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Assessment of zinc phosphide bait shyness and tools for reducing flavor aversions



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ABSTRACT

Prairie voles (*Microtus ochrogaster* Wagner) cause extensive damage in agricultural, suburban, and urban environments. Control of these animals has historically relied on the use of anticoagulant rodenticides and zinc phosphide. However, shyness to zinc phosphide baits has reduced its efficacy. The aim of this study was to evaluate the factors involved in zinc phosphide bait shyness through preference testing. Baits were made using a rolled oat base and contained various combinations of the components of zinc phosphide baits such as lecithin, magnesium carbonate and known flavor modulators sodium cyclamate and zinc sulfate. Encapsulation of zinc phosphide was also tested as a potential means to mask undesirable flavor qualities of the compound. Consumption of test baits was measured in four day laboratory feeding trials. Results demonstrated that numerous components of current bait formulations serve as salient cues during conditioned aversions and therefore may contribute to bait shyness. Vole avoidance of zinc sulfate and sodium cyclamate revealed that these potential additives would not decrease bait shyness. Encapsulation of zinc phosphide may have masked some of the negative flavor cues and therefore should be considered in future bait development. This study suggests that, since voles are able to distinguish components of current bait formulations, varying composition of zinc phosphide baits between applications may serve to reduce bait shyness.

1. Introduction

Prairie voles (*Microtus ochrogaster* Wagner) cause significant damage to orchards, ornamentals, and field crops (O'Brian, 1994). In particular, voles are a significant problem in alfalfa and artichoke fields (Salmon and Gorenzal, 2010; Schnabel, 2005). Zinc phosphide is a registered toxicant for the control of several rodents worldwide (USA, Europe, Asia-Pacific, and Australia (Eason et al., 2013)) and its use has increased as resistance to anticoagulant toxicants has become more prevalent (Salmon, 2015). Because of this widespread use, the characteristics and potential environmental impacts of zinc phosphide have been well documented. It is known to have low environmental impact when compared to other toxicants, owed largely to its instability under moist conditions and in soil. Therefore, zinc phosphide has low potential for ground and surface water contamination (EPA, 1998). The half-life in soil has been estimated to be 10–20 days (Eason et al., 2013). Also, zinc phosphide does not undergo uptake by plants (Marsh, 1987). Zinc phosphide is not specific for the target species and therefore could pose a threat to non-target species. Because it is highly labile and does not accumulate in animal tissues, the digestive tract of poisoned animals

containing zinc phosphide poses the greatest risk for secondary poisoning. However, the parts of the target animal consumed by non-targets and weather conditions may also mitigate non-target risks (Eason et al., 2013; Sterner and Mauldin, 1995).

Zinc phosphide is registered for control of voles in pastures, rangelands, sugar beets (grain baits), alfalfa, barley, dry beans, potatoes, wheat (wheat baits), and artichokes (artichoke bract baits). It is also registered for the control of pocket gophers (*Thomomys bottae* Eydox and Gervais) in croplands, rangelands, and pastures (grain baits). Marsh reported *Microtus* to be sensitive to zinc phosphide with an LD₅₀ of 12.4–18 mg/kg (Marsh, 1987). In a separate study, Sterner and colleagues examined the efficacy of zinc phosphide to gray tailed voles (*Microtus canicaudus* Miller) in alfalfa. They found baiting with 2.0% zinc phosphide on steam-rolled-oat groats reduced vole numbers in treatment enclosures by > 94% when a single pre-baiting application was used (Sterner et al., 1996). The high efficacy and low both non-target risks and environmental impact discussed above make zinc phosphide a popular choice for the control of pest species.

However, zinc phosphide baits are subject to reduction in efficacy as a result of “bait shyness”, a learned aversion of toxic baits resulting

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from sub-acute exposure to the toxicant (El Hani et al., 1998). With this type of aversions, the distinctive flavor of the bait is associated with the toxic consequences of ingestion. While the toxin itself may serve as a cue, all components of the bait can contribute to the recognized flavor. If a rodent does not succumb to the toxin, the aversion can be quite strong and it is highly unlikely that the animal will ingest similar baits in the future. Furthermore, aversions can be socially transmitted to offspring and conspecifics (Provenza, 1995). Thus, bait shyness may significantly reduce the efficacy of zinc phosphide bait in rodent populations. To mitigate bait shyness, it is recommended that zinc phosphide baits not be used in the same location more than once in any six-month period even when more frequent use is permitted (Schnabel, 2005). Overcoming the palatability issues of zinc phosphide baits will reduce sub-acute exposures and prevent the formation of bait shyness within rodent populations.

Although bait shyness has long been associated with zinc phosphide baits, few studies have been conducted to address this problem. In a recent study with ground squirrels, modification of the carbohydrate profile was proposed (Johnston et al., 2005a). Interestingly, it was reported that the binder (soy lecithin) was a significant contributor to the poor palatability of grain baits which was not overcome by addition of soluble carbohydrates (Johnston et al. 2005a, 2005b). Another possible solution is the encapsulation of the zinc phosphide prior to incorporation into the bait. Encapsulation is the enclosure of a small particle with an inert “shell”. This is a plausible solution that should be explored further. However, encapsulation of zinc phosphide fails to account for the contribution of other components (e.g. binders) to the flavor profile of the bait. Furthermore, encapsulation may affect the rate of zinc phosphide hydrolysis. Zinc phosphide is acid hydrolyzed in the stomach which results in formation of toxic phosphine gas and subsequent toxic effects. If formation of phosphine gas is hindered by encapsulation, efficacy may be significantly reduced.

Rather than encapsulating the active ingredient, an entirely different approach for reducing bitterness of pharmacological agents was reported by Keast et al. (2004). The overall bitter taste was suppressed by zinc sulfate. Zinc ions interfere with bitter taste receptors (T2Rs) and have been used to suppress bitterness of highly bitter compounds such as quinine and denatonium benzoate in humans. Zinc ions also interfere with sweet taste receptors (T1Rs). However, the sweetness of some artificial sweeteners is not suppressed by zinc (Keast and Breslin, 2005). Thus, use of the sweetener sodium (Na) cyclamate in conjunction with zinc sulfate can drastically improve palatability of bitter pharmaceuticals.

In this study, several experiments were conducted to evaluate factors involved in zinc phosphide bait shyness. In the first two experiments, bait shyness was mimicked by conditioning an aversion to the baits with intraperitoneal delivery of lithium chloride. Lithium toxicosis is commonly employed in studies of flavor aversion (Riley and Freeman, 2004). In the third experiment, zinc phosphide was encapsulated to directly alter its flavor profile.

2. Materials and methods

2.1. Subjects

Voies were caught near Fort Collins, CO (40° 33' N, 105° 4' W) and transferred to the animal facility. All animals were quarantined for two weeks and maintained on a 12 h light 12 h dark schedule throughout the experiment. Animals were individually housed in standard wire bottom cages (16" x. 9.5" x 7") with external water bottles. Wood inserts that held two Petri dishes (100 mm × 15 mm, Fisher Scientific, Waltham, MA USA) were secured to one end of each cage. Food was placed in the petri dishes. When not on test, animals received normal rodent chow pellets (Rodent diet 500, LabDiet St. Louis, MO USA) and water *ad libitum*.

2.2. Bait preparation

Baits were formulated with rolled oats (Ranch-Way Feeds, Fort Collins, CO USA). Additives included lecithin (Sigma-Aldrich, St. Louis, NJ USA), USP grade mineral oil (Spectrum Chemical MFG Corp., New Brunswick, NJ USA), magnesium carbonate (Sigma-Aldrich, St. Louis, NJ USA), zinc sulfate (Sigma-Aldrich, St. Louis, NJ USA), sodium cyclamate (Sigma-Aldrich, St. Louis, NJ USA), and zinc phosphide (Sigma-Aldrich, St. Louis, NJ USA). Zinc phosphide was also subjected to encapsulation with EPO (Sigma-Aldrich, St. Louis, NJ USA) and steric acid (Sigma-Aldrich, St. Louis, NJ USA). Zinc phosphide was added to a pan coater and an aqueous suspension of EPO and stearic acid (5:1) was sprayed until approximately 20% coating (by weight) was achieved. Coated zinc phosphide was air dried and submitted for chemical analysis.

2.2.1. Control bait (Con)

Control bait was formulated with 1.1% lecithin, 2.3% mineral oil, 3.1% magnesium carbonate, and 93.5% rolled oats; the same composition as the other bait preparations without the addition of zinc phosphate. Oats were placed in a large mixer (model A-20, Hobert MFG CO, Troy, OH USA), mineral oil was then added and allowed to thoroughly mix for approximately three minutes. Lecithin and magnesium carbonate were combined and then added to oats coated with mineral oil and mixed for two minutes. The bait was spread on trays and allowed to dry for three hours at room temperature with an average relative humidity of approximately 30%.

2.2.2. Zinc phosphide baits (ZP tech, ZP cap, or lecithin-free ZP)

Wheat baits contained zinc phosphide at 10% of the concentration used in the field baits (0.2%) and consisted of 1.1% lecithin, 2.3% mineral oil, 3.33% zinc phosphide or encapsulated zinc phosphide concentrate, and 93.3% rolled oats. A lecithin-free zinc phosphide bait was prepared similarly to the ZP tech bait, except that it did not contain lecithin. ZP cap and ZP tech baits were made as described above, but instead of magnesium carbonate either the zinc phosphate (ZP tech) or encapsulated zinc phosphate (ZP cap) was added.

2.2.3. Magnesium bait (Mg)

The magnesium bait, containing no zinc phosphide, was formulated with 2.3% mineral oil, 3.1% magnesium carbonate, and 94.6% rolled oats. As with the control baits, oats were placed in a large mixer, mineral oil was added and mixed thoroughly; magnesium carbonate was then added to oats coated with mineral oil and mixed for two minutes. The bait was allowed to dry for three hours at room temperature with an average relative humidity of approximately 30%.

2.2.4. Adulterated control bait

Zinc sulfate (0.8%) and sodium cyclamate (0.25%) were added to the ingredients of the control bait (1.1% lecithin, 2.3% mineral oil, 3.1% magnesium carbonate, and 92.45% rolled oats) to produce the adulterated bait. The same method as described for control bait was used except lecithin, magnesium carbonate, zinc sulfate, and sodium cyclamate were combined and then added to the oil coated oats. This was mixed for two minutes and dried at room temperature for three hours.

2.3. Preference testing

Prior to initiation of all taste aversion experiments, basal food ration was removed and subjects were offered plain wheat grain *ad libitum* for four consecutive days to mimic prebaiting procedures used in field applications. For each experiment, subjects were assigned to treatment groups such that mean test acclimation intake and standard deviation were similar among treatments.

Subjects had restricted access to water and food overnight (16 h). At

0900 h, subjects were presented 10 g of the conditioned stimulus (appropriate test bait) and water. The test bait was removed after 45 min and intake was recorded by mass difference. Immediately following removal of the test bait, subjects in experiment 1 or 2 received a single intraperitoneal injection of the lithium chloride solution (20 mg/mL lithium chloride in PBS (Sigma-Aldrich, St. Louis, NJ USA) or 0.01 M PBS). A 200 mg/kg dose of lithium chloride was achieved by injecting 0.010 mL per gram body mass. Food was withheld for three hours following delivery of lithium chloride, at which time subjects were provided their basal ration.

Preference testing began on the day following lithium chloride exposure. Twenty-four hour, two-choice tests were repeated for four consecutive days. Subjects were provided 20 g each of the conditioned stimulus (appropriate test bait) and an alternative food (as appropriate). The position of the conditional stimulus (left or right) was stratified among subjects and alternated daily for each subject. Diets were replaced daily and intake determined by mass difference. Subjects had *ad libitum* access to water during preference tests. Testing was performed under approved NWRC IACUC protocol QA-1781. Specifically, voles were housed in individual wire-bottom cages and provided commercial laboratory rodent chow, bedding, and water *ad libitum*. Voles exposed to zinc phosphide were carefully monitored every 30 min for four hours following removal of zinc phosphide bait or every 30 min for the first four hours bait is present.

2.4. Experimental design

2.4.1. Experiment 1. Induced bait shyness and lecithin avoidance

Twenty-four adult, mixed sex (17 male, 7 female; weight range 28.4–84.0 g) voles were used in experiment 1. Control bait (Con) was used as the conditioned stimulus for all treatments. The lecithin and cyclamate groups (8 voles per group) received *i. p.* injection of lithium chloride. The unconditioned group received *i. p.* injection of 0.01 M PBS. During preference testing, subjects were offered control bait and an alternative bait (either the magnesium or adulterated control bait) according to treatment group as previously described (Table 1). Test bait preference scores for Experiment 1 were calculated by dividing test diet intake by total intake (test diet plus control diet).

2.4.2. Experiment 2. Induced bait shyness and zinc phosphide avoidance

Twenty-four adult, mixed sex (11 male, 13 female; weight range 26.9–81.0 g) voles were used in experiment 2. Subjects in the unconditioned group of experiment 1 were employed (divided equally among experiment 2 treatments). The conditioned stimulus was either 0.2% zinc bait or control bait, depending on treatment group. Subjects in all treatment groups received a single *i. p.* injection of lithium

Table 1

Experimental treatment groups and conditions. The basis of preference score (test bait intake divided by total bait intake) is indicated with an *.

Experiment	Treatment	CS	US	Preference
1	Unconditioned	Con bait	None	Con bait vs. Mg bait*
1	Cyclamate	Con bait	LiCl	Con bait vs. adulterated con bait*
1	Lecithin-Free	Con bait	LiCl	Con bait vs. Mg bait*
2	Zinc	ZP tech bait	LiCl	ZP tech bait* vs. Con bait
2	Control	Con bait	LiCl	ZP tech bait* vs. Con bait
2	Encapsulate	ZP tech bait	LiCl	ZP cap bait* vs. Con bait
3	ZP tech	ZP tech bait	None	ZP tech bait vs. ZP cap bait*
3	ZP cap	Zp cap bait	None	ZP tech bait vs. ZP cap bait*

chloride. During preference testing, subjects were offered control bait and alternative bait (zinc phosphide technical bait or encapsulated zinc phosphide bait) according to treatment group as previously described (Table 1). Test bait preference scores for Experiment 2 were calculated by dividing test diet intake by total intake (test diet plus control diet).

2.4.3. Experiment 3. Innate bait shyness: zinc phosphide and encapsulated zinc phosphide

Sixteen adult, mixed sex (6 male, 10 female; weight range 24.0–96.5 g) voles were used in experiment 3. Zinc phosphide bait (technical material) was the conditioned stimulus for one treatment group and encapsulated zinc phosphide bait for the other. To test innate bait shyness, that not induced by injections of lithium chloride as in previous experiments, animals were given two days experience with the one of two zinc phosphide baits, according to treatment group as previously described (Table 1). Over the two day free-feeding exposure, voles consumed 0.56 ± 0.34 g ZP tech bait and 0.57 ± 0.23 g ZP cap bait.

Following bait exposure, preference tests were conducted by offering all subjects a two-choice test of zinc phosphide bait and encapsulated zinc phosphide bait for two days. Encapsulated zinc bait preference scores for Experiment 3 were calculated by dividing test diet intake by total intake (test diet plus control diet).

2.5. Statistical analysis

Intake data from two-choice tests and preference scores were analyzed by repeated measures analysis of variance (ANOVA) with treatment and day (repeated) as fixed effects and subject as the random effect using SAS[®] (PROC MIXED with default restricted maximum likelihood method and variance components covariance structure). The null hypothesis of indifference (indicated by a preference score of 0.5) was tested by using the value 0.5 minus the preference score as the response in the model. Residual plots were generated to evaluate ANOVA assumptions.

3. Results

3.1. Experiment 1. Induced bait shyness and lecithin avoidance

There were significant treatment ($F_{2,20} = 7.23$; $p = 0.0043$) and treatment*day ($F_{6,57} = 3.13$; $p = 0.0101$) effects in experiment 1 (Fig. 1). Day was not significant ($F_{3,57} = 0.09$; $p = 0.9668$). Evaluation of the residuals demonstrated that no assumptions of ANOVA were violated. Subjects offered control bait as a conditioned stimulus, in the absence of lithium aversion (Unconditioned group), demonstrated indifference between control bait and Mg bait (lecithin-free) on days 1, 3, and 4 and preference for the lecithin free Mg bait on day 2 (Table 2). Subjects in the Lecithin group, demonstrated preference for the lecithin-free Mg bait and aversion to the control bait on days 1 and 2 (Table 2), indicating association of lithium-induced toxicosis with the flavor of lecithin. Subjects in the Cyclamate group demonstrated a preference for the bait that was paired with lithium chloride administration on days 1 and 2 (Table 2). Their avoidance of zinc sulfate and sodium cyclamate was so pronounced it drove them to disregard the salient cues from the control bait to which they had been adversely conditioned.

3.2. Experiment 2. Induced bait shyness and zinc phosphide avoidance

Treatment ($F_{2,20} = 0.30$; $p = 0.746$) and day ($F_{3,54} = 0.11$; $p = 0.952$) effects were not significant in Experiment 2. The treatment*day interaction ($F_{6,54} = 3.65$; $p = 0.0041$) was the only significant effect (Fig. 2). Subjects in the Zinc treatment group that received ZP tech bait as the conditioned stimulus paired with LiCl administration demonstrated a strong aversion to ZP tech bait when it was offered with control bait for all four days of preference testing

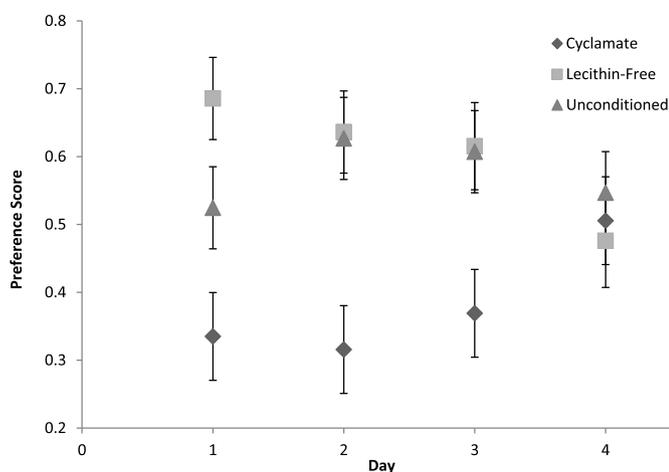


Fig. 1. Preference scores from experiment 1. Test bait preference scores (intake of test diet divided by the sum of test and control diets) from experiment 1. The unconditioned group tested innate preference for lecithin free bait. The cyclamate group was conditioned against control bait and preference tested for adulterated control bait containing zinc sulfate (0.8%) and sodium cyclamate (0.25%). The lecithin-free group was conditioned with control bait containing lecithin and preference tested for lecithin free Mg bait.

Table 2 Least square means of test response (equal to 0.5 minus preference score) and test of null hypothesis that test response equals zero for significant treatment by day interactions in Experiment 1.

Treatment Group	Day	Estimate	DF	t-value	p-value
Cyclamate	1	0.165	57	3.77	0.0004
Cyclamate	2	0.184	57	2.82	0.0066
Lecithin	1	-0.186	57	-4.54	< 0.0001
Lecithin	2	-0.136	57	-2.23	0.030
Unconditioned	2	-0.127	57	-2.07	0.0427

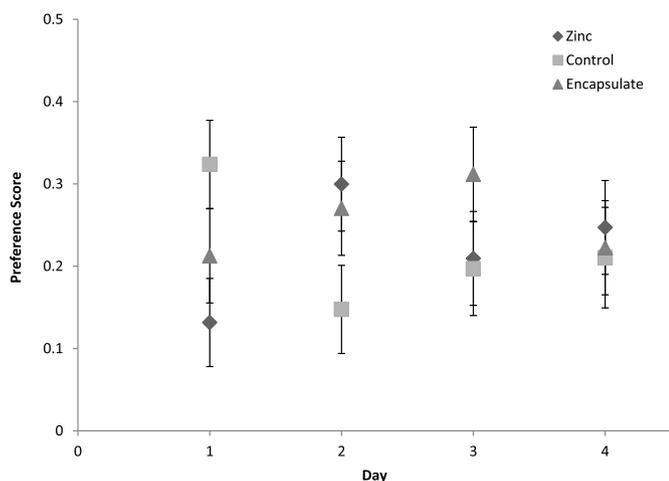


Fig. 2. Preference scores from experiment 2. Test bait preference scores (intake of test diet divided by the sum of test and control diets) from experiment 2. The zinc group tested conditioned avoidance of zinc phosphide bait when offered control bait as an alternative. The control group was conditioned against control bait and preference tested for zinc phosphide technical bait. The encapsulate group was conditioned against zinc phosphide bait and preference tested for encapsulated zinc phosphide bait and control bait.

Table 3 Least square means of test response (equal to 0.5 minus preference score) and test of null hypothesis that test response equals zero for significant treatment by day interactions in Experiment 2.

Treatment Group	Day	Estimate	DF	t-value	p-value
ZP Technical	1	0.368	54	8.54	< 0.0001
ZP Technical	2	0.200	54	4.04	0.0002
ZP Technical	3	0.290	54	4.38	< 0.0001
ZP Technical	4	0.252	54	3.94	0.0002
Encapsulated	1	0.287	54	6.23	< 0.0001
Encapsulated	2	0.230	54	4.62	< 0.0001
Encapsulated	3	0.188	54	2.84	0.0064
Encapsulated	4	0.278	54	4.33	< 0.0001
Control	1	0.176	54	4.09	< 0.0001
Control	2	0.352	54	7.59	< 0.0001
Control	3	0.301	54	4.55	< 0.0001
Control	4	0.289	54	4.20	0.0001

(Table 3). Subjects in the Control group that were offered control bait as the conditioned stimulus paired with LiCl administration preferred the control bait to the novel ZP tech bait for all four days of testing (Table 3). Day 1 preference for zinc phosphide bait was significantly greater among the Control group versus the Zinc treatment group ($t_{20} = 3.15$; $p = 0.0027$). However, these animals still preferred to consume lecithin over zinc phosphide even though they had induced bait shyness for the lecithin cue from the LiCl injection. Subjects in the Encapsulate group also preferred the control bait on all four days of preference testing (Table 3).

3.3. Experiment 3. Innate bait shyness: zinc phosphide and encapsulated zinc phosphide

Zinc phosphide exposure ($F_{1,9} = 4.92$; $p = 0.0538$) and day ($F_{1,9} = 3.74$; $p = 0.0853$) approached significance in Experiment 3. The interaction effect was not significant ($F_{1,9} = 3.08$; $p = 0.113$). Encapsulated bait preference was 0.495 for the ZP cap treatment group and 0.752 for the ZP tech group, indicating that voles with prior experience the zinc phosphide technical bait demonstrated a preference for the encapsulated bait, while voles with prior experience with encapsulated zinc phosphide bait were indifferent to the two baits, consuming an average of 0.23 g of ZP tech bait and 0.24 g of ZP cap bait. In the two day no choice experience, they consumed on average 0.56 ZP tech bait and 0.57 ZP cap bait.

4. Discussion

Bait shyness is a result of pesticide-induced toxicosis being associated with the flavor of the consumed bait. These experiments demonstrated that many components of a bait formulation may serve as salient flavor cues of conditioned aversions and contribute to bait shyness. In experiment 1, zinc sulfate failed to disrupt this association. Furthermore, voles showed a strong avoidance to the zinc sulfate/sodium cyclamate adulterated control bait. Although adding zinc sulfate and sodium cyclamate has worked to reduce bitterness in human pharmaceuticals, these data demonstrate that voles innately avoid these compounds. Their addition to formulations would not increase bait acceptance and therefore, there is no reason to include them in baits. Experiment 1 also confirmed that lecithin can be a salient cue associated with toxicosis. When lithium toxicosis was paired with a lecithin-containing control bait, voles demonstrated an avoidance of lecithin-containing control bait (Fig. 1). Preference for the lecithin-free Mg bait persisted for three days and demonstrated that while lecithin may not be avoided innately, its flavor can be easily detected and readily associated with toxicosis; its elimination from rodenticide baits should be considered to reduce identifiable flavor cues associated with toxic baits.

Zinc phosphide was also avoided without any conditioning with

lithium chloride. When control bait was paired with LiCl, voles still preferred the control baits to novel zinc phosphide technical baits in experiment 2 (Fig. 2). Interestingly, when aversion to ZP tech bait was conditioned via prior experience with encapsulated ZP bait in Experiment 3, voles demonstrated no preference when offered the choice of encapsulated zinc phosphide baits (ZP cap) and zinc phosphide technical bait (ZP tech). Conversely, voles offered ZP tech prior to preference testing demonstrated a preference for encapsulated bait (ZP cap). These data suggest that encapsulation does alter the flavor profile of the bait and reduces bait shyness.

This finding supports previous work developing baits with encapsulated zinc for controlling brushtail possums (*Trichosurus vulpecula* Kerr) and rodents (Clapperton and Porter, 2005; Ross and Henderson, 2006; Shapiro et al., 2016). These formulations have overcome some of the bait shyness associated with the use of non-encapsulated zinc phosphide as an active ingredient (Shapiro et al., 2016). In brushtail possum pen trials, microencapsulated zinc phosphide increased mortality 47% compared to non-encapsulated zinc phosphide (Ross and Henderson, 2006). Microencapsulated zinc phosphide baits have also been shown to be efficacious against ferrets (*Mustela furo* L.) in pen trials (Clapperton and Porter, 2005). Recent pen and field trials of microencapsulated zinc phosphide bait registered for use in New Zealand reported 87.5% mortality in captive brushtailed possums and 82.2% motility of possums in field trials (Shapiro et al., 2016). These studies demonstrate that microencapsulating zinc phosphide increases mortality when compared to non-microencapsulated zinc phosphide baits; however, bait avoidance by small mammals may still impact the efficacy of baiting operations.

Bait shyness has been a major obstacle to the success of zinc phosphide baits used to control mammalian pests. Bait shyness results in decreased bait acceptance and reduced efficacy of zinc phosphide baits (Jacob et al., 2010; Parshad and Kochar, 1995; Shepherd and Inglis, 1993; Sridhara and Srihari, 1980). Jacob and colleagues have examined the relationship between zinc phosphide concentration and bait shyness in vole studies where they varied zinc phosphide concentration up to 5%. They found that zinc phosphide concentration was inversely related to bait uptake and when consumption data from all zinc phosphide concentrations was examined together, greater than 50% more bait was consumed on the first day of the trials than the subsequent 4 days. They also investigated the bait base, wheat kernels or wheat based pellets, to determine if the components of the bait would affect consumption. In enclosure trials, the wheat kernels were consumed more quickly than the wheat-based pellet; however, the authors believed that the pellet may offer some benefits since the zinc phosphide is distributed throughout the pellet unlike the wheat kernel where the zinc phosphide is on the outside (Jacob et al., 2010). Other studies have tested zinc phosphide bait uptake using maize or rice as the bait base when baiting is being done in rice fields. In these studies, rice base increased zinc phosphide bait uptake by 51% compared to maize, although these studies still reported reduced uptake of baits containing zinc phosphide when compared to sham rice baits (Leung et al., 2007). This again demonstrated some aversion to zinc phosphide and further supports the use of encapsulated zinc phosphide in bait formulations.

Based on the findings of this study and others, the encapsulation of zinc phosphide will reduce bait shyness associated with both innate and conditioned avoidance. By masking the distinct flavor of the zinc phosphide, encapsulation may increase bait uptake. If animals, specifically voles, can no longer detect salient features of zinc phosphide baits, it is less likely that bait shyness will develop and therefore consumption of bait would be higher when compared to non-encapsulated zinc phosphide. Moreover, encapsulation would mask any odor associated with zinc phosphide whereby decreasing neophobia associated with the novel bait. This would increase consumption in the animals' first interaction with the bait. The increased uptake of zinc phosphide could result in an increase in the efficacy of the bait. However, additional studies need to determine if encapsulation changes the efficacy of

zinc phosphide regardless of bait uptake by altering how the compound interacts with the digestive tract. Because encapsulation may change the stability of zinc phosphide, risks to non-target species should also be examined.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical approval

All procedures performed in studies involving animal were in accordance with ethical standards of the institution or practice at which the studies were conducted.

Author contribution

KEH and BAK conceived and designed the study. KEH and NMH did data collection; BAK performed statistical analysis. KEH wrote the manuscript. All authors reviewed and edited the manuscript.

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