Determination of Zinc Phosphide Residues in the California Ground Squirrel *(Spermophilus beecheyi)* by Gas Chromatography−Flame Photometric Detection

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Zinc phosphide (Zn_3P_2) recovery from small mammal tissues is assessed for the first time by an analytical method developed for the determination of Zn_3P_2 residues in the gastrointestinal (GI) tracts of California ground squirrels *(Spermophilus beecheyi)* by gas chromatography−flame photometric detection (GC−FPD). GI tracts from field-collected squirrels were removed, and the stomachs and intestines were separated. Emptied stomachs were fortified with varying combinations of Zn_3P_2 and partially digested range grass; intestines were fortified with Zn_3P_2 only. Tissues were processed and acid hydrolyzed in a sealed flask to produce phosphine (PH_3) gas, and samples of the PH_3-containing headspace gas were analyzed by GC. Recovery data were used to develop predictive linear equations relating tissue weights and Zn_3P_2 observed with actual Zn_3P_2 residues. The validated linear range was 0.1−103.4 μg of Zn_3P_2/mL headspace (0.102−100.3 mg of Zn_3P_2/sample, $r^2 = 0.9993$). The limits of detection of the method were 0.015 mg (≈250 ppb) and 0.013 mg (≈154 ppb) in stomachs and intestines, respectively. No chromatographic interferences were observed.

Keywords: *Zinc phosphide; California ground squirrel; Spermophilus beecheyi; gas chromatography−flame photometric detection*

INTRODUCTION

Zinc phosphide (Zn_3P_2) has been extensively used as a rodenticide since the early part of this century (Chitty and Southern, 1954). Although Zn_3P_2 is virtually insoluble in any solvent, it is hydrolyzed in acidic or basic solutions, producing free zinc ions and phosphine (PH_3), the latter being a highly diffusive and extremely toxic gas. Following ingestion, Zn_3P_2 is hydrolyzed by hydrochloric acid in the stomach, and the resultant PH_3 absorbed and distributed throughout the body (Andreev et al., 1959).

With the introduction of sodium monofluoroacetate (Compound 1080), strychnine, and various anticoagulants for rodenticidal use beginning in the 1950s, interest in Zn_3P_2 usage decreased. However, because of the environmental concerns posed by the widescale use of these compounds, Zn_3P_2 re-emerged as a safer alternative. It is currently registered for use in a wide variety of agricultural and nonagricultural situations (Johnson and Fagerstone, 1994) and is the only rodenticide registered for broadcast use. Zn_3P_2 is most frequently applied in a grain bait matrix, usually adhered to oats, corn, or wheat.

Two Zn_3P_2-oat bait formulations (1.0 and 2.0% active ingredient) are currently used in the control of the California ground squirrel (*Spermophilus beecheyi*) on annual rangeland in California. To maintain the registration of these formulations, additional field efficacy studies were required by the U.S. Environmental Protection Agency. Zn_3P_2 tissue residue data were needed to assess the secondary hazards associated with the use of these baits, and this required the development and validation of an analytical method for the quantification of Zn_3P_2 residues in California ground squirrels.

Few studies have attempted to directly measure Zn_3P_2 residues in mammals. The analytical methods used in these studies required acid hydrolysis of Zn_3P_2 to produce PH_3, which was then either measured directly via gas chromatography (GC) or reacted with other compounds to form light-absorbing complexes measured colorimetrically. Values obtained were then converted to Zn_3P_2 equivalents. Terzić (1984) reported the development of a methodology involving the conversion of Zn_3P_2 hydrolysis-produced PH_3 to phosphide by precipitation with silver nitrate. This was followed by the solubilization of the precipitate and subsequent creation of a molybdenum blue complex for spectrophotometric analysis. Although characterized primarily for technical samples, the method was claimed to be effective for the determination of Zn_3P_2 residues from poisoned animals. However, no tissue recovery, repeatability, or residue data were reported. To assess Zn_3P_2 residues in the gastrointestinal (GI) tracts and corresponding carcasses of the common vole (*Microtus arvalis*), Tkadlec and Rychnovský (1990) used the colorimetric method described by Križan et al. (1981), in which PH_3 was absorbed into a 0.5% solution of the silver salt of mercaptobenzimidazole in redistilled pyridine. Tabata (1986) used a semiquantitative colorimetric assay to analyze Zn_3P_2 residues in the GI tract and various organs of a single red-backed wood mouse (*Apodemus speciosus*). Matschke et al. (1983) reported Zn_3P_2 residues in the stomachs of Richardson's ground squirrels (*Spermophilus richardsonii)* analyzed by the headspace GC−flame photometric detection (FPD) method developed by Okuno et al. (1975); this method was originally validated for the analysis of Zn_3P_2 in range vegetation. Sterner and Mauldin (1995) modified this same method to analyze both Zn_3P_2 and PH_3 residues in the carcasses of meadow (*Microtus pennsylvanicus*) and prairie voles (*M. ochrogaster*).

Method characteristics such as linearity and limit of detection were occasionally reported in these mammal studies, but matrix-specific recovery and repeatability data were never presented. Such data are important because PH_3 is highly reactive and easily adsorbed and oxidized in organic matrices, thus leading to artificially low estimates of Zn_3P_2 content. Published reports (Berck and Gunther, 1970; Robison and Hilton, 1971; Hilton and Mee, 1972; Terzić, 1984) and work in our laboratory (unpublished data) indicate that PH_3 recovery from plant products and animal tissues is variable and incomplete. Terzić (1984) reported that recovery of Zn_3P_2 residues from tissue was lower than that from Zn_3P_2 technical samples, and postulated that this result was due to degradation on first contact with wet tissues. Tkadlec and Rhychnovsky (1990) recovered an average of 57.8% (±25.4%) of the total Zn_3P_2 ingested by common voles, but did not determine the percentage of PH_3 lost to the tissue matrix during hydrolysis. Quantification of PH_3 recovery from the matrix of interest is critical to the accurate determination of Zn_3P_2 residues.

Total Zn_3P_2 tissue residues have been shown to be localized almost entirely in the GI tract in small mammals. Matschke and Andrews (unpublished data) found no significant quantities of Zn_3P_2 outside the GI tract in black-tailed prairie dogs (*Cynomys ludovicianus*) fed a 2% Zn_3P_2-oat bait. Tkadlec and Rhychnovský (1990) found only 0.3% of the total amount of a 5% Zn_3P_2-bait ingested by common voles to be located outside the GI tract. Tabata (1986) qualitatively analyzed all the organs, brain, GI tract, urine, and feces in a red-backed mouse force-fed 10 mg of Zn_3P_2 and found no Zn_3P_2 activity outside the GI tract.

Because Zn_3P_2 is primarily confined to the GI tract, these findings indicate that it is unnecessary to analyze the entire carcass to quantify Zn_3P_2 residues in rodents. To determine whole body Zn_3P_2 residues, the headspace GC−FPD method of Okuno et al. (1975) was modified and validated for the determination of Zn_3P_2 residues in the GI tracts of California ground squirrels. Headspace GC methods provide substantial advantages in sensitivity, specificity, ease of sample preparation, and PH_3 sampling when compared with colorimetric methods. The need for the assembly of an involved apparatus for the generation and quantitative collection of PH_3 common to all colorimetric methods was also eliminated.

MATERIALS AND METHODS

Sample Collection. One hundred nine ground squirrels were collected in Alameda County, California, in March 1991 and March 1994 from an area where no previous Zn_3P_2 baiting programs had been conducted. Carcasses were immediately placed in sealed plastic bags, frozen, shipped to the Denver Wildlife Research Center (DWRC), and kept frozen (−30 °C) until processed. Ground squirrels were of both sexes, ranged in size from 350 to 950 g, and were removed from annual rangeland supporting the following plant species: frying pan (*Eschscholzia minutiflora*), pink spineflower (*Chorizanthe membranacea*), mediterranian barley (*Hordeum murinum* ssp. *leporinum*), nitgrass (*Gastridium ventricosum*), wild oats (*Avena fatua*), cheatgrass (*Bromus tectorum*), soft chess (*B. hordeaceous*), ripgut brome (*B. diandrus*), coast live oak (*Quercus agrifolia*), and valley oak (*Q. lobata*). In March, annual grassland vegetation in California is green and actively growing, and the stomach contents of these ground squirrels reflected this diet.

An additional 41 ground squirrels were also collected from Alameda County, California, frozen, and shipped to the DWRC in July 1994 to complete validation. Stomach contents of these animals indicated a diet of seeds, dried grasses, and acorns.

During method development, analysis of the entire GI tract as one sample presented several technical obstacles. Superior results were obtained by separation and individual analysis of the stomach and intestines.

Sample Preparation. *General.* Prior to method validation, several carcasses collected in March were partially thawed. A small, midline thoracic incision was made in these carcasses to expose the stomach, which was then opened, and the frozen contents were removed. This procedure provided a pool of partially digested range grasses (hereinafter referred to as "grass") to be used for subsequent stomach fortifications.

To assess the interaction of varying amounts of Zn_3P_2 and stomach/intestine contents on Zn_3P_2 recovery, carcasses from the March collections were randomly assigned to a design matrix in which stomachs were fortified with one of four levels of grass (0, 15, 35, and 50 g) and four levels of Zn_3P_2 (0.5, 2.5, 10, and 50 mg). Three carcasses were randomly assigned to each of the 16 Zn_3P_2/grass combinations. Intestines were fortified with the same amount of Zn_3P_2 used in the corresponding stomach, but analyzed with original contents intact.

Prior to sample preparation, a small (≈2.5-cm diameter) circle of plastic wrap was placed in the throat of a large (100-mm diameter) glass funnel to temporarily occlude the opening. The funnel was then inserted into the mouth of a volume-calibrated 1000-mL Erlenmeyer flask.

To fortify the tissues, the preweighed carcasses were thawed, and the GI tracts were removed by severing the esophagus ≈1.5 cm anterior to the stomach and severing the distal colon as close to the rectum as possible. The entire GI tract was then weighed.

Stomachs. The stomach was separated from the intestines, weighed, opened by making an incision along the greater curvature, cleaned of all contents, and reweighed. Stomach contents were also weighed, and grass from the pool was added or original stomach contents were removed to achieve the appropriate fortification weight. Contents were then placed into the glass funnel/flask that were previously described. The stomach was spread out (exterior surface exposed), rinsed twice with water, and dried, and the appropriate amount of Zn_3P_2 was added. The stomach was then folded over and transferred to the funnel containing the grass. Funnel contents, still partially frozen, were then minced thoroughly with sharp scissors (8 in. shear-type, Fiskars) and prevented from passing through the funnel by the plastic wrap circle. When mincing was complete, the scissors were used to displace and mince the plastic, and to push the entire contents down into the flask. The scissors and funnel were then rinsed with five 10-mL aliquots of water, and the rinsate was allowed to drain into the flask.

Immediately following the rinse, 50 mL of 30% (v/v) sulfuric acid were added to the flask, which was then sealed by inserting a rubber sleeve stopper (size 15, 16-mm plug diameter, 19-mm sleeve length, Fisher Scientific) and pulling the stopper sleeves down tightly around the outside of the flask mouth. The flask was then shaken at high speed for 90 min in a mechanical shaker (Equalpoise, model 6550, $2^3/_8$ in. stroke, Eberbach, Ann Arbor, MI). Studies performed during method development showed negligible PH_3 formation in the absence of acid following the tissue mincing/rinse step. For subsequent recovery calculations, the weights of the grass and stomach tissue were added together to give total tissue weight. The volume of the combined tissues (≈1 g/mL) and the 100 mL acid/rinsate were then subtracted from the total headspace volume.

Intestines. As with the stomachs, intestinal contents were partially frozen, making complete content removal for subsequent controlled fortification extremely difficult. Therefore, intestines were not fortified with additional grass but were analyzed with original contents intact.

Each intestine was fortified with the same amount of Zn_3P_2 used to fortify the corresponding stomach. Zn_3P_2 fortification was accomplished by making a small incision in the upper large intestine, moving the contents, and splaying the tissue to create an empty, flattened pocket into which the appropriate amount of Zn_3P_2 was placed. The pocket was closed, and the

intestines were transferred to a second funnel, processed, and hydrolyzed as previously described for stomachs.

An additional level of Zn_3P_2 fortification (30 mg) was subsequently added. A shortage of March-collected carcasses made it necessary to use carcasses collected in July to complete Zn_3P_2 fortification at the new level. To determine if recovery data from July carcasses could be successfully integrated with the March recovery data to form an overall data set combining carcasses from both collection times, it was necessary to demonstrate equivalent Zn_3P_2 recoveries from both tissue matrices. An additional study was performed repeating six of the Zn_3P_2/grass combinations described previously. There were insufficient July carcasses to repeat the entire 16 block matrix, so stomachs were fortified with one of two levels of grass (15 and 35 g) and one of three levels of Zn_3P_2 (2.5, 10, and 50 mg). A pool of stomach contents was collected from several July carcasses and used for these fortifications. Two carcasses were randomly assigned to each group. Intestines were fortified with the same amount of Zn_3P_2 used in the corresponding stomach, with original contents left intact. Zn_3P_2 recoveries from stomachs and intestines in July carcasses were then compared statistically with recoveries from March tissues at corresponding Zn_3P_2/grass interaction levels.

Upon determining that Zn_3P_2 recovery was equivalent between collection time matrices, carcasses collected in July were used to assess Zn_3P_2 recovery at the additional 30 mg of Zn_3P_2 level. Stomachs were fortified with 0, 15, 35, and 55 g of grass, with three carcasses per weight. As before, intestinal contents were not modified.

Analytical Methods. A Hewlett-Packard 5890 Series II gas chromatograph (Walbronn, Germany) equipped with a flame photometric detector fitted with a phosphorus-specific filter (526 nm) was used for all analyses. The GC conditions were as follows: purge time, 1 min; injector temperature, 70 °C; detector temperature, 190 °C; oven temperature, 60 °C, isothermal; headspace volume injected, ≈10 μL, splitless; split vent flow, 100 mL/min; purge vent flow, 4.1 mL/min; carrier gas, helium, at ≈5.5 mL/min; detector gasses: auxiliary gas, nitrogen, 120 mL/min; oxygen, 28 mL/min; hydrogen, 75 mL/min, analytical column, GS-Q Megabore (J&W Scientific, Folsom, CA), 30 m × 0.54 mm i.d., 0.25-μm film thickness.

The GC was equipped with a pneumatically actuated six-port gas sampling valve (Valco Instrument Company, Inc., Houston, TX; model DC6WP) and a mechanical vacuum pump (Edwards High Vacuum, Crawley, Sussex, England; model E2M-1). A 1-m tefzel transfer line (0.3 mm) fitted with a 7-μm particulate trap and a 25-gauge disposable syringe needle was used to deliver the phosphine-containing gas sample from the sample vessel headspace to the 100-μL injection loop.

The injection loop was loaded by drawing the headspace gas through the loop with reduced pressure for 0.14 min. The gas sample was immediately swept into the GC injection port with the carrier gas by switching the six-port valve. The valve was switched back to the load position after 1 min. The GC injection port was equipped with a borosilicate glass Cyclosplitter injection sleeve.

Sample Pressure-Injection Bias. During method development, an increase in headspace pressures in flasks containing stomach and intestinal tissues from control animals following acid hydrolysis was noted. This increase was presumably due to gas generation by the sample matrix. Pressure increases (over ambient) ranged from 2.8 to 15.4 mmHg in flasks containing stomachs and from 20.8 to 64.9 mmHg for intestines. In previous work in our laboratory, a flask pressure-related signal bias was observed when performing headspace sampling by hand injection, resulting in decreased response with increasing pressure. To determine if a similar bias existed with the use of the previously-described pneumatic sampling system, an experiment was performed comparing chromatographic responses from hand versus pneumatic injections from a series of flasks with identical Zn_3P_2 standard concentrations and serially increasing headpressures. Seven empty flasks were volume-corrected with water to give identical headspace volumes. A 1.0 mg aliquot of Zn_3P_2 was added to the first flask, followed by 40 mL of 30% H_2SO_4. The flask was then stoppered and shaken for 30 min. The flask was randomly assigned to one of seven pressures ranging from ambient to ambient + 72 mmHg (in ≈12 mmHg increments). Compressed air was then injected into the flask, and pressure was monitored simultaneously with a digital manometer (model 2655; Yokogawa, Newnan, GA) until the appropriate pressure had been reached. Flask contents were shaken to mix, and then sampled in duplicate by hand and pneumatic injections. The procedure was repeated with each flask individually.

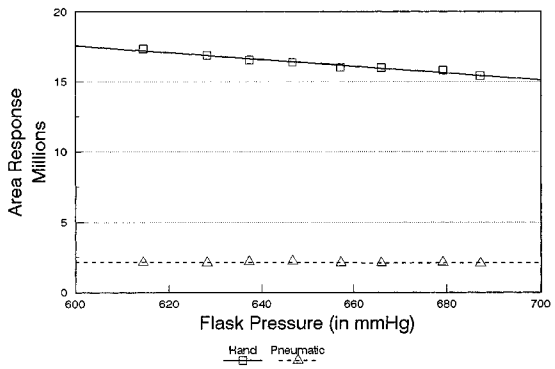

Figure 1. Injection type versus flask pressure.

Selectivity. Five ground squirrels (three, March; two, July) were prepared and analyzed by the procedures described previously. Stomach tissues were fortified with 55 g of grass from the appropriate grass pool, and intestines were analyzed with original contents intact. Zn_3P_2 was not added to either tissue.

Method Limit of Detection. For the purposes of this study, the method limits of detection (MLOD) were calculated as the concentration of Zn_3P_2 in a sample required to produce a chromatographic peak response equal to 3 times the noise observed in the baseline of non-Zn_3P_2 fortified samples at the retention time of PH_3. The MLODs for stomach and intestines were determined from the mean chromatographic response obtained from six ground squirrels. Stomachs were fortified with 55 g of grass and ≈0.056 mg of Zn_3P_2. Intestines ranged in weight from 83.04 to 94.60 g, were fortified with ≈0.170 mg of Zn_3P_2, and were analyzed with original contents intact.

Statistical Analysis. Initially, multiple regression analyses were performed on the recovery data from the March-collected ground squirrels and linear models were constructed to relate observed amounts of Zn_3P_2 and corresponding tissue weights with known Zn_3P_2 fortification levels in whole stomachs and intestines. Next, to support the inclusion of July carcass tissues in the model building process, recovery data from the July carcass fortification experiment were analyzed as a two-factor factorial experiment in a randomized block design to test effects on Zn_3P_2 recovery due to the difference in collection times (March versus July) and the interaction of collection times with grass fortification levels.

Finally, to determine if recovery data from the July-collected animals fortified with 30 mg of Zn_3P_2 could be integrated into the original March data set, prediction intervals based on the original equations were calculated for observed values from the actual 30-mg samples. Coverage by one-at-a-time confidence intervals (Graybill, 1976) was used as an acceptance test for these data. The predictive model-building procedure was then repeated, incorporating the 30-mg fortification data. Use of the resulting equations produced estimates of total Zn_3P_2 residues in unknown samples (including the 95% confidence interval for that value) based on the sample weight and quantity of Zn_3P_2 detected.

RESULTS AND DISCUSSION

Sample Pressure/Injection Bias. Results from the study comparing chromatographic responses from hand versus pneumatic sampling of pressure-varied flasks are illustrated in Figure 1. Responses from the pneumatic injections showed little variation with increasing

Table 1. Comparison of Mean Zn_3P_2 Recoveries from March versus July Carcasses[a]

matrix	wt of grass added (g)	sampling period	Zn_3P_2 fortification level (mg) 2.5	10	50
stomach	15	March[b]	83.7 (5.1)	91.7 (1.4)	96.9 (0.95)
		July[c]	80.1 (7.4)	89.6 (2.1)	96.8 (1.2)
	35	March[b]	81.6 (1.5)	88.4 (1.4)	91.1 (0.5)
		July[c]	75.5 (4.6)	86.0 (0.3)	91.2 (1.8)
intestines	0	March[d]	71.6 (5.1)	79.9 (4.5)	87.4 (4.1)
		July[e]	71.0 (5.1)	80.1 (4.8)	87.4 (2.4)[f]

[a] Values in parentheses represent one standard deviation. [b] $n = 3$. [c] $n = 2$. [d] $n = 12$. [e] $n = 5$. [f] $n = 4$.

pressure, but a definite pressure-related decrease in signal strength was observed in the hand injected samples. This decrease amounted to a 12.7% loss of response over a range of headpressures typically found in ground squirrel tissues processed by this method. A similar pressure-induced signal bias from hand injected samples was observed by Okuno et al. (1975), who attributed sample pressure buildup in vegetation to acid hydrolysis formation of carbon dioxide, and explained the signal bias as the result of syringe needle sample loss due to pressure equalization following withdrawal from the sample flask.

These problems were alleviated by the use of the pneumatic sampling system, by which headspace gas was withdrawn under reduced pressure and delivered directly to the GC column head. The sample was never exposed to ambient pressure, and resultant equalization losses did not occur.

Bias and Repeatability. Mean percent recoveries and standard deviations from the experiment comparing Zn_3P_2 recovery in stomachs and intestines from July-collected carcasses with those collected in March are shown in Table 1. No effect on Zn_3P_2 recovery due to collection times was indicated for stomachs as a main effect ($p = 0.2356$) nor in an interaction with grass fortification ($p = 0.4181$). Similarly, no effect due to collection time could be detected in the recovery data for intestines ($p = 0.5290$).

These results clearly demonstrated that there were no differences in Zn_3P_2 recovery from March versus July carcasses at various Zn_3P_2/grass interaction levels, and supported the validity of including recovery data from the July carcasses fortified at the 30-mg Zn_3P_2 level into an overall data set used in building final predictive linear equations.

Percent recovery data for all Zn_3P_2 and grass-fortified stomachs are shown in Table 2, with associated means, standard deviations, and coefficients of variation. Zn_3P_2 recoveries ranged from 55.5 to 100% and, at each grass fortification level, generally increased as Zn_3P_2 fortification levels increased. Conversely, recovery at each level of Zn_3P_2 fortification usually decreased as the amount of grass increased. Coefficients of variation ranged from 0.51 to 13.5%, with an average of 3.99 ± 3.89%, indicating a high degree of repeatability.

Using the statistical procedures described, recovery data from the July-collected ground squirrels fit very well into the interval predicted using recovery data from the March-collected animals. Combining both sets of data allowed the construction of a final predictive linear equation for the estimation of actual Zn_3P_2 residues based on those observed. The predictive equation and R^2 for the estimation of total stomach Zn_3P_2 residues are given in eq 1:

$$\text{total } Zn_3P_2 \text{ residue} = -1.0433 + 0.419\,(\text{total weight, g}) + 1.0575\,(Zn_3P_2 \text{ observed, mg}) \quad (1)$$

$$R^2 = 0.9973$$

Equation 1 indicates that total weight (grass + stomach) and amount of Zn_3P_2 were found to be important predictors of recovery.

Percent recovery data for Zn_3P_2-fortified intestines are shown in Table 3. Intestinal weights ranged from 40.12 to 129.26 g and are shown in parentheses. Zn_3P_2 recoveries ranged from 54.3 to 87.2%, generally increasing as Zn_3P_2 fortification amounts increased, regardless of tissue weight. This relationship indicated that, unlike the stomach, intestinal weight did not relate to recovery. The predictive equation and R^2 are given in eq 2:

$$\text{total } Zn_3P_2 \text{ residue} = 0.5563 + 1.1302(Zn_3P_2 \text{ observed}) \quad (2)$$

$$R^2 = 0.9965$$

Total GI tract Zn_3P_2 residues were obtained by adding stomach and intestinal residues.

Response Linearity. Prior to analysis of fortified tissue samples, a single set of seven Zn_3P_2 standards was prepared with concentrations ranging from 0.1 to 103.4 μg of Zn_3P_2/mL of headspace (equivalent to ≈0.102−100.3 mg of Zn_3P_2 in tissues). Headspace samples from each standard were injected in triplicate. The linear regression analysis of chromatographic peak area (y-axis) versus Zn_3P_2 concentration (x-axis) generated a slope of 154 171, a y-intercept of 72 869, and an r^2 of 0.9993. The y-intercept, although appearing large, was not significantly different from zero ($p = 0.10$, $\alpha = 0.05$) according to the SAS PROC REG program (SAS, Inc., 1989). Additionally, a log versus log regression of the same data yielded a slope of ≈1.00 (1.0075), indicating a linear, directly proportional response. Response factors (Zn_3P_2 concentration/response) were virtually identical across the entire Zn_3P_2 concentration range, with a mean value of 6.323×10^{-6}, a range of 5.802×10^{-6} to 6.789×10^{-6}, and a coefficient of variation of 3.37%. Regression analysis of response factors versus concentration data showed that the null hypothesis (slope = 0) could not be rejected ($p = 0.33$, $\alpha = 0.05$). These results indicated a highly linear and directly proportional relationship between detector response and Zn_3P_2 concentration, permitting the use of a single-point calibration (≈10 mg of Zn_3P_2) for sample analysis.

Selectivity and Limit of Detection. No chromatographic interferences were observed in stomach or intestine samples from any of the five ground squirrels processed. Limits of detection were 0.015 (≈250 ppb) and 0.013 mg (≈154 ppb) for stomach and intestine, respectively. Since stomachs had been fortified with 55 g of grass, the stomach MLOD represented a worst-case estimate.

Conclusion. The described method allows for the quantitative recovery and assessment of Zn_3P_2 residues in animal and ingested plant tissues. Furthermore, experience in our laboratory has shown that this method can easily be scaled down to accommodate smaller samples. The method was demonstrated to be sensitive, specific, repeatable, and highly linear over the Zn_3P_2 concentration range of interest; requiring less compli-

Table 2. Percent Zn_3P_2 Recovery from Stomachs

	zinc phosphide wt (mg)				
grass wt (g)	0.5	2.5	10	30	50
0	87.6	96.9	97.0	101	96.9
	86.5	92.3	94.6	98.7	99.7
	92.7	94.7	97.4	101	99.4
	$\bar{x} = 88.9 \pm 3.3$	$\bar{x} = 94.6 \pm 2.3$	$\bar{x} = 96.3 \pm 1.5$	$\bar{x} = 100 \pm 1.3$	$\bar{x} = 98.7 \pm 1.5$
	CV = 3.7	CV = 2.4	CV = 1.6	CV = 1.3	CV = 1.5
15	81.0	78.3	91.8	96.1	97.2
	66.9	88.5	90.2	96.1	95.8
	80.3	84.2	93.0	94.7	97.6
	$\bar{x} = 76.1 \pm 7.9$	$\bar{x} = 83.7 \pm 5.1$	$\bar{x} = 91.7 \pm 1.4$	$\bar{x} = 95.6 \pm 0.81$	$\bar{x} = 96.9 \pm 0.95$
	CV = 10.4	CV = 6.1	CV = 1.5	CV = 0.85	CV = 0.98
35	75.6	80.1	88.7	92.5	90.7
	72.1	83.1	86.8	91.6	87.6
	64.5	81.5	89.6	91.8	84.9
	$\bar{x} = 70.7 \pm 5.7$	$\bar{x} = 81.6 \pm 1.5$	$\bar{x} = 88.4 \pm 1.4$	$\bar{x} = 92.0 \pm 0.47$	$\bar{x} = 87.7 \pm 2.9$
	CV = 8.1	CV = 1.8	CV = 1.6	CV = 0.51	CV = 3.3
55	63.2	72.9	84.0	89.8	91.5
	48.2	63.6	81.2	87.7	91.2
	55.2	77.1	71.6	91.6	90.5
	$\bar{x} = 55.5 \pm 7.5$	$\bar{x} = 71.2 \pm 6.9$	$\bar{x} = 78.9 \pm 6.5$	$\bar{x} = 89.7 \pm 2.0$	$\bar{x} = 91.1 \pm 0.51$
	CV = 13.5	CV = 9.7	CV = 8.2	CV = 2.2	CV = 0.56

Table 3. Percent Zn_3P_2 Recovery from Intestines[a]

	fortification level (mg)				
	0.5	2.5	10	30	50
1	60.1 (44.21)	68.1 (70.39)	73.1 (74.38)	85.2 (51.45)	89.7 (68.33)
2	45.3 (87.64)	67.8 (119.12)	77.4 (104.96)	89.8 (50.35)	84.2 (88.10)
3	45.9 (78.45)	77.7 (70.06)	81.1 (40.16)	89.8 (40.08)	91.5 (40.46)
4	57.3 (40.12)	77.9 (40.36)	86.5 (64.90)	82.6 (52.70)	89.9 (98.37)
5	41.1 (66.76)	74.7 (85.76)	78.2 (109.18)	82.7 (40.11)	84.0 (55.71)
6	65.4 (74.13)	70.1 (90.03)	84.0 (48.09)	88.6 (35.61)	89.3 (114.3)
7	61.4 (96.10)	73.5 (68.48)	80.7 (96.74)	86.2 (39.00)	82.1 (105.3)
8	62.7 (73.06)	77.0 (67.95)	82.6 (63.39)	87.6 (33.11)	84.7 (99.39)
9	56.3 (72.10)	71.9 (64.82)	86.2 (81.26)	88.7 (48.30)	87.8 (57.23)
10	53.4 (104.80)	64.0 (74.41)	77.8 (97.23)	90.1 (50.23)	87.5 (52.20)
11	52.6 (129.3)	73.2 (72.60)	78.4 (90.64)	92.1 (35.20)	85.2 (81.80)
12	52.8 (70.08)	62.8 (115.14)	72.9 (112.72)	83.2 (56.86)	85.9 (105.2)
X (%)	54.3% (78.06)	71.6% (78.26)	79.9% (81.97)	87.2% (44.42)	86.8% (80.5)
s (%)	7.5 (24.5)	5.1 (21.8)	4.5 (24.0)	3.2 (8.1)	2.9 (24.9)
CV (%)	13.0 (31.4)	7.1 (27.8)	5.7 (29.3)	3.7 (18.1)	3.3 (30.9)

[a] Intestinal weights given in parentheses.

cated apparatus and procedures than those found in most colorimetric assays. Recovery data from July-collected ground squirrels were easily integrated into the original predictive intervals despite differing dietary components (dried grass, seeds, and acorns versus green grass) indicating a highly rugged method not easily perturbed by matrix variability.

Installation of the pneumatic gas sampling system provided a lower cost alternative to automated headspace sampling systems, and permitted the unbiased, accurate analysis of samples with headspace pressures well in excess of ambient.

The development of linear regression equations that relate recovered Zn_3P_2 and corresponding tissue weights with known Zn_3P_2 fortification levels permitted the accurate assessment of total Zn_3P_2 residues in small mammals. This method has been used to generate the required analytical chemistry data to support field efficacy and secondary hazard studies. To our knowledge, no other published Zn_3P_2 tissue method provides such recovery estimates.

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