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Zinc phosphide residues in gray-tailed voles (*Microtus canicaudus*) fed fixed particles of a 2% grain bait

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Abstract

This study measured depelted-carcass residues of zinc phosphide (Zn_3P_2, CAS #1314-84-7) in 8 (4♂ and 4♀) gray-tailed voles (*Microtus canicaudus*). Six (3♂ 3♀) voles were confined individually in 1.89 dkl (5 gal) plastic pails that contained 5, 2% Zn_3P_2 steam-rolled-oat (SRO) groats; 2 voles (1♂ and 1♀) served as analytical (unbaited) controls. Four test voles (3♂ 1♀) died within 7.5 h after bait exposure; whereas, 2 test voles showed no signs of toxicosis and were euthanized 7.0 h after bait exposure. Whole carcasses were stored frozen and depelted carcasses were analyzed within 31 days for Zn_3P_2 residues using a acid-hydrolyzation, gas-chromatographic (GC) method. Analytical controls were euthanized, with carcasses stored and analyzed the same as test voles. A mean (±SD) 4.7 (±0.8) SRO groats were consumed by the test voles; this converted to a mean (±SD) intake of 2.15 (±0.38) mg Zn_3P_2 and dose of 73.25 mg/kg (±22.95) Zn_3P_2. The mean (±SD) Zn_3P_2 residue in the 6 test vole carcasses was 0.42 mg (±0.68); control carcasses contained <0.009 mg Zn_3P_2—<method limit of detection (MLOD). Results confirm that: (1) carcass residues of Zn_3P_2 in voles are variable, but typically <50% of ingested rodenticide and (2) risks of secondary poisoning posed by Zn_3P_2-baited voles to avian and mammalian predators/scavengers are low due to the relatively high toxic thresholds (>20 mg/kg) required to affect these species. Published by Elsevier Science Ltd.

1. Introduction

Zinc phosphide (Zn_3P_2) is an acute rodenticide that has numerous applications in agriculture and public health (Gratz, 1973; Marsh, 1988; Sterner, 1994). Current U.S. Department of Agriculture registrations include: (1) Zn_3P_2 (1.82%) on wheat grains for mouse (*Microtus* spp. and *Peromyscus* spp.) control in orchards (EPA Reg. No. 56228-3), (2) Zn_3P_2 control of rats (*Rattus norvegicus*) in infested areas around homes/buildings (EPA Reg. No. 56288-7) and (3) Zn_3P_2 concentrate (63.2%) for muskrat (*Ondata zibethicus*) and nutria (*Myocaster coypus*) control, near sugarcane and rice fields (EPA No. 56288-9). The mode of action involves hydrolysis of Zn_3P_2 to phosphine (PH_3) upon reaction with stomach acids; death results from decreased electron transport and failed respiration within cells (Chefurka et al., 1976; Hazardous Substance Databank, 1993; Murphy, 1986).

That Zn_3P_2 affords high acceptance and efficacy for numerous rodent species is well documented (e.g. Marsh, 1988; Sterner et al., 1996; Tietjen, 1976). However, bait shyness (Marsh, 1988; Sterner, 1994) and broad-spectrum toxicity (Johnson and Fagerstone, 1994; Marsh, 1988) are recognized deficiencies. Mitigation of bait shyness is usually effected via pre-baiting to increase initial bait ingestion and reduce the frequency of sub-lethal dosings (Marsh, 1988) and selective application of baits is used to preclude bait foraging by non-targets (Johnson and Fagerstone, 1994; Marsh, 1988). Still, concerns remain about primary and secondary hazards of Zn_3P_2 to non-target animals. Poisonings of gallinaceous birds, passerines, waterfowl and carnivores are documented (see Johnson and Fagerstone, 1994; Tietjen, 1976). Poisonings that have occurred are mainly due to improper disposal of grain baits or inappropriate bait applications.

A number of 'direct-' (baited-rodent carcasses fed to non-target predators/scavengers) and 'indirect-Zn_3P_2-hazards' studies (residue estimates of Zn_3P_2/PH_3 in rodent carcasses) have been published (Bell and Dimmick, 1975; Chitty, 1954; Sterner and Mauldin, 1995; Tabata, 1986; Tkadlec and Rychnovsky, 1990). Key outcomes of these studies include: (1) ingestion of multiple, Zn_3P_2-laden-rodent carcasses can prove fatal to certain canids, felids, and raptors in single-choice feeding situations (Bell and Dimmick, 1975; Chitty, 1954; Tkadlec and Rychnovsky,

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1990), (2) only about half ($\leq 69\%$ or 2.2 mg) of ingested Zn_3P_2 and negligible amounts of PH_3 ($\leq 21\ \mu g$) are recovered from carcasses using current analytical techniques (Sterner and Mauldin, 1995; Tabata, 1986) and (3) $\approx 95\%$ of the undigested Zn_3P_2 resides in the gastrointestinal (GI) tracts of lethally-dosed rodents—a form of primary hazard to nontarget species (Tkadlec and Rychnovsky, 1990).

The current study estimates carcass residues of Zn_3P_2 in gray-tailed voles that were presented with 5 particles of 2% Zn_3P_2 SRO groats. Additionally, validation of the GC recovery method is described.

2. Materials and methods

2.1. Test site

The study was conducted outdoors at the Hyslop Crop Science Field Laboratory (HCSFL)—an agricultural research facility operated by Oregon State University (OSU). The HCSFL is located in west central OR, ≈ 10 km north of Corvallis. It is comprised of ≈ 81 ha (≈ 200 ac) divided into 45.7×182.8 m fields (150×600 ft.) separated by ≈ 5 m-wide grass berms.

2.2. Voles

Eight gray-tailed voles (4♂ 4♀) were used. The voles were caught the night preceding use in Sherman live traps ($7.5 \times 9.5 \times 25.5$ cm). Traps were placed in commercial cardboard milk cartons and positioned in vole runs on the grass berms. Traps were set at dusk and emptied/closed in the morning ($\approx$0700–0800 PDT).

Hall and Kelson (1959) considered the gray-tailed vole a sub-species of montane vole (*M. montanus*) that inhabited valleys in the western portions of the northern Sierras (e.g. Willamette River Valley, Hood River Valley). However, more recent taxonomic accounts designate the gray-tailed vole a separate species (Hall, 1981; Banks et al., 1987; Verts and Carraway, 1987).

2.3. Zn_3P_2 and SRO groats

The technical product was Zn_3P_2 (CAS No. 1314-84-7; Lot No. TZ 2565; Bell Laboratories, Madison, WI), with pigment black No. 6 and No. 7 amorphous carbon-crystalline CI 7726 (CAS #1333-86-4; Benbow Chemical Packaging Inc., Syracuse, NY) used as dyes. Prior to the study, the Zn_3P_2 was assayed as 88.8% (± 0.27) active ingredient (a.i.) by staff of Analytical Chemistry Project, Denver Wildlife Research Center (DWRC). The pigment black No. 6 with No. 7 was applied to blacken (deter nontarget consumption) both test and control (pre-) baits under field conditions.

Test baits were prepared as a 2% Zn_3P_2 SRO groat (Honeyville Grain Co., Honeyville, UT), 1.04% (vol:vol) vehicle, a small per cent dye, and remainder SRO groat mixture. The vehicle was prepared prior to formulation; this consisted of 50% Alcolec-S (American Lecithin Co., Woodside, NY) and 50% Medical Center Mineral Oil (Hunt Products Co., Inc., Dallas TX). Baits were prepared by staff of the Formulation Laboratory, Product Development Section, DWRC.

2.4. Zn_3P_2 dose

Because the voles were each fed 5 SRO groat particles prepared with 2% Zn_3P_2, the determination of Zn_3P_2 dose was estimated using 'particle-dose analysis' (PDA). This assessment estimates the cumulative particle-by-particle amounts of Zn_3P_2 ingested by the rodents. PDA assumes accurate nominal bait formulation and homogeneous adherence of Zn_3P_2 to each SRO groat (see Sterner, 1994). For current purposes, 100 SRO groats were weighed; the mean ($\pm$SD) weight of groat particles was 23 (± 9) mg. Assuming homogeneous distribution of Zn_3P_2 on groats, the PDA formula was:

$$\text{Particle } Zn_3P_2 = \bar{x}\ \text{Particle Weight (mg)} \times 2.0\% Zn_3P_2.$$

Thus, mean particle Zn_3P_2 was estimated at 2.0% (0.02) of 23 mg or 0.46 mg.

2.5. Procedures

On Day 1 ($\approx$1130 PDT, Oct. 5, 1993), 6 voles (3♂ and 3♀) were confined individually in large plastic pails (1.89 dkl; 5 gal). The pails were located outdoors on a table adjacent to a trailer used for equipment storage at HCSFL; the table was shaded by the trailer (maximum temperature 16°C). Each bucket contained 5, 2%-Zn_3P_2-SRO groats covered by a ≈ 6 cm layer of pulled grass (cover).

Each vole was observed hourly until signs of toxicosis occurred (e.g., lethargy, labored respiration) but then was monitored continuously until death—exact time of death was recorded. Upon death, carcasses were weighed, sealed in a 0.38 dkl lock-top plastic freezer bag, placed in an ice-filled, insulated plastic container (Tote Six® Ice Chest; Rubbermaid®, Medina, OH), transferred to a freezer at OSU, and frozen until transport to DWRC. Voles that showed no signs of toxicosis after ≈ 7 h (1800 h) were euthanized via carbon dioxide (CO_2) inhalation in a polystyrene chamber ($32.5 \times 23 \times 12.5$ cm) with a plastic lid; these carcasses were then iced, transferred to the freezer, and frozen the same as the previous voles.

On Day 3 ($\approx$1200 h PDT), 2 additional gray-tailed voles (1♂ and 1♀) were also euthanized for residue analysis as controls. These voles were not fed baits, but were

euthanized, frozen, maintained and transported the same as the test voles.

On Day 10, all vole carcasses were removed from the freezer and placed in the insulated plastic container with dry ice. The carcasses were then transported to DWRC via automobile (5 day trip). All carcasses arrived frozen at DWRC (dry ice present) on Day 15 and were again placed into a freezer. Between Days 23–31, carcasses were analyzed for Zn_3P_2 residues.

2.6. Residue analyses

2.6.1. Carcass preparation

Carcass Zn_3P_2 residues were quantified using a modified version of the analytical method described by Mauldin and Mishalanie (In Press). The pelt, paws and tail were removed from each frozen vole carcass, which was then thawed and weighed. A volume of deionized water (mL) equal to 4 × the carcass weight (g) was measured. The carcass was placed in a blender jar (Waring, New Hartford, CT), three-fourths of the water added and the carcass blended for 1 min. Next, the jar was removed, repeatedly inverted to free the blades of tissue, replaced, and the contents blended for another 4 min.

To homogenize this mixture, the carcass slurry in the jar was transferred to a beaker and a stir bar having no median rib and a length slightly shorter than the internal diameter of the beaker was inserted. The remaining one-fourth of the water was added to the blender jar and shaken vigorously to rinse the jar. The rinsate was then emptied into the beaker which was placed on a stir plate located beneath a Polytron PT3000 Homogenizer (Brinkmann Instruments, Westbury, NY). This rinsate and carcass slurry was homogenized at 15,000–20,000 rpm for 5 min while the contents were stirred to prevent settling of Zn_3P_2.

Triplicate 9 mL aliquots of the homogenate were removed using a 10 mL pipettor (Eppendorf, Germany) and transferred to separate 125 mL narrow-mouth Erlenmeyer flasks. Following the addition of 40 mL of 30% sulfuric acid (H_2SO_4; vol:vol), each flask was sealed with a rubber sleeve stopper and shaken for 30 min at low speed on a mechanical shaker (Eberbach, Ann Arbor, MI). Next, using a glass syringe, 5 μL of flask headspace gas was removed and injected into a Model 5890, Hewlett-Packard (Walbron, Germany) gas chromatograph (GC) that was equipped with a flame photometric detector having a phosphorus-specific filter (526 nm). The GC conditions were as follows: Column, GS-Q Megabore, 30 m × 0.53 mm i.d., 0.25 μm film thickness (J&W Scientific, Folsom, CA). Temperatures during analysis were: injection port = 70°C; oven = 60°C (isothermal); detector = 200°C. Gas flows were: carrier (helium), 7.5 mL/min, split vent, 68 mL/min; purge vent, 2.0 mL/min; auxiliary nitrogen, 120 mL/min; hydrogen, 75 mL/min; oxygen, 20 mL/min. The retention time of PH_3 was ≈2 min.

2.6.2. Analytical method validation

To assess response linearity, 7 Zn_3P_2 standards ranging in concentration from 0.090 to 8.96 μg Zn_3P_2/mL headspace (8 to 806 μg Zn_3P_2) were prepared. The appropriate amount of Zn_3P_2 standard material was weighed on a microbalance (Model C-31, Cahn Instruments, Inc., Cerritos, CA) and transferred to separate 125 mL Erlenmeyer flasks which were then prepared and analyzed in triplicate as described for homogenate aliquots. Linear regression analysis of standard chromatographic response (y-axis) versus Zn_3P_2 concentration (x-axis) yielded an r^2 of 0.9987, with a slope of 1060.85 (SAS Institute, 1989). The y-intercept was -21.42 ± 53.67, and was not significantly different from zero ($p = 0.5551$).

Nevertheless, examination of response factors (Zn_3P_2 concentration/response) indicated a non-linear trend across the concentration range. For sample quantification, 5 Zn_3P_2 standards ranging from 0.09 to 8.96 Zn_3P_2/mL headspace were prepared and analyzed daily; the y-intercept was used to calculate Zn_3P_2 content in carcass homogenate aliquots run concurrently.

To assess bias and repeatability, 10 control carcasses were assigned to each of 3 fortification levels, and fortified by placing 0.7, 2.5, or 8.0 mg Zn_3P_2 into the stomach. Carcasses were then processed as previously described. Mean (±SD) per cent recoveries for the 0.7, 2.5, and 8.0 mg Zn_3P_2 levels were 38 (±17)%, 48 (±12)%, and 64 (±13)%, respectively. Confidence intervals (95%) for the 3 fortification levels were 26–48%, 39–57%, and 55–73%, respectively.

The MLOD, defined as the amount of sample Zn_3P_2 required to produce a PH_3 signal equal to 3 × the baseline noise, was determined to be 0.03 mg Zn_3P_2/vole carcass. No matrix-related chromatographic interferences were observed.

2.7. Data analysis

Summary statistics ($\bar{x}$, SD, minimum-maximum) of vole weight, bait-ingestion, time-to-death and carcass-residue data were used to characterize amounts of Zn_3P_2 found in carcasses. Analytical method validation utilized the Statistical Analysis System (SAS) PROC REG Program (SAS Institute, 1989).

3. Results and discussion

Table 1 presents the pelted/depelted body weight (g), ingested SRO groats (#), time-to-death (h:min), Zn_3P_2 dose estimates (mg/kg) and carcass Zn_3P_2 residue (mg) for each vole.

3.1. Mortality

Four (3♂ 1♀) Zn_3P_2-baited voles died in an average of 6 h 24 min (±1:43) post-bait exposure; the remaining 2

Table 1
Body weights (g), SRO groats ingested (#), time-to-death (h:min), Zn_3P_2 dose estimates (mg/kg) and depelted-carcass-ZN_3P_2 residues for Zn_3P_2-baited and analytical (control) gray-tailed voles

Vole (gender)	Body Weight		SRO Groats Ingested (#)	Time to Death (h:mm)	Zn_3P_2 dose estimates (mg/kg)	Carcass ZN_3P_2 (mg)[1]
	Death (pelted) (g)	Analysis (depelted) (g)				
1♂	36	30.2	3	7:29	38.2	0.16±0.01
2♀	28	24.7	5	7:00[2]	82.1	0.25±0.02
3♂	23	20.1	5	3:55	100.0	1.80±0.12
4♀	25	21.1	5	7:15	92.0	0.15±0.00
5♂	33	29.0	5	7:18	69.7	0.11±0.09
6♀	40	35.2	5	7:00[2]	57.5	0.07±0.01
Analytical (unbaited)						
1♂	29	24.3	—	—	—	<MLOD[3]
2♀	37	31.5	—	—	—	<MLOD

[1] Mean ± SD of triplicate specimen analyses; quality control analyses of 3 ZN_3P_2-spiked carcasses yielded ± SD recoveries of 18±3.8, 21±5.7, and 40±2.0% — highly variable estimates reflecting on the precision of the current validated method.
[2] Euthanized; showed no signs of toxicosis at 7 h post baiting.
[3] MLOD =0.009 mg Zn_3P_2.

test voles (♀) were euthanized at 7 h post bait presentation because of darkness and decreased temperatures. Mean time-to-death was almost identical to the 6 h 22 min(±2:52) observed by Sterner and Mauldin (1995) in an earlier residue study involving prairie voles (*M. ochrogaster*) fed *ad libitum* 2% SRO groat bait.

3.2. Zn_3P_2 doses

Although no median-lethal-dose value (LD_{50}) of Zn_3P_2 has been reported for gray-tailed voles, acute-oral LD_{50} values in meadow and prairie voles are 15.2–18.0 and 16.2 mg/kg, respectively (see Bell, 1972; Hood, 1972).

A mean (±SD) 4.7 (±0.8) particles were consumed by the test voles (i.e., Vole 1 ate 3 groats and Voles 2–6 ate 5 groats each). Based on PDA, Vole 1 was estimated to ingest 1.38 mg of Zn_3P_2 and Voles 2–6 ingested 2.30 mg Zn_3P_2. Mean (±SD) estimated Zn_3P_2 doses for test voles equaled 73.25 (±22.9) mg/kg, with minimum and maximum ingested doses of 38.2 mg/kg (Vole 1) and 100.0 mg/kg (Vole 3), respectively. Assuming a LD_{50} of 18.0 mg/kg for gray-tailed voles (see Bell, 1972; Hood, 1972), dosage data showed that the test animals ate between 2.1 (Vole 1) and 5.6 (Vole 3) LD_{50s} of Zn_3P_2.

3.3. Zn_3P_2 depelted-carcass residues

A mean (±SD) 0.42 (±0.68) mg Zn_3P_2 residue was found in the 6 test vole carcasses, with minimum-maximum Zn_3P_2 residues of 0.07 (Vole 6) and 1.80 mg (Vole 3). Analyses of control (unbaited) vole carcasses yielded Zn_3P_2 residues <MLOD.

Results add to the existing database of Zn_3P_2 residues in voles (see Tkadlec and Rychnovsky, 1990; Sterner and Mauldin, 1995). Research has shown that the remainder of ingested Zn_3P_2 is typically low in baited vole carcasses. Using a colorimetric method to quantify Zn_3P_2, Tkadlec and Rychnovsky (1990) reported that ≈58% (±25%) of total ingested Zn_3P_2 was found in the GI tracts of Zn_3P_2-killed voles; only 0.3% (±0.3%) of ingested Zn_3P_2 was found in the remainder of carcasses. Whereas, Sterner and Mauldin (1995) observed that whole-carcass Zn_3P_2 residues averaged 1.73 mg (min-max: 0.31–4.95)—≈25–50% of ingested amounts—under conditions of *ad libitum* feeding.

Minimum-maximum LD_{50} or approximate lethal dose (ALD) values cited for some mammalian and avian groups are: rodents 6.8–40.0 mg/kg, canids 40.0–93.0 (ALD) mg/kg, felids 40 mg/kg (ALD), gallinaceous birds 8.8–26.7 mg/kg, passerines 23.7–178.0 mg/kg, raptors >20.0 mg/kg, and waterfowl 7.5–67.4 mg/kg (see Johnson and Fagerstone, 1994). Although depawed/depelted residue analysis affords improved chemical analysis and homogeneity of tissue aliquots, these data could distort actual estimates of Zn_3P_2 available to scavengers/predators. That is, scavenger/predator ingestion of paws, pelts, and tails of voles adds bulk and slows carcass consumption by these animals/birds. Sterner and Mauldin (1995) and Sterner (1996) have already reported that high numbers of vole carcasses (>5) would have to be consumed by even lightweight (1–2 kg) cats and dogs during relatively continuous feeding to deliver a lethal dose of Zn_3P_2/PH_3. The 'worst case' residue of 1.80 mg Zn_3P_2 observed in the current sample would equate to the required short-term ingestion of over 100 carcasses for a 5 kg dog (ALD=40 mg/kg).

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