Bull Environ Contam Toxicol (2007) 79:459–461
DOI 10.1007/s00128-007-9252-1

Measuring Degradation of Zinc Phosphide Residues in Possum Stomach Contents

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Received: 29 March 2007 / Accepted: 18 July 2007 / Published online: 14 September 2007

Abstract Zinc phosphide (ZnP) has been identified as a potentially cost-effective vertebrate pesticide for possum (*Trichosurus vulpecula*) control in New Zealand. We established methodology for analysis of microencapsulated ZnP formulations (MZP) and investigated the half-life of residual ZnP in the stomach contents of poisoned possums. An interlaboratory study was conducted to compare results of ZnP analysis in stomach contents. The half-life of ZnP was 3.4 days for ZnP in possum stomach contents and 6.7 days in vomit. Subsequent estimates were made of 34 and 67 days, respectively, for residual ZnP to decline to concentrations below the 1 μg/g method detection limit.

Zinc phosphide (ZnP) is widely used as a rodenticide in many parts of the world. Its oral toxicity seems mostly due to hydrolysis on contact with stomach acids, which releases phosphine (Krishnakumari et al. 1980; Casteel and Bailey 1986). A microencapsulated ZnP formulation (MZP) is undergoing registration for control of the brushtail possum (*Trichosurus vulpecula*) in New Zealand. While ZnP is generally considered to present a low risk of secondary poisoning to non-target wildlife (e.g. Johnson and Fagerstone 1994), the greatest hazard presented to a carnivore or scavenger is consuming the digestive tract or vomitus of a recently poisoned animal, which may contain unreacted ZnP (Savarie 1981). Establishment of a validated method for sample preparation and analysis was an important step in this process, and also to ensure a reliable analytical capacity to monitor environmental effects of future field applications of MZP for possum control.

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Materials and Methods

Possums were housed in controlled-environment rooms at the Landcare Research (LCR) animal facility, Lincoln, New Zealand in individual wire cages with removable nest boxes. They were acclimatised for 4 weeks to a palatable loose, cereal-based diet containing 0.1%w/w cinnamon, which is the basis of pelleted bait formulation that would be used to deliver MZP to possums in pest control operations. This was supplemented with fruit and vegetables with water freely available. Stomach contents from four possums fed on this diet were divided into four portions of approximately 125 g each, and fortified with microencapsulated zinc phosphide (MZP, 26.5% ± 1.5% w/w, Pest-Tech Ltd, NZ) to produce treatment concentrations of 0 (control), 54, 108, and 213 μg/g. Each sample was homogenised with a Janke & Kunkel ultra-turrax (IKA-Labortehnik) for 1 min (at 9,500 rpm). The LCR toxicology laboratory, Lincoln, carried out analyses of these samples based on the method of Corley et al. (1998). A 1-g sample was weighed into a 35 mL glass centrifuge tube, to which was added 25 mL of toluene and 10 mL of 5%v/v sulphuric acid. The tube was capped and shaken for 75 min on a horizontal shaker at 180 cycles per minute. The sample was then centrifuged for 5 min at 4°C (1,750× rcf) and placed into a GC vial for analysis. ZnP content was determined by comparison to a known analytical standard. This assay had a stated method detection limit of 1 ppm. Zinc phosphide content was determined using an HP 5890 gas chromatograph coupled nitrogen–phosphorus detector with a rubidium bead. The helium and nitrogen were set at 1 and 30 mL per minute, respectively (105 and 220 kPa) and the hydrogen and air were set at 4 and 110 mL per minute, respectively (138 and 275 kPa). A Hewlett-Packard (HP-5) column was used with a 0.25 mm ID and

0.25 μm film thickness. The GC was held at 35°C for 1 min, then ramped at 20°C per minute to 120°C, then held for 5 min. The injector temperature was 120°C and the detector was 240°C with an injection volume of 1 μL and an analyte retention time of 1.3 min. Toluene, (Mallinckrodt, USA), propylene glycol and sulphuric acid (98%, specific gravity 1.84) were all analytical grade >98% (BDH, UK). Zinc phosphide analytical standard (certified as 80%w/w pure) was purchased from Hacco Inc. Maddison, Wisconsin, USA. All gases used were instrument grade and supplied by BOC New Zealand.

As part of an interlaboratory comparison, 10-g subsamples of the homogenised, MZP-spiked possum stomach contents (eight) were sent for analysis by Environmental Science and Research Ltd., Porirua, NZ (ESR), using a different methodology to that of LCR. Subsamples were placed into separate plastic containers (10mL, SARSTEDT) with no headspace to ensure minimal phosphine loss and stored at –20°C. All samples were frozen at –20°C and were received by ESR within 24 h. ESR used a method described by Heenan et al. (2003).

Fifteen possums were euthanased four hours after ingesting an effective lethal dose (50 mg/kg) of MZP in 20 g food. Stomach contents were removed immediately from three of these animals, and homogenised together as a baseline from which to assess the rate of degradation of residual ZnP in stomach contents over time. Eight subsamples of this baseline mix were analysed for ZnP content. Simulated weathering regimes were established in enclosed plastic compartments in a controlled-temperature room (19 ± 4°C). Four intact possum carcasses, and 50 g of mixed stomach contents (to simulate vomitus) were placed in each of three treatments; wet (daily watering with 10 mL), moderate (watering every fourth day with 10 mL) or dry (no watering). At 2, 4, 10, and 14 days, a 10 g subsample of the 'vomitus' was taken, and the entire stomach and contents removed from one carcass in each treatment. Each subsample of the mixed stomach contents was homogenised and stored in plastic sealable containers at –20°C until analysis. Intact stomachs from carcasses were blended for 1 min and tested in duplicate within 4 h. All samples were analysed by the LCR laboratory using the method described.

The humidity of the weathering enclosures and the pH of exposed, mixed stomach contents were also measured at each sampling time. The pH of stomach contents was assessed by adding equal parts tissue (5 g each) to deionised water, vortexed, and shaken for 1 h at 180 cycles per minute. The extract was centrifuged at 1,500×*g* (rcf) for 5 min and subsequently measured on a Jenway 3310 pH meter. The pH meter was calibrated between 4.0 and 7.0 ± 0.02 pH units (at 20°C) with certified BDH 'Colourkey' buffer solutions. A thermo-hygro humidity meter was used to assess atmospheric water content. All statistical analyses on resulting data were performed using GenStat version 6, VSN International, Oxford, UK (2002). ZnP loss was determined using an exponential decay model ($ZnP = ae^{b*time}$), and half-life was determined from the regression as ($\log_e(2)/b$), where b is the slope of the regression of $\log_e(ZnP)$ against time.

Table 1 Zinc phosphide concentrations (μg/g) measured in an interlaboratory comparison between Landcare Research (LCR) and Environmental Science and Research (ESR), in eight replicates of each of three treatments of possum stomach contents spiked with MZP

	54 μg/g spike		108 μg/g spike		213 μg/g spike	
	LCR	ESR	LCR	ESR	LCR	ESR
	50	13.8	126	20.4	255	77.9
	49	7.67	112	28.8	222	53.4
	47	11.4	106	22.6	190	58.0
	47	7.3	113	32.5	208	38.9
	48	38.1	116	40.2	197	51.1
	43	14.5	97	51.8	183	59.6
	49	12.4	117	37.6	208	74.3
	47	16.8	101	39.9	194	64.9
Mean	48	15.2	111	34.2	207	59.8
SD	2.10	9.8	9.5	10.3	23.0	12.6
% Diff		68		69		71

Results and Discussion

The method used by LCR indicated recoveries of 89%, 103% and 97% compared to recoveries of 28%, 32%, and 28% by ESR. All control blanks were below the detection limit. Concentrations measured by ESR were significantly lower across all three treatments (Table 1). Bias ranged between 3% and 12% for LCR with a precision (coefficient of variation) of 4%–11% and method uncertainty (95% CI) not greater than 16% (6%–16%). In general, as the concentration of ZnP increased so did the precision and bias. The results from ESR had less precision (cv) ranging between 21% and 64%, a large bias (68%–72%), and a method uncertainty (95% CI) ranging between 30% and 91%. The average difference between the results obtained by LCR and ESR (32.44, 76.89, and 147.4 μg/g) was highly significant ($p < 0.001$) for all test groups based on a one sample *t* test (Table 2). Spiked samples were tested within 48 h of preparation by LCR and within 10 working days by ESR. The latter results indicated consistent, substantial losses of phosphine from the samples over this time, so that in future testing for residual ZnP, loss of analyte should be minimised by prompt (within 48 h) analysis. Another cause

Table 2 Statistical differences (one sample *t* test) in zinc phosphide concentrations (μg/g) measured in an interlaboratory comparison between Landcare Research and Environmental Science and Research, in eight replicates of each of three treatments of possum stomach contents spiked with MZP

Spike (μg/g)	Mean difference (95% CI)	Variance	Standard deviation	Standard error of mean	Probability (*p*)
54	32.44 (23.87–41.02)	105.2	10.26	3.626	<0.001
108	76.89 (62.01–91.78)	316.9	17.80	6.294	<0.001
213	147.4 (129.6–165.3)	455.8	21.35	7.548	<0.001

of analyte loss may have been differences in analytical technique. Both laboratories employed sulphuric acid for phosphine evolution from sample matrices, and the gas chromatograph parameters were very similar. However, LCR used toluene to absorb the phosphine whereas ESR used a headspace technique, which required preheating of the sample (40°C) prior to injection on the GC. Given the volatility of ZnP, certain losses maybe attributed to phosphine loss post-acidification.

The mean ZnP concentration of the baseline stomach contents mix ($n = 8$) was 976 μg/g. Previous authors have reported only about half of ingested ZnP and negligible amounts of phosphine were recovered from carcasses, using analytical techniques described by Sterner and Mauldin (1995) and Tabata (1986). Possums fed lethal doses of ZnP gave an average concentration of 783 μg/g (unpub. data).

Log-transformed ZnP concentration was regressed against time to estimate half-life (days) of ZnP in the possum carcasses (half lives with 95% confidence intervals, for stomach contents of carcasses in the dry, moderate and wet treatments, 3.2 (2.1–8.2), 3.9 (1.2–2.9), and 3.0 (1.4–30.6) days, respectively. In the 'vomitus' placed in the dry, moderate and wet treatments half-lives were 7.6 (3.6–69.8), 5.7 (3.7–12.4), and 7.1 (4.2–25.1) days, respectively. The only significant relationship ($p = 0.02$) between ZnP concentration and time was in carcasses in the dry treatment, however, the overall effect in the carcasses (ignoring moisture levels) was highly significant (slope = –0.2050, SE = 0.0345, $t_{10} = 5.95$, $p < 0.001$), with an average half-life of 3.4 days. This effect was also highly significant in the 'vomitus' (slope = –0.1034, SE = 0.0103, $t_{10} = 10.04$, $p < 0.001$), with an average half-life of 6.7 day. Regression analysis of differences in slope and intercepts found no significant differences between any treatments (dry, moderate and wet) either for stomach contents of possum carcasses or 'vomitus'. Using the average half-life for both whole intact possum carcasses (3.4 days) and 'vomitus' (6.7 days) it would take 34 and 67 days, respectively, for residual ZnP to decline to concentrations below the 1 μg/g method detection limit. These durations can be used as a basis for estimating secondary exposure window periods for non-target wildlife, and determining no-access periods e.g. for domestic dogs in areas where MZP has been applied for possum control.

Acknowledgments This was contracted research carried out for the New Zealand Animal Health Board (Project R-10656). We thank Julie Turner, Karen Washbourne, Ryan Moffat and Grant Morriss for assisting with the possum trials, Guy Forrester for statistical advice and Christine Bezar for editing. Thanks also to Phil Cowan and two anonymous reviewers for improvements to the manuscript.

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