Development of Non-prey Baits for Delivery of Acetaminophen to Brown Treesnakes *(Boiga irregularis)* on Guam

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List of Acronyms

ANOVA – Analysis of Variance
BTS – Brown Treesnake
DNM – Dead Neonatal Mouse/Mice
GC/MS – Gas Chromatography/Mass Spectrometry
IUPAC – International Union of Pure and Applied Chemistry
NIST – National Institute of Standards and Technology
NWRC – National Wildlife Research Center
PVC – polyvinyl chloride
SERDP – Strategic Environmental Research and Development Program
USA – United States of America
USDA – United States Department of Agriculture
U.S. EPA – United States Environmental Protection Agency
VOCs – Volatile Organic Chemicals

Keywords: bait, *Boiga irregularis,* brown treesnake, dead neonatal mouse, fatty acids, GC/MS, Guam, headspace analysis

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Mention of commercial names is for identification only and does not constitute endorsement by the United States Department of Agriculture. All work was performed following approval by the NWRCs Institutional Animal Care and Use Committee under QA-1745.

Abstract

Objective: Dead neonatal mice (DNM) are the bait substrate for delivery of acetaminophen to brown treesnakes *(Boiga irregularis,* BTS*)* on Guam. Acetaminophen is a registered oral toxicant for BTS. DNM serve as a lure attractant and are well accepted by snakes; however, for logistic and economic reasons, it is necessary to develop an effective bait substrate to replace DNM. The objective of this project is to evaluate extracts from aged DNM that when applied to non-prey baits will enhance the bait take (consumption) by BTS.

Technical Approach: Decomposition products from DNM carcasses aged for 48 h in an environmental chamber and in headspace volatiles from DNM aged for 24 h and 48 h were fractionated and chemicals in the fractions were characterized by gas chromatography/mass spectrometry. Beef and Pup-Peroni®, a commercial dog food snack (used as surrogates for non-prey food items) were treated with formulations of these chemical extracts and decomposition products from 48h aged DNM. These substrates were then evaluated as bait enhancers for BTS under field conditions on Guam. Solvent extracts from DNM carcasses aged for 48 h applied to beef baits also were evaluated as bait take enhancers.

Results: In this study 29 chemicals were identified from the decomposition products of DNM carcasses aged for 48 h. Three chemical extract formulations and decomposition products from these 48h aged DNM were evaluated as bait enhancers for BTS. Each of the three extracts tested contained three to seven chemicals that represented fatty acids and volatile organic chemicals. None of the beef baits treated with the three chemical extract formulations from 48h aged DNM enhanced consumption of treated beef baits. In addition, volatile chemical recipes from 24h and 48h aged DNM were not effective bait enhancers. Beef and the commercial dog food snack were treated with decomposition products from 48h aged DNM. Treated beef consumption was enhanced, but not the treated dog food snack. Diethyl ether, pentane , and water extracts from decomposition products of DNM aged for 48 h enhanced bait consumption, but chemicals in these extracts have not been identified. Finally, acetone and ethanol extracts did not enhance bait consumption.

Benefits: The data from this study indicate the importance of the whole odiferous complement from aged dead neonatal mice to promote bait consumption by brown treesnakes and that bait consumption may be substrate specific. The high bait take efficacy with the diethyl ether, pentane, and water extracts indicate that bait enhancer chemicals could be identified and evaluated in future research. The odiferous plume from dead mice probably consists of several chemical signatures that change over time, and the attractiveness of these temporal chemical fingerprints probably also change. Although the preceding results are important findings, they also indicate that the path to developing a cost-efficient chemical signature enhancement for non-DNM bait will be difficult and success is not certain.

Objective

In 2007, a study was completed which showed that a non-prey bait, beef, treated with the total decomposition products from 48h aged DNM enhanced bait take (consumption) by brown treesnakes under field conditions (Savarie 2008). The significance of these results suggests that the decomposition products could be chemically fractioned, identified by gas chromatography/mass spectrometry (GC/MS), and formulated into recipes that could be applied to non-prey baits to replace DNM. The objective of this project is to conduct bait evaluations with some of the chemicals identified from the total decomposition products from 48h aged DNM and chemicals identified in the volatile headspace from 24h and 48h aged DNM. The project objective relates to the SERDP Statement of Need (SISEED 10-01) for developing innovative approaches for control of BTS on Guam.

Background

The accidently introduced invasive brown treesnake *(Boiga irregularis*, BTS*)* has caused the extirpation of 10 of the 12 native forest bird species and declines of native lizards, major power outages, and human health emergencies on the island of Guam (Savidge 1987, Fritts 1988, Rodda and Fritts 1992, Rodda et al 1992, Fritts and Rodda 1998). Operational procedures to control snakes include trapping, nighttime searches on fences around cargo areas, and detector dogs to deter snake dispersal via Guam's cargo network to other at risk areas such as Hawaii (Engeman and Vice 2001, Vice and Vice 2004). Dead neonatal mice (DNM) treated with acetaminophen, an oral toxicant for BTS (U.S. EPA Reg. No. 56228-34), is an emerging technique for controlling snakes on a landscape scale on Guam. DNM serve as a lure attractant and bait substrate and are very well accepted by BTS (Jojola-Elverum et al. 2001, Savarie et al. 2001), and are the bait of choice for delivery of acetaminophen to BTS in the field. For logistical and economic reasons, there is a need to develop an effective bait matrix to replace DNM for control and eradication of BTS. DNM are relatively expensive (about $0.34 each), have to be shipped frozen from the continental United States and maintained frozen until applied in the field, and have a field life of only 2-3 days. An artificial bait substrate that is less expensive, can be stored at room temperature before application in the field, and has a longer field life would be more efficient for operational use.

USDA/National Wildlife Research Center (NWRC) scientists have evaluated numerous candidate substrate and chemical lure attractants for BTS including commercial snake steak sausages, cotton rolls, plastic lizards, commercial canned meats, shrimp, chicken meat, and beef, but none were as effective as DNM (Savarie and Clark 2006). In comparative tests with caged snakes, bait take (consumption) of DNM and beef baits was 90% and 80%, respectively. Under field conditions, bait take for DNM was 100% but only 27% for beef. Bait take of snake steak sausages by caged BTS is also substantially greater than under field conditions (J. Shivik, pers. comm.). These data indicate that beef and sausage baits are palatable to snakes (otherwise they probably would not have been consumed under laboratory caged conditions), and it is hypothesized that the poor field bait take results occurred because the baits did not have enough attractive odor to entice snakes to the baits. Bait take evaluations have also been conducted with beef treated with decomposition products from dead mice. Bait take of untreated beef was low (7%), whereas bait take of untreated DNM and treated beef were 97% and 67%, respectively (Savarie 2008). The significance of these results opens the possibility that other types of non-prey food items, that are stable without being maintained refrigerated or frozen before field application, could be developed as practical bait substrates for snakes.

Previous research indicated that 48h aged DNM carcasses were highly preferred by BTS and that bacterial degradation of the skin produces odiferous chemical volatiles that promote consumption (Jojola-Elverum et al. 2001). Volatiles from 24h and 48h aged DNM have been identified (B. Kimball, pers. comm.) and were evaluated in the present study. Additionally, 48h aged DNM carcasses were extracted with solvents and the extracts were evaluated, as well as chemicals identified by GC/MS analyses of aged DNM, and decomposition products of DNM. The volatiles, solvent extracts, chemicals identified by GC/MS, and decomposition products were evaluated for their ability to enhance consumption of beef baits. Beef was selected as the non-prey bait because it is marginally accepted by BTS.

Materials and Methods

The experiments addressed three categories of investigation. The first category describes the procedures used to prepare 48h aged DNM under environmental chamber conditions and GC/MS analyses that were funded by SERDP. The second category describes the headspace analyses of volatile chemicals from 24h and 48h aged DNM aged under field conditions on Guam and was not funded by SERDP. The third category describes seven bait evaluation experiments conducted under field conditions on Guam that were all funded by SERDP.

Category 1

Environmental Chamber 48h Aged DNM

Prior to GC/MS fatty acid and volatile organic chemical (VOC) analyses, DNM were aged 48 h in a Conviron environmental chamber at NWRC. DNM were placed in open glass jars for 48 h on a 24 h cycle ranging from about 24^{o}C to 31^{o}C and 80% relative humidity to approximate the ambient environmental conditions on Guam. DNM were stored frozen at approximately -20^{o}C.

Fatty Acid and Volatile Organic Chemical Analyses

Fatty acid profiles for control DNM, 48h aged DNM, control beef, and beef treated with the decomposition products from 48h aged DNM were determined by GC/MS following one of two organic solvent extractions of a tissue sample. All tissue samples were obtained following homogenization with a cryomill in liquid nitrogen. The first extraction was a modified microwave assisted Folch extraction (Hamilton et al. 1992) where 0.5 g of tissue was extracted in 10 mL of 2:1 chloroform/methanol. The microwave applied 400 W and ramped the extraction vessel temperature from 30 to 70^{o}C at 10^{o}C per min, holding for 30 min. The extract was filtered and rinsed with water. The organic solvent was concentrated to dryness under a mild stream of nitrogen at 40^{o}C. The sample was reconstituted in 10 mL of hexane. A 1 mL aliquot was derivatized with 1 mL of 3N HCl in methanol. Tridecanoic acid methyl ester was added as an internal standard.

The GC/MS used to analyze these samples of the first extraction was an Agilent 6890 GC and a 5973 MS. The GC column used was an Agilent DB-225, 20 m x 0.1 mm, with 0.1 mm film thickness. The conditions under which these samples were analyzed were to inject 1 mL, with the inlet at 85 psi and 240^{o}C, with a split ratio of 8:1. The oven was held at 35^{o}C for 0.5 min and then ramped to 195^{o}C at 25^{o}C/min, ramped to 205^{o}C at 30^{o}C/min, and then ramped at 8^{o}C/min to a final temperature of 230^{o}C. This temperature was held constant for 8min. The MS scanned from 50 to 550 m/z in positive ion mode. The MS source was set at 230^{o}C, the MS quadrapole was set at 150^{o}C, and the transfer line at 280^{o}C. Total run time was 21.4 min. Peak identification/quantification was determined with a 37 compound fatty acid standard mixture (Supelco, Bellefonte, Pennsylvania, USA).

The second extraction was also a microwave assisted extraction but used 10 mL of a 1:1 methylene chloride/ acetone solvent added to 0.5 g of tissue. The microwave applied 400 W and ramped the extraction vessel temperature from 30 to 70^{o}C at 10^{o}C/min, holding for 30 min. The water was removed from the extract by the addition of 1 g sodium sulfate. To establish a volatile compound profile the samples were analyzed directly by GC/MS. To establish a fatty acid

profile for the 1:1 methylene chloride/acetone extract, solvent samples were evaporated to dryness under a mild stream of nitrogen at 40^{o}C and then reconstituted in 2 mL of hexane; 1 mL was derivatized and analyzed as above. Tridecanoic acid methyl ester was added as an internal standard to these samples.

The GC/MS used to analyze these samples of the second extraction was an Agilent 6890 GC and a 5973 MS. The GC column used was a Zebron DB-1HT, 30 m x 0.25 mm, with 0.1 mm film thickness. The conditions under which these samples were analyzed were to inject 1 mL, with the inlet at 14 psi, and 240^{o}C, with a split ratio of 10:1. The oven was held at 30^{o}C for 2 min and then ramped to 300^{o}C at 10^{o}C/min. This temperature was held constant for 5 min. The MS scanned from 10 to 500 m/z in positive ion mode. The MS source was set at 230^{o}C, the MS quadrapole was set at 150^{o}C, and the transfer line at 280^{o}C. Total run time was 34 min. Peaks were identified by ion fragmentation match with a NIST database and confirmed by injecting a standard of the identified compound.

Fatty acid profiles were determined for three control DNM and three 48h aged DNM. Fatty acid profiles were also determined for three individual pieces of control beef and three individual pieces of beef that had been treated by tumbling with 48h aged DNM (2 beef : 1-48h aged DNM) in a closed glass jar on a benchtop roller for 1 h at ambient room temperature (≈ 21^{o}C).

The volatile organic chemical (VOC) profiles were determined from three subsamples from either a single control DNM or a 48h aged DNM. The VOC profiles from control beef or 48h aged DNM-treated beef were determined from composite samples obtained from two beef pieces. For the treated beef, two pieces were tumbled with one 48h aged DNM in a closed glass jar on a benchtop roller for 1 h at about 21^{o}C. Additionally, the VOC profile was determined for four 48h aged DNM to examine the variance across different mice.

The fatty acid profile for the lipid fraction extracted in the VOC extraction was determined for three subsamples from either a single control DNM or a single 48h aged DNM. The corresponding fraction from control beef or 48h aged DNM treated beef was determined for three subsamples of a composited from two beef baits, one control and one 48h aged DNM treated.

To assess the effect of different solvent polarities on the VOC that can be extracted from a 48h aged DNM, DNM were extracted in acetone, ethanol, diethyl ether, pentane, and water. In each case a single DNM was placed in 4 mL of the solvent at room temperature for 15 min. From this, 1 mL of solvent was extracted according to the VOC procedure above in dichloromethane/acetone at 1:1. The water was removed from the extract by the addition of 1 g sodium sulfate. To establish a volatile compound profile the samples were analyzed directly by GC/MS as above.

Category 2

Headspace Volatile Analyses from 24h and 48h Aged DNM

Headspace analyses of volatile chemicals from 24h and 48h aged DNM were conducted in 2001 (Kimball, NWRC unpublished data). Although not funded by SERDP, the results of these analyses are included in this report as a supplement to the analyses of the fatty acid and volatile organic chemical analyses and total decomposition body products from 48h aged DNM. These results formed the basis for the design of Experiments 6 and 7 below. Carcasses of DNM were subjected to headspace volatiles collection. Collections were made on the island of Guam to ensure that carcasses were naturally inoculated with a relevant population of microbes. DNM were placed in a side-arm flask equipped with a rubber stopper through which a length of glass tubing had been placed. With the stopper in place, an air-pump could be attached to the glass tubing such that ambient air was drawn in the side-arm and evacuated the headspace contents of the flask through the glass tube. Volatiles were collected on a thermal desorption tube placed in-line between the flask and the pump. Samples were collected for one or two hours. A unique desorption tube was used for each unique sample. DNM were analyzed over a time-course from 0 to 48 hours. The desorption tubes were placed in sealed glass storage tubes and transported to Fort Collins, CO, for analysis at the NWRC analytical chemistry laboratory. The thermal desorption tubes were placed (individually) into a Tekmar 3000 Purge and Trap analyzer attached to an Agilent 5972 gas chromatograph/mass spectrometer (GC/MS). Analyses were conducted according to previously described procedures (Kimball et al. 2000).

Category 3

Bait Evaluation Experiments

Bait evaluation experiments were conducted in July-September 2010 and July-August 2011 on U.S. military land on Guam. On Andersen Air Force Base the test sites were the Tarague Beach and Sanitary Landfill Pipeline areas. On Navy Base Guam the test sites were the Haputo Beach and Tenjo Vista Tank Farm (July-August 2011) areas. The metric of bait take (i.e., bait acceptance, consumption) by BTS was the disappearance of DNM, the positive control, and test baits from PVC tubes. All bait evaluation experiments were funded by SERDP.

PVC tubes (5.1cm diameter x 30.5cm long, hereafter called bait stations) were bisected with a 0.64cm diameter bolt, 2.5cm from each end, to mitigate entry of non-targets animals such as crabs (Figure 1). Scoring of over 5,000 h of video tape indicate that BTS are almost exclusively the only animal responsible for bait disappearance (Savarie and Clark 2006, P. Savarie, personal observation).

Figure 1. (Top) Standard bait station. (Bottom) Standard bait station modified with a glass vial and cotton wick for delivery of volatile chemicals inside the tube.

Before each experiment bait stations were cleaned and the inside scrubbed with a chlorine bleach solution (1:50 chlorine:water) and rinsed with water. Bait stations were placed along the forest perimeter adjacent to roads at 20 m intervals about 1.5 m high in vegetation in straight line transects. Each transect contained 10 baits per bait treatment with the number of treatments varying for each experiment. Bait take in each transect was recorded daily for two days and baits missing were not replaced. All baits were randomly assigned (Research Randomizer, http://www.randomizer.org/form.htm) to the bait stations in each transect. After two days, each transect was moved to a new location in each experiment. Beef baits (≈ 5 g) were prepared from U.S. Chill bottom round roast; DNM were 4-7 g. Seven experiments were conducted and Table 1 summarizes the design for each experiment. Procedures for each of the seven experiments follow Table 1. Bait take of each treated bait type was compared to DNM, the positive control, and control beef in each experiment using the analytical methods described below in the section "Statistical Analyses for Bait Evaluation Experiments."

Table 1. Design summary for the seven bait take experiments.

Experiment no.	Objective	No. of transects	Bait types (n = 10 bait types for each transect)
1	Test decomposition products from 48h aged DNM as bait enhancers	6	DNM Beef Pup-Peroni® Beef 48h aged DNM Pup-Peroni 48h aged DNM
2	To determine attractiveness of beef treated with acetone (a), diethyl ether (de), ethanol (e), pentane (p), and water (w)	2	DNM Beef aBeef deBeef eBeef pBeef wBeef
3	Test beef treated with solvent extracts (acetone = ae, diethyl ether = dee, ethanol = ee, pentane = pe, water = we) and decomposition products from 48h aged DNM as bait enhancers	5	DNM Beef aeBeef deeBeef eeBeef peBeef weBeef Beef 48h aged DNM
4	Evaluate attractiveness of beef treated with volatile organic chemicals and fatty acid extracts from 48h aged DNM using soybean oil as the carrier	3	DNM Beef Beefo BeefE1 BeefE2 BeefE3

Table 1. (Continued). Design summary for the seven bait take experiments.

Experiment no.	Objective	No. of transects	Bait types (n = 10 bait types for each transect)
5	Evaluate attractiveness of beef treated with volatile organic chemicals and fatty acids extracts from 48h aged DNM using ethanol as the carrier	3	DNM Beef Beefe BeefE1 BeefE2 BeefE3
6	To evaluate beef treated with headspace volatiles from 24h and 48h aged DNM applied by either soaking (s) or by wick (w)	3	DNM Beef sBeef24h sBeef48h wBeef24h wBeef48h
7	To evaluate beef treated with headspace volatiles from 24h and 48h aged DNM by direct application of 0.25 mL	2	DNM Beef Beef24h Beef48h

Experiment 1: Importance of Decomposition Products for Bait Attractiveness
The objective of this experiment was to determine the attractiveness of the decomposition products from 48h aged DNM to beef and Pup-Peroni® (≈ 3.7 g; a commercial beef flavored dog food snack that can be stored at ambient room temperature without refrigeration). DNM decomposition products were obtained by aging 5 DNM for 48 h in 470 mL open glass jars under ambient field conditions on Guam. The jars were placed in snake-proof enclosures (10.2 cm diameter x 30.5 cm long plastic tubes with ends closed by fine nylon mesh). The plastic tubes were positioned horizontally and hung about 1.5 m high in forest vegetation. The goal was to produce decomposition products that are attractive to BTS (Jojola-Elverum et al. 2001). Five bait types were tested: control DNM, beef, and Pup-Peroni® ; and beef and Pup-Peroni® each treated with decomposition products from 5-field aged 48h DNM by tumbling in 470 mL closed glass jars on a benchtop roller for 1 h. This experiment consisted of 5 bait types with 10 baits per bait type in each of 6 transects for a total of 300 bait stations.

Experiment 2: Attractiveness of Beef Baits Treated with Solvents
The objective of this experiment was to determine the attractiveness of beef treated with each of the 5 solvents used in experiment 3 for extracting 48h field aged DNM. The beef baits were treated by adding 20 mL of each solvent (acetone, diethyl ether, ethanol, pentane and water) to individual 470 mL glass jars each containing 10 beef baits. The treated beef baits were stored in the closed glass jars until placed in bait stations on the same day of treatment. Seven bait types were tested: control DNM and beef; beef treated with acetone, diethyl ether, ethanol, pentane, and water (aBeef, deBeef, eBeef, pBeef, and wBeef, respectively). This experiment

consisted of 7 bait types with 10 baits per bait type in each of 2 transects for a total of 140 bait stations.

Experiment 3: Bait Take of Beef Baits Treated with Solvent Extracts and Decomposition Products from 48h Field Aged DNM

In this experiment bait take of beef treated with solvent extracts and decomposition products from 48h field aged DNM was evaluated. Individual closed 470 mL glass jars containing 5-48h aged DNM were extracted for 15 min with 20 mL in each of 5 solvents (acetone, diethyl ether, ethanol, pentane, and water). Each jar was swirled 4-6 times during the 15 min extraction. The aged DNM were removed and 10 beef baits added to each of the 5 jars. The treated beef baits were stored in the closed glass jars until placed in bait stations on the same day of extraction. Decomposition products were transferred to beef by tumbling 10 beef baits with 5-48h aged DNM for 1 h in a closed glass jar. Eight bait types were tested: control DNM and beef; beef treated with acetone, diethyl ether, ethanol, pentane, and water extracts (aeBeef, deeBeef, eeBeef, peBeef, and weBeef, respectively), and beef treated with 48h aged DNM decomposition products (Beef48h). This experiment consisted of 8 bait types with 10 baits per bait type in each of 5 transects for a total of 400 bait stations.

Experiment 4: Attractiveness of Beef Baits Treated with Fatty Acids and Volatile Organic Chemicals (Soybean Oil as Carrier)

The objective of this experiment was to determine the attractiveness of fatty acids (Tables 3, 5, and 7) and volatile organic chemicals (Tables 4, 6, and 8) identified by GC/MS from 48h aged DNM. Seven chemicals were tested. All 4 of the volatile organic chemicals were tested except that the cis- isomer of 3, 4-dimethyl-2-pentene was inadvertently tested instead of the trans - isomer identified by GC/MS. Each of the 4 volatile organic chemicals showed large concentration increases in 48h aged DNM as compared to control DNM (Table 6), which may be a signal for attractiveness of aged DNM. Except for hydrocinnamic acid, the other 3 VOCs transferred from 48h aged DNM to beef (Table 6). Only 3 of the 25 fatty acids (capric-C10:0, lauric-C12:0, and myristic-C14:0) were tested and each showed large concentration increases in 48h aged DNM as compared to control DNM (Table 7). The chemicals and amounts for each chemical tested are listed in Table 2. Each extract was mixed in 10 mL soybean oil and tumbled with 50-5 g beef baits for 10 min. The control for the treated beef extracts was 50-5 g beef baits mixed in 10 mL soybean oil for 10 min. All baits were stored frozen until used for bait evaluations. Six bait types were tested: control DNM and beef, and beef treated with soybean oil, Extract 1, Extract 2, and Extract 3 (Beefo, BeefE1, BeefE2, and BeefE3, respectively). This experiment consisted of 6 bait types with 10 baits per bait type in each of 3 transects for a total of 180 bait stations.

Table 2. Chemicals and amounts in Extracts 1, 2, and 3 from DNM aged in an environmental chamber for 48 h.

Extract no.	Chemical	Amount in formulation
1	cis-3,4-dimethyl-2-pentene	110 µL
	2-methylbutyric acid	60 µL
	4-methylpentanoic acid	430 µL
	Hydrocinnamic acid	108 mg
	Capric acid	62 mg
	Lauric acid	62 mg
	Myristic acid	62 mg
2	cis-3,4-dimethyl-2-pentene	110 µL
	2-methylbutyric acid	60 µL
	4-methylpentanoic acid	430 µL
	Hydrocinnamic acid	108 mg
3	Capric acid	62 mg
	Lauric acid	62 mg
	Myristic acid	62 mg

Experiment 5: Attractiveness of Beef Baits Treated with Fatty Acids and Volatile Organic Chemicals (Ethanol as Carrier)

The methods for extract formulations, extracts tested, and bait types tested were identical to experiment 6 above except that 10 mL ethanol was used as the carrier for each of the 3 extracts mixed with beef (Table 2). Ethanol was tested as an alternate to increase bait take efficiency because the consumption of baits with the soybean oil carrier in experiment 4 were low. The beef controls were mixed with 10 mL ethanol (Beefe). Six bait types were tested: control DNM and beef, and beef treated with ethanol, Extract 1, Extract 2, and Extract 3 (Beefe, BeefE1, BeefE2, and BeefE3, respectively). This experiment consisted of 6 bait types with 10 baits per bait type in each of 3 transects for a total of 180 bait stations.

Experiment 6: Attractiveness of Chemical Volatiles from 24h and 48h Field Aged DNM

Two beef treatments were made with recipes from the 24 h and 48 h field aged DNM chemical volatiles (Table 9): 1) beef (≈ 5-7 g, n = 30 per recipe) soaked in 30 mL in each of the recipes for 10 min in a closed glass jar, and 2) glass vial containing 7 mL of each recipe with a cotton wick protruding inside the plastic bait tube adjacent to a control beef bait (n = 30 per recipe). The soaked and wicked applications of these volatiles exposed snakes to two different presentations of the chemicals. This experiment was conducted with 6 bait types: control DNM and beef, and beef treated by soaking in chemical volatile formulations from 24 h and 48 h field aged DNM (sBeef 24 and sBeef 48); and control beef exposed to the volatiles from 24 h and 48 h field aged DNM by cotton wick (wBeef 24 and wBeef 48). This experiment consisted of 6 bait types with 10 baits per bait type in each of 3 transects for a total of 180 bait stations. This experiment was funded by SERDP.

Experiment 7: Attractiveness of Direct Application of Chemical Volatiles from 24h and 48h Field Aged DNM

The rationale for direct application of the chemical volatiles was that the soaked and wicked beef treatments of chemical volatiles in experiment 6 may have been repellent to the BTS. Four bait types were tested: control DNM and beef, and beef treated by direct application of 0.25 mL from each of the chemical volatile formulations from 24h and 48h field aged DNM (Beef 24 and Beef 48). The recipes for the 24h and 48h aged DNM are in Table 9. This experiment consisted of 4 bait types with 10 baits per bait type in each of 2 transects for a total of 80 bait stations. This experiment was funded by SERDP.

Statistical Analyses for Bait Evaluation Experiments

Statistical analyses were performed on the mean two-day rate of bait take. The analyses were the same format for each of the seven bait evaluation experiments. For each of the seven experiments, the statistical analysis was a randomized block ANOVA where the individual transects formed the blocks. The measurements analyzed for each bait type on each transect was the proportion of baits taken. As part of the ANOVA procedures the efficacy of all baits were individually compared against control DNM as the standard for comparison using *a priori* linear contrasts. All analyses were conducted using SAS PROC MIXED with restricted maximum likelihood estimation (SAS Institute 2004).

Results and Discussion

Category 1

Fatty Acid and Volatile Organic Chemical Analyses

Chromatograms of fatty acids extracted from control beef, beef treated with 48h aged DNM, control DNM, and 48h aged DNM, using the microwave assisted modified Folch method, are presented in Figure 2. The DNM have much higher concentrations of fatty acids than the beef bait substrate. The fatty acids identified in the samples in Figure 2 are listed in Table 3.

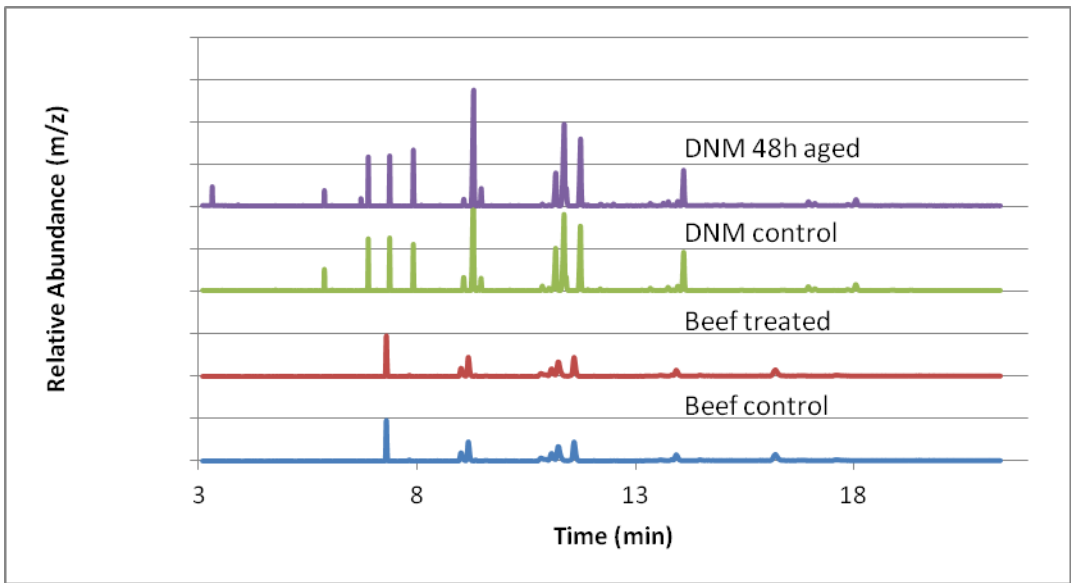

Figure 2. Chromatograms of fatty acids profiles determined for beef, control and treated, compared to control DNM and 48h aged DNM. The tick marks on the y axis relative abundance (m/z) scale are 1e6 in magnitude.

Table 3. Fatty acids identified in the microwave assisted modified Folch extraction.

Peak #	IUPAC nomenclature	Common name	Retention time (min)
1	C8:0	Caprylic acid	4.95
2	C10:0	Capric acid	6.07
3	C12:0	Lauric acid	7.08
4	C14:0	Myrisitic acid	8.15
5	C16:0	Palmitic acid	9.56
6	C16:1	Palmitoleic acid	9.77
7	C17:0	Heptadecanoic acid	10.43
8	C17:1	cis-10 heptadecanoic acid	10.69
9	C18:0	Stearic acid	11.42
10	C18:1n9c	Oleic acid	11.63
11	C18:2n6	Linoleic acid	12.01
12	C18:3n6	γ-Linolenic acid	12.22
13	C18:3n3	Linolenic acid	12.5
14	C20:0	Arachidic acid	13.42
15	C20:1n9	cis-11-Eicosenoic acid	13.62
16	C20:2n6	cis-11,14-Eicosadienoic acid	14.06
17	C20:3n6	cis-8,11,14-Eicosatrienoic acid	14.3
18	C20:4n6	Arachadonic acid	14.44
19	C20:3n3	cis-11,14,17-Eicosatrienoic acid	14.65
20	C20:5n3	cis-5,8,11,14,17-Eicosapentanoic acid	15.07
21	C22:0	Behenic acid	15.8
22	C22:1n9	Erucic acid	16.12
23	C22:2	cis-13,16-Docosadienoic acid	16.78
24	C22:6n3	cis-4,7,10,13,16,19-Docosahexaenoic acid	18.64
25	C24:0	Lignoceric acid	19.38

Chromatograms of the chemicals extracted in the microwave assisted VOC extraction method from control beef, beef treated with 48h aged DNM, control DNM, and 48h aged DNM are presented in Figure 3. The compounds identified in the VOC extraction are listed in Table 4.

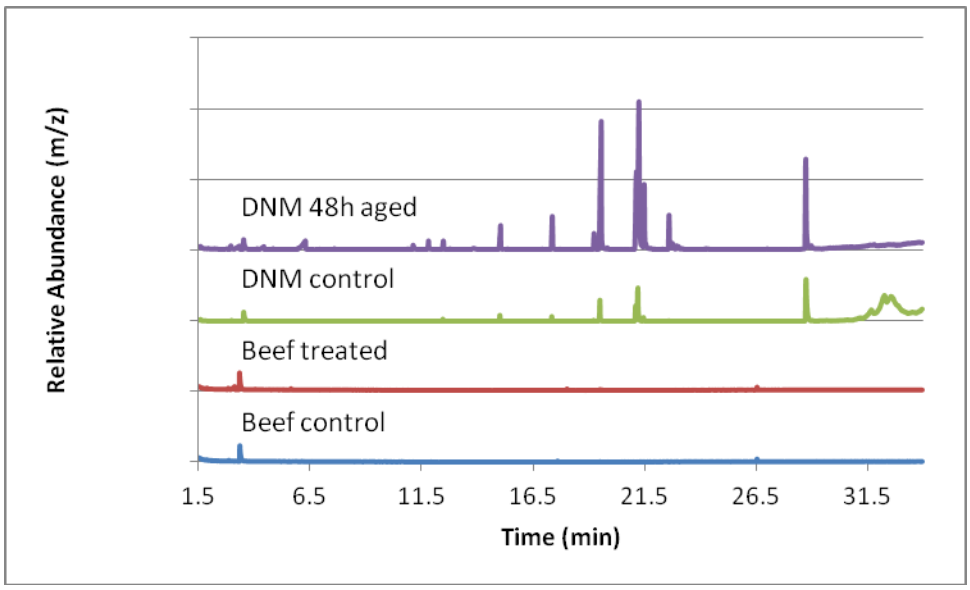

Figure 3. Chromatograms for the extractable volatile organic chemicals for beef baits, control and treated, compared to control DNM and 48h aged DNM. The important volatiles elute in the chromatogram before 11.5 minutes. The tick marks on the y axis relative abundance (m/z) scale are 0.5e6 in magnitude.

Table 4. Volatile organic chemicals isolated from DNM and beef baits.

Peak #	Chemical name	Retention time (min)
1	trans-3,4-dimethyl-2-pentene	2.8
2	2-methyl-butanoic acid	4.3
3	4-methyl-pentanoic acid	5.8
4	Hydrocinnamic acid	11

Chromatograms of the fatty acids isolated in the VOC extraction from control beef, beef treated with 48h aged DNM, control DNM, and 48h aged DNM are presented in Figure 4. The fatty acids identified in these samples are the same as listed in Table 5.

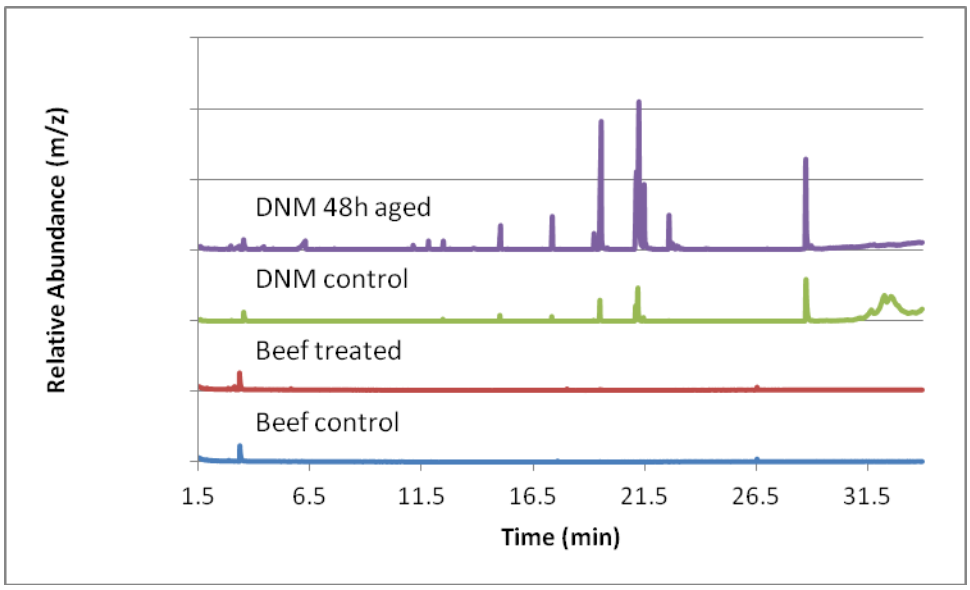

Figure 4. Chromatograms of the fatty acid fraction isolated during the volatile component extraction for beef, control and treated, compared to control DNM and 48h aged DNM. The tick marks on the y axis relative abundance (m/z) scale are 3e6 in magnitude.

Results from the fatty acid modified Folch extractions for the mice and beef baits are presented in Table 5. Large increases in the levels of the fatty acids C8:0, C10:0, C14:0, C16:0, C16:1, C18:0, C18:1n9c, and C18:2n6c were observed in the 48h aged DNM treated beef baits compared to the control beef baits. Generally, aging mice increased certain fatty acid concentrations in the tissue but not in statistically significant levels given the very high variances observed across the samples.

Table 5. Concentrations of fatty acids determined in the microwave assisted modified Folch extraction for control beef, 48h aged DNM treated beef, control DNM, and 48h aged DNM.

IUPAC nomenclature	Beef				DNM			
	Control		Treatetd		Control		Aged	
	mean (µg/g)	std dev	mean (µg/g)	std dev	mean (µg/g)	std dev	mean (µg/g)	std dev
C8:0	0.64	0.10	1.88	0.46	5.13	4.40	2.96	1.32
C10:0	1.28	1.42	35.60	2.23	136.32	109.99	91.13	32.38
C12:0	0.00	0.00	0.00	0.00	280.71	205.85	255.31	88.15
C14:0	14.71	3.18	88.74	5.28	297.22	197.75	353.50	122.55
C16:0	333.81	49.54	570.04	57.45	1190.26	587.89	1409.74	369.60
C16:1	15.28	3.13	35.31	3.64	105.73	50.59	133.12	34.83
C17:0	6.01	3.58	6.83	2.67	7.38	3.48	8.50	2.79
C17:1	0.59	0.41	1.36	0.16	1.25	0.73	1.17	0.35
C18:0	183.21	31.94	254.67	28.00	542.85	253.49	534.34	172.42
C18:1n9c	232.24	53.33	412.34	64.23	1051.63	387.70	1269.10	255.71
C18:2n6	469.66	63.16	650.76	42.81	758.26	267.54	856.30	138.84
C18:3n6	0.50	0.26	0.86	0.84	9.85	4.10	11.92	2.35
C18:3n3	2.98	3.66	0.64	0.47	19.33	8.66	23.78	2.92
C20:0	0.32	0.05	1.85	0.58	4.61	2.87	6.53	3.31
C20:1n9	5.27	1.32	5.48	2.00	29.70	11.99	35.51	11.11
C20:2n6	0.77	0.29	1.98	0.79	48.20	23.87	57.12	13.64
C20:3n6	179.40	25.05	170.31	138.84	60.47	29.87	71.34	20.18
C20:4n6	0.48	0.84	0.60	1.04	557.11	247.39	549.41	156.73
C20:3n3	1.55	1.59	1.42	1.52	2.13	1.81	2.00	0.24
C20:5n3	0.50	0.26	2.09	0.88	3.39	2.29	1.81	0.88
C22:0	0.23	0.39	0.87	0.19	0.09	0.04	0.06	0.06
C22:1n9	6.82	4.38	4.22	1.43	0.76	0.29	1.56	0.19
C22:2	4.95	2.93	2.81	2.38	1.69	0.38	1.77	0.48
C22:6n3	2.44	0.83	6.05	5.10	140.36	67.06	181.52	53.34
C24:0	0.00	0.00	5.24	0.47	0.91	0.21	0.30	0.07

Results from the VOC extractions for the mice and beef baits are presented in Table 6 with the measured concentrations in the extractable VOCs increasing in the 48h aged DNM. Comparing replicates from a composite to the concentrations observed across animals did not indicate that the variability was increased across animals. Three of the VOCs, trans-3, 4-dimethyl-2-pentene, 2-methyl-butanoic acid, and 4-methyl-pentanoic acid were transferred from 48h aged DNM to beef during the treatment where the two were tumbled together in a closed jar.

Table 6. Concentration of volatile organic chemicals in control and 48h aged DNM-treated beef baits, control DNM, and DNM aged for 48 h. Beef was treated with the decomposition products from DNM aged for 48 h in an environmental chamber.

Chemical	Beef control		Beef treated with 48h aged DNM decomposition products	
	composite sample (3 replicates)		composite sample (3 replicates)	
	mean (µg/g)	std. dev.	mean (µg/g)	std.dev.
trans-3,4-dimethyl-2-pentene	37.34	10.51	61.14	12.62
2-methyl-butanoic acid	0	0	31.06	5.24
4-methyl-pentanoic acid	0	0	99.46	10.70
Hydrocinnamic acid	62.47	108.20	0	0
	DNM control		48h aged DNM	
	composite sample (3 replicates)		composite sample (3 replicates)	
	mean (µg/g)	std. dev.	Mean (µg/g)	std. dev.
trans-3,4-dimethyl-2-pentene	62.72	46.54	318.81	69.25
2-methyl-butanoic acid	0	0	337.74	69.77
4-methyl-pentanoic acid	2.31	3.99	1922.09	262.95
Hydrocinnamic acid	0	0	441.34	209.46
	48h aged DNM			
	individual samples (n=4)			
	mean (µg/g)	std. dev.		
trans-3,4-dimethyl-2-pentene	309.8	54.98		
2-methyl-butanoic acid	233.57	130.54		
4-methyl-pentanoic acid	1619.24	709.15		
Hydrocinnamic acid	429.94	199.75		

The concentrations of fatty acids determined in the mice and beef baits in the VOC extract are presented in Table 7. The trends for increasing concentrations of the fatty acids: C8:0, C10:0, C14:0, C16:0, C16:1, C18:0, C18:1n9c, and C18:2n6c were observed in the 48h aged DNM-treated beef baits compared to the control beef baits as was observed in the fatty acid extractions. The levels of fatty acids extracted from the 4 matrices using this solvent system were not appreciably different than those observed for the matrices extracted by the microwave assisted Folch extraction.

Table 7. Concentration of fatty acids determined in the volatile organic chemical extracts for control beef, 48h aged DNM treated beef, control DNM, and 48h aged DNM.

IUPAC Nomenclature	Beef				DNM			
	Control		Treated		Control		Aged	
	mean (µg/g)	std dev	mean (µg/g)	std dev	mean (µg/g)	std dev	mean (µg/g)	std dev
C8:0	0.33	0.08	0.83	0.18	3.50	0.75	6.70	0.03
C10:0	0.37	0.06	12.69	1.39	78.08	6.52	150.16	3.56
C12:0	0.48	0.13	32.40	3.40	163.14	9.36	305.17	19.74
C14:0	5.35	0.88	46.32	5.38	173.49	11.50	373.55	37.54
C16:0	232.08	14.55	322.90	32.24	903.41	45.85	1420.12	196.12
C16:1	1.51	0.68	1.61	0.47	105.89	7.60	153.68	17.08
C17:0	5.15	0.35	5.13	0.38	7.80	0.68	6.98	5.68
C17:1	5.14	0.30	5.31	0.49	6.70	1.24	8.47	0.49
C18:0	118.68	8.15	137.24	16.47	394.64	15.29	500.63	72.16
C18:1n9c	145.92	4.43	210.34	32.38	762.53	148.70	797.28	102.38
C18:2n6	343.28	6.04	362.41	52.91	949.16	207.85	965.24	90.28
C18:3n6	2.76	0.33	4.14	0.11	12.93	0.78	15.31	1.29
C18:3n3	6.86	0.69	8.69	0.45	20.27	1.24	29.36	1.72
C20:0	0.39	0.38	2.01	0.22	4.46	0.57	8.62	1.70
C20:1n9	0.74	0.36	5.26	1.54	30.05	2.37	37.92	6.10
C20:2n6	2.71	0.31	6.64	1.54	44.14	3.29	55.65	7.33
C20:3n6	26.52	1.51	28.98	3.06	59.74	4.18	76.46	10.27
C20:4n6	135.02	10.74	154.44	12.73	585.89	22.15	521.85	50.66
C20:3n3	1.45	0.46	1.27	0.60	2.99	0.57	4.78	2.46
C20:5n3	18.70	1.88	18.41	0.95	6.54	0.31	15.78	5.29
C22:0	0.09	0.03	0.23	0.21	2.90	1.23	6.88	1.34
C22:1n9	0.56	0.09	0.91	0.65	2.45	0.90	5.16	2.10
C22:2	0.33	0.07	0.49	0.04	5.06	0.30	6.64	1.23
C22:6n3	4.37	0.74	17.00	1.94	164.41	7.34	219.41	19.16
C24:0	0.13	0.07	0.68	0.50	6.70	2.90	11.54	7.53

The quantity of the 4 VOCs extracted using the solvents pentane, diethyl ether, acetone, ethanol, and water are presented in Table 8. Only acetone and ethanol could extract all 4 VOCs in detectable amounts. Diethyl ether and water extracted 3 of the VOCs and pentane extracted only 1 of the VOCs. The concentrations observed reflect that the solvents were extracting compounds from an intact mouse, and the differential solubility of a compound in a given solvent.

Table 8. Volatile organic chemical concentrations in solvents of different polarity exposed to a 48h aged DNM for 15 minutes. Solvents are listed in order of increasing polarity.

Chemical (µg/g)	Solvent				
	Pentane	Diethyl ether	Acetone	Ethanol	Water
trans-3,4-dimethyl-2-pentene	4.66	3.06	37.51	91.59	3.38
2-methyl-butanoic acid	0	7.41	28.00	14.67	6.27
4-methyl-pentanoic acid	0	88.74	58.24	59.21	70.80
Hydrocinnamic acid	0	0	208.26	43.77	0

Category 2

Headspace Volatile Analyses from 24h and 48h Field Aged DNM

Chromatographic data were subjected to linear discriminant analysis using models previously created for evaluation of coyote attractant volatiles (Kimball et al. 2000). A primary finding was that 24h aged DNM volatiles and 48h aged DNM volatiles were classified into differing cluster categories. Following the procedures applied for preparing artificial lures on the basis of headspace analyses (Kimball et al. 2000), recipes were calculated for 24h and 48h aged DNM using only seven components which represented the major chemical functional groups represented in the headspace samples. One chemical from each functional group was chosen to represent the group as shown in Table 9. This table was developed from unpublished data not funded by SERDP (Kimball, NWRC unpublished data). The rationale for testing headspace volatile chemicals is based on the study by Jojola-Elverum et al. (2001). These investigators inferred that bacterial decomposition of DNM produced odors that were attractive to BTS.

Table 9. Recipes of chemical ingredients of functional groups from the headspace volatiles from 24 h and 48 h field aged DNM.

Functional group	Ingredient	24h aged DNM	48h aged DNM
Fatty acid	Isobutyric Acid	40 mL	15 mL
Alcohol	n-Butanol	14 mL	9 mL
Aldehyde	Hexanal	11 mL	13.5 mL
Alkaloid	2,6-Dimethyl Pyrazine	0.5 g	0.2 g
Ester	Ethyl Butyrate	0.3 mL	0.2 mL
Sulfide	Methyl Disulfide	1.2 mL	1.0 mL
Solvent	Ethanol	14 mL	52 mL

Category 3

Bait Evaluation Experiments

Experiment 1: Importance of Decomposition Products for Bait Attractiveness
Only the Beef48h bait treated with decomposition products from 48h aged DNM was not statistically distinguished from DNM in the proportions taken by BTS (Table 10). The Pup48h bait was also treated with decomposition products but was not preferred by snakes. These results suggest that the bait substrate is important since beef treated with decomposition products from 48h aged DNM (Beef48h) were well accepted but treated Pup-Peroni ® (Pup48h) baits were not. The high rate of bait take of beef treated with 48h aged DNM confirms the previous study (Savarie 2008).

Table 10. Mean two-day rate of bait take by brown treesnakes in experiment 1for control DNM, control beef (Beef), control Pup-Peroni ® beef dog snack (Pup), and beef and Pup-Peroni® treated with the total decomposition products from 48h aged DNM (Beef48h and Pup48h). Means and p-values for 1 df contrast comparing mean proportion of DNM to each bait tested (n = 10 for each bait type on each of 6 transects).

Bait	Two-day total bait take	
	mean	p-value
DNM	.717	
Beef	.300	.0001
Pup	.067	<.0001
Beef48h	.683	.7120
Pup48h	.383	.0013

Experiment 2: Attractiveness of Beef Baits Treated with Solvents
None of the 5 treated beef treatments (aBeef, deBeef, eBeef, pBeef, wBeef) enhanced bait consumption by snakes and all, including beef, were significantly distinguished from DNM in the proportions taken by BTS (Table 11). This indicates that these solvents, directly applied to

the beef baits, did not influence snake appetitive behavior and probably had little effect on bait consumption when they were used as solvent extracts of 48h aged DNM in experiment 3.

Table 11. Mean two-day rate of bait take by brown treesnakes in experiment 2 for control DNM, control beef (Beef), and beef treated with solvents (a = acetone, de = diethyl ether, e = ethanol, p = pentane, w = water). Means and p-values for 1 df contrast comparing mean proportion of DNM take to each bait tested (n = 10 for each bait type on each of 2 transects).

Bait	**Two-day total bait take**	
	mean	p-value
DNM	.700	
Beef	.100	.0018
aBeef	.150	.0029
deBeef	.200	.0045
eBeef	.150	.0029
pBeef	.200	.0045
wBeef	.250	.0074

Experiment 3: Bait Take of Beef Baits Treated with Solvent Extracts and Decomposition Products from 48h Field Aged DNM

Four baits (deeBeef, peBeef, weBeef, Beef48h) were not statistically distinguished from DNM in the proportions taken by BTS, while 3 baits (Beef, aeBeef, eeBeef) were statistically distinguished from DNM (Table 12). These results indicate that components in the extracts of diethyl ether, pentane, and water from 48h DNM, and decomposition products from 48h aged DNM are attractive to snakes and enhance prey searching and appetitive behavior.

Table 12. Mean two-day rate of bait take by brown treesnakes in experiment 3 for control DNM, control beef (Beef), beef treated with solvent extracts from 48h aged DNM (ae = acetone, dee = diethyl ether, ee = ethanol, pe = pentane, we = water), and beef treated directly with the total decomposition products from 48h aged DNM (Beef48h). Means and p-values for 1 df contrast comparing mean proportion of DNM take to each bait tested (n = 10 for each bait type on each of 5 transects).

Bait	**Two-day total bait take**	
	mean	p-value
DNM	.780	
Beef	.200	<.0001
aeBeef	.380	.0002
deeBeef	.660	.2154
eeBeef	.400	.0004
peBeef	.660	.2154
weBeef	.620	.1022
Beef48h	.820	.676

Experiment 4: Attractiveness of Beef Baits Treated with Fatty Acids and Volatile Organic Chemicals (Soybean Oil as Carrier)

Five baits (Beef, Beefo, and beef treated with Extracts 1, 2, and 3 from 48h aged DNM using soybean oil as the carrier) were all statistically distinguished from DNM in the proportions taken by BTS (Table 13). These data indicate that the chemical ingredients formulated in soybean oil for each of the three extracts listed in Table 2 do not enhance consumption of treated beef.

Table 13. Mean two-day rate of bait take by brown treesnakes in experiment 4 for control DNM, control beef (Beef), beef treated with soybean oil (Beefo), and beef treated with three chemical extracts (E1, E2, E3) from 48h aged DNM using soybean oil as the carrier. Means and p-values for 1 df contrast comparing mean proportion of DNM to each other bait tested (n = 10 for each bait type on each of 3 transects).

Bait	**Two-day total bait take**	
	mean	p-value
DNM	.800	
Beef	.533	.0128
Beefo	.233	<.0001
BeefE1	.233	<.0001
BeefE2	.200	<.0001
BeefE3	.300	.0002

Experiment 5: Attractiveness of Beef Baits Treated with Fatty Acids and Volatile Organic Chemicals (Ethanol as Carrier)

Five baits (Beef, Beefe, and beef treated with Extracts 1, 2, and 3 from 48h aged DNM using ethanol as the carrier) were all statistically distinguished from DNM in the proportions taken by BTS (Table 14). These data are similar to the results in experiment 4 and show that the ethanol formulations of the chemical ingredients for each of the three extracts listed in Table 2 do not enhance consumption of treated beef.

Table 14. Mean two-day rate of bait take by brown treesnakes in experiment 5 for unadulterated dead neonatal mice (DNM), unadulterated beef (Beef), beef treated with ethanol (Beefe), and beef treated with three chemical extracts (E1, E2, E3) from 48h aged DNM using ethanol as the carrier. Means and p-values for 1 df contrast comparing mean proportion of DNM take to each bait tested (n = 10 for each bait type on each of 3 transects).

Bait	**Two-day total bait take**	
	mean	p-value
DNM	.900	
Beef	.233	<.0001
Beefe	.367	.0002
BeefE1	.100	<.0001
BeefE2	.267	<.0001
BeefE3	.333	<.0001

Experiment 6: Attractiveness of Chemical Volatiles from 24 h and 48 h Field Aged DNM
All 5 bait types (Beef, sBeef24h, sBeef48h, wBeef24h, wBeef48h) were statistically different from DNM in the proportions taken by BTS (Table 15). These data indicate that the headspace volatile recipes in Table 9 from 24h and 48h aged DNM do not elicit snake prey search and appetitive behavior.

Table 15. Mean two-day rate of bait take by brown treesnakes in experiment 6 for control DNM, control beef (Beef), and beef treated with chemical volatiles from 24h (recipe 1) and 48h (recipe 2) aged DNM. Chemical volatiles were applied by either soaking (s) the beef before field application, or were applied by a wick (w) adjacent to unadulterated beef inside the bait tube. Means and p-values for 1 df contrast comparing mean proportion of DNM to each bait tested (n = 10 for each bait type on each of 3 transects).

Bait	**Two-day total bait take**	
	mean	p-value
DNM	.767	
Beef	.233	.0002
sBeef24h	.033	<.0001
sBeef48h	.100	<.0001
wBeef24h	.100	<.0001
wBeef48h	.167	<.0001

Experiment 7: Attractiveness of Direct Application of Chemical Volatiles from 24h and 48h Field Aged DNM
The 3 bait types (Beef, Beef24h, Beef48h) compared to DNM were statistically different from the proportions taken by BTS (Table 16). These data indicate that these chemical volatiles are not attractive to snakes and support the data in experiment 6 that the headspace volatile recipes in Table 9 from 24h and 48h aged DNM do not enhance bait consumption of treated beef.

Table 16. Mean two-day rate of bait take by brown treesnakes in experiment 7 for control DNM, control beef (Beef), and beef treated by direct application of 0.25 mL of chemical volatiles from either 24h (recipe 1) or 48h (recipe 2) aged DNM. Means and p-values for 1 df contrast comparing mean proportion of DNM to each bait tested (n = 10 for each bait type on each of 2 transects).

Bait	**Two-day total bait take**	
	mean	p-value
DNM	.600	
Beef	.150	.0069
Beef24h	.200	.0097
Beef48h	.200	.0097

Conclusions and Implications for Future Research

Chemicals identified in the carcasses of aged DNM and volatile headspace evaluated as bait enhancers in brown treesnakes were not effective. Fourteen chemicals were tested. Seven chemicals (four volatile organic chemicals and three fatty acids) of interest were from 48h aged DNM. The seven chemicals were tested as a single extract and as separate extracts for the four volatile organic chemicals and the three fatty acids. Large increases in each of the four volatile organic acids was observed in 48h aged DNM but apparently were not the signal for promoting prey searching and appetitive behavior. Only three of the 25 fatty acids identified from 48h aged DNM were tested. Concentrations of these three fatty acids (capric, lauric, and myristic) increased in the 48h aged DNM and were transferred to beef baits in high concentrations. Of the 22 fatty acids not tested, four (palmitic, arachidic, cis-11-eicosenoic, and cis-4,7,10,13,1610-docosahexaenoic) would be good candidates for future testing because they were relatively high in 48h aged DNM and there was good transfer of each to beef. Seven chemicals, each representing a distinct functional group from the volatiles in the headspace of 24h and 48h aged did not show promise as bait enhancers. A future area of investigation would be to evaluate other chemicals in these functional groups.

Beef baits treated with diethyl ether, pentane, and water extracts from the decomposition products of 48h aged DNM were also well accepted by snakes (Table 11). These data indicate that each of these three solvents extracted chemical components that promoted bait consumption. This is the first indication that the decomposition products can be fractionated into products that enhance bait consumption. Only one of four volatile organic chemicals was common in each of three solvent extracts (Table 7). Since the four organic volatile chemicals did not enhance consumption of treated beef, this indicates that other chemicals in these extracts enhance consumption. Future work would identify these chemicals and evaluate them as bait enhancers.

The bait substrate is very important as shown by the equivalent rate of consumption of beef treated with the whole decomposition products of 48h aged DNM and unadulterated DNM. In contrast, there was no evidence that the treated commercial dog food snack, Pup-Peroni®, was a bait enhancer. A possible reason that Pup-Peroni® was not accepted is because it contains flavors and preservatives that may not be attractive to the majority of snakes. The present form of beef is not practical because it has to be maintained frozen or refrigerated before application in the field. Another area of investigation would evaluate freeze-dried and dehydrated beef that could be reconstituted with water before application in the field.

The underlying hypothesis of this study was that the whole odiferous complement from aged DNM contains specific chemical cues that stimulate appetitive foraging behavior. This study was the first attempt to test serial fractions of the whole odiferous complement from dead mice as bait enhancers. Whole odors and decomposition products were effective as bait enhancers to BTS but the fatty acids and volatile organic chemicals identified and tested from serial fractions were not. There is validity in testing additional serial fractions as diethyl ether, pentane, and water extracts of dead mice did enhance bait consumption. Each of these extracts probably contain unique chemical cues. Identification, formulation, and evaluation of these chemicals would be included in the next phase of future research.

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