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Chapter 13

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Field studies were conducted in California to assess efficacy of chlorophacinone-treated steam-rolled oats for controlling rangeland rodents. An objective of these studies was to assess the potential hazards of chlorophacinone residues in rangeland rodent carcasses and livers to mammalian and avian scavengers, especially raptors. Belding's ground squirrels, valley pocket gophers and *Microtus spp.* carcasses collected during the efficacy studies were analyzed for chlorophacinone residues. The method limit of detection (MLOD) for liver and carcass tissue samples averaged 0.036 μg/g and 0.034 μg/g, respectively. Chlorophacinone residues in Belding's ground squirrel (n=62) liver and carcass tissue ranged from <MLOD to 0.82 μg/g and <MLOD to 0.55 μg/g, respectively. Chlorophacinone residues in valley pocket gopher (n=8) liver and carcass tissue ranged from <MLOD to 0.42 μg/g and <MLOD to 1.21 μg/g, respectively. Chlorophacinone residues in whole body *Microtus* sp. (n=3) tissue ranged from 0.26 to 4.1 μg/g, respectively. Risk assessment indicated acute risk for mammalian scavengers and negligible to minimal risk for avian scavengers consuming rangeland rodents exposed to chlorophacinone baits.

INTRODUCTION

Chlorophacinone (2-[[(Chlorophenyl)phenylacetyl]-1H-indene-1,3(2H)-dione] and diphacinone [2-(Diphenylacetyl)-1H-indene-1,3(2H)-dione] are registered late-first generation or early-second generation anticoagulant rodenticides commonly used to control populations of rats and mice in urban areas. These anticoagulants are also effective in the control of other rodents such as pocket gophers (*Thomomys bottae*), Belding's ground squirrels (*Spermophilus beldingi*), and California ground squirrels (*Spermophilus beecheyi*). The acute oral toxicity (LD_{50}) for rats of both chlorophacinone and diphacinone is approximately 2 mg/kg, compared to the acute oral toxicity for other anticoagulants such as warfarin and pindone which is ~ 59 mg/kg.

Monetary damage to range grasslands attributed to pocket gophers and ground squirrels is difficult to estimate. Rangeland rodents can reduce vegetation by 20 to 40 percent, which results in less plant material for livestock grazing (*1,2*). Additionally, the combination of grazing by pocket gophers, ground squirrels, and livestock can lead to severe soil erosion. Damage to earthen irrigation ditches and dams has been observed in areas where pocket gopher and ground squirrel populations are excessive (*1,2*). Control methods for ground squirrels and pocket gophers include exclusion, shooting, trapping, flooding, use of acute toxicants including anticoagulants, and fumigants (*3*). California uses steam-rolled oat baits fortified at 0.005% (w/w) and 0.010% (w/w) chlorophacinone or diphacinone to control rangeland rodent populations.

When considering the use of pesticides to control rangeland rodents, the risk of secondary toxicity to scavengers potentially consuming target species such as Belding's ground squirrels and valley pocket gophers should be evaluated. Therefore, an objective of this study was to determine if residues of chlorophacinone were significantly high to pose a risk to scavengers. Following Environmental Protection Agency (EPA) procedures, incurred residues were compared to accumulated chlorophacinone toxicity values for various species to assess the risk of secondary toxicity for scavengers/predators. Studies reporting the toxicity of diphacinone were also cited when characterizing risk to secondary consumers, since their toxicities are generally similar.

Typically, only liver and serum are analyzed for residues of anticoagulants as anticoagulants are metabolized and accumulate in the liver. However, whole ground squirrel carcasses containing chlorophacinone residues are available to scavengers, not exclusively the liver. Based on the assumption that a majority of the chlorophacinone residue would be in the liver, the liver was removed from the carcass in this study and analyzed separately. The remaining carcass, with the appendages, head, and pelt removed, was homogenized and analyzed as an additional sample.

Several methods have been developed for analysis of indandiones in baits, formulations, and tissues. A gas chromatographic method with derivatization (*4*) is sensitive and selective, but suffers from low recoveries and is time consuming. Spectrophotometric methods (*5,6*) have been utilized for baits and formulations, but are not selective when assaying multi-residue samples. Thin-layer chromatography (*7-9*) methods are not suited for determining low levels of residues in complex matrices such as plant and animal tissues. Reversed-phase high-performance liquid chromatography (HPLC) methods (*10-14*) provide sufficient sensitivity, but often produce poor chromatographic resolution for the indandiones. Ion-pair reversed-phase HPLC (*15-20*) is sensitive and selective, but column lifetime is often short, due to adsorption of the ion-pairing reagent onto the stationary phase of the column packing material. In this sudy, reversed-phase ion-pair HPLC was used because good chromatographic resolution can be achieved and column lifetime can be extended with regular washing. Sample extraction utilized solid phase extraction (SPE) for sample cleanup with high sample throughput.

METHODS

Sample Collection

During field efficacy studies for the use of chorophacinone steam-rolled oat bait by spot baiting and with bait stations in alfalfa fields in Siskiyou County, California, carcasses of Belding's ground squirrels and *Microtus* were collected above ground *(21, 22)*. These studies were conducted in May to June of 1996. Additionally, during a field efficacy study for the use of chlorophacinone and diphacinone treated steam-rolled oat bait use by spot baiting in burrow systems in Siskiyou County, California the carcasses of valley pocket gophers were located and collected underground *(23)*. This study was conducted in October to November of 1997. Whole rodent carcasses were collected and placed in an individual plastic bags, sealed, labeled, and frozen in a portable freezer at -5 °C. The samples were stored in a freezer until shipped to our laboratory where they were stored in freezers at -20 °C until assayed. Method validation and analyses were completed under U.S. EPA Good Laboratory Practice guidelines (40 CFR 160).

Sample Preparation

Whole animal carcasses were weighed. Carcasses were weighed a second time after removal of the pelt, head, and appendages. Finally, after removing and weighing the liver, each carcass was weighed a third time. The liver was weighed separately. Individual livers and carcasses (minus head, pelt, and appendages) were frozen and homogenized with a cryogenic mill (*24*). Homogenization was completed by freezing

the tissue with liquid nitrogen in a stainless steel cylinder and crushing the sample with a stainless steel piston until the tissue became a powder. The powdered, frozen liver sample was transferred to a 35-mL glass sample bottle and the powdered, frozen carcass sample was transferred to a 500-mL polyethylene bottle. The homogenized samples were stored at -20 °C and assayed within two weeks.

Reagents

Acetone, chloroform, hexane, ethyl acetate, and methanol were liquid chromatography grade reagents (Fischer Scientific, Denver, CO). Deionized water was purified using a Milli-Q water purification system (Millipore, Bedford, MA). Concentrated phosphoric acid (Fischer Scientific, Denver, CO) was used to make 4 N phosphoric acid in water. Concentrated formic acid (Fischer Scientific, Denver, CO) was used to prepare 1% formic acid in 1:1 acetone:chloroform extraction solution. Anhydrous sodium sulfate (Fischer Scientific, Denver, CO) was mixed with tissue samples to remove water.

Chlorophacinone (98.9%) was obtained from LiphaTech (Milwaukee, WI). Concentrated stock standards of chlorophacinone were prepared by first drying the technical grade compound for 4 hours at 110 °C, then dissolving 10.000 mg in 10.0 mL of ethyl acetate. Working standards, ranging in concentration from 0.030 μg/mL to 10.0 μg/mL, were prepared by dilution of stock solutions with mobile phase. All standard solutions were stored at 5 °C.

Tetrabutylammonium dihydrogen phosphate (97%) was purchased from Aldrich (Milwaukee, WI) and was used to prepare a 5 mM solution in methanol. An aqueous solution of 5mM tetrabutylammonium dihydrogen phosphate with 50 mM potassium dihydrogen phosphate buffer (Alltech, Inc.; Deerfield, IL) was prepared.

Liver and Carcass Tissue Sample Extraction and Cleanup

Sample Extraction

Homogenized tissue samples were weighed (1.0 - 1.1 g) into a mortar and 10.0 g of anhydrous sodium sulfate was added. The tissue and sodium sulfate were ground together with a pestle for five minutes. The solid mixture was transferred to a 50-mL tube with a powder funnel. The mortar was rinsed with three 5 mL aliquots of extraction solution and transferred to the 50 mL tube. Sample tubes were vortex mixed thoroughly and shaken horizontally on a mechanical shaker at high speed for 20 minutes. Sample tubes were centrifuged at approximately 2500 rpm for 5 minutes.

The extract was transferred to a 50-mL glass tube. The extraction was repeated twice following two subsequent 10 mL additions of extraction solution. Extract solvent was removed by placing the tubes in a warm water bath ($\leq$ 60 °C) and allowing nitrogen gas to flow over the surface of the extract until no solvent remained.

The residue was reconstituted with 5.0 mL of hexane, gently vortex mixed, and sonicated for 10 minutes.

Analyte Concentration

Each silica SPE (2 g) column was conditioned with approximately 5 mL of hexane. The packing material was not allowed to dry. The reconstituted sample extract was added to the SPE column with a Pasteur pipet. The entire solution was passed through the column at 1 to 2 mL/min (vacuum was typically not necessary). The eluate was collected in a 25-mL glass tube. Each SPE column was rinsed with hexane by adding 5 x 2.5 mL aliquots (12.5 mL total volume) to the 50-mL tube and transferring the solution to the SPE columns. This eluate was discarded. Each SPE column was rinsed with 20 mL (8 x 2.5 mL) of 1:1 ethyl ether:hexane and this eluate discarded.

Liver Sample Analyte Elution

A clean 15-mL screw top centrifuge tube was placed under each SPE column in the manifold. The analyte was eluted from each SPE column by adding 15 mL (6 x 2.5 mL) of 12% (v/v) methanol in ethyl ether. After the last 2.5 mL aliquot of eluant passed through the SPE column, vacuum was used to collect eluant that remained in the SPE packing material.

Carcass Sample Analyte Elution

A clean 15-mL screw top centrifuge tube was placed under each SPE column in the manifold. The analyte was eluted from each SPE column by adding 20 mL (8 x 2.5 mL) of 15% (v/v) methanol in ethyl ether. After the last 2.5 mL aliquot of eluant passed through the SPE column, the vacuum was applied to collect eluant remaining in the SPE packing.

Sample Reconstitution

The volume of eluate was reduced by placing tubes in a warm water bath and blowing a stream of nitrogen over the solution until the solvent was removed (early in the solvent removal procedure the tube was kept out of the water bath). The residue was redissolved with 1.0 mL of 75:25 methanol:water (with 5 mM tetrabutyammonium phosphate), vortex mixed and sonicated for 5 minutes. The reconstituted samples were filtered through a 0.45 μm Teflon syringe filter into a vial and capped before HPLC analysis.

High Performance Liquid Chromatography

The HPLC system consisted of a Hewlett-Packard 1090 liquid chromatograph (Palo Alto, CA) and a Hewlett-Packard 1050 variable wavelength detector. The mobile phase was prepared by mixing aqueous and methanolic solutions of 5 mM tetrabutylammonium dihydrogen phosphate (32:68 v/v) and adjusting the pH to 8.0 with 4 N phosphoric acid. The mobile phase was degassed by sparging with helium. At the end of each set of analyses, the column was washed with a mixture of 1:1 (v/v) methanol:water for 40 minutes. Each tissue sample was analyzed in duplicate. The HPLC parameters are listed in Table I.

Quality Control Samples and Fortification of Controls

Belding's ground squirrels and valley pocket gophers were trapped and euthanized by California Department of Food and Agriculture representatives at two sites in Siskiyou County, California prior to any baiting operations (*25*). These animal carcasses and livers were processed and screened for chlorophacinone and diphacinone prior to combining control samples into a composite. Control liver and carcass tissue samples were fortified at 0.10, 1.0, and 10 ppm chlorophacinone with aliquots of fortification standards of chlorophacinone in ethyl acetate. The quality control samples were then assayed with the appropriate method described previously.

Table I. HPLC parameters for the analysis of liver and carcass extracts

Parameter	*Conditions*
Mobile Phase:	Combine the aqueous IPC solution and methanolic IPC solution in the ratio 68:32 (Methanol:Water)
Column Conditioner:	1:1 Methanol:Water
Flow Rate:	1.0 mL/min
Injection Volume:	100 μL
Column:	Keystone ODS/H (C18), 5 μm, 250 mm x 4.6 mm i.d. or equivalent (use guard column containing identical HPLC packing)
Column Temp.:	35 °C
Detector:	UV @ 285 nm and 325 nm
Run Time	Standard: 20 minutes Samples: 35 minutes

Chlorophacinone concentrations were determined by comparing the area of the chlorophacinone peak in the sample extract to a working standard. The retention time of chlorophacinone over the dates of analyses (2/7/97 to 3/27/97) varied from 15.2 to 17.5 min, respectively.

Microtus Analysis

During the collection of animal carcasses for the field portion of one of the studies, several *Microtus spp.* carcasses were found and collected. These samples were handled and stored under the same conditions as the ground squirrels and pocket gophers. Each whole animal was homogenized and assayed as described previously.

RESULTS AND DISCUSSION

Analytical Methods

Mean recoveries of chlorophacinone of liver (n = 24) and carcass (n = 28) quality control samples were 80.4 ± 17.2% and 75.5 ± 10.0% (Table II). Two lots of the silica solid phase extraction columns were used to complete the analyses, with no difference in recoveries observed between the two lots.

Table II. Analytical recoveries of chlorophacinone in Belding's ground squirrel and valley pocket gopher tissues for quality control samples

Fortification Levels (ppm)	*Tissue*	*Range (%)*	*Mean (%)*	*Std. Dev. (%)*	*CV (%)*
Belding's Ground Squirrel					
0.010 - 10	Carcass (n = 17)	60 - 134	83	17	21
0.010 - 10	Liver (n = 17)	55 - 89	74	10	14
Valley Pocket Gopher					
0.010 - 1.0	Carcass (n = 7)	70 - 87	76	5.4	7.1
0.010 - 2.5	Liver (n = 11)	62 - 98	79	11	14

Response Linearity

Two sets of six calibration standard solutions were prepared ranging in concentration from 0.030 to 10 μg/mL. Each standard solution was injected two times and a linear regression performed on the data set. The relation between chromatographic response and concentration was linear. The response was directly proportional to concentration over the range of interest.

Method Limit of Detection

The method limit of detection (MLOD) was calculated as the concentration of chlorophacinone required in the sample to generate a signal equal to 3 times the baseline noise (peak to peak) observed in the chromatogram of the control extract. The MLOD was estimated from the chromatographic response in height of a control tissue extract and an extract from a control tissue sample fortified at 0.10 μg/g. The MLOD for all liver and carcass tissue samples averaged 0.036 μg/g and 0.034 μg/g, respectively. Chromatograms of carcass sample extracts with positive and negative chlorophacinone responses are shown in Figure 1.

Carcass and Liver Residues

Chlorophacinone residues in Belding's ground squirrel (n = 62) liver and carcass tissue ranged from <MLOD to 0.82 μg/g and <MLOD to 0.55 μg/g, respectively (Table III). Chlorophacinone residues in valley pocket gopher (n = 8) liver and carcass tissue ranged from <MLOD to 0.42 μg/g and <MLOD to 1.21 μg/g, respectively. Total residue was calculated by multiplying the analyte concentration in the liver or carcass by the liver or carcass weight and summing the quantities, respectively. For samples containing <MLOD, the MLOD was used to calculate mean residue and total residue.

The primary wavelength for quantitative analysis was 285 nm, though absorption at 325 nm was also determined. The ratio of absorbance at 285 and 325 nm was used to qualitatively confirm presence of the analyte. The molar absorptivity of chlorophacinone at 285 nm is twice the molar absorptivity at 325 nm.

Microtus Residues

Chlorophacinone residues in 3 whole animal *Microtus* sp. were 0.26, 0.36 and 4.1 μg/g, respectively.

Secondary Toxicity Assessment

Many factors determine whether rodenticide residues in poisoned animals pose a secondary hazard to non-target (scavenger) species. These include the chemical and toxicological properties of the active ingredient, composition of the formulated bait and how it is applied, behavior of the non-target species at risk, local environmental factors, and the variability of residue concentrations among carcasses (*26*). For example, ground squirrels which are diurnal may not be preyed upon by nocturnal predators, however, their carcasses may be available to either nocturnal or diurnal scavengers. A study of the anticoagulant brodifacoum on plains pocket gophers

Determination of Chlorophacinone - Ground Squirrel Carcass Tissue

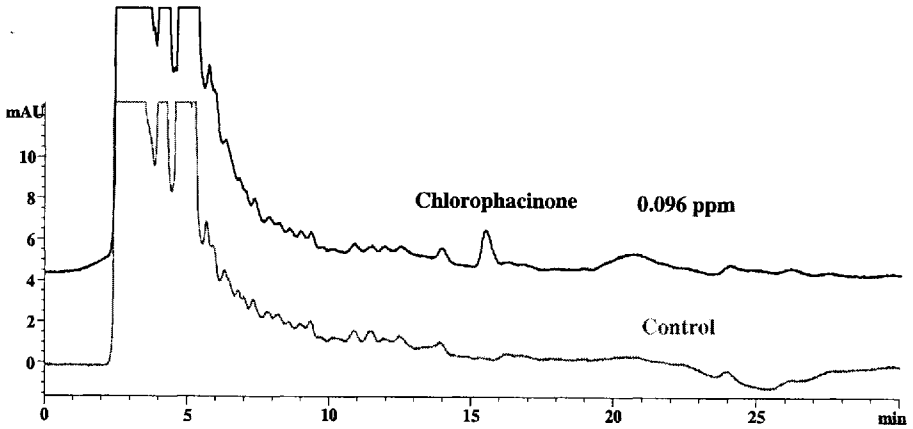

Figure 1. Chromatograms of a control blank and a 0.096 μg/g chlorophacinone-fortified control carcass tissue samples with ultraviolet detection at 285 nm.

showed that 90% of radio-equipped animals expired underground (*27*), which minimizes the risk of secondary poisoning. Environmental factors play a role in determining how long a carcass is available to scavengers. During hot and dry weather, carcasses above ground are often dessicated and consumed by insects in less than two days (*28*).

Table III. Residues of chlorophacinone in Belding's ground squirrel and valley pocket gopher tissues

Sample Type (# analyzed)	*Range of Residues (μg/g)*	*Mean Residue* (μg/g)*	*Range of Total Residue (μg)*	*Mean Total Residue (μg)*
Ground Squirrel				
Livers (62)	<MLOD - 0.82	0.133	0.10 - 11	1.8
Carcass (62)	<MLOD - 0.55	0.131	1.1 – 123	20
Pocket Gopher				
Liver (8)	<MLOD - 0.42	0.161	0.060 - 2.4	0.92
Carcass (8)	<MLOD - 1.21	0.357	1.0 – 126	23
Microtus spp.				
Carcass (3)	0.26 - 4.1	1.58	2.1 – 57	21

*To calculate the mean residue for samples reported as <MLOD, the MLOD was used as the value for these samples.

A commonly used approach for evaluating non-target hazards is the calculation of a risk quotient (RQ) (*29*). Acute risk quotients predicting the potential for lethal exposure are routinely calculated using the median lethal dietary dose (LC50) of the most sensitive species in relation to the expected pesticide concentration in the diet. This method divides the expected environmental concentration (EEC) (in the case of secondary toxicity this would be the maximum observed tissue concentration) by the LC50 of the most sensitive species.

$$RQ = EEC / LC50$$

Acute dietary toxicity to mammals is normally not known. In conducting a screening level assessment for mammals, the LC50 is determined by dividing the median lethal acute oral dose (LD50) value (usually the rat LD50) by the animal's daily dietary intake in relation to the animal's body weight. A risk quotient is then determined as above, by dividing the EEC by the derived LC50 value.

*RQ = EEC / [LD50 * % Body Wt. Consumed per day]*

Significant risk to non-target avian and mammalian species is predicted if the RQ is greater than 1. However, a value between 0.5 and 0.1 usually requires that the product be used under specified restrictions. The following risk assessment focuses on direct mortality resulting from the secondary exposure to chlorophacinone contaminated carcasses. Sub-lethal effects leading to indirect mortality will not be addressed.

The residues found in ground squirrel livers collected in this study were used to represent the maximum expected environmental concentration for conducting a secondary hazard assessment for predators and scavengers. Although higher residues were found in the pocket gopher, these carcasses had to be dug out of the ground and obviously presented little hazard to scavenging species. Acute toxicity data for chlorophacinone and diphacinone were obtained from the EPA (*30, 31*) and RTECs (*32*) databases (Table IV). Risk quotients calculated using the maximum residues found in Belding's ground squirrel livers indicate little risk for avian species (Table V). However, acute risk is predicted for all three weight classes of mammals. If these (Table V) calculations were made for more typical feeding situations where the entire carcass is consumed and the maximum residue for ground squirrel carcasses being 0.55 μg/g, risk is lower but still indicated. The highest chlorophacinone residue found in a *Microtus* carcass was 4.1 μg/g. Risk quotients calculated on a EEC of 4.1 μg/g are 5 times higher than shown in Table V. Even at the higher EEC no risk for avian species is predicted. However, risk quotients for mammals would be increased by 5 and indicate that all weight classes of mammals and the coyote are potentially at risk from acute exposure if only *Microtus* were consumed. The most realistic approach may be to use the mean residue value for the Belding's ground squirrel carcasses as the EEC. Most scavengers will not specifically consume the liver and very few carcasses will be at the maximum concentration (Table V).

It is possible a scavenging or predatory mammal's diet may consist solely of contaminated *Microtus* for a period long enough to obtain a lethal dose, but it is more likely *Microtus* would only be scavenged occasionally. In this study treated colonies were exhaustively searched to locate carcasses. *Microtus* are approximately 7 times smaller than *Belding's* ground squirrels. Because of the small size of a *Microtus*, carcasses might have been overlooked. This may explain why 20 times more ground squirrel carcasses were found than *Microtus* carcasses despite the fact ground squirrels are fossorial and many may die underground. The dessication rate of a smaller carcass will be much quicker than that of the ground squirrel, reducing the length of time it will be attractive to vertebrate scavengers. Because *Microtus* live above ground, the potential for scavenging a carcass is high. In a treated ground squirrel colony with large numbers of squirrel carcasses above ground, there may be a tendency for scavengers to focus on the abundant food source and overlook small *Microtus* carcasses.

Table IV. Toxicity data for Chlorophacinone and Diphacinone

Species	LD50 (mg/kg) Chlorophacinone	LD50 (mg/kg) Diphacinone
Rat (*Rattus spp.*)	2.1 ‡	1.5 ‡
Mouse (*Mus. Spp.*)	1.1 ‡	28 ‡
Rabbit (*Oryctolagus spp.*)	50 ‡	35 ‡
Mallard(*Anas platyrhynchos*)	100 ‡	3160 ‡
Northern bobwhite (*Colinus virginianus*)	260 †, 430 ‡	1200 †
Dog (*Canis domesticus*)	---	3.0 ‡
Cat (*Felis catus*)	---	15 ‡
Pig (*Sus spp.*)	---	150 ‡
Coyote (*Canis latrans*)	---	0.6 (*33*)
Species	**LC50 (ppm) Chlorophacinone**	**LC50 (ppm) Diphacinone**
Coyote (*Canis latrans*)	0.95 (*36*)	---
Mallard (*Anas platyrhynchos*)	170 †, 430 †	910 †
Northern bobwhite† (*Colinus virginianus*)	56 †, 240 †	4500 †
Golden eagle (*Aquila chrysaetos*)	---	2.7* NOEL (*33*)
Barn owl (*Tyto alba*)	1.3^* NOEL (*34*)	—
American kestrel (*Falco sparverius*)	5.7^* NOEL (*35*)	—

* no mortality ^ extrapolated (reference) † EPA Data (*30, 31*) ‡ RTECS (*32*)
NOEL = (No Effect Level)

Studies (*33-35*) to assess the secondary hazards of indandione anticoagulants on raptors can be evaluated and compared to the RQs values calculated with our data. One study investigated the effects of secondary exposure of golden eagles to sheep muscle laced with 2.7 ppm diphacinone (*33*). Of seven golden eagles fed this diet, none expired (four eagles were fed for 5 days and three were fed for 10 days).

Hematocrit and prothrombin levels indicated subacute symptoms of toxicity and two of seven eagles treated demonstrated sublethal symptoms. Assuming that chlorophacinone and diphacinone toxicities are similar (Table IV), the total quantity of residue consumed by the eagles was approximately three times higher than found in one *Belding's* ground squirrel analyzed. The highest residue detected in Belding's ground squirrel tissue was 50% lower than that fed to the eagles and no mortality occurred. Therefore, risk appears to be even lower than estimated by previous studies *(33).*

Table V. Risk quotients for avian and mammalian species using maximum observed residue in liver tissue

			Liver		*Carcass*	
Birds		*LC50*	*Max Residue (μg/g)*	*RQ*	*Mean Residue (μg/g)*	*RQ*
Northern bobwhite		56	0.82 ^	0.015	0.131	0.0023
Mallard		170	0.82	0.005	0.131	0.0008
Mammals	*% BW Consumed*	*Estimated LC50 #*	*Max. Residue (μg/g)*	*RQ*	*Mean Residue (μg/g)*	*RQ*
15 g	95	1.15	0.82	0.71	0.131	0.11
30 g	66	1.67	0.82	0.49	0.131	0.078
1000 g	15	7.33	0.82	0.11	0.131	0.018
Coyote	-	0.95*	0.82	0.86	0.131	0.14

Estimated LD50 is based upon the mouse LD50 of 1.1 mg/kg

^ Belding's Ground Squirrel maximum liver residue and mean carcass residue observed

* *(36)*

Mendenhall and Pank assessed anticoagulant rodenticides (6 compounds including chlorophacinone) hazards to owls *(34)*. For ten days, two barn owls were fed tissue from rats that had been poisoned with a mean consumption of 12.6 mg of chlorophacinone in treated bait. Mortality of rats occurred within 6 days on average. Neither bird expired and no symptoms of toxicity were observed. No residue analysis was completed on the rat tissue. If a "worst case" scenario is adapted and no excretion or metabolism occurred for the poisoned rats, one barn owl consumed 712 g of rat tissue containing 9.2 mg of chlorophacinone, the concentration of chlorophacinone in the rat tissue would have been 13 μg/g. Metabolism studies with domestic rats administered 1.4 mg of chlorophacinone have shown that after two days, 90% of chlorophacinone was metabolized or excreted *(37)*. If it is then assumed that 10% of the chlorophacinone was retained by the rats, the concentration of chlorophacinone in the rat carcass would have been 1.3 μg/g. This no effect level is

greater than the highest residue concentration of chlorophacinone in poisoned Belding's ground squirrels or pocket gophers.

A third publication reports the effect of chlorophacinone poisoned *Microtus* on American kestrels *(35)*. For 21 days, four American kestrels were fed *Microtus* that were poisoned with an average of 1.14 mg chlorophacinone in a treated bait. On average, *Microtus* mortality occurred within 6 days. None of the birds expired and no external toxic symptoms were observed, but several birds subsequently euthanized and examined internally showed evidence of hematomas. Unfortunately, no residue analysis was completed on the *Microtus*. Based on the previous assumption that after two days 90% of chlorophacinone is metabolized or excreted, the concentration of chlorophacinone in the *Microtus* tissue with an average body mass of 40 g would have been approximately 5.7 μg/g. This is almost 500% greater than the maximum concentration observed for chlorophacinone in pocket gophers and 39% greater than the maximum residue observed in the three *Microtus* carcasses analyzed. These studies indicate that secondary hazards to birds consuming chlorophacinone burdened rodent carcasses are minimal.

Two references *(33, 36)* yielded information on indanedione hazards to mammals. In one study *(33)*, acute toxicity was noted when pairs of coyotes were orally gavaged with diphacinone at seven levels from 0.16 to 10.0 mg/kg. This study yielded an LD_{50} of approximately 0.6 mg/kg. An experiment investigating the secondary chlorophacinone exposure of coyotes *(36)* was conducted with California ground squirrels exposed to chlorophacinone fortified bait. Coyotes were fed one California ground squirrel a day for five consecutive days. Three of the seven exposed coyotes died. Residues in the ground squirrels ranged from 0.16 to 2.8 μg/g with a mean value of 0.95 μg/g. The mean residue concentration reported by Marsh and Howard *(36)* was seven times higher than the mean residue observed in this study for Belding's ground squirrel tissues. The maximum residue concentration was three times higher than the maximum residue reported for Belding's ground squirrel tissues in this study. The RQ calculated using an LC50 of 0.95 μg/g and the maximum residue observed in Belding's ground squirrel livers in our study (0.82 μg/g) is 0.86. If the mean carcass residue is used as the EEC, (0.131 μg/g) the RQ is lowered to 0.14, indicating the actual field risk of chlorophacinone exposure may be lower than that simulated in the previous study *(36)*. These results indicate coyotes consuming a diet of only contaminated Belding's ground squirrels are at risk for acute affects.

CONCLUSION

The methodology developed for analyzing chlorophacinone liver and whole body tissue proved to be reliable, efficient and simple. The same method was used to analyze tissue from three different species. Chlorophacinone residues in Belding's ground squirrel (n=62) carcass and liver tissues ranged from <MLOD to 0.55 μg/g and <MLOD to 0.82 μg/g. Chlorophacinone residues in valley pocket gopher (n=8) carcass and liver tissues ranged from <MLOD to 1.21 μg/g and <MLOD to 0.42 μg/g. Chlorophacinone residues in whole body *Microtus* spp. (n=3) tissue were 0.26, 0.36,

and 4.1 μg/g, respectively. In estimating potential secondary hazards for proposed use of indandione rodenticides, every likely scenario cannot be investigated. Three studies combined with the residue data from this work reinforce the avian risk quotients and suggest that the potential chlorophacinone secondary hazards to avian scavengers are minimum to negligible. However, the implications from the two studies with coyotes and indandione secondary toxicity are reinforced by the residue data from this work and the associated mammalian risk quotients. These studies suggest potential secondary hazards for chlorophacinone to some mammalian scavengers.

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