?	Yes; all ferrets were observed daily for general health and inspected by a veterinarian prior to testing
Pre-conditioning:	3 days prior to testing, ferrets were fasted 3.5 hours and each presented with a thawed, untreated rat carcass in a no-choice presentation
Treatment group:	2 replicates; 5 ♂ and 5 ♀ randomly assigned per replicate
Control group:	5 ♂ and 5 ♀ fed untreated dead rats
Test duration:	5 days treated; 21 day post-test observation period
Preparation of test material offered to ferrets:	Treated rats were fed pelleted bait (no-choice feeding) for 5 consecutive days; After 5 days, rats were euthanized with CO_2 and individually bagged and frozen (-1 to -20°C); Bait consumption was determined daily for each rat;
Presentation of test material:	Each ferret received a thawed, weighed rat carcass on the test day 0 (test onset); To ensure that most rat tissues and organs were eaten, a second rat carcass was offered to a ferret only if most of the first carcass was eaten; Uneaten rat carcass was collected and weighed
Tissue analysis of rats and ferrets:	Four rat carcasses were analyzed for whole-body chlorophacinone residue; Livers of 2 dead ferrets were analyzed for residue

Materials and Methods	
Observations:	Mortality, morbidity, and signs of intoxication were observed daily; Ferret body weight was measured at day 0 (test onset), day 5 (test completion) and day 21 (end of post-test observations); Dead ferrets were necropsied for evidence of anticoagulant poisoning

Reported Results	
Mortality:	Control: 0 of 10 Treated: 11 (55%) of 20 rep. 1: 5 of 10 rep. 2: 6 of 10
Days to death from onset of exposure:	5 to 14
Signs of intoxication:	Control: none Treated: morbidity and hemorrhage in ferrets that died
Mean body-weight gain after 26 days:	Control: 32.5% Treated: rep. 1: 12.4% rep. 2: 10.9%
Rat whole-body chlorophacinone residue (n = 4):	mean = 453 ppb (805, 614, 218, and 175 ppb)
Mean consumption of rat carcass during the 5-day treatment period:	Control: 881 g (per kg ferret body weight) Treated: rep. 1: 687 g/kg bw rep. 2: 775 g/kg bw
Necropsy findings for ferrets:	"Gross pathological symptoms, such as hemorrhaging in the throat, thorax and abdomen, were consistent with anticoagulant poisoning."
Ferret liver-tissue analysis:	Chlorophacinone concentrations in the 2 ferret livers analyzed were 0.600 and 0.483 ug/g

Reviewer's Conclusions:

The study demonstrates that mammalian predators feeding on rodents poisoned with 0.005% ai chlorophacinone bait may be killed. Eleven (55%) of 20 ferrets exposed to poisoned rats died during the study. Most ferret deaths occurred on days 7 to 11, but 1 ferret died on day 5 and 1 on day 14. The authors report that "It was evident in the necropsies that all ferrets that died had hemorrhaged." All control ferrets remained healthy throughout the test.

The liver from each of two ferrets that died during the study was analyzed for residue to confirm that dead ferrets had been exposed to chlorophacinone. Although this was done for only two of the 11 dead ferrets, it provides useful information on residue load in the liver of predators that died from consuming chlorophacinone-poisoned rodents.

This study focused on mortality as the measure of adverse effects. All treated and untreated ferrets also were observed daily for signs of poisoning, but necropsy was performed only on animals that died. Necropsy of survivors could provide useful information about sublethal intoxication. Because anticoagulants interfere with the synthesis of blood-clotting factors, measurements of blood coagulation also would be extremely useful and should be incorporated in such studies.

Only four rats were analyzed for whole-body chlorophacinone residue. Because of variability in bait consumption and residue loads, no fewer than 10 individuals should be analyzed. An additional five rats were analyzed in the secondary hazard study with magpies.

Some deficiencies exist with this study. No information was provided on the individual necropsies of the ferrets or the qualifications of the person who performed them. Was this done by a veterinarian or a wildlife pathologist? A description of the findings for each ferret should be included as a requirement to submit the raw study data. Additionally, no information was provided on blood coagulation times of the treated and control ferrets. Although blood coagulation time has not typically been required for secondary hazard studies, such information is extremely useful in determining potential adverse sublethal effects of anticoagulants. Such effects could be important if surviving ferrets were subsequently exposed to chlorophacinone-poisoned rodents.

The report does not state how long treated rats were frozen prior to being thawed and offered to ferrets. The report also does not state how long the four rats and two ferret livers were frozen prior to residue analysis. Storage stability data are required for samples stored frozen for 30 days or more (Pesticide Reregistration Rejection Rate Analysis, Residue Chemistry Follow-up Guidance, EPA 737-R-93-001, 1993).

Chlorophacinone: Avian Secondary Hazard and Target Species Residue Study With 0.005% ai Bait

Study Title: Secondary hazard study using chlorophacinone-killed laboratory rats fed to black-billed magpies (*Pica pica*)

Date: June 4, 1997

Author: Baroch, J.

Laboratory: Genesis Laboratories, Inc.
10122 N. E. Frontage Road
Wellington, CO 80549

Sponsor: LiphaTech, Inc.
3101 W. Custer Avenue
Milwaukee, WI 53209

Lab. Study No.: 96019

MRID No.: 446314-02

Reviewer: William Erickson, Biologist, ERB3/EFED W. Erickson 4/18/01

Approved by: Henry T. Craven 4/18/01

Results Synopsis: 20 black-billed magpies survived after feeding for five consecutive days on laboratory rats poisoned with 0.005% ai pelleted chlorophacinone bait. Whole-body residue in 5 rats ranged from 0.21 to 0.93 ppm.

Study Classification: Supplemental for 70-B-SS (Secondary poisoning, bird) for 0.005% ai bait
Supplemental for 70-C-SS (Residue in target species) for 0.005% ai bait

Materials and Methods	
Test species:	Black-billed magpie (*Pica pica*); adults
Source:	Trapped wild in Larimer County, CO
Test material:	Laboratory rats (Sprague Dawley strain) fed Rozol Paraffinized Pellets (0.005% ai) for 5 consecutive days

Materials and Methods	
Housing conditions and pre- and post-test food adequate?	Magpies were housed individually in plastic-coated wire pens (61 x 76 x 46 cm) suspended over metal collection pans; Moistened, dry dog food was provided ad libitum during pre- and post-treatment periods
Pre-test acclimation period	17 days
Birds healthy pre-test?	Yes; magpies were observed daily for general health and inspected by a veterinarian prior to testing
Pre-conditioning:	3 days prior to testing, magpies were fasted 17.5 hours and each presented with a thawed, untreated rat carcass in a no-choice situation
Treatment group:	2 replicates of 10 birds each were exposed to chlorophacinone-poisoned rats; birds were randomly assigned to replicates
Control group:	10 birds were fed untreated dead rats
Test duration:	5 days treated; 21 day post-test observation period
Preparation of test material:	Treated rats were fed pelleted bait (no choice feeding) for 5 consecutive days; those that died were individually bagged and frozen (-1 to -20°C); survivors were euthanized with CO_2, and bagged and frozen
Presentation of test material:	Each magpie received a rat carcass (thawed and weighed) on day 0 (test onset) and an additional fresh carcass on day 3 to ensure ad libitum feeding; All uneaten rat carcass was collected and weighed at the end of the exposure period
Tissue analysis	Five rat carcasses were analyzed for whole-body chlorophacinone residue

Materials and Methods	
Observations:	Daily observations were made for mortality, morbidity, and signs of intoxication; Magpie body weight was measured at day 0, day 5 (test completion) and day 21 (end of post-test observations); A gross necropsy was performed on all treatment-group magpies after the post-treatment observation period

Reported Results	
Mortality:	Control: 0 of 10 Treated: 0 of 20
Days to death from onset of exposure:	n/a
Signs of intoxication:	Control: none Treated: none
Mean body-weight gain after 26 days:	Control: 4.3% Treated: rep. 1: 3.0% rep. 2: 4.7%
Rat whole-body chlorophacinone residue levels (n = 5):	Mean = 0.4673 ug/g (0.2107, 0.3030, 0.4248, 0.4709, and 0.9272 ug/g)
Mean consumption of rat carcass during the 5-day treatment period:	Control: 1891 g (per kg magpie body weight) Treated: rep. 1: 1492 g/kg bw rep. 2: 1618 g/kg bw
Necropsy findings:	Possible signs of toxicosis were slight discoloration or yellowing of the liver in 4 treated birds; the spleen of one of these birds was not uniform in color

Reviewer's Conclusions:

All magpies ate substantial portions of the rat carcasses provided. Most magpies consumed the major organs of the abdomen and thorax first, followed by the large limb muscles. The head, hide, and bones usually were not eaten. No abnormal behavior or signs of toxicosis were observed in any magpies during the feeding or observation periods.

All 46 treated rats displayed signs of anticoagulant poisoning and 15 died. Signs of toxicosis included morbidity, hemorrhage, lack of appetite, hyporeactivity, rapid breathing, and labored breathing. Hemorrhage was reported from various places on the body, including nose, ears, eyes, and left and right forelimbs.

Some deficiencies exist with this study. No information was provided on the individual necropsies of the magpies or the qualifications of the person who performed them. Was this done by a veterinarian or a wildlife pathologist? A description of the findings for each magpie should be included as a requirement to submit the raw study data. Additionally, no information was provided on blood coagulation times of the treated and control magpies. Although blood coagulation time has not typically been required for secondary hazard studies, such information is extremely useful in determining potential adverse sublethal effects of anticoagulants. Such effects could be important if magpies were to be subsequently exposed to chlorophacinone-poisoned rodents.

Only five rats were analyzed for whole-body chlorophacinone residue. Because of variability in bait consumption and residue loads, no fewer than 10 individuals should be analyzed. An additional four rats were analyzed in the secondary hazard study with the domestic ferret.

Storage stability data are required for samples stored frozen for 30 days or more (Pesticide Reregistration Rejection Rate Analysis, Residue Chemistry Follow-up Guidance, EPA 737-R-93-001, 1993). Part of the study did include a storage-stability test in which spiked rat tissue was frozen for 21 days, which was stated to be the longest time that any of the rats fed to magpies were stored in the freezer. Recovery in the thawed tissue was low, ranging from only 9.84 to 48.3%. The author attributes the poor recoveries to experimental error in the method used, rather than instability of chlorophacinone in the frozen rat tissues, but does not clarify what experimental errors occurred.

This study is upgraded to "Supplemental" for guideline no. 70-B-5S (secondary poisoning, bird)

W. Erickson, EFED 4/18/01

Accession No. 407514-02

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorophacinone.
   Shaughnessey Number: 067707

2. **TEST MATERIAL:** CPN (Rozol™), a chlorophacinone-treated rodenticide pellet, 0.005% ai.

3. **STUDY TYPE:** Secondary Hazard Evaluation in Raptors. Species tested: Red-tailed hawk (Buteo jamiacencis), great horned owl (Bubo virginianus).

4. **CITATION:** Askham, L. 1988. Chlorophacinone - Secondary Hazard Evaluation of chlorophacinone in raptors. Submitted by Chempar, Lilpha Chemicals, Inc., Milwaukee, WI. Conducted by Washington State University, Pullman, WA. Laboratory Study No. 88-1 EPA Accession No. 407514-02.

5. **REVIEWED BY:**

   Mark R. Roberts
   Wildlife Biologist
   Ecological Effects Branch

   Signature: Mark R. Roberts
   Date: 1/11/90

6. **APPROVED BY:**

   Ann Stavola
   Acting Head, Section III
   Ecological Effects Branch

   Signature: Ann Stavola
   Date: 3/1/90

CONCLUSIONS: The study, as designed, is not a scientifically sound method of evaluating the secondary hazards of chlorophacinone in raptors because there was no control group. Problems in quality control of samples (thawed, decayed carcasses) prevented reliable assessment of dosages. The small sample size (n=1) of great horned owls prevented a valid conclusion as to the effects of chlorophacinone on this species. Furthermore, without addressing the secondary hazards to mammalian species, data requirement 71-5 can not be fulfilled.

8. **RECOMMENDATIONS:** It is recommended that the registrant consult with EEB and/or submit a field study protocol to EEB for review, prior to study initiation.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A

11. **MATERIALS AND METHODS:**

A. Test Animals: Three female and 2 male red-tailed hawks (Buteo jamaicencis) and a single male great horned owl (Bubo virginianus) were used in the study. The birds were obtained from the U.S. Fish and Wildlife Service and were nonreleasable because of previous injury. All birds were acclimated to the facility for 30 days prior to study initiation. During the acclimation period, each bird was fed 2-3 voles (Microtus montanus) daily, and provided with water ad libitum.

B. Test System: All birds were housed in 45 X 120 X 90 cm cages. (Although not specified in the report, it is assumed that all birds were caged separately). Lighting, temperature, and humidity were not reported.

C. Dosage: The raptors were dosed by feeding them voles that had ingested various amounts of 0.005% CPN bait in pellet form. Each raptor was fed 2 voles per day for 6 consecutive days. The average dose was estimated by analyzing CPN residue levels in 10 voles that were fed 0.005% CPN pellets for 9 consecutive days.

D. Design: Voles were live-trapped and acclimated to communal cages for 2 weeks. They were then individually caged in plexiglass and stainless steel containers with woodchip nesting material. Food consisted of vegetable matter and grain. Each vole was observed for 7 days to access its general health before the test was initiated. Each vole then received 10 g per day of 0.005% fresh pelleted bait, in a no choice presentation, for 9 consecutive days or until mortality occurred. During this period, the cages were cleaned each day and all of the bait that remained from the previous feeding was removed (including food cached in the nesting material) and weighed to the nearest 0.1 g. Another 10 g of fresh bait was placed in the cage and the process was completed. At death, each vole was stored at -20°C until fed to the raptors.

The raptors were fed 2 CPN treated voles per day for 6 consecutive days. Water was available ad libitum and no additional food was provided during the six-day feeding period. The birds were fed a combination diet (not explained) from days 7-35. The birds were observed daily for any signs of anticoagulant poisoning.

All birds were euthanized with CO_2 on day 37. The entire carcass of each bird was analyzed for CPN residue using modified gel permeation chromatography (GPC) and high performance liquid chromatography (HPLC). In order to determine average residue levels, 10 voles fed 0.005% CPN pellets for 9 days were assayed using the same procedures and equipment. Agrisearch Inc., Frederick, MD, performed the assays of bird and vole carcasses and bait pellets. The detection limits were 150 ppb in the voles and 60 ppb in the raptors. Detection limits and results for the CPN analysis in the bait were not reported.

E. Statistics: No statistical analyses were performed, other than descriptive statistics of CPN residue levels.

12. **REPORTED RESULTS**: "By the end of the six day test, each bird had consumed 12 voles, of which a cumulative average of 8.05 mg of CPN had been ingested." CPN residues ranging from less than 60 to 249 ppb were detected in the birds (Table 3, attached). Based upon the average CPN consumption of the 10 assayed voles, and the subsequent analyses of their carcasses, CPN elimination in the voles averaged 89.61% (Table 1, attached). The average CPN residue level per bird was 164 ppb (Table 2, attached), an average reduction of 73.21% between ingestion and analysis. No correlation was found between the amount of CPN residue in the raptors and the amount of CPN ingested by the voles.

Voles were completely ingested within 20 minutes of presentation to the raptors. No change in feeding was noted during or after the testing period. With the exception of 1 bird that appeared lethargic on days 24 and 25 after the exposure period, no abnormal behavior was noted. No birds showed signs of discomfort, stress, or external bleeding. "Several birds injured the soft portion of their upper mandibles when pecking at the cages, however, the lacerations quickly healed. Blood coagulation occurred quickly, with no prolonged bleeding."

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES**: "The potential for secondary toxicity to two species of birds (red-tailed hawks and great horned owls) appears to be low. None of the treated birds died, nor showed signs of stress nor prolonged bleeding. The significant reduction of active ingredient between ingestion and analysis indicated a rapid degradation and elimination of the compound in both vole and bird systems."

A GLP statement page was included stating that "This study meets the requirements for 40 CFR Part 160" and was signed by the sponsor and study director.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS**:

A. Test Procedure: The test was designed primarily to quantify the amount of CPN ingested by raptors. The test would have been an effective method of achieving that objective, but problems discussed in Section 14C are of concern.

B. Statistical Analysis: No statistical analyses were conducted, other than descriptive statistics of CPN residue levels.

C. Discussion/Results: Based upon the analysis of residue in the bird and vole carcasses, the author concluded that an average of 81.61% of the CPN ingested by the voles was eliminated through excretion and metabolism, as was 73.21% of the CPN ingested by the raptors. Although not mentioned by the author, carcasses were "thawed and rotten" upon arrival at Agrisearch, Inc. for residue analysis (Agrisearch receipt, attached). In the absence of data indicating otherwise, it must be assumed that the decayed condition of the carcasses would result in degradation of CPN through microbial decomposition and other processes. The residue levels used by the author to estimate the dosages received by the raptors and the elimination values are therefore erroneous.

Even if the residue analysis had been acceptable, the conclusion of "... a rapid degradation and elimination of the compound in both the vole and bird systems" could not have been accepted. The birds were not euthanized until 30 days after the six-day testing period. Elimination after 30 days hardly qualifies as rapid.

Several errors in reporting results made interpretation very difficult. For example, the following was reported on page 5. "Birds No. 5 and 6 consumed voles with the highest amounts of CPN (9.28 and 9.69 mg), but contained the lowest residues (69 and .60 ppb). The average residue level per bird was 0.164 ppb;..."
The correct version should have been Birds No. 6 and 4 consumed voles with the highest amounts of CPN (9.28 and 9.69 mg), but contained the lowest residues (49 and 69 ppb). The average residue level per bird was 164 ppb;..."

Although the author used terms such as "significant reduction" and "no correlation" there were no statistical analyses to verify these phrases.

Raw data on the portion of the 10 g fresh bait consumed and cached daily by voles was not reported.

The report stated that the bait fed to the voles was analyzed for confirmation of CPN levels. The results of this analysis were not reported.

The report stated that the results indicate the potential for secondary poisoning to great horned owls appears to be low. However, only one great horned owl was used in this study. A larger sample is definitely needed before conclusions concerning the species can be justified.

Furthermore, behavioral observations of caged, presumably crippled raptors, are of limited value in an assessment of potential hazards of CPN to free-ranging raptors. Extrapolation of the behavior of these birds to wild avian species should be done with extreme caution.

Several very important components of testing for secondary poisoning effects by anticoagulant rodenticides were lacking in this study:

1) a control group.
2) pre-treatment vs. post-treatment bodyweights.
3) pre-treatment vs. post-treatment blood coagulation times.
4) complete necropsies.

D. **Adequacy of the Study:**

(1) **Classification:** ~~Invalid~~ Supplemental WZ 4/18/01

(2) **Rationale:** A determination of the amount of CPN ingested by the raptors (the stated objective of the study) was impeded by the decayed condition of the carcasses used for residue analysis. Data obtained from a single owl is insufficient to estimate toxicity to the entire species. Data on secondary hazards to mammalian predators/scavengers was lacking.

(3) **Repairability:** No