Poster Presentation

Benzoate Adjuvant to Increase the Utility of Methylxanthine Pesticides: Identification of a Potential Rodenticide Formulation for Organic Food Production

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ABSTRACT: There is growing interest in the use of natural products such as caffeine and theobromine for control of a variety of pest species. We hypothesized that the limited water solubility of these compounds limits their effectiveness. To overcome this hurdle, we evaluated the use of sodium benzoate as an adjuvant to increase the solubility and potency of methylxanthines. Our results indicated that sodium benzoate increased the toxicity of a methylxanthine mixture to rodents but not to canids. These results indicate that sodium benzoate has potential for increasing the efficacy and selectivity of methylxanthine-based rodenticides. As methylxanthines and sodium benzoate are plant-derived natural products, potential applications for methylxanthine and sodium benzoate based pesticides include organic food production.

KEY WORDS: caffeine, organic food, rodenticide, theobromine

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INTRODUCTION

The number of farmers and the acreage dedicated to organic farming has increased steadily during the past decade as organic food sales in the U.S. have increased by about 20% annually. In the U.S., the USDA-National Organic Program regulation requires that pesticides used in conjunction with organic food production must be natural or synthetics that appear on the National List (7CFR 205.600-607). In general, nearly all synthetic pesticides are prohibited and nearly all naturals are permitted (Caldwell et al. 2005). Additionally, inert formulation additives must be classified as 4A (inert ingredients of minimal concern) or 4B (other formulation ingredients that will not adversely affect public health) by the U.S. Environmental Protection Agency (US EPA).

Organic farming and new trends toward the use of safer pesticides for crop protection have created new opportunities for botanical pesticides (Casanova et al. 2002). Botanical pesticides are often promoted as an alternative to conventional pesticides due to their low toxicity to non-target species, limited persistence in the environment, and selectivity to target species. Such qualities conform to the guidelines expressed in the US EPA Food Quality Protection Act (Dev and Koul 1997:3-12; Isman 2000). While there are a large number of botanicals with potential pesticidal activity, the only 4 such compounds that have found widespread use for crop protection are pyrethrum (chrysanthemum), rotenone (derris tree), nicotine (tobacco), and azadirachtin (neem tree) (Sugavanam and Copping 1998).

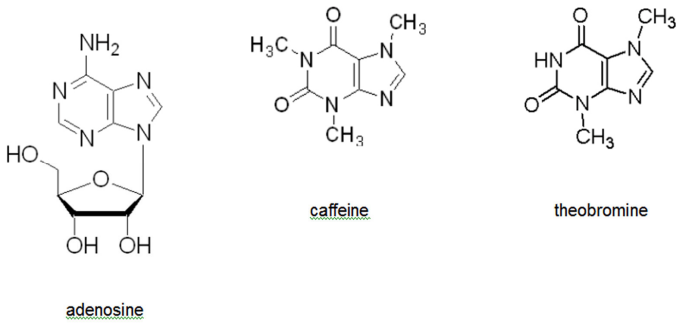

Figure 1. Structures of adenosine and the methylxanthines theobromine and caffeine.

Methylxanthines such as caffeine and theobromine (Figure 1) are components of a variety of plants including coffee, tea, cocoa, and kola. While methylxanthines are commonly used as stimulants by humans, these compounds have demonstrated pesticidal activity with respect to a variety of species including canids, rodents, birds, and insects (Johnston 2005; Avery et al. 2005, Nathanson 1984).

The mode of action of methylxanthines is linked to their structural similarity to adenosine (Figure 1). Adenosine is an inhibitory neurotransmitter that effects a decrease in neuron stimulation. Many methylxanthines can bind to the adenosine synapse binding site, thereby preventing the decrease/termination in nerve stimulation that would normally accompany adenosine binding. Additionally, methylxanthine binding increases the release of excitatory neurotransmitters (nor-adrenaline and dopamine) while decreasing the release of inhibitory neurotransmitters GABA and serotonin. The net result is an increase in neuron activity (Xie et al. 2007).

Methylxanthines also act as phosphodiesterase inhibitors. Phosphodiesterases break down cAMP and cGMP. These cyclic nucleotides transmit signals from the cell surface to intracellular proteins. In the absence of phosphodiesterases, cAMP and cGMP continuously transmit signals that increase cell activity (Rall 1980: 592).

The effects of increased neuron/cell activity are numerous. Such effects include increased release of adrenaline and increased blood sugar. Excess intake of methylxanthines may result in nausea and vomiting, restlessness, diarrhea, muscle tremors, increased urination, and/or cardiac arrhythmia.

As a vertebrate pesticide, methylxanthines have many desirable attributes, including social acceptability. Given the nearly ubiquitous consumption of coffee, tea, and chocolate, methylxanthines such as caffeine and theobromine are compounds with which the general populace is quite familiar. These compounds are also naturally occurring and are relatively innocuous to humans (Johnston 2005, Fagerstone et al. 2004).

Evaluation of methylxanthine mixtures as a toxicant for pest canids indicated that a 5:1 ratio of theobromine:caffeine represents an optimal compromise between the potent toxicity of theobromine and the desirable qualitative (no adverse symptoms preceding mortality) toxicity associated with theobromine. While methylxanthines have potential as vertebrate pesticides, the toxicity of caffeine and theobromine to mammals suggests that a significant dose would be required to achieve the desired pesticidal efficacy. Our previous studies with coyotes indicated that delivering the theobromine:caffeine mixture in a larger volume of water increased the toxicity of the mixture and decreased the time to mortality. As methylxanthines have limited solubility in water, the increased amount of water increased the quantity of methylxanthines in solution. Since benzoate salts increase the solubility of theobromine and caffeine in water, we hypothesized that the co-administration of benzoate salts (and the subsequent increase in theobromine: caffeine solubility) would also increase the quantity of methylxanthines in solution, resulting in increased toxicity and speed of action.

Additionally, benzoate salts are listed as a 4A (minimal risk) inert ingredient. Ingredients rated as 4A are recognized as safe ingredients for use in all pesticide products. Thus, addition of sodium benzoate would be acceptable for a pesticide formulation to be used in organic food production and would likely have minimal impact on registration requirements. We evaluated the use of sodium benzoate as an adjuvant to potentially increase the solubility and associated potency of methylxanthines.

MATERIALS AND METHODS

Materials

Wistar Rats were obtained from Simonsen Labs (Gilroy, CA). Theobromine, caffeine, and sodium benzoate were obtained from Sigma Chemical Co. (St. Louis, MO). Dormitor (1 mg medetomidine hydrochloride/mL) and Antisedan (5 mg/mL atipamezole hydrochloride) was obtained from Pfizer Animal Health, Exton PA); Ketaved (100 mg ketamine hydrochloride/ mL) was obtained from Vedco Inc. (St. Joseph, MO). Coyotes were obtained from the USDA APHIS WS National Wildlife Research Center (NWRC) Predator Research Field Station in Logan, UT and the University of Wyoming Coyote Colony in Laramie, WY.

Rat Toxicity Testing

Rats were orally gavaged with aqueous solutions of methylxanthines and/or sodium benzoate. Each group was dosed with a unique amount of the theobromine: caffeine mixture to permit the construction of a dose vs. percent mortality curve.

In Test One, the toxicity of sodium benzoate was determined. Three groups of 5 rats were dosed with sodium benzoate. The sodium benzoate was dissolved in water to deliver the target dose levels (3.5, 7, and 14 g sodium benzoate/225-g rat) in 3 mL water.

In Test Two, the toxicity of the methylxanthine mixture (theobromine:caffeine, 5:1) was determined. Five groups of 5 rats were dosed with methylxanthines. The target dose levels were 600, 900, 1100, 1300, and 1550 mg/kg. The volume of the dosing solution was 3 mL. Following a 48-hour observation period, percent mortality was calculated for each treatment group.

In Test Three, the toxicity of methylxanthines: sodium benzoate (1:1) was determined. Four groups of 5 rats were dosed with methylxanthines:sodium benzoate 1:1. The target dose levels were identical to Test Two. The volume of the dosing solution was 3 mL. Following a 48-hour observation period, percent mortality was calculated for each treatment group.

Rats were monitored at 1-hour intervals for the first 8 hours post dosing, at 2-hour intervals for the next 16 hours, and at 8-hour intervals for the next 24 hours. All surviving rats were euthanatized shortly thereafter.

Coyote Toxicity Testing

Individually caged coyotes were transported from the NWRC Millville Research Station or the University of Wyoming Laramie Coyote Research Station to NWRC headquarters in Fort Collins.

Upon arrival at NWRC, coyotes were transferred to individual outdoor pens and quarantined for at least 1 week prior to dosing. Immediately prior to methylxanthine dosing, each coyote was sedated via intramuscular injection with 1 mL Dormitor (1 mg medetomidine hydrochloride) and 0.5 mL Ketaved (50 mg ketamine hydrochloride). Coyotes were orally gavaged with the aqueous suspensions of the test materials (250 mg methylxanthines/mL). To ensure quantitative transfer of the test mixtures, each coyote was subsequently gavaged with 60 mL water. Test mixture dose and subsequent water gavage was delivered at a rate of approximately 5 mL per second.

The toxicity of the methylxanthine (theobromine: caffeine, 5:1) mixture to coyotes was determined by dosing 16 coyotes; 4 groups (4 coyotes per group) were each dosed with a unique dose of the methylxanthine mixture. Following dosing, coyotes were returned to their pens and sedation reversed with 1 mL Antisedan (5 mg atipamezole hydrochloride).

The theobromine:caffeine (5:1) methylxanthine mixture was also evaluated on coyotes. Coyotes were orally gavaged with methylxanthines:sodium benzoate (1:1). Each of 4 treatment groups (5 coyotes per group) received a unique dose of the test mixture. Dose levels were 330, 340, and 350 mg/kg. The control group

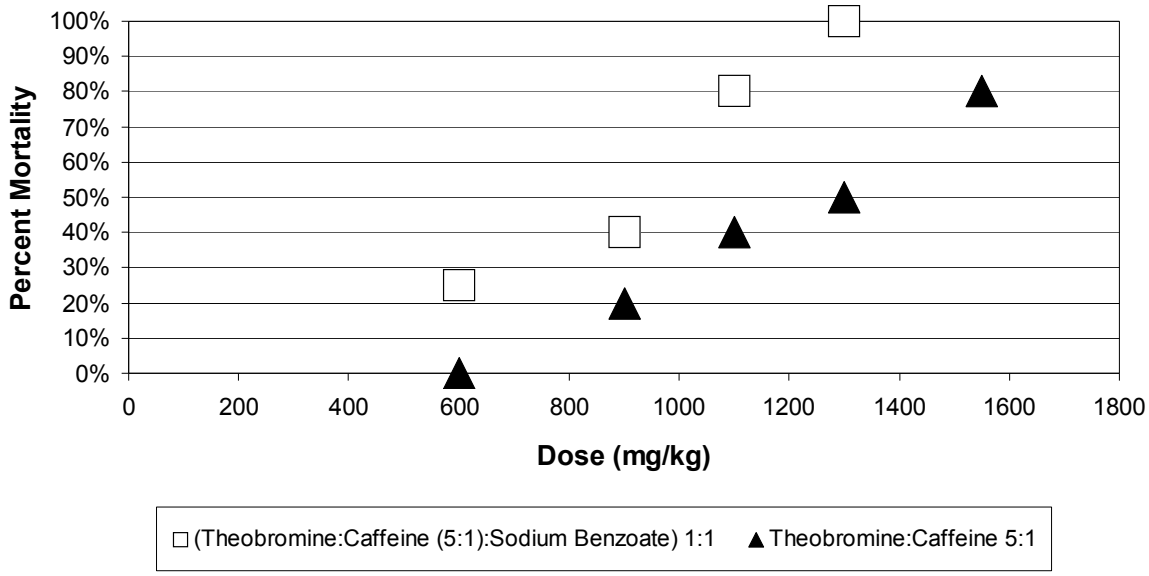

Figure 2. The effect of benzoate co-administration on methylxanthine toxicity to rats.

received only aqueous sodium benzoate solution (5 coyotes).

For all coyote dosing experiments, post-dosing observations were accomplished using cameras and remote monitoring. Post-dosing observations were at 30-minute intervals for the first 6 hours post dosing, once an hour observation for the next 12 hours, at 2-hour intervals for the next 6 hours, and 12-hour intervals for the next 24 hours. Each observation period consisted of 3 minutes per coyote.

Data Analysis

Dose vs. percent mortality curves were constructed for each toxicity test (Finney 1971). From these curves, common toxicity parameters (e.g., LD_{50}, LD_{99}) were determined for the methylxanthine and methylxanthines: sodium benzoate mixtures.

To evaluate the potential synergistic effect of sodium benzoate on oral methylxanthine toxicity, the dose vs. mortality curves for each treatment (with and without sodium benzoate) were compared via analysis of covariance.

To evaluate the potential effects of sodium benzoate on methylxanthine induced time to mortality, the dose vs. time to mortality curves for each treatment were compared via analysis of covariance.

RESULTS

In rats dosed only with sodium benzoate, toxicity was observed in the two highest dose levels. No toxicity was noted at lowest dose level of 3.5 grams sodium benzoate per rat. To avoid toxicity induced by sodium benzoate, we limited the subsequent evaluation to a 1:1 ratio of methylxanthines:sodium benzoate.

Analysis of covariance indicated that the two methylxanthine rat toxicity curves (with and without sodium benzoate) are significantly different ($p \leq 0.05$) (Figure 2). The addition of sodium benzoate increased the toxicity of the methylxanthines to rats. For rats, the LD_{50} and LD_{99} values of the methylxanthine mixture was estimated to be 1237 and 1823 mg/kg, respectively. The addition of sodium benzoate yielded rat LD_{50} and LD_{99} values of 732 and 1385 mg/kg, respectively. These comparisons indicate that the addition of benzoate salts increased the toxicity of the methylxanthines by about 25-40%.

Analysis of covariance indicated that the two methylxanthine coyote toxicity curves (with and without sodium benzoate) were not significantly different (Figure 3). For coyotes, the addition of sodium benzoate did not increase the toxicity of the methylxanthines. The LD_{50} and LD_{99} of test mixtures to coyotes were 336 and 385 mg/kg, respectively.

Analysis of the two rat methylxanthine (with and without sodium benzoate) dose vs. time to mortality curves were not significantly different. While the addition of sodium benzoate increased the potency of the methylxanthine mixtures, the time to methylxanthine-induced mortality was not affected by sodium benzoate co-administration.

DISCUSSION

Organic food production is increasing by about 10 to 50% per year in many developed countries. Plant-derived natural products are often used as pesticides in organic food production and packaging facilities. Rodent control in food production facilities is an omnipresent challenge. Rodent control options in organic food production facilities are currently limited to mechanical devices. Methylxanthines represent a class of naturally derived compounds that have potential for pest rodent control associated with organic food production.

Our results illustrate that the oral toxicity of the methylxanthines theobromine and caffeine is potentiated by co-ingestion of sodium benzoate; sodium benzoate increased the toxicity of a mixture of these compounds by approximately 40%.

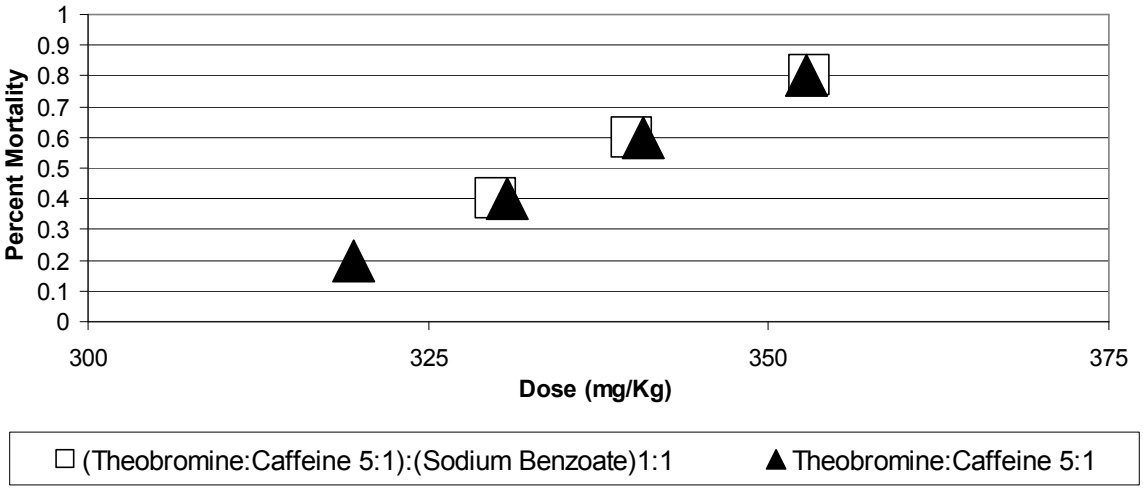

Figure 3. The effect of benzoate co-administration on methylxanthine toxicity to coyotes.

As canids are potentially exposed to rodenticides, it is fortunate that sodium benzoate did not increase the toxicity of the methylxanthines to canids. The addition of sodium benzoate to a methylxanthine-based rodenticide increased the toxicity of the rodenticide to the target species without increasing toxicity to at least some potentially exposed non-target species. The net result is that the addition of sodium benzoate to a methylxanthine rodenticide bait would decrease the amount of methylxanthines required to prepare an efficacious bait. This would decrease the non-target hazards to canids.

This preliminary investigation suggests that methylxanthines have potential utility for pest control in organic food production. However, future studies are needed to determine if methylxanthine baits can be prepared which are both efficacious and palatable to target species.

As sodium benzoate is a plant-derived natural product, it has potential for use in organic food production applications. Sodium benzoate is listed as a 4A (minimal risk) inert ingredient. This class (4A) of inert ingredients is regarded by US EPA as safe ingredients for use in all pesticide products. Addition of sodium benzoate to a pesticide formulation likely would have minimal impact on registration requirements while increasing the potency and selectivity of a methylxanthine-based rodenticide product.

ACKNOWLEDGEMENTS

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