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PRODUCT MANAGER NO. W. Miller (16)

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COMPANY NAME

SUBMISSION PURPOSE Submission of lab audit for brodifacoum and 1080 studies

SHAUGHNESSEY NO.	CHEMICAL, & FORMULATION	% A.I.

EEB REVIEW

Chemical(s): Compound 1080 and Brodifacoum

100.0 Submission Purpose

Review of laboratory audits, conducted at the Animal Damage Control Laboratories at the Denver Wildlife Research Center (DWRC) on ecological effects and secondary poisoning studies for Compound 1080 and Brodifacoum, respectively.

101.0 Discussion

From July 14-16, 1986, an interagency inspection team *conducted a data audit and Good Laboratory Practice (GLP) conformance inspectiion, at the Animal Damage Control Laboratories at the DWRC. This audit was requested by the Office of Compliance Monitoring (OCM), Office of Pesticides and Toxic Substances (OPTS).

The studies were audited through review of available raw data and reports, interviews with senior study personnel and visits to the laboratory areas where the studies were conducted (with the exception of the skunk study conducted in Logan, Utah).

The studies audited were identified by the EEB as data requirements to support Federal Registrations for these chemical (see previous reviews).

The following ecological effects studies were identified by the OPTS for auditing:

I. "Secondary Toxicity Hazards of the Anticoagulant Brodifacoum to American Kestrels (Falso sparverius," September 12, 1979.

II. "Secondary LC_{50} study of the toxicityof the Anticoagulant Brodifacoum to American Kestrels (Falco sparverius)," June 1981.

III. "Estimated Doses of Sodium Fluoracetate (Compound 1080) Delivered to Coyotes by Toxic Collars"' November 15, 1984.

IV. "Primary Hazard of the 1080 Toxic Collars to Skunks and Golden Eagles," Dec. 21, 1984.

101.1 Results of Laboratory Audits

The following excerpts were taken directly from the Ecological Effects Study Audit Inspection Report.

I. Secondary Toxicity Hazard of the Anticoagulant Brodifacoum to American Kestrels (Falco sparverius), September 12, 1979.

This report provied the data for several studies.

A. An acute oral LC_{50} study (to determine the toxicity of the brodifacoum to voles)

B. Three day chronic feeding study using voles (to exposure voles to known amounts of brodifacoum which then could be fed to the kestrels).

C. Secondary poisoning study (to determine the toxicity of brodifacoum treated voles to kestrels).

A. Acute oral toxicity of brodifacoum to voles

Biological Audit Discussion

The laboratory had raw data that supported the values for the toxicity of the chemical to voles at doses of 1.0, 0.75, 0.37, 0.18 and the controls.

During the set up of this study the staff discovered that the balance being used to weigh the animals for the 0.045 mg/kg doses of brodifacoum was malfunctioning. They made the corrections in the weight measurements for this concentration which were documented in the raw data. The initial incorrect measurements for 0.045 mg/kg dose were erased. The staff realized the malfunctioning balance also affected the weights of voles for the 0.09 mg/kg exposure level. They apparently made the adjustment in data and corrected the weights on the data sheet after erasing the original pencilled data. However the amounts of chemical administered to the animals as shown in Table 1 for dose 0.09 mg/kg could not be verified with raw data. (attachment F-1 and F-2). The dosages may have been calculated for the incorrect animal weights. The laboratory staff could not be sure what actual dosages were applied to the test animals in the 0.09 mg/kg test group.

Chemistry Audit Discussion

The batch of brodifacoum that was reported to have been used as the toxicant in this study (technical batch 3/4/5) was analyzed by ICI Americas, inc., Biological Research Center, Goldsbora, North Carolina (ICI) and the results reported to DWRC in a memo

dated May 21, 1979. This memo reported the percentage of active ingredient as 94.43%, but it did not identify the remainder of the material. The memo also gave the number of the method used in the analysis (GAM 012), but it did not include a description of the method, copies of any chromatograms generated by the method, or any quality control information related to the analysis. These data should have been required by the DWRC in order to substantiate the reliability of the analyses.

A very sketchy description of the preparation of the dosing solution was given at the top of the "Toxicity Record" (see Exhibit G-1). These records were dated, but not signed. Most of the data on these sheets were entered in pencil and appeared to be in, at least, two distinctively different handwritings. The raw weights from the balance were not given nor was the balance identified. It was simply stated that for the 1.0 mg/kg dose group, 15 mg of the active ingredient was placed in 75 ml of PEG-400 (polyethylene-glycol) to yield a 0.2 mg/ml solution. There were not standard operation procedures (SOPs) for dose preparation which would have explained this procedure in more detail. There was one mistake on these formulation sheets (see Exhibit G-2). For the 0.045 mg/kg dose group, the sheet indicated that 10 mg of a 0.1875 mg/ml solution was diluted to 20 ml to give a 0.09 mg/ml solution. At 0.1 ml/20 gm body weight, this would have given a 0.45 mg/kg dose level. The most likely explanation of this descrepancy is that it was actually a 0.01875 mg/ml solution which was diluted to a 0.009 mg/ml solution whcih would have given the indicated 0.045 mg/dose level. This example of the dosage calculations also indicated a rather rough approach to rounding, the 0.009 mg/ml solution was actually 0.0094 mg/ml which gives an actual dosing level of 0.047 mg/kg. At least, two significant figures should have been carried through the calculations.

B. Three day chronic bioassay of brodifacoum bait to voles.

Biological Audit Discussion

The second stage of this three part study was the exposure of voles to brodifacoum bait for 3 days so the laboratory would have treated animals to fed the kestrels.

The raw data for this part of the study was available but difficult to follow since the staff had used the original data as work sheets to rank the voles by the amount of food consumed.

With the following exceptions I was able to verify the accuracy of the values presented in Table. 2.

1. In the 15 ppm test group, vole 3M originally weighed 62 g, not 58 g, according to the raw data sheet.

2. The weight of surviving voles in test group 3 (1 ppm brodifacoum) and the controls were not available on raw data sheets, but were given in a summary table.

3. The initial weight of control animal 2M and 60 g not 58 g
   The initial weight of control animal 8F was 51 g not 41 g
   The initial weight of control animal 10F was 48 G not 36.g

The laboratory staff could not explain the errors in initial body weights, but it was suspected they may be due to substitution of animals.

Chemical Audit Discussion

The same technical batch of brodifacoum was used in this part of the study as in the acute experiment. However, in this part of the study the toxicant was added to oat bait for oral ingestion instead of being gavaged into the voles. The report states that the brodifacoum was dissolved in 25 ml of acetone in a graduated cylinder and that the acetone and two washings of the cylinder were applied to 500 gm of oats which were air dried overnight. There are no raw data records concerning how, when or who performed this particular preparatiion, nor was there an SOP for bait preparation in general. After preparation of six dosing levels by the DWRC, aliquots of the bait were analyzed for levels of brodifacoum by ICI. The results were reported to the DWRC in a memo dated December 14, 1979 (see Exhibit G-3). There was a significant discrepancy between the analytical results and the nominal dosing levels prepared by DWRC. At the highest level of dosage, which was theoretically 50 ppm, ICI reported that the bait contained only 36 ppm. This descrepancy is largest at the higher concentration levels at

which most of the voles died and it might not significantly affect the reported LC_{50} of 1.4 ppm, but it does indicate a problem with either the residue analysis or the bait preparation method. Since these analytical results were, like the toxicant analysis, reported without any method description, chromatograms or quality control data, it is impossible to judge their quality. The analytical report memo did state that "the method has not yet been completely perfected" and in a previous memo dated November 27, 1979, (Exhibit G-4) ICI had stated that they "have not been able to adequately analyze the brodifacoum oat groat samples you provided due to poor extraction from the oats". This issue was addressed at the DWRC in a handwritten memo from Dr. Peter Savarie to Keith LaVoie dated December 27, 1979 (see Exhibit G-5) in which it was decided that "our designated ppm is more accurate than the assayed ppm". There is not enough evidence available to resolve this issue either way.

C. Secondary poisoning of brodifacoum fed voles to kestrels

Biological Audit Discussion

Two and 6 day feeding of voles to kestrels

The laboratory staff fed individually caged voles a diet of 50 ppm brodifacoum for three days at which time the voles were sacrificed and frozen. The voles were than ranked by the amount of treated food they consumed during the three day exposure period. The kestrels were then also ranked by weight. The DWRC staff then preselected the voles that would be fed to each kestrel with the largest kestrels being fed the voles that consumed the largest amount of treated bait. The laboratory assumed the voles eating the largest amount of the toxicant would contain the largest residues of the brodifacoum. When birds failed to eat the voles offered to them or a bird died, etc. it created problems with the experiment.

The staff reranked the remaining voles so that the voles believed to have the largest residues were fed to the largest birds. The reranking of the voles and changes in the schedule were done on the original raw data sheets creating serious problems when the audit team tried to reconstruct the sequence of events. It also appeared to create problems for the staff when they were preparing the final report.

Exhibit F-1 is a copy of the table (raw data) used by the lab to record the basic data on food consumption of the voles, and to rank the voles by amount of food consumed. Erasures and unsigned entrees etc. are obvious. Attachment F-2 is an intermediate table prepared by the researcher that indicated the weights of the kestrels; the amount of bait eaten by the voles and the staff's estimates of the exposure of kestrels to brodifacoum. Attachment F-3 is a copy of the laboratory's conversion chart of grams of bait vs miligrams of toxicant.

Attachment 4 identifies the voles that were fed to the kestrels for the 2 to 6 day exposure periods. Attachment F-4 indicates a false start occurred when the staff used untreated voles. Unlabeled cross outs, special notations etc. were not explained, initialed or dated.

Peter Savarie of DWRC and I worked through the raw data for this study and verified (documented) many of the statistics given inthe report. We found the following inconsistancies in the kestrel feeding study:

1. We were unable to find the raw data for body weights given in Table 3 for the two day exposure test.

2. We were unable to document the weights of kestrels exposed for 6 days.

3. I examined the data in Table 3 concerning the total amount of 50 ppm bait eaten by the voles and I was unable to verify any of the weights of bait eaten in the column in the table for the six day exposure period. Disregarding the crossout information on attachment 4 we were able to document the amount of bait eaten by the voles in attachment F-2a (work sheet by the DWRC staff). However, the totals of the bait eaten in attachment F-2a did not agree with the corresponding figures in Table 3 of the report. We were not able to document why the amount of bait eaten by the voles as reported in Table 3 were from one to 18 grams higher than were recorded in the raw data (attachment F-4 and F-2a). In other words the raw data supported the amount of brodifacoum bait eaten by the voles in the laboratory's intermediate summary table, but did not support the information in the final report.

I made no effort to verify the data given in Table 6 on Prothromin time analysis.

In summary the inability of the auditor to separate original raw data from apparenlty copied raw data recorded in the same format as the Tables in the final report made the auditing of this study very difficult. There were also a number of data discrepancies, the most notable of which were; the dosage of voles at 0.09 mg/kg in the acute study; the identity of treated voles fed to kestrels and the weight of bait eaten by voles at the 50 ppm exposure level.

Chemical Audit Discussion

The initial toxicant used in this study was a 50 ppm brodifacoum pelletized bait formulation, number 5072A batch reference 782619. This batch was analyzed by ICI which reported to DWRC in a May 21, 1979 memo that the bait contained 0.0046% (46 ppm) active ingredient. As in the ICI toxicant analysis for the acute and chronic studies, ICI did not provide a method description, chromatograms or quality control data.

This bait was fed to voles which were subsequently fed to kestrels. DWRC sent 10 voles and tissue from the four kestrels that died to ICI for brodifacoum residue analysis. The kestrel tissue was apparently never analyzed. The vole residue analysis was reported to DWRC in a memo dated November 27, 1979. Chromatograms and quality control information were provided by ICI for this analysis. Two of the ten vole samples were lost due to a malfunction of the high-pressure liquid chromatograph used in this analysis. Also, the analyst felt that the spike results were not valid due to a separate analytical problem. A detection limit was not given with the results, but the lowest result reported was 0.41 ppm and the spike level was 0.5 ppm. The chromatograms show the presence of possible interfering peaks that would have made detection limit of 1 ppm more appropriate for this analysis (see Exhibit G-6, in which the results for B-3 and B-4 were 3.2 and 3.0 ppm while B-2 is reported to contain 0.41 ppm; the different isomer ratio in B-2 indicates that interferences are affecting the brodifacoum peaks). The spiking level should have been at a level nearer 2 ppm and an analytical blank or control sample should have been analyzed

with the samples. Also, the report did not state the type fo sample on which the spiked analyses were performed.

Conclusion

While there were a number of significant and minor problems with the chemicl analyses associated with this study, including missing raw data, these problems by themselves probably would not affect Agency evaluation of the study's final conclusions.

II Secondary LC_{50} Study of the Toxicity of the Anticoagulant Brodifacoum to American Kestrels (Falco sparverius), June 1981, work unit 920.17.

Biological Audit Discussion

In this study five groups of 8 kestrels were fed one of four concentrations of brodifacoum in vole tissue for 5 days to determine an LC_{50}.

I examined the large volume of data that was in the file folder for this study and confirmed most of the data given in the report tables. Much of the data base was in pencil, however. The data was in a format (neat columns, clean data sheets etc.) that suggested transcribed or calculated data rather than original raw data [i.e. the bait eaten column was given in hundredths of a gram (4 digits) with no available records of pre or post exposure weights (amount of food offered or amount food not eaten)]. The staff was unable to explain how they arrived at four digit consumption figures without recording starting and ending weights. There was no evidence of raw notes that may have provided pre and past exposure data from which they could calculate the food consumption statistics.

The data in Table 1 was supported by information that had been recorded on the work sheets. The raw data supported the distribution of kestrels by sex and place of capture and their placement into the six treatment levels as indicate in Table 2.

The raw data supported the data presented in Table 3 with the following exceptions:

1. The data indicated the 129g kestrel in the control group was actually in the 6.0 ppm Test Group instead of one of the 125g birds. A 116g kestrel should have been listed in the control group.

The raw data indicates the bird weighing 158g in the control group actually weighed 138g.

A recalculation of the average weight of the birds in the 6.0 ppm and the control groups did not change appreciably when the correct weights used. The lack of the unit of measurement (ppm) and the failure to identify test chemical in the captions of Table 3 of the report are indications of the lack of labeling and descriptive information that were evident on much of the "raw" data sheets found in the files for the kestrel studies. The available "raw data" did document the ratio of dead birds to exposed birds indicated in table 5.

With the exception of the death date for bird #56 the information in Table 6 was supported by raw data and pathology reports. According to DWRC data, Kestrel #56 died on 11/16/80 not 11/14/80.

In summary, there appeared to be raw data and other records to support the findings for this study, but the lack of adequate titles, and labels on raw data sheets left it up to the reader or auditor to determine what really was the data. By comparing the columns on the raw data sheet (attachment H-2) with the labeled tables in the report, it was generally possible to determine the purposes for which the information was intended.

It required the assistance of the DWRC biologist, Peter Savarie, who was familiar with the study, to reconstruct the data base before the audit team could verify the accuracy of the study report. At times it was difficult to discriminate between original raw data and summary tables prepared for the final report.

Chemistry Audit Discussion

The brodifacoum which was used as a toxicant in this study was reported to be from the concentrate batch 97/9 which had been prepared on 5/28/80. This concentrate was analyzed by ICI and the result was reported to the DWRC by memo dated June 4, 1980. The active ingredient was reported to 0.25% brodifacoum. The method number for the analytical method (GAM 012/78A) was referenced, but no description of the method, chromatograms or quality control information was given. A complete analysis of the concentrate was reported which included the nonactive ingredients. This analysis accounted for 100.00% of the weight of the concentrate.

The 0.25% brodifacoum concentrate was used in the preparation of 100 ppm oat groat bait which was fed to voles. There are no records of this bait preparation and no analyses were performed on the bait.

The dosed voles were ground whole to form a single pooled sample, five aliquots of which were sent to ICI for brodifacoum analysis. ICI analyzed a single sample of vole meat for brodifacoum. There is no indication whether this was a composite of the five aliquots of vole meat or just one of the aliquots. The result was concentration of 6.7 ppm. ICI did provide chromatograms, quality control information and a calculation sheet for this analysis.

The vole meat was then fed to kestrels. Tissue residues from the five kestrels that died and one salvaging magpie that had died beneath the kestrel cages were analyzed by Analytical Bio Chemistry Laboratories Inc. (ABC) in Columbia, Missouri for brodifacoum. Chromatograms, data sheets and quality control information were all provided by ABC. A quality assurance statement signed by the Quality Assurance Officer was also provided. There was a minor mistake in ABC's calculations (see attachment I-1). The detection limit for the control also, the rounding off procedures were not consistent between samples.

ABC and ICI quantified brodifacoum differently for the vole and the kestrel analyses. ICI calculated separate response factors for the cis and trans isomers while ABC assumed a consistent response factor by summing the peak heights. This difference did not appear to affect the results significantly. It did point out that there is a major difference between the isomer ratios in the brodifacoum standard and in the vole and kestrel residues. The trans isomer becomes less significant as the brodifacoum passes through the food chain. This could have some affect on the secondary toxicology of brodifacoum and should have been mentioned in the final report.

Conclusion

In spite of some record keeping problems with the bait preparation, the chemical analyses associated with this study were adequat for the purposes of the study. The two sets of residue analyses by ICI Americas and ABC appeared to be complete and accompanied by an adequate level of quality control.

III "Estimated Doses of Sodium Fluoroacetate (Compound 1080) Delivered to Coyotes by Toxic Collars"; 11/15/84, Acc. No. 144402

Biological Audit Report

There was no one protocol available to cover the information gathered for this report, because it was a summary of data made available as a result of numerous previously conducted studies. The data came from pen and field observations made on coyotes killed by 1080 from July 1977 through August 1984 as part of several projects. Because of the type of exposures and accumulation of data data (field notes and a variety of data sheets) and incomplete data base was collected. Time of death, body weights, age of animals, amount of exposure to toxic material were frequently estimated. Obviously the significance of reported chemical analyses have to be affected by the best estimates made by the field observers. Missing analytical data further affects the verification of the findings or estimates in the report.

The attached copy (Exhibit B-1) of the field note book of Guy Connelly for Monday, September 24, 1979 describes the raw data for wild coyote TX #3, listed in table 2 of the Agency submitted report. The second wild coyote numbered TX #3 (8/24/74) in Table 2 was the record for another animal.

The typical raw data sheets used in 1984 to record the penned Coyote exposure data (Exhibit B-2) provide a copy of the pencilled notes on coyote #2894-95 that died on 6/21/84.

I looked at the raw data for the coyotes described in this study and found at least some field information (raw data) on all animals. I examined about 25 percent of the records in more detail (animals #'s D96, 2732, listed in table 1 and 2928, 1895, 2704, 2722, 2894, 2898 and TX #3 (9/24/79) listed in table 2 and confirmed the dates of death and body weights (sometimes estimated). Some weight estimates were made on site and recorded. Some estimates were made later using unspecified criteria that frequently were not documented by raw data.

The estimated body weights of animals TX #3 (9/24/79) and 1895 were not documented in the raw data I examined. The weights for animals 2732, 2928 and TX #3 (8/24/79) appeared to be estimated not measured.

I discussed the report with researchers Peter Savarie and Howard Tietjen. They indicated the staff had to draw a lot of rough raw data together from a variety of sources (and studies) to get enough information for this report. The report indicates it is the laboratory's best estimate of the relationships between exposure levels and residue levels in the dead coyotes.

Chemical Audit Report

The audited study did not involve an active in-life component, all data were derived from previously conducted toxic collar experiments and trials intended for evaluation of efficacy or for other purposes, some of the work dating back to the late 1970's. It was decided to limit the audit to confirmation of the analytical values contained in Tables 1 and 2 of the Agency submitted report as there did not appear to be any raw data on the characterization of the the technical batches of sodium fluoroacetate and the preparation of the formulations used to generate the dosage levels in Tables 1 and 2. The only information related to dosages was represented in individual coyote reports (Examples given in Exhibit C-1) and a DWRC peer reviewed publication (Exhibit C-2).

The sodium flouroacetate levels reported for muscle tissue in Tables 1 and 2 of the Agency submitted report were audited and evaluated on the basis of availability of reports, notebook data and chromatograms (summarized on the next page) and the overall quality or reliability of this data.

It may be seen from the summary table that not all raw data, particularly notebook entries and chromatograms were recoverable by DWRC personnel. This missing raw data is not only inconsistent with FIFRA GLP Stanards requirements, it is also a deviation from pesticide requirements [40 CFR 169.2(k)] in that all supporting raw data for a product registration have not been retained. Copies of typical DWRC analytical reports (Exhibit C-3), notebook entries (Exhibit C-5) and chromatograms (Exhibit C-5) were taken to provide examples of the quality of documentation for data and records that were recoverable for this st;udy. It may be seen that the notebook entries (Exhibit C-4) are all rather scant, generally providing only a data sample identification and a result. Some sample sizes for analysis were also provided. Available

chromatograms were reasonably well identified. It should be noted from the summary table footnotes that one reported sample value [coyote TX #3 (9/24/79)] could not be reconciled with the raw data and two coyote samples (#2761 and 2898) appeared to be misidentified in the available raw data. If coyote #2761 is misidentified #2671 as claimed by P. Savarie, it should be noted also that the muscle sample appears to have been received at the laboratory on 2/16/83 (Exhibit C-3) the day before the coyote is reported to have died (2/17/83).

Review and evaluation of the available raw data indicated a number of deficiencies that significantly impact on the reliability of the reported muscle tissue values. These deficiencies are summarized as follows:

1. Minimal precision data (in terms of true replicate results) were apparent for any of the available sodium flouroacetate analyses in muscle tissue that were reviewed. Although results were reported in duplicate for some of the coyotes, these values appear only to represent duplicate injections of single extracts into the gas chromatograph. There were no notes in the notebook data to conclude otherwise and the DWRC personnel were also in agreement that there were no apparent sample analysis precision data.

ANALYTICAL DATA COMPLETENESS SUMMARY

TABLE 1.

Coyote No.	Date of Death	Report	Notebook Ref.	Chromatogram
458	4/18/79	No	Yes (5/14/79)	No
462	4/20/79	No	Yes (9/17/81)	No
448	4/24/70	No	Yes (9/16/81)	No
A	10/19/81	Yes (A)	Yes (12/15/81)	No
B	10/20/81	Yes (A)	Yes (12/15/81)	No
C	10/21/81	Yes (A)	Yes (12/15/81)	No
D	10/22/81	Yes (A)	Yes (12/15/81)	No
E	10/23/81	Yes (A)	Yes (12/15/81)	No
2617	2/9/83	Yes	Yes (6/9/83)	Yes
2761	2/17/83	Yes (B)	Yes (6/14/83)(B)	Yes
2945	2/4/83	Yes	Yes (6/7/83)	Yes
2947	2/2/83	Yes	Yes (E)	Yes
2971	1/27/83	Yes (C)	Yes (C)	No
2977	1/27/83	Yes	Yes (11/8/83)	Yes
3091	1/27/83	Yes	Yes (5/31/83)	Yes
D96	8/16/82	Yes	Yes (E)	Yes
2583	8/10/82	Yes	Yes (E)	Yes
2928	8/9/82	Yes	Yes (E)	Yes
2561	8/9/82	Yes	Yes (E)	Yes
2585	8/17/82	Yes	Yes (E)	Yes
D341	10/29/82	Yes	Yes (E)	Yes

ANALYTICAL DATA COMPLETENESS SUMMARY (CONT.)

Table 2.

Coyote No.	Date of Death	Report	Notebook Ref.	Chromatogram
1895	7/24/77	Yes	Yes (12/27/79)	No
2704	6/16/79	Yes	Yes (E)	No
2722	7/11/79	Yes	Yes (E)	No
DM385	7/11/79	Yes	Yes (E)	No
2894	6/21/84	Yes	No	No
2898	7/3/84	Yes (F)	No	No
2589	7/3/84	Yes	No	No
2261	7/17/84	Yes	No	No
2939	8/6/84	Yes	No	No
MT#2 (G)	9/23/78	Yes	Yes (6/11/79)	No
TX#3 (8/24/79)	8/24/79	Yes	Yes (2/8/80)	No
TX#3 (9/24/79)	9/24/79	Yes	Yes (2/8/80)	No

Notes:
(A) Reported in Okuno, I et. al., J.A.O.A.C. 67, 549 (1984) [Exhibit C-2].
(B) Appears to be mislabelled in analytical report and data as coyote #2671.
(C) This is likely to be the same coyote as #2972 due to dual numbering by DWRC.
(D) Value in given notebook (0.23 ppm) cannot be reconciled with value in final report (0.46 ppm).
(E) Dates were not recorded during the audit for all notebook entries.
(F) Appears to be mislabelled in analytical report as #2989.
(G) Given in analytical report and notebook as GEC-102.

2. No accuracy data (spike sample recoveries, reference sample analyses or other information) were available for any of the reported results, thus the day-to-day analytical reliability cannot be determined.

3. No reagent, sample or container blanks were in evidence as having been performed to verify noninterference from possible artifacts during any of the analyses. This deficiency becomes particularly notable for several sample analyses analyses for which the fluoroacetate derivative response was just barely measureable.

4. Confirmation of fluoroacetate derivative as the measured analyte was not performed for all positive samples for which raw data were recoverable, thus giving rise to the possibility of false positives amonth the reported values. Dual column gas chromatographic confirmation was performed for some samples, however, it should be noted that a result of 0.39 ppm was reported for coyote #1895 (Exhibit C-4), which was recorded as not detected with the alternate confirmation column.

5. Specific methodology employed was not referecned in any of the reports, raw data or other records. The DWRC scientists stated that they were certain that any coyote tissue analyses performed during 1982 and later were more than likely to have been performed according to the procedure described in Okuno, et al. J.A.O.A.C. 65, 1102 (1982) [Exhibit C-6] and that any reported analses performed prior to 1982 would have been conducted according to Okuno, et al. J.A.O.A.C. 63, 49(1980) [Exhibit C-7]. These two methods are similar in overall approach, however, repeatability and recovery. Without additional documentation, it must be assumed that these procedures were followed, as appropriate.

6. The analytical reference standards of sodium flouroacetate that were used for the analyses (and their preparation) were nowhere referenced in the raw data or other records. Thus, there is no means of verifying the source, age, purity, or quality of any of the standards against which the sample response values were measured. It also cannot be determined how

often the standards were prepared, by whom, how they were stored, or in what type of container.

7. Several coyote tissue samples (e.g., coyotes #448, #462, and #1895) were analyzed more than two years after the date of recorded death. Stability of fluoroacetate in frozen tissue was demonstrated for only up to 60 days by DWRC (Eh=xhibit C-2). There was no indication of any of the raw data that any of the tissue samples were actually frozen during storage prior to analysis, however, the DWRC personnel were sure that freezing would have been standard practice.

Thus, it may be seen that not only are there considerably missing raw data for this study, the analyses appeared to be conducted with such minimal quality control and recorded in such an incomplete fashion, that the reliability of the reported results cannot be effectvely evaluated.

IV. "Primary Hazards of the 1080 Toxic Collar to Skunks and Golden Eagles"; 12-21-84, Acc. No. 144401.

A. Skunk Feeding Portion of the Study

The field portion of this study was performed in Logan, UT. The individuals conducting the field portion of the study were not available to assist in the review of the data base. The raw data had been sent to DWRC from Utah. Howard Tietjen and Peter Savarie were familiar with the study and helped me sort through the data.

The raw data were recorded mostly in pencil with relatively few apparent corrections. The data were out of order when the file was first examined by DWRC by the auditor, but was easily reassembled by the DWRC scientists because the data base was fairly complete and the data sheets were adequately labeled. Some of the "raw data" sheet looked more like they may have been prepared as tables for the final report rather than original data. In general, I was able to reconstruct the skunk portion of the study and verify information provided in the tables in this report. I could not find data to verify the dates that the collard sheep were killed by coyotes (Table 2). I was able to find and verify the diet consumption data in Table 3 and 4.

The raw data supported by sheep/skunk combinations and the feeding levels for days 1-7 given in Table 5 of the report. However, I was not able to document the initial weights of any of the lamb carcasses.

With the following exceptions the laboratory was able to document the data given in Table 6. I could find no data for skunk 12 F. According to the raw data, the weight of the sheep carcass fed to skunk 21M weighed 4.25 lbs not the reported 4.75 lbs.

I examined about 43% of the pretest food consumption data for all skunks and found no discrepancies in reporting. The remaining 57% of the data appeared available but it was not checked for accuracy in reporting.

The laboratory data document the weather conditions reported for the skunk portions of the test. (Table 11).

This skunk portion of the overall study appeared adequately documented and generally accurately reported by the scientists except for the dates of the sheep deaths. Nothing found in the biological portion of skunk study should adversely affect the usefulness of this part of the study.

B. Golden Eagle Portion of the Study

The Golden Eagle portion of this test was conducted at the Denver Wildlife Research Center in Denver, CO. Biologists familiar with the study were present durng the audit to aid in locating and interpretting available raw data. Almost all of the raw data were recorded in pencil. Many of the raw data sheets lacked titles or complete identification heading on the columns of data. The data sheets did appear to document the procedures and reported information.

The environmental conditions listed in Table 15 of the report and the consumption of the 1080 treated lambs by golden eagles (Table 13) were supported by raw data. The beginning and end treatment weights of the eagles given in Table 14 were supported by the raw data except that the data for eagle 9F and 10M were reversed. According to the raw data, the information given for eagle 9F were for bird 10M and visa versa.

Numerous reporting errors were found in the "average daily consumption before stabilization" columns in Table 12. The data for birds 7M and 11F appeared to be reported correctly. The average daily consumption for eagle 10M for before stabilization and after stablization had been reversed according to the raw data. The average daily consumption before stabilization for eagle 8M for the lambs diet could only be supported by the raw data (as suggested by laboratory personnel) if 75 gram portions of lamb rather than 50 gram portions were weighed out. It was not documented that this had actually occurred. There was no indication that 75 grams were weighed out for any of the other birds either. The possiblility that the laboratory staff could actually have used another dosage level (75 g portions) could significantly impact the reliability of this portion of the study. The laboratory could not document the portions of food prepared for this study.

The laboratory staff and I were not able to recompute the average amount of lamb reported to have been eaten by eagles 11F and 9F, using available data for the before stabilization period. The records did not support the theory that the error for bird 8M was due to their weighing out 75 g portions of food.

The laboratory's raw data for the before stabilization portion of the study documents food consumption levels below those listed in Table 12. Using the avaiable data for birds 8M, 11F, and 9F, it was just not possible to substantiate the pretest stabilization diet weights in table 12.

The audit team recommended that the staff reanalyze their raw data and that they provide the Registration Division and Dean Hill of the audit team with corrected tables. They should correct their reported data only if they have actually documented the fact that the original data submissions were incorrect, otherwise they should consider dropping reported data that can not be supported by raw data currently in the possession of the lab.

Chemistry Audit Report

Summary of Study:

This study was conducted by DWRC to supplement other sodium fluoroacetate toxicity studies for which reprots had previously been submitted to the Agency in support of toxic collar registration. The subject study report consisted of two components or experiments, one describing toxicity of sodium fluoroacetate exposed in lamb carcasses to striped skunks and the other summarizing similiar exposure experiments using golden eagles. The in-lite portion of the skunk experiment was conducted at Loga, Utah, whereas, the eagle study was conducted at DWRC in Denver.

The skunk experiment was carried out in two trials, one involving exposure of skunks to coyote killed collar-bearing lamb carcasses, and the other involving exposure of skunks to simulated kills, i.e., lamb carcasses that had been artificially treated with sodium fluoroacetate solution to mimic field kills. The simulated kills were dosed with an amount of sodium fluoroacetate which had been established through analysis of collared Angora goats that were killed by coyotes in other field trials. None of the skunks died after feeding on the carcasses for up to seven days, and although several lost weight, there were no other reported signs of intoxication. No analyses were reported to have been performed on either the treated lamb carcasses of the skunk tissues after sacrifice at the end of the expermiment.

The golden eagle phase of the study consisted of the exposure (via feeding) of five birds for seven days to lamb carcasses treated with sodium fluoroacetate solution so as to simulate death by coyote after rupture of a toxic collar. The eagles were preconditioned by feeding untreated lamb carcasses, no eagles died, although some toxic signs were reported, such as body tremors, erected feathers and lethargy. The symptoms were reported to have disappeared once exposure ceased. No sodium fluoroacetate analyses were reported to have been performed on either the lamb carcasses or the eagle tissue after exposure.

The following chemistry related aspects of the two studies were audited:

1. Assay, Characterization, Stability, and Formulation of the Test Substance Used to Evaluate Toxicity to Skunks of Coyote Killed Collared Lambs.

2. Assay, Characterization, Stability, and Preparation of Test Substance Used in Striped Skunk "Simulated Kill" Experiment.

3. Assay, Characterization, Stability, and Preparation of Test Substance Used in "Simulated Kill" Golden Eagle Experiment.

4. Determination of Sodium Fluoroacetate in Angora Goat Skin Used to Calculate Lamb Carcass Dose for "Simulated Kill" Skunk and Eagle Experiments.

The audit findings related to these topics are discussed in detail as follows:

1. <u>Assay, Characterization, Stability, and Formulation of the Test Substance Used to Evaluate Toxicity to Skunks of Coyote Killed Collared Lambs.</u>

   No information was provided in the report submitted to the Agency regarding the identification or analyses of the batch(es) of test substance used in the collar efficacy studies from which the lamb carcasses were derived. Nor were there any raw data or other apparent records available at DWRC for any of this information. Also, there were no specific formulation records or related data. There was no indication of any analyses having been performed to ascertain fluoroacetate levels in the lamb carcass skin or other tissues.

2. <u>Assay, Characterization, Stability, and Preparation of Test Substance Used in Striped Skunk "Simulated Kill" Experiment.</u>

   Page 4 of the submitted report of this study states that the technical sodium fluoroacetate used to prepare the dosing solution was 90% nominal purity, however, four samples of this technical material

(Batch No. unspecified) were analyzed at DWRC and found to contain an average of 94.5% active ingredient. The amount of sodium fluoroacetate applied to the simulated kill carcasses was thus reported as 45 mg rather than 43 mg as planned.

No raw data (notebook entries, benchsheets or chromatograms) could be located in DWRC files to substantiate the reported finding of 94.5% purity for the technical sodium fluoroacetate used to prepare the carcass dosing solution. Although a report of analysis from the chemistry laboratory was found for a 94.5% material (Exhibit E-1), this could not be unequivocally referenced to the subject study. None of the senior DWRC investigators could recall the circumstances of the analysis or locate any possible remaining material.

Also no data or other records could be found that would substantiate any analyses for characterization or stability (either neat or in aqueous solution) that may have been performed to determine these GLP recommended parameters. There were also no raw data documenting preparation of the reported dosing solution (11.1 mg/mL sodium fluoroacetate and 3 mg/mL Rhodamine B) used to dose the lamb carcasses, i.e., when, by whom, and how conducted. There was no indication that any analyses had been performed to substantiate the sodium fluoroacetate levels in the dosing solution(s).

The missing raw data for the reported determination of test substance purity is not only inconsistant with GLP Standards data requirements, but it is also a major deviation from registration regulations [40 CFR 169.2(k)] in that raw data supporting a pestiicde registration have not been retained.

3. <u>Assay, Characterization, Stability, and Preparation of Test Substance Used in "Simulated Kill" Golden Eagle Experiment.</u>

The technical sodium fluoroacetate used in the eagle portion of the study is given as 87.0% active ingredient in a footnote on page 7 of the Agency submitted report. No

other information regarding batch number, characterization, stability, methodology, quaility control, and so forth, is provied. It is also not mentioned in the footnote where the sodium fluoroacetate assay was performed, however, the DWRC scientists stated that the assay(s) would have been performed at the DWRC laboratory in Denver by either W. Okuno or D. Meeker.

No raw data (notebok entries, benchsheets or chromatograms), records, reports or other sustantiating evidence could be found in DWRC files that would document this reported analytical result. Likewise, no additional data or other information could be located at DWRC regarding characterization or stability of the sodium fluoroacetate used in the eagle experiment. There were aslo no raw data documenting the preparation of the dosing solution, i.e., when, by whom, and how conducted. No analyses were apparently performed by DWRC personnel to substantiate the concentration(s) of sodiumm fluoroacetate in the dosing solution(s).

The missing raw data for the reported sodium acetate purity is not only inconsistant with GLP Standards requirements, it is also a major deviation [40 CFR 169.2(k)] from the pesticide registration regulations which require retention of all supporting raw data for the life of a registration.

4. <u>Determination of Sodium Fluoroacetate in Angora Goat Skin Used to Calculate Lamb Carcass Dose for "Simulated Kill" Skunk and Eagle Experiments.</u>

Page 4 of the Agency submitted report states:

"... The amount sprayed on each lamb was arbitrarily established as the upper 95% confidence limit derived from measurements of the amount of 1080 containation on necks of toxic-collared Angora goats killed by wild coyotes. Six measurements (2 replicate determinations for each of three goats) yielded a mean of 37 mg FAC with upper 95% confidence limit of 43 mg (Burns et al. 1984: 18-20)..."

A copy of a report memo was located in the DWRC files (Exhibit E-2) that appears to represent the transmittal of the results from the laboratory to the DWRC field unit for the above referenced Angora goat skin analyses. Even though the report copy is unassigned, the data (6-8-83) is consistant with other study related documents. A laboratory notebook page was also located which appears to reflect a reference to the analyses (Exhibit E-3). This notebook reference provides sample weights and extract volumes along with calculated results which are in agreement with those given in the report memo (Exhibit E-2) and as given in the Agency report, i.e., 37 mg. Chromatograms for these determinations were also located in DWRC files, however, copies were not collected.

Although there were some notebook data, chromatograms and a report memo, the audited analyses still were determined to suffer from a number of shortcomings that may have affected the reliability of the reported results. The method used was not specifically referenced, however, H. Tietjen and J. Gillis were sure that the method utilized was that described in Okuno et al. J.A.O.A.C. 65 1102 (1982), (Exhibit E-4). However, the sample sizes were not consistant with this procedure and skin/hide analyses are not specifically mentioned traceable and the preparation of the working standards could not be found in documented form. There were also no apparent quality control analyses perfomred, i.e., replicate analyses (to determine precision), spiked sample recovery (to evaluate accuracy), blank analysis (to verify identity of the measured analyte). The replicate analyses reported appear to be simply the results of duplicate injections of the same extract into the gas chromatograph and not true replicate analyses uding multiple tissue samples. A method detection limit was also not provided.

Thus, although there were some data and a report for the Angora goat skin determinations, these results must still be considered suspect due to lack of supporting quality control data nad traceability of reference standards.

Conclusion:

There were a number of problems associated with the chemistry aspects of this study. Amoung the more significant are the missing raw data for the reported assays of the two batches of technical sodium fluoroacetate used for preparation of the dosing solutions used in the skunk and eagle portions of the study. This missing raw data is also a deviation from FIFRA registration regulations in addition to not conforming to FIFRA GLP Standards requirements.

Although some raw data were located for the reported Angora goat skin analyses, these data must be considered suspect due to their apparent lack of accompanying quality control data so as to be able to effectively evaluate their reliability.

102.0 Data Adequacy

Table 2 is a summary of the study dificiencies and audit conclusions for the reported studies. Based upon this information the EEB has determined that the only study suitable to support registration is:

"Secondary LC50 study of the toxicity of the Anticoagulant Brodifacoum to American Kestrels".

103.0 Test Study Repairability

Provided that the DWRC can resubmit the raw data and/or other missing information, pertinent findings, etc., specific to the study deficiencies described in the Inspection Audit Report, the following studies may be reclassified as suitable to support registration:

a. "Secondary Toxicity Hazards of the Anticoagulent Brodifacoum to American Kestrels.

b. Primary Hazards of the 1080 Toxic Collars to Skunks and Golden Eagles.

c. "Estimated Doses of Sodium Fluoroacetate delivered to Coyotes by Toxic Collars."

104.0 Conclusions

As a result of a change in workload priorities, and the urgency to complete the review, the EEB did not have sufficient time to conduct a data evaluation for each of the studies. As such, the EEB relied heavily on those study deficiencies, conclusions and recommendations reported in the audit report in making its decision on the adequacy and repairability of the data to support a registration.

Based upon these findings, the EEB concludes that only 1 of the 4 studies are adequate to support registration requirements (See Sction 102.0 Data Adequacy). If however, the DWRC can locate and resubmit the raw data and other pertinent missing information, as specified, and provided such information does not significantly alter the reported results, the 3 other studies may be found to be adequate to support registration. We suggest that copies of the Inspections Audit be sent to the DWRC so that the laboratory personnel involved can address the specific deficiencies identified for each study.

Table 2: Summary of Study Deficiencies and Conclusions

Study	Study Deficiencies		General Conclustions
	Bilogical	Chemical	
I. Secondary Toxicity Hazards of the Anticoagulant Brodifacoum to American Kestrels:			
a. Acute oral LD50	a. Inconsistent reporting of data.	a. Insufficient data. Data descrepancies. Miscalculations. Roundings-off errors.	The inability of the auditor to seperate original raw data from apparently copied raw data recorded in the same format as the Table in the final report made auditing of this study difficult. There was also a number of data descrepancies, the most notable of which were; the dosage of voles at 0.09 mg/kg in the acute study; the identy of treated voles fed to voles at the 50 ppm exposure level. Problems with chemical analysis probably would not affect evaluation of study conclusions. conclusions.
b. 3-day chronic bioassay	b. Errors in body weight data.	b. Missing raw data; decrepancies between analytical results and nominal dosing levels; Problems with residue analysis and bait prep.; and poor quality control	
c. Secondary poisoning of brodifacoum fed voles to Kestrels	c. Missing body wt. raw data; reporting descrepancies; reporting errors; study design problems.	c. Equipment problems; Invalid spike results; Missing raw data.	
II. Secondary LC50 study of the toxicity of the Anticoagulant Brodifacoum to American Kestrels	Poor record keeping	No analysis of bait; record keeping problem	Although there were some minor problems, the audit team could verify accuracy of the biological report. The Chemistry residue analsis appeared to be complete and accompanied by an adequate level of quality control

Table 2: Summary of Study Deficiencies and Conclusions (Continued)

III. Estimated Doses of of 1080 Delivered to Coyotes by Toxic Collars	Incomplete data base; Missing analytical data; Data Descrepancies; Just a best estimate.	Missing raw data; poor quality control; incomplete data records.	Missing analytical data affects the verification of the finding or estimates in the report. Not only are there missing raw data, the analyses appeared to be conducted with such minimal quality control and recorded in such an incomplete fashion that the reliability of the reported results cannot be effectively evaluated.
IV Primary Hazards of the 1080 Toxic Collar to Skunks and Golden Eagles:			
a. Skunk Study	a. Some missing data.	a. Missing raw data of test Substance identification, and purity; no residue analysis; missing data on preparation of dosing material.	a. The biological portion of the study appeared adequately documented. Nothing found in this portion should adversely affect usefulness of the study. However, missing raw data on test substance purity is inconsistent with GLP and a major deviation from registration regulations.
b. Golden Eagle Study	b. Incorrect Tables; Missing data; Incomplete records.	b. No raw data records; missing raw data on test substances; no data documenting test solution; lack of supporting quality control data and traceability of reference standards.	b. The audit team recommended that the raw data be re-analyzed and data tables corrected. Corrections should be made only if they have raw data to document facts that this original data submissions were incorrect, otherwise, reported data that cannot be supported by raw data should be dropped from the report. There were a number of problems associated with the chemistry aspects of this study. Amoung the more significant are the missing raw data for the reproted assays of the two batches of technical 1080 used for preparation of the dosing solutions used in the skunk and eagle studies. Data reported for the goat skin part of the study must be considered suspect for lack of quality control data.

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