

STUDY TITLE

Determination of Chlorophacinone and Diphacinone Residues in California Ground Squirrels and Non-Target Animals

DATA REQUIREMENTS:

70-A-SS Secondary Poisoning Mammal
70-B-SS Secondary Poisoning Bird
70-C-SS Whole Body Residue, Target Species

AUTHORS

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STUDY COMPLETED

December 23, 2002

PERFORMING LABORATORY

Analytical Chemistry Project
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CITATION

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STATEMENT OF DATA CONFIDENTIALITY

No claim of confidentiality is made for any information contained in this study on the basis of it falling within the scope of FIFRA 10(d)1(A), (B), or (C).

Submitter: California Department of Food and Agriculture

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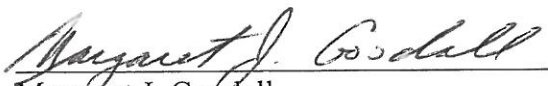
Signature: Duane L. Schnabel

Title: State Biologist


Date: 12-26-2002

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Analytical determination of Chlorophacinone and Diphacinone in California Ground Squirrels was performed under QA 976, Invoice No: 01-052 in accordance with NWRC QA procedures, Methods 114A and 114B and the Good Laboratory Practices (GLPs) as outlined in 40 CFR Part 160, August 19, 1989. Exceptions to GLPs were as follows: A signed protocol from California Department of Food and Agriculture was not received until after all the samples were analyzed.


Margaret J. Goodall
Study Director

12/23/02
Date


Sponsor

12-26-2002
Date


QUALITY ASSURANCE STATEMENT

This study (QA-976, Invoice No: 01-052) was inspected by the NWRC QA Program on the dates listed below. Periodic Inspection Reports were submitted to the Principal Investigator, and Test Facility Management as follows:

Phase	Inspection Date	Date to PI	Date to PI Management
Protocol	No inspection	No Inspection	No inspection
Sample Preparation	03/08/02	03/14/02	03/14/02
Sample Preparation/extraction	04/01/02	04/05/02	04/05/02
Final Report/Raw Data Review	5/14/02-6/14/02 12/19/02-12/20/02	6/14/02 12/20/02	6/14/02 12/20/02

All QA inspections were submitted to Study Director and Study Director's Test Facility Management on 12-23-02.

Submitted By:


Catherine M. Bens
Quality Assurance Manager

12/23/02
Date

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ABSTRACT

The objective of this study was to determine the magnitude of residues for diphacinone and chlorophacinone in whole, ground California ground squirrels and non-target animals collected in a field efficacy study to compare the use of 0.005% and 0.01% diphacinone and 0.005% and 0.01% chlorophacinone baits. The methodology developed proved to be reliable and produced mean recoveries \pm standard deviation in quality control samples of $85.6 \pm 12\%$ for chlorophacinone ($n = 100$) and $81.1 \pm 11\%$ for diphacinone ($n = 91$), respectively.

INTRODUCTION

California Ground Squirrel carcasses were collected from two field studies. The first entitled "Field Efficacy Studies Comparing 0.005% and 0.01% Diphacinone and Chlorophacinone Baits for Controlling California Ground Squirrels (*Spermophilus beecheyi*)" was conducted by Dr. T.P. Salmon, University of California, Davis. The second entitled "A camera and hook system for viewing and retrieving rodent carcasses from burrows" was conducted by Dr. Kurt VerCauteren, National Wildlife Research Center, Ft. Collins, Colorado.

The animal carcasses were submitted to the Analytical Chemistry Project at the National Wildlife Research Center (NWRC). Diphacinone and Chlorophacinone residue determinations in the whole ground squirrels and several non-target animals were performed using National Wildlife Research Center (NWRC) Analytical Chemistry Project Method 114A and 114B. (Appendix D)

TECHNICAL MATERIALS

Summary of Reference Standards

The diphacinone technical material was obtained from Bell Laboratories (Madison, Wisconsin).

Lot #:	T1595
Received:	9-20-96
Opened:	9-20-96
Certified:	1-9-02
Purity:	100.0%

The chlorophacinone technical material was obtained from Lipha Tech, Inc. (Milwaukee, Wisconsin).

Lot #	CLOM 085
Received:	10-22-97
Opened:	10-23-97
Certified:	6-8-01
Purity:	99.74%

SAMPLES

Receipt and storage

Frozen field samples were received, inventoried and logged in according to the appropriate Standard Operating Procedures (SOPs). They were stored at $-20 \pm 5^{\circ}\text{C}$ until processed.

Preparation

Samples judged suitable for analysis were removed from the freezer and either partially or fully thawed. The carcass was weighed prior to skinning. The head, feet and pelt were removed. The animal was placed in a newly labeled plastic bag and returned to the freezer.

The skinned animal was later removed from the freezer, weighed and ground twice using a Univex meat grinder Model # MG 8912 (Serial # P006368). The ground animal was placed in a Food Saver LacLoc® plastic bag and returned to the freezer. When frozen, the air was displaced from the storage bag using a Food Saver Professional II Vacuum Packaging system™ by Tilia Professionals.

Analytical methodology

The ground animal carcasses were assayed initially using NWRC Analytical Method 114A, "Determination of Chlorophacinone Residues in Whole Body California Ground Squirrels". Some of the initial chromatographic results indicated that diphacinone might also be present in animals collected from chlorophacinone treated fields. This method was replaced with NWRC Analytical Method 114B, "Simultaneous Determination of Diphacinone and Chlorophacinone Residues in Whole Body California Ground Squirrels" on January 18, 2002. (Appendix D).

Duplicate one gram weighings of each sample of ground tissue were extracted with a solution of 1.5% ammonium hydroxide in 1:1 acetone:chloroform, and cleaned up by solid phase extraction using a normal

phase mechanism. The solvent was removed again and the sample reconstituted with 1.0 mL of mobile phase and analyzed by reversed-phase ion-pair High Performance Liquid Chromatography (HPLC). The observed concentration of analyte in the two samples had to agree within 25% or a third weighing was analyzed.

Two lots of aminopropyl solid phase extraction columns were used to complete the analyses. The analyte recovery dropped below 70% when the second lot of columns was used on April 11, 2002. The solid phase extraction clean-up procedure was modified by changing the concentration of the eluant from 5 to 10 mM tetrabutylammonium dihydrogen phosphate (TBA) with resulting improvement in analyte recovery. Sample results from the analyses performed on April 11th are not reported. The samples were re-analyzed on May 1, 2002.

Non-Target Animals

Of the 21 non-target animals submitted for analysis, 16 were analyzed. The samples were removed from the freezer and the head, legs, tail and pelt of the mice and rats were removed while the animal was still frozen. (Appendix E) The remaining sample was homogenized with a SPEX CertiPrep 6850 Freezer/Mill (liquid nitrogen automated homogenizer). The powdered tissue was transferred to a Food Saver LacLoc® plastic bag. The air was displaced from the storage bag using a Food Saver Professional II Vacuum Packaging systemTM by Tilia Professionals. The sample was returned to the freezer. The samples were analyzed according to the procedures outlined in Method 114B. The five samples not analyzed consisted of only skin, bone and/or feathers.

As control tissue of the non-target animals was not available, control ground squirrels were fortified with chlorophacinone and diphacinone and analyzed with the non-target animals.

Qualitative Confirmation of Diphacinone and Chlorophacinone Residues

The primary wavelength for quantitative analysis was 325 nm. Comparing the retention time of the analyte chromatographic response in calibration standards versus those observed in sample extracts is typically used to qualitatively confirm the presence of each analyte. However, the background of the chromatograms varied from one carcass to the next due to such factors as length of time the carcass was exposed to environmental factors and the affect of scavengers. Therefore in addition to retention time, the ultra-violet/visible spectrum for diphacinone and chlorophacinone were used to confirm the presence

of the analyte in each sample (Figure 1). The UV spectra consist of a maxima at 285, 315 and 325 nm.

Quality control samples

For each set of samples assayed, a minimum of 6 quality control (QC) samples were prepared. Animals used for QC samples were collected in untreated areas. They were skinned, ground and screened for the presence of chlorophacinone and diphacinone. The levels chosen for fortification were 0, 0.2 and 2.5 $\mu\text{g/g}$. As some animals were found to contain analyte concentrations >2.5 $\mu\text{g/g}$, fortifications were also made at 5.0 $\mu\text{g/g}$.

Storage Stability Study

One gram samples of control ground squirrel and one gram samples of control ground squirrel fortified at 2.5 $\mu\text{g/g}$ chlorophacinone and diphacinone were prepared on 4/3/02 according to the procedures outlined in NWRC Analytical Method 114B. Approximately five 50-mL glass tubes containing the control or fortified tissues were placed in several Food Saver LacLoc® plastic bags and returned to the freezer. When frozen, the air was displaced from each storage bag using a Food Saver Professional II Vacuum Packaging system™ by Tilia Professionals. The samples were initially analyzed on 4/3/02 and again after approximately four and seven months of freezer storage. Unanalyzed samples were repackaged and immediately returned to the freezer. Freezer temperature charts are archived at the NWRC archives.

RESULTS

Quality Control Samples

Mean recoveries of chlorophacinone ($n=100$) and diphacinone ($n=91$) in all quality control samples were $85.6 \pm 12\%$ and $81.1 \pm 11\%$, respectively. This indicates that the analyses were performed correctly and that the resulting residue data are valid. The mean recoveries for chlorophacinone fortified controls at 0.2 $\mu\text{g/g}$ and 2.5 $\mu\text{g/g}$ were $86.3 \pm 14\%$ ($n=49$) and $85.3 \pm 10\%$ ($n=41$) respectively. The mean recovery for chlorophacinone fortified controls at 5.0 $\mu\text{g/g}$ were $83.7 \pm 12\%$ ($n=10$). The mean recoveries for diphacinone fortified controls at 0.2 $\mu\text{g/g}$ and 2.5 $\mu\text{g/g}$ were $80.6 \pm 11\%$ ($n=44$) and $81.7 \pm 9.7\%$ ($n=37$), respectively. The mean recovery for diphacinone fortified controls at 5.0 $\mu\text{g/g}$ was $81.4 \pm 13\%$ ($n=10$). Recoveries at 5.0 $\mu\text{g/g}$ for both analytes were comparable to those at the 2.5 $\mu\text{g/g}$ level.

The quality control samples analyzed with the non-target samples are not included in these results but are listed in Appendix B.

Method Limit of Detection

The method limit of detection (MLOD) was calculated as the concentration of chlorophacinone or diphacinone required in the sample to generate a signal equal to 3 times the baseline noise (peak to peak) observed in the chromatograms of the control extracts. The MLOD was estimated from the chromatographic response of a control ground squirrel extract and an extract from a control ground squirrel fortified at 0.2 µg/g. The MLOD for all chlorophacinone fortified ground squirrels averaged 0.077 µg/g (n= 26) with a range of 0.024 to 0.22 µg/g. The MLOD for all diphacinone fortified ground squirrels averaged 0.071 µg/g (n= 24) with a range of 0.035 to 0.19 µg/g.

The method limits of detection reported for the non-target analyses are not included in these summaries but are listed in Appendix B.

Storage Stability Study

After approximately seven months of freezer storage, mean chlorophacinone residues in the fortified samples were 84.5% of original values and mean diphacinone residues were 89.9 % of original values. (Table 1)

Chlorophacinone and Diphacinone Residues in Whole Ground California Ground Squirrels and non-target Animals.

Of the 313 animals submitted for analysis, 187 ground squirrels were assayed, 101 ground squirrels were deemed unsuitable for analysis and 4 were used as controls for Quality Control fortifications. Twenty-one non-target animals are included in that total number. Sixteen non-target animals were assayed, five were deemed unsuitable for analysis.

Diphacinone and Chlorophacinone residue results in whole ground California Ground squirrels and non-target animals are reported in Appendix A. The observed concentrations were not adjusted for QC recoveries. The sample weights are provided in Appendix D.

Dr. Salmon's report, "Field Efficacy Studies Comparing 0.005% and 0.01% Diphacinone and Chlorophacinone Baits for Controlling California Ground Squirrels (*Spermophilus beecheyi*)" contains a summary of data by treatment, plot, site and species.