

Evaluation of Cocoa- and Coffee-Derived Methylxanthines as Toxicants for the Control of Pest Coyotes

JOHN J. JOHNSTON

USDA/APHIS/Wildlife Services, National Wildlife Research Center, 4101 LaPorte Avenue,
 Fort Collins, Colorado 80521

Methylxanthines were quantified in coffee, tea, and chocolate products. Tarajulie tea from India, cocoa powder, and cocoa nibs contained the highest levels of methylxanthines. Theobromine, caffeine, and theophylline combined in the ratios observed in tea and chocolate were ingested by coyotes. Although both mixtures induced acute toxicity, the symptoms accompanying the chocolate methylxanthine mimic were preferable. Manipulation of the ratios of methylxanthines in the chocolate mimic led to the identification of a 5:1 theobromine/caffeine mixture as a promising coyote toxicant. This mixture was then administered to coyotes using the coyote lure operative device (CLOD). Mortality occurred in every coyote that ingested any portion of the CLOD contents. These results indicate that mixtures of theobromine and caffeine have the potential to be developed into a selective, effective, and socially acceptable toxicant for the control of pest coyotes.

KEYWORDS: Methylxanthine; theobromine; caffeine; CLOD; coyote toxicant

INTRODUCTION

In 2000, U.S. farmers and ranchers reported \$51.6 million in cattle and calf losses from animal predators. Canids (coyotes, dogs, wolves) were responsible for 83.4% of these predatory losses (1). That same year, U.S. farmers and ranchers suffered \$16.5 million in sheep and goat losses from animal predators. Canids were responsible for 75.8% of these losses (2). Coyotes were responsible for ~80% of livestock predation attributed to canids. Overall, coyotes were responsible for \$44 million in U.S. livestock losses.

Other damage caused by coyotes include collisions with aircraft (3, 4), attacks on pets (5) and children (6), damage to fruit and vegetable crops (7), predation on game species such as elk and deer (8), and predation on poultry (9). In addition to directly damaging fruit and vegetable crops, coyotes also contribute to crop losses via damage to hose irrigation systems (10). Coyotes have also been implicated in the transmission and spread of epizootic rabies in the United States (11).

Ranchers and pest control specialists use a variety of control techniques to minimize damage caused by coyotes. These techniques include exclusion (fencing), guard animals, scaring devices, trapping, shooting, and toxicants (12). The broad-spectrum mammalian toxicants sodium cyanide and sodium fluoroacetate (Compound 1080) are the only oral toxicants registered for predator control in the United States. In 1998, California voters passed Proposition 4, which severely restricted the use of sodium cyanide and sodium fluoroacetate for the control of livestock predators such as coyotes. As toxicants are a critical component of nearly all integrated pest management strategies (13–15), these bans severely restrict the ability of ranchers and pest control specialists to limit losses caused by

coyotes. Since the passage of Proposition 4 in California, similar initiatives have been passed in Colorado and Arizona. It is likely that this trend will expand to other states. As the development of pesticide products and subsequent registration with the U.S. Environmental Protection Agency (EPA) typically require more than a decade of work, it behooves the agricultural community to proactively develop new, more selective, and socially acceptable toxicants for pest (especially predatory) coyotes.

Because most predator-induced livestock losses in the United States are due to canids, a predator control substance should exhibit a high degree of toxicity to canids and, ideally, a low toxicity to nontarget animals and humans. The propensity for dogs to overdose on chocolate is documented in the veterinary literature (16–18). The methylxanthines theobromine and, to a lesser extent, caffeine are believed to be responsible for the toxicity of chocolate to dogs (Figure 1) (19, 20). In addition to chocolate, significant quantities of theobromine and caffeine are found in tea, coffee, and cola beverages. Although it is unknown what levels of caffeine and theobromine are acutely toxic to humans, given our significant and constant exposure to these compounds, toxicity to humans is likely quite low. However, 40 kg (100 pound) dogs have been poisoned by the amounts of these compounds contained in 0.5 pound of cocoa powder or 1 pound of dark chocolate (18). These findings suggest that theobromine and caffeine are much more toxic to canids than to humans—a desirable characteristic for any predacide. Theobromine and caffeine oral LD₅₀ (median lethality) values for dogs are 200–500 and 140 mg/kg of body weight (BW), respectively (21, 20). Theobromine and caffeine LD₅₀ values for rats are 1265 and 355 mg/kg of BW, respectively (22, 23). Additionally, for theobromine, the oral LD₅₀ value is 837 mg/kg for mice (22). For caffeine, there is a reported LD₅₀ value

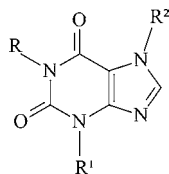


Figure 1. Methylxanthines: caffeine (1,3,7-trimethylxanthine), $R = R^1 = R^2 = \text{CH}_3$; theobromine (3,7-dimethylxanthine), $R = \text{H}$, $R^1 = R^2 = \text{CH}_3$; theophylline (1,3-dimethylxanthine), $R = R^1 = \text{CH}_3$, $R^2 = \text{H}$.

of 246 mg/kg of BW for rabbits (23). A comparison of these toxicity values suggests that methylxanthines are more toxic to dogs than to many other animal species.

The enhanced toxicity of these compounds to canids is believed to be related to a metabolic pathway that produces an unidentified methylxanthine metabolite that is unique to dogs. Additionally, unique ratios of *N*-demethylase-derived detoxification enzyme activities have been observed in dogs. These metabolism idiosyncrasies likely contribute to the relatively long half-life of methylxanthines in dogs (24). Methylxanthine toxicosis is associated with diuresis and inhibition of cellular calcium reuptake, which increase free calcium concentration and enhance skeletal muscle contractions. The resulting symptoms of methylxanthine toxicosis typically include central nervous system stimulation and tachycardia (25).

Although it would be impractical to deliver several pounds of chocolate to coyotes, the methylxanthines theobromine and caffeine are routinely extracted and concentrated from cocoa manufacturing waste and coffee beans, respectively. These compounds can be reformulated to duplicate the methylxanthine ratios in chocolate. This methylxanthine mixture could then be evaluated for its potential as a canid-specific toxicant. Additionally, it may be possible to manipulate the methylxanthine ratios to improve the potency, selectivity, symptoms, and/or cost of the pest coyote toxicant. Furthermore, it is possible that familiarity with coffee, tea (caffeine), and chocolate (theobromine) would minimize public perception of risk and possible opposition to such a product.

The objective of this research was to determine if methylxanthines have potential as active ingredients for a selective toxicant to control pest coyotes. To accomplish this goal, the following experiments were conducted to (1) identify natural products that contain significant quantities of methylxanthines, (2) develop two prototype pest coyote toxicants containing the methylxanthine ratios in the natural products identified in the first step, (3) conduct toxicity tests to determine the optimal combination of methylxanthines for development as an improved toxicant for pest coyotes, and (4) administer the pest coyote toxicant to captive coyotes using a proven field delivery device (26–28). All study procedures and experimental design were reviewed and approved by the WS/NWRC Institutional Animal Care and Use Committee.

MATERIALS AND METHODS

Materials. Ground (espresso grind) Arabian Mocha Sanani, Ethiopian Fancy, Kenya, Colombia, Costa Rica, Guatemala, Nicaragua las Hermanas, New Guinea, Sulawesi-Kalosi, and Sumatra coffee beans and green (Gunpowder) tea (China) were purchased from Peet's Coffee (Berkeley, CA). Tarajulie orange pekoe [Thakubari, (northeastern) India], Nonsuch [Nilgiri, (south-central) India], Kambaa Estate Kenya (Africa), and St. Coombs pekoe (Sri Lanka) black teas were purchased from Jolly Good Tea and Gifts (Vader, WA). Cocoa nibs, cocoa powder, bittersweet chocolate bars, and dark chocolate chunks were obtained from Italco Food Products (Denver, CO). Cocoa hulls (cocoa mulch) were obtained from Mirana International Resources (Palos Verde

Peninsula, CA). Lard (Morrell Co., Cincinnati, OH), Crisco vegetable (soybean) oil (J. M. Smuckers, Orrville, OH), bacon (Shure Fine International, North Lake, IL), and canned Alpo Prime Cuts dog food (Purina, St. Louis, MO) were purchased from local supermarkets. Acetonitrile (Optima grade) was obtained from Fisher Scientific (Fair Lawn, NJ). Caffeine, theobromine, and theophylline for analytical standards were obtained from Sigma Chemical (St. Louis, MO). Natural caffeine and theobromine extracts were obtained from Pechiney World Trade USA (Stamford, CT).

Coyotes. Adult coyotes (mixed sex) were obtained from the U.S. Department of Agriculture (USDA)/Animal and Plant Health Inspection Service (APHIS)/Wildlife Services (WS)/National Wildlife Research Center (NWRC) field station in Millville, UT, and from the University of Wyoming's predator colony in Laramie, WY. Coyotes were transported to and housed in the USDA/APHIS/WS/NWRC outdoor pen facility in Fort Collins, CO. Coyotes were quarantined for at least 2 weeks prior to toxicity testing. Coyotes were maintained on a daily ration of 350 g of Mazuri Exotic Canine Diet (PMI Nutrition International, Brentwood, MO) and water ad libitum.

Methylxanthine Analyses. In a 50 mL beaker, 10 ± 1 mg of ground coffee beans, tea leaves, chocolate, cocoa powder, cocoa nibs, or cocoa hulls was added to 10 mL of boiling water and covered with a watch glass. Each sample was simmered for 5 min and subsequently permitted to cool at room temperature for 20 min. The aqueous extract was filtered through a $0.45 \mu\text{m}$ filter for subsequent injection of $10 \mu\text{L}$ aliquots into a Hewlett-Packard (Palo Alto, CA) high-performance liquid chromatograph equipped with an H-209 4.6×250 mm column. Methylxanthines were separated using an acetonitrile/water (11:89) mobile phase at a flow rate of 1 mL/min. Methylxanthines were detected by ultraviolet absorption (245 nm) and quantified versus external standards.

Methylxanthine Dosing. *Test 1.* Caffeine and theobromine were mixed together at a ratio of 1:13 to prepare a methylxanthine chocolate mimic. A methylxanthine tea mimic was prepared by mixing a 20.5:1.3:1 ratio of caffeine/theobromine/theophylline. The mimics (15 g) were individually mixed with ~ 40 mL of lard/rendered bacon fat/soybean oil (5:1.5:1). Two coyotes were offered the lard mixture containing the chocolate mimic, and two coyotes were presented the lard mixture containing the tea mimic. The mixtures were offered in tared stainless steel bowls. After 3 h, the bowls and unconsumed mimics were removed and weighed to determine consumption.

Test 2. Aqueous suspensions of methylxanthines were prepared by combining 1 part theobromine or theobromine/caffeine mixture with 24 parts water. A variety of theobromine/caffeine ratios were evaluated. Coyotes were sedated with 1 mL of Dormitor (1 mg of medetomidine hydrochloride) (Pfizer Animal Health, Exton, PA) and 0.5 mL of Ketaved (50 mg of ketamine hydrochloride) (Vedco, St. Joseph, MO). Methylxanthine suspensions were administered via oral gavage, followed by 60 mL of water to ensure quantitative delivery of the desired quantity of methylxanthines. For each methylxanthine mixture evaluated, methylxanthines were administered to 16 coyotes; 4 coyotes were dosed at each of 4 different dose levels. After the coyotes had been replaced in their cages, the sedation was reversed by the administration of 1 mL of Antisedan (5 mg of atipamezole hydrochloride) (Pfizer Animal Health). Coyotes were observed intermittently for at least 12 h postdosing.

Toxicity data were analyzed by constructing log dose versus probit mortality curves for each theobromine/caffeine mixture evaluated. The median lethal dose (LD_{50}) and 99% lethal dose (LD_{99}) were calculated for each mixture (29).

Test 3. Canned dog food was mixed 2:1 with water and blended to achieve a "pourable" homogenate. The dog food homogenate was blended with corn syrup and the 5:1 theobromine/caffeine mixture to give a final ratio of 3:3:2 (dog food homogenate/corn syrup/theobromine/caffeine mixture). It was hoped that the corn syrup would mask the bitterness of the methylxanthines and that the dog food would serve as an incentive for the coyotes to consume the mixture. Fifty grams of this mixture was added to the 60 mL reservoir of a coyote lure operative device (CLOD) (Figure 2), a device that is being developed to deliver a variety of active ingredients to coyotes under field conditions (30–33). No lure or attractant was placed on the CLOD. A single

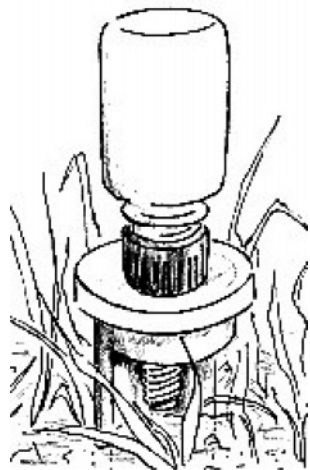


Figure 2. Coyote lure operative device (CLOD). Below-ground stake is not shown. Adapted from Berentsen et al. (33).

CLOD was then secured to the floor of each of six coyote pens at 7:00 a.m. Coyotes were observed at 0.5 h intervals. Unconsumed CLODs and CLOD remnants were removed at 10:00 a.m. This experiment was repeated with another six coyotes using a methylxanthine/dog food formulation containing a 4:1 ratio of theobromine/caffeine.

RESULTS AND DISCUSSION

Methylxanthines were quantified in a variety of coffee, tea, and chocolate extracts from samples grown at various locations to evaluate the possibility of a source that was particularly rich in methylxanthines. Caffeine was the only methylxanthine identified in coffee (**Figure 3**). Regardless of the country of origin, coffee samples from around the world contained ~27 mg of caffeine/g of coffee beans (**Figure 4**). Caffeine, theobromine, and theophylline were detected in tea samples from around the world. Methylxanthine levels were highest in Tarajulie tea from India. Caffeine, theobromine, and theophylline ratios in aqueous Tarajulie tea extracts averaged 20.5:1.3:1, respectively (**Figure 4**). Theobromine and caffeine were detected in chocolate products. The highest methylxanthine concentrations were detected in cocoa nibs and cocoa powder. Theobromine and caffeine were detected in these products at a ratio of 13:1, respectively (**Figure 4**).

Test 1. On the basis of these findings, tea and chocolate methylxanthine mimics were prepared by combining caffeine, theobromine, and theophylline at the ratios observed in Tarajulie tea and cocoa powder. Lard/bacon fat/soybean oil-based mixtures of each mimic were consumed by two coyotes each. For each mimic, one coyote vomited shortly after consumption of the mimic-fortified lard mixture. These two coyotes survived. Both coyotes that retained the mimic/lard mixture died within 4 h of consumption. The coyote that retained the tea mimic received a dose of 204 mg of caffeine/kg of BW, 13.3 mg of theobromine/kg of BW, and 9.9 mg of theophylline/kg of BW. This animal died about 1.5 h after the onset of symptoms, which included trembling, increased salivation, and seizures. The coyote that retained the chocolate mimic received 31.6 mg of caffeine/kg of BW and 413 mg of theobromine/kg of BW. This animal died following about 15 s of relatively minor symptoms, which included recumbent posture and labored breathing. On the basis of the symptoms preceding mortality, it was decided to further pursue a toxicant based on the chocolate mimic formulation.

Test 2. Because an extract of cocoa beans or powder would likely be prohibitively expensive to provide the active ingre-

dients for a pest coyote toxicant, we sought an economical source of these active ingredients. As reports in the veterinary literature indicated that dogs had been poisoned following consumption of cocoa mulch (cocoa hulls), cocoa hulls were obtained for methylxanthine analysis (34). The HPLC analysis of aqueous extracts of ground cocoa hulls indicated that the hulls contained the same ratios of methylxanthines as were detected in cocoa powder and cocoa nibs (**Figure 4**). Following estimation of the resources required to extract the several hundred grams of methylxanthines required for toxicity testing, kilogram quantities of cocoa hull extracted theobromine and coffee bean extracted caffeine were obtained from a commercial supplier.

Theobromine and caffeine were combined in the proportions observed in the cocoa extracts, 13:1 theobromine/caffeine, to permit an evaluation of the methylxanthine cocoa mimic toxicity to coyotes. Four coyotes were dosed at 450 mg/kg of BW; two coyotes were dosed with a water-based suspension, and two coyotes were dosed with a soybean oil based suspension. The coyotes dosed with the oil-based suspension regurgitated the suspension shortly after dosing; both of these animals exhibited no signs of toxicosis and survived. The animals dosed with the water-based suspension retained the dosing solution. Both animals exhibited relatively mild signs of toxicosis: increased salivation and slight trembling for several minutes. One animal died ~4 h after dosing. The other animal survived. On the basis of these results, it was decided to pursue water-based suspensions for subsequent experiments.

Dose versus response toxicity testing was conducted with water-based solutions using four groups of sedated coyotes (four coyotes per group) that were administered the cocoa mimic at 400, 450, 650, or 850 mg/kg of BW. Premortality symptoms were relatively mild, and mortality ranged from 50 to 100% (**Table 1**). Analysis of log dose versus probit mortality curve yielded LD₅₀ [50th percentile (median) lethal dose] and LD₉₉ (99th percentile lethal dose) estimates of 424 and 640 mg/kg, respectively.

Assuming that it would be more economical to pursue U.S. EPA registration of a single active ingredient rather than a two active ingredient product, we evaluated the toxicity of theobromine to coyotes. Four groups of sedated coyotes (four coyotes per group) were administered aqueous suspensions of theobromine at 400, 450, 650, or 850 mg/kg of BW. Premortality symptoms were relatively mild, and percent toxicity ranged from 0 to 75% (**Table 1**). Analysis of the log dose versus probit mortality curve yielded LD₅₀ and LD₉₉ estimates of 516 and 618 mg/kg, respectively.

Although theobromine or the cocoa mimic could be used to render an apparently humane mortality to coyotes, I felt that a more potent mixture of theobromine and caffeine would be needed to deliver a lethal dose under field conditions. Because caffeine is more toxic to canids than theobromine, different theobromine/caffeine ratios were evaluated in an attempt to identify a product that displayed the higher toxicity associated with caffeine while retaining the minimal premortality symptoms associated with theobromine. Coyotes were dosed with theobromine/caffeine mixtures at 600 mg/kg of BW. One coyote each was dosed via oral gavage with an aqueous suspension containing a 1:1, 1:2, 2:1, 4:1, 5:1, or 6:1 ratio of theobromine/caffeine. Coyotes dosed with the 1:1 and 1:2 mixtures exhibited undesirable symptoms of toxicosis and were euthanized. Coyotes dosed with the other mixtures died during the postdosing observation period. For these coyotes, premortality symptoms were considered to be acceptable, although the duration and

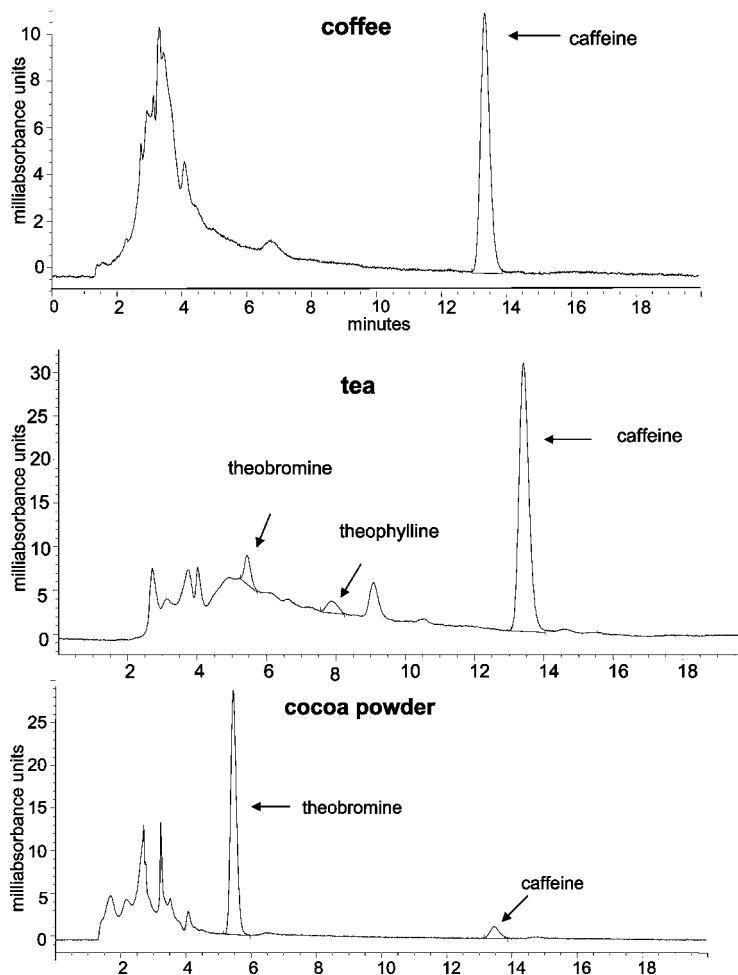


Figure 3. Chromatograms illustrating methylxanthine analyses of coffee (top), tea (Tarajulie) (middle), and cocoa powder (bottom).

magnitude of premortality symptoms generally decreased with increasing proportion of theobromine in the mixture.

On the basis of these findings, dose versus response toxicity testing was conducted with the 5:1 mixture of theobromine/caffeine. Four groups of sedated coyotes (four coyotes per group) were administered aqueous suspensions of theobromine at 250, 350, 450, or 650 mg/kg of BW. Percent toxicity ranged from 0 to 100% (**Table 1**). Premortality symptoms appeared to be nonexistent to minimal. Analysis of the log dose versus probit mortality curve yielded LD_{50} and LD_{99} estimates of 336 and 385 mg/kg, respectively. The increased toxicity of the 5:1 theobromine/caffeine mixture accompanied by minimal premortality symptoms suggested that this ratio of theobromine and caffeine has potential as a natural and socially acceptable toxicant for pest coyotes.

Test 3. To evaluate the potential of a methylxanthine-based pest coyote toxicant in a proven field delivery device, a 5:1 theobromine/caffeine formulation was added to CLODs and offered to six coyotes. All coyotes that bit the CLOD consumed some of the contents. Three of six coyotes consumed the CLOD contents. Consumption ranged from ~50 to 100% of the CLOD contents. Even though one of these coyotes vomited 15 min after consumption, all three coyotes died. Death occurred approximately between 2 and 7 h after the CLODs were offered to the coyotes. Premortality symptoms were extremely minimal and were of several seconds in duration. Estimated doses ranged from 700 to 1200 mg/kg.

Given the absence of undesirable symptoms noted in the coyotes dosed with the 5:1 theobromine/caffeine mixture, the

CLOD experiment was repeated with a 4:1 theobromine/caffeine mixture and six coyotes (the higher proportion of caffeine should give a more potent and less expensive product). In this experiment, two coyotes consumed the CLOD contents. One animal consumed the entire CLOD contents and vomited shortly thereafter; this animal died 3 h after the CLOD had been offered. The second animal consumed only 10% of the CLOD contents; it died 8 h after being offered the CLOD. Both animals staggered before becoming recumbent. Labored breathing preceded mortality. Estimated doses ranged from 140 to 1300 mg/kg. Although no formal control experiments were conducted (e.g., coyotes dosed with mixtures containing no theobromine or caffeine), the fact that no coyotes died in the lowest test 2 treatment groups (**Table 1**) indicates that the dosing procedure did not contribute to mortality observed during these experiments. Also, given the limited numbers of coyotes available for this testing, it was not possible to detect sex- or age-related trends in sensitivity to methylxanthines.

To decrease the cost of active ingredients, several modifications could be considered including using smaller (30 mL) CLOD reservoirs with synthetic theobromine and caffeine. This would lower the cost of active ingredients to approximately \$0.40 per delivery device. Also, because methylxanthine toxicosis is mediated by an increase in intracellular calcium concentrations, it is possible that the potency of methylxanthine mixture may be increased by simultaneous administration of inorganic calcium. Alternatively, observations in our laboratory indicate that sodium benzoate increases the solubility and toxicity of caffeine. The addition of relatively inexpensive

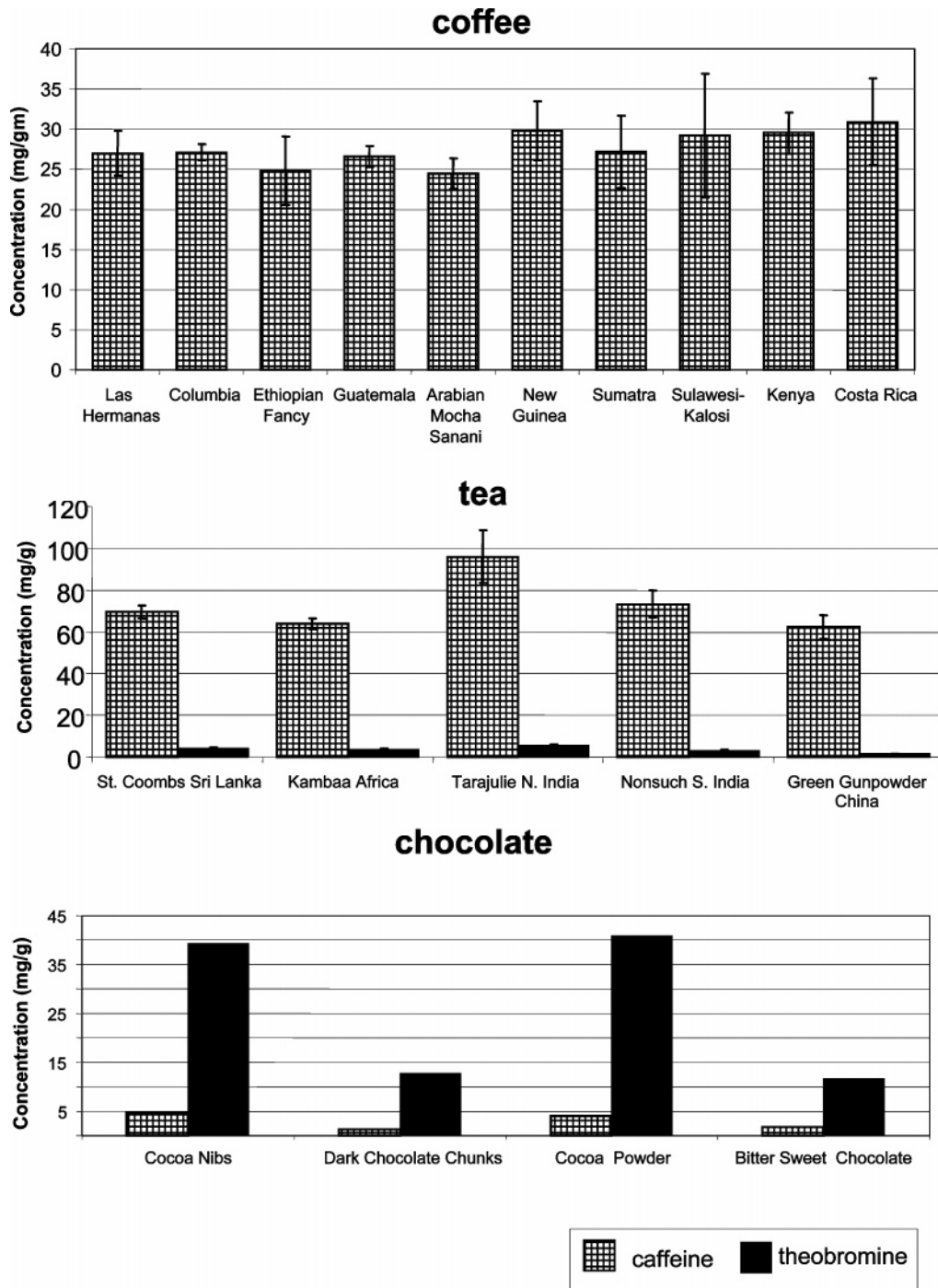


Figure 4. Theobromine and caffeine composition of various coffee (top), tea (middle), and chocolate (bottom) samples.

calcium and/or sodium benzoate to the formulation may permit the use of smaller quantities of the more expensive methylxanthines, which would decrease the cost of the required dose of the pest coyote toxicant.

In 1983, criteria for the selection and development of predator toxicants were summarized by Savarie and Connolly (35). These criteria included high effectiveness, acceptable taste and odor, rapid speed of action, low hazards to humans, availability of an antidote, acceptable level of environmental safety, minimal regulatory concerns, low cost, and reasonable availability. The data from this study clearly indicate that methylxanthine mixtures can effectively induce acute toxicity in coyotes and meet the criteria cited by Savarie and Connolly. Savarie and Connolly stated that substances with noxious tastes or odors are likely to be rejected by coyotes; the fact that captive coyotes

ingested methylxanthine formulations from CLODs indicated that methylxanthines can be formulated in a manner that is palatable to coyotes. Given the nearly ubiquitous occurrence of caffeine and theobromine in the diets of numerous human societies, it appears that the theobromine and caffeine hazards to humans are minimal. With respect to antidotes, given the low toxicity of theobromine and caffeine to humans, it is unlikely that a human antidote for the methylxanthine pest coyote toxicant would be required. Veterinary clinics are well aware of potential methylxanthine poisoning in dogs, and supportive therapy for inadvertently dosed dogs (vomiting, oral charcoal administration, oral saline solutions) is readily available. This is not a likely scenario for cyanide- or fluoroacetate-poisoned dogs. Environmental safety with respect to effects on nontarget wildlife are likely acceptable for methylxanthines as

Table 1. Toxicity Summary for Methylxanthine-Dosed Coyotes

methylxanthine mixture	dose (mg/kg of BW)	no. exposed	% mortality	LD ₅₀ (mg/kg)	LD ₉₉ (mg/kg)
13:1 (T:C) ^a	400	4	50	424	640
	450	4	75		
	650	4	100		
	850	4	100		
1:0 (T:C)	400	4	0	516	618
	450	4	25		
	650	4	50		
	850	4	75		
5:1 (T:C)	250	4	0	336	385
	350	4	75		
	450	4	100		
	650	4	100		

^a Theobromine/caffeine.

these compounds appear to exhibit increased toxicity to canids compared to most other species tested. Additionally, selectivity can be enhanced by the mode of application. For example, if delivered via the livestock protection collar, only animals that attack livestock would be directly exposed to the toxicant. In field studies, nontarget interest in CLODs has been minimal. Consumption by other species would be minimized because curious nontarget wildlife would need to puncture the polypropylene bottle to consume the contents. At \$0.40 per unit, the cost of the current formulation is higher than optimal. However, the addition of inexpensive synergists might significantly decrease the required amount and associated cost of the active ingredients. Regulatory requirements and associated costs for U.S. EPA registration for any predator toxicant are significant. Additionally, each of the two active ingredients in this product will likely have to be evaluated by the EPA. However, registration criteria focus on efficacy and safety. On the basis of these criteria, a methylxanthine-based pest coyote toxicant should fare well. Finally, given the ability of citizens to effectively regulate the availability of predator control measures through voter initiative options available in many states, potential social acceptability should be considered. Although there will invariably be a segment of society that is uncomfortable with any predator or pest coyote toxicant, most people's high degree of familiarity and comfort with chocolate and coffee should minimize opposition to a pest coyote toxicant based on mixtures of theobromine and caffeine.

This study demonstrated that theobromine and caffeine can be combined to deliver a potent and humane toxicant for coyotes. Such mixtures can be delivered to coyotes via the CLOD. Future research needs include the identification of the optimal quantity of theobromine and caffeine mixture to be included in a CLOD and the subsequent evaluation of this toxicant and delivery system under field conditions. Evaluation of this toxicant in the livestock protection collar could expand the types of delivery devices compatible with this product and further increase the selectivity of this coyote toxicant (36). However, the limited volume of the ingested livestock protection collar contents may need to be increased for use with methylxanthine coyote toxicant. This may be accomplished by formulating the collar contents to contain a material that functions as a taste attractant for predatory coyotes. The results from this research clearly demonstrate that theobromine/caffeine mixtures have potential as a pest coyote toxicant that is effective, selective, and potentially more socially acceptable than fluoroacetate or sodium cyanide.

ACKNOWLEDGMENT

The donation of coyotes by the USDA/APHIS/WS/NWRC Logan Utah Field Station and the University of Wyoming Predator Research Colony was essential for completion of this research. Cooperation of the NWRC Animal Care Staff greatly facilitated this study. Research support by NWRC Analytical Chemistry personnel Dennis Kohler and Elisha Kosak is greatly appreciated. Last and certainly not least, this research would not have been conducted without the tremendous support and input of Russ Mason, former NWRC Mammals Program Manager and current Science Advisor for the International Association of Fish and Wildlife Agencies.

LITERATURE CITED

- (1) U.S. Department of Agriculture, Agricultural Statistics Board, National Agricultural Statistics Service. *Cattle Predator Loss*; 2001; 11 pp.
- (2) U.S. Department of Agriculture, Agricultural Statistics Board, National Agricultural Statistics Service. *Sheep and Goat Predator Loss*; 2001; 11 pp.
- (3) Barras, S. C.; Wright, S. E. Civil aircraft collisions with birds and other wildlife in Ohio, 1990–1999. *Oh. J. Sci.* **2002**, *102*, 2–7.
- (4) Lensch, C. M.; Tedrow, C. A.; Scanlon, P. F. Mammal collisions with U.S. Air Force aircraft, 1987–1996. *J. Sci.* **2001**, *52*, 108.
- (5) Grinder, M.; Krausman, P. R. Coyotes in urban areas: conflicts and solutions. In *U.S. Forest Service Proceedings Rocky Mountain Research Station*; Gottfried, G. J., Edminster, C. B., Dillon, M. C., Eds.; 1998; Vol. 5, pp 235–243.
- (6) Cabyn, L. N. Coyote attacks on children in western North America. *Wildl. Soc. Bull.* **1989**, *4*, 444–446.
- (7) Armstong, J. B.; Phillip, M. C.; Walters, N. *Assessment of Coyote Damage to Crops and Livestock in Alabama*; Alabama Game and Fish Final Report, Project AL W-044/Job 20-A/Study 20; 1993; 58 pp.
- (8) Geese, E. M.; Grothe, S. Analysis of coyote predation on deer and elk during winter in Yellowstone National Park, Wyoming. *Am. Nat.* **1995**, *133*, 36–43.
- (9) Althoff, D. P.; Philip, S. G. Coyote family spatial relationships with reference to poultry losses. *J. Wildl. Manag.* **1981**, *45*, 641–649.
- (10) Werner, S. J.; El-Hani, A.; Mason, J. R. Repellent coatings for irrigation hose: Effectiveness against coyotes. *J. Wildl. Res.* **1997**, 146–148.
- (11) Fearneyhough, M. G. Texas oral rabies vaccination project and the experimental use of Raboral V-RG rabies vaccine in south Texas coyote rabies epizootic. *Proc. Vertebrate Pest Conf.* **1996**, *17*, 62.
- (12) Andelt, W. F. Carnivores. In *Rangeland Wildlife*; Krausman, P. R., Ed.; The Society for Range Management: Denver, CO, 1996; pp 133–155.
- (13) Lawson, A. B.; Dahlsten, D. L. Evaluation of systemic insecticides as a treatment option in integrated pest management of the elm leaf beetle, *Xanthogaleruca luteola*. *J. Econ. Entomol.* **2003**, *9*, 1455–1462.
- (14) Doerr, M. D.; Brunner, J. F.; Schrader, L. E. Integrated pest management approach of a new pest, *Lacanobia subjuncta* in Washington apple orchards. *Pest Manag. Sci.* **2004**, *60*, 1025–1034.
- (15) Fagerstone, K. A.; Johnston, J. J.; Savarie, P. J. Predicides for canid predation management. *Sheep Goat Res. J.* **2004**, 1976–1979.
- (16) Hornfeldt, C. S. Poisonings in animals. *Mod. Vet. Practice* **1987**, *68*, 25–27.
- (17) Hooser, S. *Chocolate Poisoning in Dogs*; Veterinary Professional Topics; Cooperative Extension Service, University of Illinois: Champaign-Urbana, IL, 1984; Vol. 9, pp 1–3.
- (18) Gwaltney-Brant, S. Chocolate intoxication. *Vet. Med.* **2001**, *96*, 108–111.

- (19) Gans, J. H.; Korson, R.; Cater, M. R.; Ackerly, D. D. Effects of short-term and long-term theobromine administration to male dogs. *Toxicol. Appl. Pharmacol.* **1980**, *53*, 481–496.
- (20) Farbman, D. B. Death by chocolate? Methylxanthine toxicosis. *Vet. Tech.* **2001**, March, 146–147.
- (21) Carson, T. L. Methylxanthines. In *Small Animal Toxicology*; Peterson, M. E., Talcott, P. A., Eds.; Saunders: Philadelphia, PA, 2001; pp 563–570.
- (22) Anonymous. *Labor, Hyg. Occupational Dis.* **1982**, *26*, 59.
- (23) Budavari, S. *The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals*, 12th ed.; Merck & Co.: Rahway, NJ, 1996; p 1674.
- (24) Miller, G. E.; Radulovic, L. L.; Dewit, R. H.; Brabec, M. J.; Tarka, S. M.; Cornish, H. H. Comparative theobromine metabolism in five mammalian species. *Drug Metab. Disposition* **1984**, *12*, 154–160.
- (25) Serafin, W. E. Drugs used in the treatment of asthma. In *Goodman and Gilman's The Pharmacologic Basis of Therapeutics*, 9th ed.; Hardman, J. G., Ed.; McGraw-Hill: New York, 1999; pp 672–678.
- (26) Ebbert, S. M. Field evaluation and improvement of a new system for delivering substances to coyotes. Thesis, Texas A&M University, College Station, TX, 1988.
- (27) Hein, E. W.; Andelt, W. F. Evaluation of coyote attractants and an oral delivery device for chemical agents. *Wildl. Soc. Bull.* **1994**, *22*, 651–655.
- (28) Stolzenburg, H. W.; Howard, V. W. Activation of liquid bait devices by coyotes in southern New Mexico. *Wildl. Soc. Bull.* **1989**, *17*, 306–312.
- (29) Finney, D. J. *Probit Analysis*, 3rd ed.; Cambridge University Press: Cambridge, U.K., 1971.
- (30) Marsh, R. W.; Howard, W. E.; McKenna, S. M.; Butler, B.; Barnum, D. A.; Teranishi, R. A new system for delivery of predacides or other active ingredients for coyote management. *Proc. Vertebrate Pest Conf.* **1982**, *10*, 229–223.
- (31) Ebbert, S. M.; Fagre, D. B. Importance of attractant qualities for improving a new coyote delivery system. *Proc. Great Plains Wildl. Damage Control Workshop* **1987**, *8*, 189–194.
- (32) Berentsen, A. R. Behavioral responses of coyotes to the coyote lure operative device. M.S. Thesis, Utah State University, Logan, UT, 2004.
- (33) Berentsen, A. R.; Schmidt, R. H.; Timm, R. M. Behavioral responses of coyotes to the CLOD in familiar and unfamiliar environments. *Proc. Vertebrate Pest Conf.* **2004**, *21*, 58–63.
- (34) Anonymous. ASPCA study finds cocoa bean mulch could harm your dogs. *J. Am. Vet. Med. Assoc.* **2003**, *11*, 1545.
- (35) Savarie, P. J.; Connolly, G. E. Criteria for the selection and development of predacides. In *Vertebrate Pest Control and Management Materials*; Fourth Symposium, ASTM STP 817; Kaukeinen, D. E., Ed.; American Society for Testing and Materials: Philadelphia, PA, 1983; pp 278–284.
- (36) Blejwas, K. M.; Sacks, B. N.; Jaeger, M. M.; McCullough, D. R. The effectiveness of selective removal of breeding coyotes in reducing sheep predation. *J. Wildl. Manag.* **2002**, *66*, 451–462.

Received for review January 24, 2005. Revised manuscript received March 15, 2005. Accepted March 16, 2005. This research was funded by grants from the Vertebrate Pest Control Research Advisory Committee, California Department of Food and Agriculture, and the Wyoming Animal Damage Management Board. Mention of companies or commercial products does not imply recommendation or endorsement by the U.S. Department of Agriculture. Product names are mentioned solely to report factually on data and to provide specific information.

JF050166P