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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* Eydoux and Gervais) in croplands, rangelands, and pastures (grain baits). Marsh reported *Microtus* to be sensitive to zinc phosphide with an LD_{50} 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

Voles 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