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Analysis of Zinc Phosphide in Baits, Water, Soil, and Biological Specimens

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Possums (trichosurus vulpecula) cause major damage to native forests in New Zealand and can transmit bovine tuberculosis to livestock (Meenken et al 1997). Sodium fluoroacetate (1080) is used extensively for possum control but there has been major public concern about its use particularly in relation to secondary poisoning of dogs.

Zinc phosphide is widely registered throughout the world as a toxicant primarily for rodent control. In 1998 it was recognised as a potential alternative to 1080 for possum control in New Zealand (Wickstrom & Eason 1998) and a paste bait formulation containing zinc phosphide was shown to be as least as palatable and effective as pellet bait containing 1080 (*Morgan*). Consequently a recommendation has been made that a bait containing zinc phosphide be registered for possum control (*Morgan*).

Information required to register baits for vertebrate pest control includes the need to accurately measure small amounts of toxicants in media such as bait material, animal tissue, serum, urine and soil and water (Ministry of Agriculture & Forestry 1999). In this study we have validated procedures to measure zinc phosphide levels in the above matrices with a view to assisting companies wishing to register products containing zinc phosphide for possum control in New Zealand.

A number of studies have been conducted to measure phosphide levels in a variety of matrices (Guale et al 1994, Goodall et al 1998, Mauldin et al 1996, Mauldin et al 1997, Sterner et al (1998) by converting phosphide to phosphine. None of the procedures discussed in the above papers however covered the same range of matrices as the present study. In addition while those studies employed gas chromatographic analysis they used flame photometric detection. The present study used a nitrogen phosphorus detector which has also been used successfully for the analysis of phosphide residues in post mortem specimens after suicide by ingestion of aluminium phosphide (Chan et al 1983) and in plant material (Corley et al 1998).

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MATERIALS AND METHODS

Zinc phosphide (Zn_3P_2) powder was provided by Riedel-de Haen (Seelze, Germany) and silica gel 230-400 mesh ASTM by Merck. (Darmstadt, Germany).

The silica gel (20g) was pulverised in a ball mill for 15 minutes. It is advisable to wear a powder mask when pulverising or weighing the powdered mixtures to avoid inhalation of the dusts. Shaking or sudden movements of open containers that could mobilise dust should be avoided. To enable spiking of matrices for preparation of calibration standards and reproducibility determinations, the solid zinc phosphide powder was diluted with silica gel to produce a range of dilutions as given in Table 1. In order to achieve complete mixing, the zinc phosphide and silica were combined with a tumbling and twisting action of the wrist for at least 5 minutes. Shaking or tapping actions that could cause segregation of the dense zinc phosphide particles were avoided. The powder mixtures were re-tumbled just prior to weighing operations. To ensure adequate homogeneity at the higher dilutions (lower concentrations) of zinc phosphide, the dilution 2 mixture (Table 1) was ground to a fine powder by pulverising in the ball mill for 15 minutes. Further dilutions were carried out using this ground mixture and the pulverised silica.

Table 1. Preparation of Zn_3P_2 dilutions in silica gel.

Dilution Stage	Weight Taken, g	Weight Silica Taken, g	Zn_3P_2 Content µg/mg
Stock Dilution	Zn_3P_2 powder, 2.0	10	167
Dilution 1	Stock dilution, 0.3	4.7	10.0
Dilution 2	Dilution 1, 0.4	9.6	0.4
Dilution 3	Dilution 2, 0.5	4.5	0.04
Dilution 4	Dilution 3, 0.5	4.5	0.004

Calibration standards (including a zero point) were then prepared by adding aliquots of the above dilutions to water, urine, plasma, liver and soil at the concentrations detailed in Table 2 following matrix pre-treatment as described below. The analyses of cereal baits and paste were performed using calibration standards prepared by the addition of silica gel Zn_3P_2 dilutions to blank cereal bait or paste. The nominal concentration of Zn_3P_2 in the cereal baits and pastes was 2% (20,000 ppm).

Table 2. Preparation of calibration standards.

Matrix	Matrix Volume /Weight	mg Zn_3P_2 Dilution 2	mg Zn_3P_2 Dilution 3	mg Zn_3P_2 Dilution 4	$[Zn_3P_2]$ range, (ppm)
Water	1mL	50	50, 100, 250	50, 100, 250	0.2 to 20
Urine	2.5mL	50	50, 100, 250	50, 100, 250	0.08 to 8
Plasma	2.5mL	50	50, 100, 250	50, 100, 250	0.08 to 8
Liver Homogenate	5.0mL	50, 100, 250	50, 100, 250		0.4 to 20 (ie 1 to 50 in liver)
Soil	1.0g	50, 100	50, 100, 250	250	1 to 40
Cereal or paste bait	100mg of silica diluted bait	50, 100, 250			200 to 1000 (ie 10,000 to 50,000 in undiluted bait)

For liver about 20g of tissue was accurately weighed into a clean beaker and chopped up to small pieces with scissors. The chopped tissue was carefully transferred to a Waring Blendor along with 20mL water and blended alternately at high and low speeds until a homogenous paste was obtained. The paste was transferred to a clean jar. The blendor was rinsed with a further 10mL water and the rinsings transferred to the paste in the jar. This mix was then capped tightly and mixed until a homogenous mixture was obtained. Homogenates should only be prepared on the day of use and temporarily stored in a cool place.

Obvious plant material such as roots and stalks, and large stones were removed from soil samples by hand. The soil samples were then ground in a mortar to remove any lumps. The ground soils were then mixed well to guard against the settling out of any dense zinc phosphide particles. Portions were then transferred to the ball mill and ground to a fine powder. Just prior to weighing operations the powdered soils were re-mixed by the tumbling and twisting action previously described.

A 10g portion of paste bait was cut into small pieces and mixed. About 2g of the small pieces were randomly selected, weighed and together with 8g of silica gel were transferred to the ball mill and ground until a fine powder was obtained. About 0.5g of this powder was accurately weighed into an Erlenmeyer flask and diluted with a weighed amount (about 4.5g) of powdered silica gel. The flask was stoppered and the contents were mixed thoroughly.

A 10 to 15g portion of cereal bait was broken into pieces in a mortar. About 10g was then ground in the ball mill until a fine powder the consistency of cornflour

was obtained. About 0.1g of the powdered bait was accurately weighed into an Erlenmeyer flask and diluted with a weighed amount (about 4.9g) of powdered silica gel. The flask was stoppered and the contents were mixed thoroughly. If the cereal baits were appreciably wet they were treated in the same way as the paste baits. Allowance was made for the water content by drying a separate bait sample to constant weight under vacuum.

The portions of the prepared samples or calibration standards were transferred to 20mL head-space vials by pipetting (liquid samples) or by direct weighing into the vials (solid samples and Zn_3P_2 dilutions) as in the Table 3. Sulphuric acid was then added to the vials as in Table 3 and the vials were immediately capped with a butyl rubber septum and crimp sealed. The final concentration of sulphuric acid in each vial was approximately 1 Molar.

Table 3. Make up of the head-space vials for phosphine generation.

Sample	Sample Volume/ Weight	1M H_2SO_4 added (mL)	2M H_2SO_4 added (mL)
Water	1mL	8	1
Urine	2.5mL	5	2.5
Plasma	2.5mL	5	2.5
Liver Homogenate	5mL (equivalent to 2g liver)	-	5
Soil	1g	-	10
Cereal Bait Preparation	0.1g (equivalent to 0.002g bait)	10	-
Paste Bait Preparation	0.1g (equivalent to 0.002g bait)	10	-

The vial contents were thoroughly mixed by swirling the vial, while avoiding contaminating the underside of the septum. Vials were then placed in a heating block at 40°C for 1 hour prior to sampling for injection on the GC. The mixing was repeated at 10 minute intervals during the heating period.

At the end of the heating period the contents were mixed again. A 50µL sample of the head-space vapour was immediately withdrawn from the vial using a 100µL gas-tight injection syringe for GC analysis. The vial was returned to the heating block and a second head-space sample for GC analysis was withdrawn after about 5 minutes.

The gas chromatograph used for the analysis was a Hewlett Packard 5890A model

fitted with a nitrogen phosphorus detector and a J & W Scientific GS-GasPro column (30 x 0.32mm i.d.) was operated isocratically at 200°C under the following conditions: manual split flow injection (1:40 split), nitrogen carrier and make-up gases at 30mL/minute, 30mL/minute hydrogen flow to detector, 100mL/minute air flow to detector, 240°C injection temperature, 230°C detector temperature.

Initial investigations to develop an analytical method encountered considerable problems with repeatability of reponses for the standards. Such problems have been discussed previously (Mauldin et al 1997) and have led to the use of standards containing Zn_3P_2 suspended in ethylene glycol as standards. In the present study the Zn_3P_2/silica gel standards prepared as described proved very satisfactory. The repeatability problems were not an issue provided the standards were not allowed to settle. This demixing of mixtures is to be expected when the components have, as in this case, very different densities (Burtally et al 2002, Mullin 2002).

RESULTS AND DISCUSSION

Linear regression curves for determination of phosphine levels were plotted from phosphine chromatogram peak areas versus Zn_3P_2 concentrations for the calibration standards.

Coefficients of determination (r^2) for the various standard graphs obtained were found to be greater than 0.98.

Limits of detection of zinc phosphide in the various matrices tested and coefficients of variation (CV%) at specified zinc phosphide levels are given in Table 4.

Table 4. Limits of detection and coefficients of variation for the assays of zinc phosphide.

Matrices	Limit of detection (ppm)	Coefficient of Variation (Zn_3P_2 level, ppm)	Coefficient of determination (r^2) of linear fit
Water	0.1	10%, (1.0)	0.995
Soils	1.0	13% to 15% (10)	0.991
Liver	1.0	20%, (10)	0.980
Plasma	0.1	11%, (1.0)	0.994
Urine	0.1	10%, (1.0)	0.987
Cereal Bait	Not determined	2%, (20,000)	0.999
Paste Bait	Not determined	7%, (20,000)	0.986

The limit of detection was defined as the concentration at which the phosphine signal to noise ratio was five. The coefficients of variation were determined from the variation in five replicates of each sample measured at the concentrations listed in Table 4.

Relative responses of phosphine released from the various matrices were not determined as these were run on different days. It is considered important to measure phosphine concentrations by using calibration curves constructed by spiking matrices similar to the sample matrix.

In conclusion, the sensitivity and repeatability of the analysis procedures are comparable to results obtained in previous studies and will meet the analytical needs of those seeking registration of baits for possum control.

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