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An assessment of the potential hazards of anticoagulant rodenticides to Plethodontid salamanders

Gary Witmer, Ph.D., Supervisory Research Wildlife Biologist
USDA/APHIS Wildlife Services
National Wildlife Research Center
4101 Laporte Avenue, Fort Collins, CO 80521-2154

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The USDA/APHIS National Wildlife Research Center conducted an assessment of the hazards of the anticoagulants diphacinone and brodifacoum to salamanders of the family Plethodontidae or lungless salamanders. This was done in anticipation of an attempt to eradicate the invasive house mouse (*Mus musculus*) from the Farallon Islands National Wildlife Refuge, California where the endemic subspecies Farallon arboreal salamander (*Aneides lugubris farallonensis*) occurs. Live-captured salamanders of three species (*Aneides lugubris*, *Ensatina eschscholzii xanthoptica*, and *Batrachoseps attenuatus*) were exposed to each of the anticoagulant rodenticides by both oral and dermal exposure routes. Each trial had an exposure period of ten days, followed by a ten day post-exposure period with no rodenticide exposure. There were some deaths (9 of 37 treated salamanders; 24.3% mortality). By species this was 25% of the *Aneides*, 0% of the *Ensatina*, and 75% of the *Batrachoseps* treated salamanders. It appeared that dermal exposure posed the greatest hazard; however, it is important to note that the level of dermal exposure used in this trial was much higher than what would be expected in a rodent eradication project. In essence, this was a worst-case scenario. We did not note the sub-lethal effects of weight loss or reduced food (cricket) consumption that has been observed in studies of other taxa. Skin sloughing and sores on the undersides of certain salamanders exposed to rodenticide as well as some controls left it unclear whether or not this affect was caused by the anti-coagulant. However, for salamanders in the exposure groups, it appeared that skin sloughing and sores began to recede during the post-exposure period, suggesting that some animals began to recover after rodenticide exposure. Following trial completion, samples of salamanders were analyzed for rodenticide residues. Residue concentrations were very low (in parts per billion) when compared with results from some other studies (parts per million). We concluded that while anticoagulant rodenticide pose some hazards (both lethal and sub-lethal) to salamanders, the level appears to be relatively low, especially given the very high exposure rates applied in this study compared to the exposure they would encounter in an aerial broadcast of rodenticide baits in an invasive rodent eradication project.

Introduction

House mice (*Mus musculus*) cause many types of damage and when introduced to islands, house mice can cause significant damage to natural resources, including both flora and fauna (Witmer and Jojola 2007, Howald et al. 2015). For example, on Gough Island in the South Atlantic, house mice fed on nestling albatross chicks (Cuthbert and Hilton, 2004). Invasive house mice are also negatively impacting bird populations on the USFWS's Midway Atoll (USFWS 2018). Additionally, Witmer et al. (2012) documented seedling damage by house mice in a pen study. House mice are omnivores, yet their diet is largely dominated by insects (at least on tropical Pacific islands), some of which are likely plant pollinators (Shiels et al. 2013; Shiels and Pitt 2014). Diet, however, varies depending on habitat, environmental conditions, and food availability. Because of the damage caused by mice on islands, there have been numerous attempts to control or eradicate them. The U.S. Fish and Wildlife Service (USFWS) is conducting plans for an eradication of the house mouse on the Farallon Islands National Wildlife Refuge off the coast of central California (USFWS 2013).

There have been numerous successful eradications of invasive rodents on islands (Howald et al. 2007, Witmer et al. 2011) and these projects have relied upon rodenticides for their completion (Witmer et al. 2007). The U.S. Department of Agriculture - Animal and Plant Health Inspection Service (APHIS) maintains the registrations for two rodenticide active ingredients for invasive rodent eradication: diphacinone and brodifacoum. In most eradication efforts, these are aerially applied by helicopter at an application rate of 18 kg/ha or less. This results in about two rodenticide pellets per m^2. However, in some cases, the project personnel request a higher application rate because of rodenticide pellet consumption by non-target animals and, in particular, land crabs. The rodenticide labels also allow for a second aerial application to help assure that all targeted rodents are exposed to a lethal dose of the rodenticide. Rodenticides can pose hazards to non-target animals so careful considerations and measures must be taken to reduce those risks (Witmer et al. 2007, van den Brink et al. 2018). In the case of salamanders, they could be exposed to rodenticides during an eradication project by contact with the material (dermal exposure) or by consuming invertebrates that have consumed baits (secondary oral exposure). Because salamanders respire through the skin, dermal exposure may be of greater concern than with other vertebrates.

Invasive house mice are present on the Farallon Islands National Wildlife Refuge (Refuge) and are causing damage to seabirds, the endemic arboreal salamander (*Aneides lugubris farallonensis*), terrestrial invertebrates, and native plants. The USFWS would like to eradicate the invasive mice from the Refuge and in their analyses of action alternatives for the mouse eradication, the USFWS would like an assessment of the potential hazards of brodifacoum and diphacinone to salamanders. They requested that NWRC conduct the assessment based on our extensive animal research facilities and staff and our previous experience of assessing hazards of anticoagulants to reptiles (Witmer and Mauldin 2012).

This study was conducted because of concerns about the potential hazards of anticoagulant rodenticides to salamanders. No scientific literature could be located on this topic, however, the potential hazards to reptiles has been studied extensively (e.g., Hoare and Hare 2006, Weir et al. 2016). The objective of this study was to assess the potential hazards of the rodenticides

brodifacoum and diphacinone to Farallon arboreal salamanders (family *Plethodontidae*, the lungless salamanders), using conspecifics from another population of closely related salamanders as surrogates because of the Farallon population's relatively small and endemic status. Ultimately, three closely related species of Plethodontid salamanders were used in the study: yellow-eyed ensatina (*Ensatina eschscholzii xanthoptica*), arboreal salamander (*Aneides lugubris*; mainland variety), and California slender salamander (*Batrachoseps attenuatus*); see Figures 1-3. For a description of the phylogenetic relationships of the largest family of salamanders, the *Plethodontidae*, see Vieites et al. (2011). Salamanders were exposed to rodenticides through two routes: 1) oral exposure, and 2) direct dermal exposure. It was assumed that these would be the main routes of exposure in a rodent eradication project. We hypothesized that the rodenticide exposure would cause some mortality, internal or external bleeding, or other sub-lethal effects (e.g., decline in food consumption and/or loss of weight).

Methods

The salamanders used in this study were live-captured in California and shipped to NWRC, Fort Collins, CO, by the herpetology lab of Dr. Vance Vredenburg of San Francisco State University (SFSU). Dr. Vredenburg has considerable experience in capturing and maintaining salamanders for research purposes. He acquired the permits required to capture, maintain, and transport salamanders. Personnel of SFSU operated under a separate contract with the USFWS to conduct those activities. The salamanders are not sexually dimorphic and we did not know the age or genders of the salamanders brought to NWRC.

Salamanders were housed individually in plastic mouse shoebox cages (26.5 cm long, 15.5 cm wide, 20.5 cm high) and fed small crickets (5-7 crickets twice weekly). Although salamanders eat a variety of invertebrates, crickets were used because they are readily available from a variety of commercial sources, are easily maintained, and are readily consumed by captive salamanders (V. Vredenburg, pers. comm.). The floor of each cage was lined with wet paper towels to provide needed moisture and a plastic tube for shelter (Fig. 1-3). Paper towels were kept saturated with water at all times. Cages were cleaned/changed weekly throughout the study unless mildew became obvious at which time the cage was changed. Salamanders were maintained as per the university-approved Standard Operating Procedure on salamander maintenance that was provided by San Francisco State University. Salamanders were quarantined for two weeks to help assure their healthy condition before starting the trials. We presumed that this also allowed the salamanders to stabilize in body mass prior to initiation of the trials.

Two anticoagulant rodenticides (diphacinone and brodifacoum) were tested for their potential hazards to salamanders. Two U.S. Environmental Protection Agency registered products, Brodifacoum-25D Conservation and Diphacinone-50 Conservation, were used in the study. Initially, we planned to have a control and two treatment groups for each of these two rodenticides with each providing a different route of exposure (oral exposure and direct dermal exposure). However, because of a shortage of salamanders captured for the study, we had to modify these plans as explained below. Because of their known abundance in the San Francisco Bay area and close relationship with *Aneides*, initially we planned to use *Ensatina* as our main

sample species with a smaller sample of the less abundant and harder to obtain *Aneides* for confirmation of results with *Ensatina*. However, when both of these species proved more difficult to obtain than expected, we added the more abundant but somewhat less similar (to *Aneides*) *Batrachoseps* to the study.

We had planned to use 10 salamanders in each group; however, because we did not obtain enough of the first two species of salamanders (*Aneides* and *Ensatina*), we combined the two routes of exposure and had some of each species in each group. This was called Trial 1. The control group had no rodenticide exposure, but was otherwise maintained like the treatment groups. See Table 1 for the number of salamanders used in these groups. Because we had enough *Batrachoseps* salamanders, we were able to have separate treatment groups for each route of exposure along with a control group (Trial 2).

Next we describe the methods used in Trial 2 for the two separate exposure routes used for the *Batrachoseps* salamanders. See Table 2 for the number of salamanders used in each group. The methods used in Trial 1 for the groups of *Aneides* and *Ensatina* salamanders were the same except that the two exposure routes were combined. That is, there was only one treatment group for each rodenticide.

Treatment 1 Procedures; oral exposure. Ten *Batrachoseps* were to be used in this treatment group for each rodenticide. However, group size varied somewhat because of the number of salamanders available at the start of the study. In this trial, the salamanders were to be fed crickets that had been exposed to the rodenticide by only allowing the crickets to feed on powdered/crushed rodenticide pellets for about 10 days. However, when we first fed rodenticides to the crickets, they all died shortly thereafter. (But note that when we later fed rodenticides to a different batch of crickets, all the crickets survived; see below.) Consequently, we amended the study protocol so that the powdered rodenticide was sprinkled on the crickets just before putting them in with the salamanders. This was done by placing crickets in a small plastic container containing the powdered rodenticide, replacing the cover, and then gently shaking the container. We did not try to quantify the amount of rodenticide on the crickets, but relied on the chemical residue analyses to give an idea of the burden. Additionally, we presume that much of the powdered rodenticide on the underside of the crickets came off quickly in the salamander cages as they walked around on the wet paper towels. Initially, some crickets were fed to salamanders twice weekly. However, because many salamanders ate the crickets very quickly, they then went several days without any food (crickets) available. This was a concern because they might then start losing body mass which we might interpret as an anticoagulant effect. Hence, we began feeding crickets to the salamanders more frequently to assure that they always had crickets available in their cages. The treated crickets were fed to the salamanders for 14 days. At the end of the 14-day exposure period, salamanders were placed in clean cages and observed for another 14 days (post-exposure period). During this period, they were fed "clean" crickets that had not been exposed to rodenticide.

Treatment 2 Procedures; direct dermal exposure. Ten *Batrachoseps* salamanders were to be used in this treatment group for each rodenticide. However, group size varied somewhat because of the number of salamanders available at the start of the study. In this trial, the salamanders were exposed dermally to powdered/crushed pellets sprinkled on the ground cover material and

by spraying the ground cover paper towels with water in which crushed pellets were allowed to dissolve for 7 days. With this treatment group, there may also have been some direct oral exposure if the salamanders chose to eat some of the crushed pellets. As in the other treatment group, the salamanders were exposed to the crushed pellets and treated water for 10 days. At the end of the 10-day exposure period, salamanders were placed in clean cages and observed for the 14-day post-exposure period. During this entire treatment, the salamanders were fed crickets that had not been exposed to the rodenticide.

The control groups were maintained with no rodenticide exposure during Trials 1 and 2.

Salamanders were fed 5-7 crickets twice weekly. Staff monitored cricket consumption over the course of the trials to determine if there was a decline in food consumption as the trial progressed from the exposure period to the post-exposure period. Additionally, salamanders were weighed at the start and end of the trials to determine if a change in weight occurred. These data provided measures of potential sub-lethal effects. Generally, mammals that have consumed enough anticoagulants to exhibit signs of toxicosis will stop feeding and lose weight as the signs of toxicosis advance (e.g., Witmer 2011). With birds, however, they typically do not show weight loss when fed sub-lethal doses of anticoagulants, but birds that are severely intoxicated (and perhaps succumbing/dying) stop feeding and lose weight (Rattner et al. 2012).

Salamanders were examined twice daily by laboratory staff and their condition and any mortalities were recorded. Animals were examined more frequently as signs of toxicity progressed, but frequency of examination depended on how quickly the signs progressed. If any animal was observed to be experiencing more than momentary pain or distress, laboratory staff contacted the Study Director and/or the Attending Veterinarian to have the animal examined and possibly euthanized. Signs of severe pain and distress and of a moribund condition that was used as criteria for humane killing of study animals listed by the Organisation for Economic Co-operation and Development (OECD 2000) and included abnormal vocalization, persistent labored breathing, prolonged impaired ambulation preventing the animal from reaching food or water, persistent convulsions, and significant blood loss. Dead salamanders were rinsed in clean water, weighed and placed in individual, labeled re-sealable bags and frozen for later rodenticide residue determination by the Analytical Chemistry Unit (ACU) staff. See Appendix A for the methods used by the ACU. All surviving salamanders were euthanized at the end of the study using a liquid formulation of MS222 (which also served to rinse the animals of surface residues) for later submission to ACU staff. *Aneides* and *Ensatina* salamanders were necropsied at the end of the study to check for signs of internal hemorrhaging (Stone et al. 1999). Because of their very small size (see Fig. 3), we did not necropsy the *Batrachoseps* salamanders. Additionally, some unrinsed crickets dusted with rodenticide powder and some control crickets were submitted for rodenticide residue analyses along with samples of the water that had been exposed to the powdered pellets. We also had a sample of rodenticide pellets analyzed for the concentration of active ingredients in them.

For each treatment and control group, we compared salamander weights at the start of the trial with their weights at the end of the trial using ANOVA statistical tests. We also compared cricket consumption during the rodenticide exposure period to cricket consumption during the post-exposure period. We used a significance level of $P \leq 0.05$. Other ANOVAs included

comparisons of starting weights of the groups of salamanders in Trial 1 and again in Trial 2. We also compared brodifacoum residue levels in dusted versus fed crickets. Finally, we compared brodifacoum residue levels between salamanders that died during trial 2 versus those that lived.

Results

Trial 1

Table 1 summarizes the results of Trial 1. Because of the relatively small number of *Aneides* and *Ensatina* salamanders available for this trial, we combined the two exposure routes for each treatment group. The starting weights of the 3 groups of salamanders in Trial 1 were not significantly different ($F = 1.87$, $P = 0.18$). In the brodifacoum group, two (both *Aneides*) of the seven salamanders died (28.6% mortality); while one of these salamanders had skin sloughing and external bleeding, the other showed none of these symptoms. The 2 salamanders that died appeared to have higher brodifacoum residue levels than the 5 that lived, but these levels were not significantly different ($F = 5.82$, $P = 0.06$). We noted a sloughing of skin in some animals (four of seven; 57.1%) and sores, mainly on the underside of animals (one of seven; 14.3%). An NWRC chemist noted that the pellets for both brodifacoum and diphacinone are rather acidic so this may have been responsible for some skin sloughing and sores.

There was a considerable difference in cricket consumption by the salamanders. During the brodifacoum exposure period, individual cricket consumption ranged from 3-14 crickets, while in the post-exposure period consumption by remaining salamanders ranged from 1-32 crickets. There was an increase in cricket consumption in the post-exposure period in 3 of 4 salamanders. However, overall cricket consumption was not significantly ($F = 3.83$, $P = 0.08$) different between the two periods. The total cricket consumption for the 3 groups of salamanders is presented in Table 3. Additionally, the presence and severity of skin sloughing and sores seemed to decrease in the post-exposure period. Over the course of the trial, there was some loss of weight in the treatment salamanders (0.4-3.4g) and this was marginally significant ($F = 4.80$, $P = 0.05$). Upon necropsy of the two dead *Aneides* salamanders, internal hemorrhaging was noted. After euthanasia of the surviving salamanders, necropsy revealed no internal bleeding. Brodifacoum residues in salamanders were quite variable, but low (see discussion for comparisons with other studies): *Aneides* 42.7-226 ng/g or parts per billion (ppb); *Ensatina* 48.3-101 ppb.

In the diphacinone group, one (*Aneides*) of the seven salamanders died (14.3% mortality); this individual exhibited sores and external bleeding and was euthanized. We noted a sloughing of skin in three of seven salamanders (42.7%) and sores on two of these individuals (mainly on the underside of animals; 28.6%). During the diphacinone exposure period, salamanders consumed 3-24 crickets, while in the post-exposure period they consumed 5-38 crickets. There was an increase in cricket consumption in the post-exposure period in 4 of 6 salamanders. However, overall cricket consumption was not significantly different ($F = 1.40$, $P = 0.26$) between the two periods. Additionally, the presence and severity of skin sloughing and sores decreased in the post-exposure period. Over the course of the trial, the change in weight of the salamanders was not significant ($F = 0.50$, $P = 0.49$). Upon necropsy of the dead *Aneides* salamander, internal hemorrhaging was noted. After euthanasia of the surviving salamanders, necropsy revealed no

internal bleeding. Diphacinone residues in salamanders were quite variable, but low: *Aneides* 10.8-174 ppb (parts per billion); however, no residues were detected in the *Ensatinas*.

There were no deaths in the control group and we did not note any sloughing of skin or sores. However, one of the six salamanders in the control group showed some internal bleeding upon necropsy. Cricket consumption increased some over the course of the trial in this group, but the difference was not significant ($F = 2.20$, $P = 0.17$). However, the control salamanders ate more crickets than the other 2 groups of salamanders ($F = 4.43$, $P = 0.03$). Over the course of the trial the weight loss in salamanders was not significant ($F = 0.14$, $P = 0.71$). While all salamanders in the 3 groups tended to lose a little weight, the differences between groups was not significant ($F = 1.02$, $P = 0.38$).

Trial 2

In trial 2, we used *Batrachoseps* salamanders only. Because we had considerably more salamanders in trial 2 than in trial 1, we were able to divide the exposure routes, resulting in four treatment groups. The starting weights of the salamanders in the 5 groups were not significantly different ($F = 0.41$, $P = 0.80$). One brodifacoum group ($n= 7$) received oral exposure (dusted crickets) only, while the second brodifacoum group ($n= 8$) received dermal exposure. Similarly, one diphacinone group ($n = 8$) received oral exposure only, while the second diphacinone group ($n = 8$) received dermal exposure. This was done to compare toxicity between the exposure routes. The control group ($n = 7$) received no rodenticide exposure.

Table 2 summarizes the results of Trial 2. In the brodifacoum oral exposure group, no salamanders died. There was no skin sloughing or sores observed. Cricket consumption was quite variable: 13-70 per individual during the exposure period and 4-59 in the post-exposure period, but the differences were not significant ($F = 0.01$, $P = 0.92$). The total cricket consumption for the 5 groups of salamanders is presented in Table 4. Salamanders mostly maintained the same weight over the duration of the trial; the most substantial change was 0.1g in one individual. Weight changes were not significantly different ($F = 0.15$, $P = 0.71$) over the course of the trial. Brodifacoum residues in the oral exposed salamanders ranged from 51.3-91.1 ppb.

In the brodifacoum dermal exposure group, six of eight salamanders died (75.0%). There was no skin sloughing or sores observed in any of the salamanders including those that died. The salamanders that died tended to have higher brodifacoum residue levels than the ones that lived, but these levels were not significantly different ($F = 0.98$, $P = 0.37$). Cricket consumption was somewhat variable: 9-27 in the exposure period, but increased in the two surviving salamanders (44 and 55) in the post-exposure period. This was a significant increase ($F = 20.9$, $P = 0.002$) in cricket consumption between the two periods, but it should be noted that this statistic is based on only two data points in the post-exposure period. Salamanders mostly lost a small amount of weight from the start to the end of the trial, but the differences were not significant ($F = 0.49$, $P = 0.50$). Brodifacoum residues in the dermal exposed salamanders ranged from 16.5-95.1 ppb. While the salamanders fed dusted crickets tended to have somewhat higher brodifacoum residue levels, these differences were not significant ($F = 1.02$, $P = 0.33$).

No animals died in the diphacinone oral exposure group. Skin sloughing or sores on the salamanders was not observed. Cricket consumption was somewhat variable: 6-68 in the exposure period, but stayed about the same (range of 4-66) in the post-exposure period. These differences were not significant ($F = 0.31$, $P = 0.58$). Weight gain in this treatment group ranged from 0.02-0.15g and were not significantly different ($F = 0.39$, $P = 0.54$). There were no diphacinone residues detected in the oral exposed salamanders.

In the diphacinone dermal exposure group, no animals died, but 50% of salamanders had some skin sloughing. Cricket consumption ranged from 6-57 during the exposure period, but stayed about the same (range of 5-59) in the post-exposure period. These differences were not significant ($F = 1.89$, $P = 0.19$). Salamander weights were mostly stable over the course of the trial, with changes ranging from -0.11-0.11g. The differences between the start and end of the trial were not significant ($F = 0.05$, $P = 0.83$). There were no diphacinone residues detected in the dermal exposed salamanders.

There was one death (20% mortality) in the control group. Interestingly, 20% of the control animals had sloughing skin and sores. Cricket consumption was also variable in the control group, ranging from 18-145 per salamander, but these differences were not significant ($F = 0.56$, $P = 0.47$) during the two periods (treatment versus post-treatment). Overall, there was no significant difference in the cricket consumption between the 5 groups of salamanders ($F = 0.84$, $P = 0.51$). Control animals also showed only small changes in weights during the study period: -0.02-0.43g and these differences were not significant ($F = 0.28$, $P = 0.61$). However, there was a significant difference in weight changes in the 5 groups of salamanders ($F = 3.47$, $P = 0.02$) with the brodifacoum salamanders losing the most weight and the control salamanders losing the least amount of weight.

Analyses of crickets, water, bait pellets and other findings

In Trial 1 and 2, we fed crickets that had been dusted with rodenticide powder rather than using crickets that had been fed powdered rodenticides (see explanation near the end of the Discussion section). Brodifacoum residue concentrations in crickets fed brodifacoum pellets (ranging from 296-688 ppb) were much lower than the residue concentrations in crickets dusted with powdered brodifacoum (2887-3340 ppb) ($F = 330.8$, $P = 0.0001$).

Diphacinone residues in crickets fed diphacinone pellets were quite variable (954-2930 ppb), as were crickets dusted with powdered diphacinone (1823-3980 ppb). Differences in residues between the two groups were not significant ($F = 1.78$, $P = 0.25$).

Residues in water used to soak crushed and powder rodenticide pellets were very low, likely due to the low water solubility of brodifacoum and diphacinone. Brodifacoum residues in water varied from 5.75-29.7 ppb. Diphacinone residues in water were similar to brodifacoum levels and varied from 0.08-17.7 ppb.

Because of the low rodenticide residue levels in the salamanders (i.e., ppb instead of ppm), we tested the brodifacoum and diphacinone pellets for rodenticide concentrations. These were very close to the label concentrations. For the diphacinone pellets, the mean concentration was 46.4

µg/g (= ppm) which is 93% of the desired 50 ug/g. For the brodifacoum pellets, the mean concentration was 26.3 ug/g (= ppm) which is 105% of the desired 25 µg/g.

Rodenticide residues were found in some samples where they were not expected. For example, very low concentrations of brodifacoum were found in two control *Batrachoseps* salamanders and one *Batrachoseps* salamander fed diphacinone-exposed crickets had a low concentration of brodifacoum residues. However, those concentrations of rodenticides were so low as to be considered unquantifiable (i.e., below the limit of quantitation). In addition to a few salamanders, the three groups of crickets dusted with brodifacoum had very low levels of diphacinone residues; but, again, these concentrations were not quantifiable. One possible explanation for these findings is cross-contamination of samples, processing equipment, or from latex gloves used to handle samples. Because the quality control samples are within acceptable limits and relatively few samples appeared potentially contaminated, we do not think such low level contamination of these samples compromises the quality of the analytical results.

All the residue analyses results are presented in Appendix A.

Discussion

From our Trial 1 results, it appears that rodenticide exposure poses some risk to salamanders, but that hazard appears to be relatively low in terms of mortality and sub-lethal effects, especially considering the experimental design optimized salamander exposure to rodenticides. It also appeared that salamanders can begin recovery after exposure ceases, as suggested by reduced skin sloughing and fewer sores during the post-exposure period. However, because some skin sloughing and sores were also noted in control salamanders, it is unclear whether or not skin damage was caused by anticoagulant exposure. One must also realize that in this trial there was a very high exposure rate in the treatment groups which combined oral and dermal exposures. In the brodifacoum group, the high exposure rates were from the feeding of dusted crickets instead of crickets that had fed on the rodenticides; the former had much higher concentrations of rodenticide residues. Additionally, the level of dermal exposure was much higher than it would be in an eradication project (see Figure 1). Hence, this trial presents, in essence, a worst case scenario. In an actual aerially-applied rodenticide baiting operation, using the U.S. Environmental Protection Agency's label application rate, there is generally only about two rodenticide pellets per m^2. Given that this was a worst-case scenario, the low residue concentrations in the salamanders suggests that there would be a relatively low risk to predators or scavengers consuming a salamander.

The Trial 2 results basically confirmed the results from Trial 1. However, Trial 2 seems to suggest that the higher hazard to *Batrachoseps* salamanders from anticoagulants is from dermal exposure versus oral exposure based on mortality. This could be determined because we had enough *Batrachosep*r salamanders to separate the two types of exposure into separate groups. It is cautioned, however, that we gave very high exposure rates to the salamanders in this study (Figure 1). Aerial broadcast baiting as part of an invasive rodent eradication project would likely result in much lower dermal exposure to all animals. Hence, Trial 2 also presents a worst case scenario.

The residue concentrations in this study were so low that our Analytical Chemistry Unit had to modify the normal method of detection. Normally they use High Performance Liquid Chromatography (HPLC) or the more sensitive mass spectrometer (MS). In the case of this study, they combined those methods (HPLC-MS) which greatly increased the sensitivity and probability of detecting residues.

With regard to the residue concentrations in crickets fed rodenticides, we need to clarify an early assumption that we made. When we first tried to feed powdered/crushed rodenticides to crickets, all the crickets died shortly thereafter. We assumed crickets might be sensitive to anticoagulants even though most invertebrates are known to not be sensitive to anticoagulants. Because of that early result, for the study we chose to dust crickets with powdered anticoagulants just before feeding them to the salamanders. However, when we later fed rodenticides to a different batch of crickets, all the crickets survived; those were the crickets used for residue analyses. We now surmise that we got a bad batch of crickets early on in the study. This is consistent with the scientific literature which has shown little or no impacts to invertebrates from anticoagulants even though some have been found to have substantial residues in them (Hoare and Hare 2006; Loof et al. 2011). It should be noted that dusted crickets were the only ones used in the salamander exposure trials.

A search of the scientific literature revealed no publications concerning the toxicity of anticoagulants to amphibians. Thus, little is known about the risk of anticoagulants to amphibians, but it is generally considered to be low (Eason, 1995; Chris et al., 2010). The native *Batrachoseps* salamanders on Anacapa Island are thriving 10 years after the invasive rats were eradicated using Brodifacoum-25D (Newton et al. 2016). There is considerable uncertainty regarding the toxicity of rodenticides to amphibians, but based on the fate and transport of the two rodenticides in the environment, we would anticipate relatively low risk to amphibians/salamanders under most island rodent eradication exposure scenarios. Published studies have focused on risks to mammals, birds, invertebrates, and to a lesser extent, on reptiles. These taxonomic groups are thought to be either the most sensitive or the groups most likely to consume either baits (primary exposure) or animals that have consumed baits (secondary exposure).

As such, we have little to compare our results with salamanders to with the exception of the taxonomic groups listed above. This information and residue levels comes from eradication projects with non-target monitoring before and after rodenticide application. The following paragraphs provide a brief synopsis of relevant and readily available literature for reptiles and other island fauna, where rodenticide body burdens have been used to demonstrate rodenticide accumulation potential and associated with acute toxicity, often lethality.

Witmer and Mauldin (2012) assessed the potential hazards of anticoagulant rodenticides to reptiles and reported concentrations of diphacinone and brodifacoum residues in whole bodies of captive snakes, turtles, and lizards that had been twice orally gavaged with solutions containing those anticoagulants. Body residues ranged from lows of 0.07 µg/g (= ppm) to highs of 1.58 µg/g. They also noted that 5 of 37 (13%) *Ameiva* lizards died during the study with one showing external hemorrhaging. One of 38 (3%) green iguanas died and it had external hemorrhaging.

Pitt et al. (2015) also reported concentrations of brodifacoum residues in various taxonomic groups and in environmental substrates after the rat eradication project on Palmyra Atoll in the tropical Pacific Ocean. While the concentrations were higher than they expected, they note that there were very high application rates of the rodenticide in that project (6 times higher than the EPA recommended label rate). Using whole body carcasses found after the baiting operation, they reported concentrations of 0.10-0.76 µg/g (= ppm) in birds, 0.34-0.44 µg/g in fish, and below the detection level to 0.97 µg/g in crabs. These concentrations are much lower than those found in rats that died from brodifacoum exposure: 3.75 µg/g. Pitt et al. (2015) also reported that only one fresh water sample had a residue concentration (0.05 µg/g (= ppm) above the detection level and none were detected in the salt water samples. They also reported very low soil residue concentrations of 0.007-0.018 µg/g (= ppm).

Shiels et al. (2017) reported concentrations of brodifacoum residues in various taxonomic groups and in environmental substrates after the rat eradication project on Desecheo Island in the Caribbean. Most fresh carcasses found from various taxonomic groups (rats, birds, lizards, crabs) had detectible residues of brodifacoum. Liver residues were quite variable, but rats had higher levels (8,930-27,700 ng/g (= ppb) than non-target animals (127-2,780 ng/g). They also live-harvested various lizard species about 3 weeks after the baiting operation. While all these animals appeared healthy, 65-100% had detectable residue concentrations ranging from 12.2-1100 ng/g (= ppb). Additionally, some insect and crabs had detectable residue concentrations ranging from 10.3-1580 ng/g.

This preliminary study suggests relatively low risk to salamanders from anticoagulant rodenticides. Additionally, it does appear that there would not be population-level effects on the salamander population if a mouse eradication was carried out. Because of the low residue levels in salamanders, it also appears that the hazard to animals preying or scavenging on salamanders would be low. However, more information and studies are needed to confirm these findings and to clarify various aspects. A study with larger sample sizes of animals per group might help reduce the wide variability observed in this study and would allow for more robust statistical analyses. There is also a need to fill information gaps (e.g., better exposure and robust toxicity data and histopathology data). Further study could also better explain the reason(s) behind skin sloughing and sores in salamanders. Trials with other species of amphibians would also be useful to compare with the results of this study. Finally, a small scale field application of anticoagulant rodenticides in an area containing amphibians might provide better insight to the real risk of these toxins to amphibians in a rodent eradication.

Acknowledgments

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Figure 1. *Aneides* salamander in its plastic cage showing the high level of dermal exposure in this study.

Figure 2. *Ensatina* salamander in its plastic cage in dermal exposure trial.

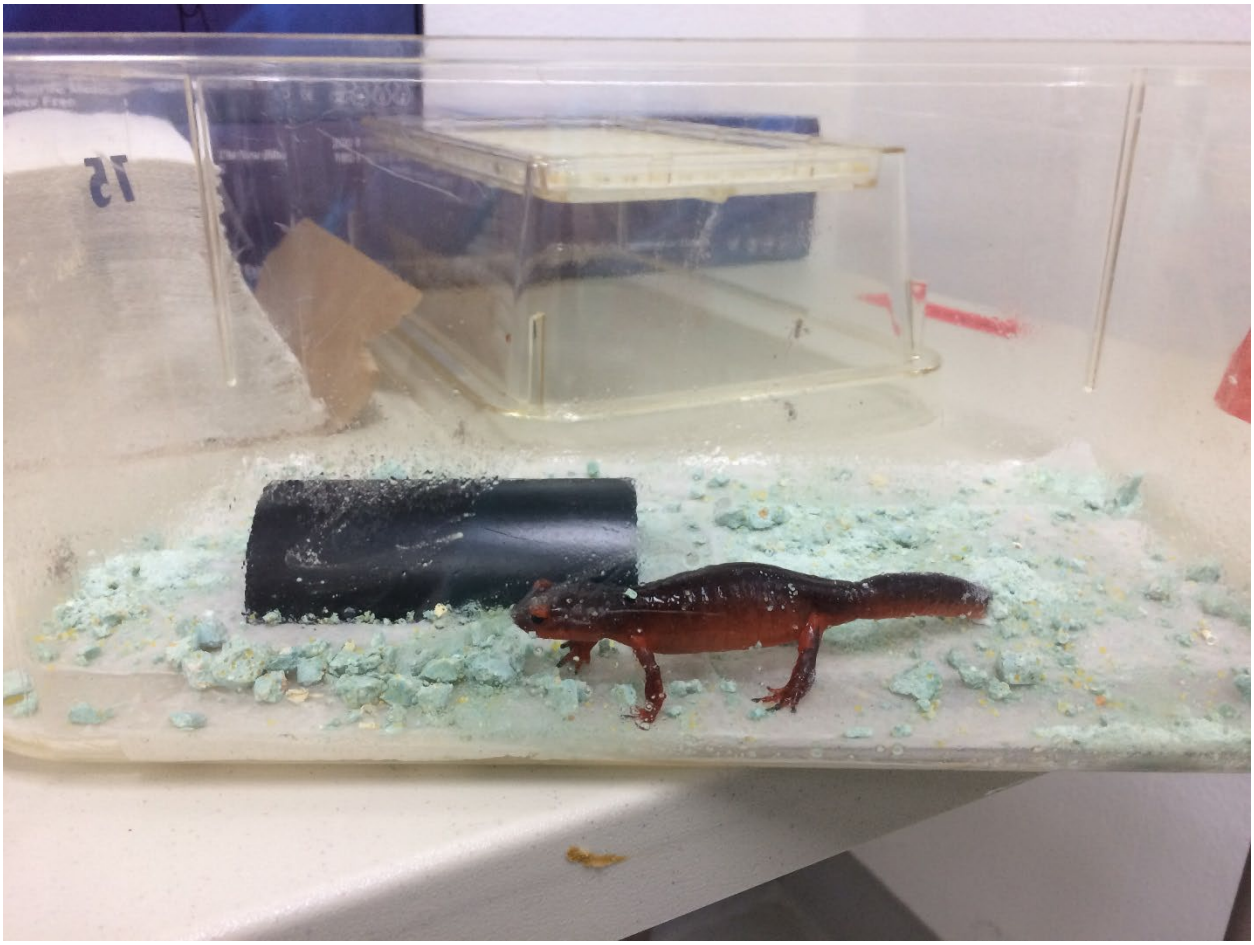

Figure 3. *Batrachoseps* salamander in its plastic cage. This was a control salamander, hence no rodenticides are present.

Table 1. Summary of the *Aneides* and *Ensatina* trial (Trial 1). Animals coded QO are *Aneides*; those coded QP are *Ensatina*.

Treatment	ID #	Initial Weight (g)	Final Weight (g)	Weight Change (g)	Comments	% Sloughing Skin	% Sores	% Mortality
Brodifacoum /oral & dermal exposure	QO1	9.4	6.1	-3.3	Died	57.14%	14.29%	28.57%
	QO4	9.0	7.8	-1.2	Euthanized at end of trial			
	QO7	9.7	7.5	-2.2	Euthanized at end of trial			
	QO10	9.4	6.0	-3.4	Died			
	QP1	7.7	6.8	-0.9	Euthanized at end of trial			
	QP4	7.3	6.9	-0.4	Euthanized at end of trial			
	QP7	13.0	10.5	-2.5	Euthanized at end of trial			
Diphacinone /oral & dermal exposure	QO2	10.5	7.7	-2.8	Euthanized due to condition	42.86%	28.57%	14.29%
	QO5	17.3	15.8	-1.5	Euthanized at end of trial			
	QO8	12.9	12.2	-0.7	Euthanized at end of trial			
	QO11	20.7	17.3	-3.4	Euthanized at end of trial			
	QP2	9.6	8.6	-1.0	Euthanized at end of trial			
	QP5	9.3	8.1	-1.2	Euthanized at end of trial			
	QP8	8.0	6.8	-1.2	Euthanized at end of trial			
Control	QO3	19.4	18.5	-0.9	Euthanized at end of trial	0.00%	0.00%	0.00%
	QO6	10.8	10.4	-0.4	Euthanized at end of trial			
	QO9	20.3	18.2	-2.1	Euthanized at end of trial			
	QO14	10.4	10.0	-0.4	Euthanized at end of trial			

QP3	6.0	4.8	-1.2	Euthanized at end of trial
QP6	15.4	13.3	-2.1	Euthanized at end of trial

Table 2. Summary of the *Batrachoseps* trial (Trial 2).

Treatment	Animal ID	Initial Weight (g)	Final Weight (g)	Weight Change (g)	Days Until Death	% Sloughing Skin	% Sores	% Mortality
Brodifacoum /oral exposure	QS5	0.73	0.73	0.00		0.00%	0.00%	0.00%
	QS10	0.45	0.55	0.10				
	QS19	0.84	0.94	0.10				
	QS27	0.52	See footnote	N/A				
	QS35	0.46	0.54	0.08				
	QS42	1.17	1.21	0.04				
	QS56	0.78	0.83	0.05				
Brodifacoum /Dermal exposure	QS6	0.52	0.42	-0.10	2	0.00%	0.00%	75.00%
	QS11	1.03	0.97	-0.06	9			
	QS30	0.81	0.60	-0.21	14			
	QS36	0.41	0.34	-0.07	10			
	QS38	0.30	0.23	-0.07	10			
	QS43	0.52	0.52	0.00				
	QS51	0.80	0.67	-0.13	10			
	QS57	0.58	0.57	-0.01				
Diphacinone /oral exposure	QS7	0.50	0.64	0.14		0.00%	0.00%	0.00%
	QS13	0.69	0.79	0.10				
	QS23	0.56	0.70	0.14				
	QS31	1.15	1.27	0.12				
	QS39	0.30	0.32	0.02				
	QS44	0.89	1.04	0.15				
	QS52	0.29	0.34	0.05				
	QS58	0.56	0.61	0.05				
Diphacinone /Dermal exposure	QS8	0.31	0.36	0.05		50.00%	0.00%	0.00%
	QS14	0.39	0.48	0.09				
	QS24	0.88	0.88	0.00				
	QS33	0.88	0.92	0.04				
	QS40	0.83	0.89	0.06				
	QS48	0.86	0.97	0.11				
	QS53	0.82	0.71	-0.11				
	QS55	0.93	0.89	-0.04				
Control	QS9	0.45	0.55	0.10		20.00%	20.00%	20.00%
	QS17	0.75	0.81	0.06				
	QS22	0.54	0.52	-0.02	6			

	QS26	0.90	0.94	0.04				
	QS34	0.38	0.40	0.02				

This carcass was lost.

Table 3. Total cricket consumption by salamanders in trial 1 by group and time period.

Brodifacoum Group	Treatment Period	Post-treatment Period	Total/Both Periods
QO1	13	X	X
QO4	3	1	4
QO7	14	32	46
QO10	11	X	X
QP1	6	13	19
QP4	8	29	37
QP7	12	22	34
Diphacinone Group			
QO2	10	X	X
QO5	24	38	62
QO8	12	9	21
QO11	6	5	11
QP2	3	9	12
QP5	9	21	30
QP8	3	14	17
Control Group			
QO3	22	10	32
QO6	22	28	50
QO9	24	60	84
QO14	25	64	89
QP3	19	17	36
QP6	23	37	60

QO = *Aneides*; QP = *Ensatina*
X = died

Table 4. Total cricket consumption by *Batrachoseps* salamanders in Trial 2 by group and time period.

Brodifacoum Oral Group	Treatment Period	Post-treatment Period (X = died)	Total/Both Periods (X = died)
QS5	59	54	113
QS10	54	47	101
QS19	50	59	109
QS27	13	7	20
QS35	28	48	76
QS42	13	4	17
QS56	70	59	129
Brodifacoum Dermal Group			
QS6	1	X	X
QS11	9	X	X
QS30	13	X	X
QS36	11	X	X
QS38	10	X	X
QS43	9	44	53
QS51	31	X	X
QS57	27	54	81
Diphacinone Oral Group			
QS7	64	55	119
QS13	57	60	117
QS23	29	46	75
QS31	68	66	134
QS39	8	4	12
QS44	25	46	71
QS52	64	45	109
QS58	25	57	82
Diphacinone Dermal Group			
QS8	6	40	46
QS14	57	59	116
QS24	23	54	77
QS33	10	5	15
QS40	34	57	91
QS48	34	55	89
QS53	10	8	18
QS55	12	20	32
Control Group			
QS9	70	54	124
QS17	48	42	90
QS22	2	X	X

QS26	74	71	145
QS34	18	19	37

Appendix A. Residue report of the NWRC Analytical Chemistry Unit.

Wildlife Services
NWRC
National Wildlife Research Center

To: Dr. Gary Witmer
Research Wildlife Biologist
NWRC

Subject: Determination of Diphacinone and Brodifacoum in Salamanders, Crickets, Water, and Baits (QA-2688); Invoice #17-019, Nov. 6, 2017

Methods: Non-GLP (salamanders, crickets, water); Method 163A (baits)

Analysis Dates: 9/12, 9/13, 9/14, 9/19, 9/25, 9/27, 9/28, 10/13, 10/27, and 10/30/2017

Notebook Reference: AC-161, pp. 86-109

QC Notebook Reference: QC-33, p. 137; AC-162, p. 4

Analyst: Steve Volker

Sample Descriptions:

Ensatina salamanders (n=8), *Aneides* salamanders (n=14), *Batrachoseps* salamanders (n=36), crickets (n=24 composite samples), water (saturated with ground bait, n=12), and baits (n=4) were received between 6/2/2017 and 9/25/2017 for analysis of diphacinone and brodifacoum. All samples were stored at -20°C until time of analysis.

Sample Preparation and Extraction:

Homogenization:

Baits and salamanders (whole bodies) were homogenized with a SPEX 6875D liquid nitrogen freezer mill. Homogenized samples were transferred immediately to vacuum sealable bags while still frozen and stored at -20°C. Cricket samples, consisting of between 11 and 27 individual crickets, were ground into a paste using a glass rod and stored at -20°C.

Extraction of salamanders and crickets:

Homogenized sample (70-80 mg) was weighed into a 1.5-mL microcentrifuge tube, 50 µL DI water added, and the sample vortex mixed 4-5 s to form a suspension. Surrogate analytes (20 µL, 16 µg/mL D_4-diphacinone and 17 µg/mL chlordifacoum in acetonitrile) and 1.180 mL of acetonitrile (ACN) were added and the sample vortex mixed twice for 15-20 s. An excess of NaCl (~120 mg) was added to produce a water:ACN phase separation and the sample vortex mixed twice for 15-20 s. The extract was clarified by centrifugation (12,000 RCF) and 0.900 mL of supernatant transferred to a dispersive solid-phase extraction (dSPE) tube containing $MgSO_4$ (150 mg), C18 sorbent (25 mg), and primary-secondary amine (PSA) sorbent (25 mg). The extract was exposed to the sorbents and $MgSO_4$ by vortex mixing for 4-5 s followed by centrifugation at 12,000 RCF for 2-3 s to clarify the supernatant. 0.400 mL of supernatant was then transferred to a 1.5-mL microcentrifuge tube and the solvent removed in a 60°C N-Evap with a gentle flow of nitrogen. The analytes were reconstituted with 100 µL ACN followed by 400 µL pH 9.5 20-mM ammonium acetate, with vortex mixing after each addition. The sample was then transferred to an autosampler vial for LC/MS analysis.

Extraction of Water:

Water samples (10-50 mL) were warmed to room temperature (overnight in a hood), vortex mixed 4-5 s, centrifuged at 1400 RCF for 2 minutes, and then 8-10 mL of supernatant filtered through a 0.7-µm glass fiber syringe filter into a 15-mL polypropylene tube. A portion of the filtered sample (1.5 mL) was transferred to a 10-mL glass tube and surrogate analytes (10 µL) added. Acetonitrile (2.0 mL), 1M HCl (0.5 mL), and excess NaCl (~1 g) were added and the sample vortex mixed 4-5 s. Chloroform (0.5 mL) was added and the sample vortex mixed 4-5 s, let set for 5-10 minutes, and then vortex mixed again. The sample was then centrifuged at 1400 RCF for 1 minutes and 1.5 mL of the upper ACN/chloroform layer transferred to a 1.5-mL microcentrifuge tube. The solvents were removed in a 45°C N-Evap with a gentle flow of nitrogen. The analytes were reconstituted with 90 µL ACN followed by 360 µL pH 9.5 20-mM ammonium acetate, with vortex mixing after each addition. The sample was then transferred to an autosampler vial for LC/MS analysis.

Baits:

All baits were assayed by NWRC Method 163A. To assess trace level residues of rodenticides, 0.600 mL of microwave extract from Method 163A procedure was transferred to a 1.5-mL microcentrifuge tube and the solvent removed in a 60°C N-Evap with a gentle flow of nitrogen. The analytes were reconstituted with 300 µL ACN followed by 1200 µL pH 9.5 20-mM ammonium acetate, with vortex mixing after each addition. The sample was then transferred to an autosampler vial for LC/MS analysis.

Instrument methods:

Salamanders and Crickets:

Agilent 1290 Infinity II HPLC with G6470A QQQ

Column	Xbridge C18, 2.5-µm, 2.1 x 50 mm, Waters P/N 186003085
Mobile phase A	90%(pH 9.5 20-mM ammonium acetate)/10%(Acetonitrile)
Mobile phase B	Acetonitrile
Flow rate	0.800 mL/min
Column temp.	60°C
Injection volume	7.5 µL
Run time	4.0 min
Source	AJS ESI, negative mode
Gas temp.	300°C
Gas flow	5 L/min
Nebulizer	45 psi
Sheath gas	250°C, 7 L/min
Capillary	-4500 V
Nozzle	-500 V

Time (min)	%A	%B
0.00	90%	10%
0.50	90%	10%
3.00	20%	80%
3.01	0%	100%
3.50	0%	100%
3.51	90%	10%

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Fragmentor (V)	Collision Energy (V)
Diphacinone	339.1	**167.1** 145	100	23 18
D4-Diphacinone	343.1	167.1	120	23
Chlordifacoum	477.1	135.1	61	37
Brodifacoum	522.9	135.0 **80.9**	165	44 50

BOLD = product ion used for quantitation

Water:

Same conditions as for salamanders and crickets with the following changes:

Flow rate	0.650 mL/min
Run time	3.5 min

Time (min)	%A	%B
0.00	85%	15%
0.50	85%	15%
2.30	30%	70%
2.31	0%	100%
2.90	0%	100%
2.91	85%	15%

Baits (LCMS):

Same conditions as for water, but 1.5 µL injection volume.

Baits (Method 163A):

Agilent 1100 Series HPLC with G1315B Diode Array Detector (DAD) and G1321A Fluorescence Detection (FLD)

Column	Gemini C18, 3-µm, 3 x 150 mm, Phenomenex P/N 00F-4439-Y0
Mobile phase A	5-mM tetrabutylammonium phosphate (TBAP) in 50%(pH 8.5 6-mM phosphate)/50%(methanol)
Mobile phase B	5-mM TBAP in methanol
Flow rate	0.650 mL/min
Column temp.	60°C
Injection volume	10 µL
Run time	26 min
Detector	UV (DAD); 325 nm
Detector	Fluorescence (FLD)
Excitation	310 nm
Emission	390 nm

Time (min)	%A	%B
0.00	85%	15%
1.00	85%	15%
17.00	45%	55%
17.01	0%	100%
23.00	0%	100%
23.01	85%	15%

Detection and Quantitation Limits:

The Detection Limit (DL) is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified as an exact value. The Quantitation Limit (QL) is the lowest concentration of brodifacoum that can be quantitatively determined with suitable precision and accuracy. The signal-to-noise (S/N) ratio was used to determine the DL and QL for each analyte. This was performed by comparing the analyte response observed in fortified control matrix with the baseline noise observed at the same retention time in control matrix. The DL and QL are defined as analyte concentrations corresponding to S/N ratios of 3 and 10, respectively. The following table presents the average DL and QL concentrations for diphacinone and brodifacoum in each control matrix.

Detection Limit (DL) and Quantitation Limit (QL)

	Diphacinone		Brodifacoum	
Control Matrix	DL	QL	DL	QL
Ensatina Salamanders (whole body)	5.9 ng/g	19.6 ng/g	6.6 ng/g	21.9 ng/g
Aneides Salamanders (whole body)	7.5 ng/g	25.1 ng/g	8.6 ng/g	28.6 ng/g
Batrachoseps Salamanders (whole body)	8.9 ng/g	29.8 ng/g	8.9 ng/g	29.7 ng/g
Crickets	4.9 ng/g	16.2 ng/g	5.9 ng/g	19.7 ng/g
Water (saturated with ground bait)	0.080 ng/mL	0.267 ng/mL	0.13 ng/mL	0.419 ng/mL
Baits (Method 163A)	2.8 µg/g	9.40 µg/g	0.043 µg/g	0.142 µg/g
Baits (LCMS)	0.0072 µg/g	0.0241 µg/g	0.0081 µg/g	0.0270 µg/g

Results:

Triplicate preparations of all samples were prepared, except when sample size was insufficient. Rodenticide residues for salamanders and crickets are reported in units of ng/g, equivalent to parts per billion (ppb). Water results are reported in units of ng/mL, also equivalent to ppb. Rodenticide concentrations in bait formulations are reported in units of µg/g, equivalent to parts per million (ppm).

If no analyte response was recorded by the data acquisition software or if the observed concentration was less than the DL, an entry of "ND" is reported to indicate that the analyte was not detected. Results that are greater than the DL, but less than the QL are identified by an asterisk "*". Care should be taken when evaluating results below the QL as the variability will be significantly greater than the variability observed in quality control (QC) samples. Results above the QL are reported to three significant figures.

***Ensatina* salamanders (whole body)**

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170602-13	QP3 (Control)	9/14/2017	ND	ND
S170602-14	QP6 (Control)	9/14/2017	ND	ND
S170602-19-A	QP1 (Brodifacoum, Dermal + Cricket)	9/14/2017	ND	101
S170602-19-B		9/14/2017	ND	95.9
S170602-19-C		9/14/2017	ND	100
S170602-20-A	QP4 (Brodifacoum, Dermal + Cricket)	9/14/2017	ND	86.9
S170602-20-B		9/14/2017	ND	85.7
S170602-20-C		9/14/2017	ND	85.5
S170602-21-A	QP7 (Brodifacoum, Dermal + Cricket)	9/14/2017	ND	50.1
S170602-21-B		9/14/2017	ND	50.7
S170602-21-		9/14/201	ND	48.3

C		7		
S170602-26-A		9/14/2017	ND	ND
S170602-26-B	QP2 (Diphacinone, Dermal + Cricket)	9/14/2017	ND	ND
S170602-26-C		9/14/2017	ND	ND
S170602-27-A		9/14/2017	ND	ND
S170602-27-B	QP5 (Diphacinone, Dermal + Cricket)	9/14/2017	ND	ND
S170602-27-C		9/14/2017	ND	ND
S170602-28-A		9/14/2017	ND	ND
S170602-28-B	QP8 (Diphacinone, Dermal + Cricket)	9/14/2017	ND	ND
S170602-28-C		9/14/2017	ND	ND
		DL (ng/g) =	5.9	6.6
		QL (ng/g) =	19.6	21.9

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

* Results reported with an asterisk denote concentration less than the Quantitation Limit (QL).

***Aneides* salamanders (whole body)**

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170602-09	QO3 (Control)	9/19/2017	ND	ND
S170602-10	QO6 (Control)	9/19/2017	ND	ND
S170602-11	QO9 (Control)	9/19/2017	ND	ND
S170602-12	QO14 (Control)	9/28/2017	ND	ND
S170711-31-A	QO13 (Control)	9/28/2017	ND	ND
S170711-31-B		9/28/2017	ND	ND
S170711-31-C		9/28/2017	ND	ND
S170711-32-A	QO12 (Control)	9/28/2017	ND	ND
S170711-32-B		9/28/2017	ND	ND
S170711-32-C		9/28/2017	ND	ND
S170602-15-A	QO1 (Brodifacoum, Dermal + Cricket)	9/28/2017	ND	108
S170602-15-B		9/28/2017	ND	98.0
S170602-15-C		9/28/2017	ND	103
S170602-16-A	QO4 (Brodifacoum, Dermal + Cricket)	9/28/2017	ND	45.6
S170602-16-B		9/28/2017	ND	46.6
S170602-16-C		9/28/2017	ND	38.8
S170602-17-A	QO7 (Brodifacoum, Dermal + Cricket)	9/28/2017	ND	85.5
S170602-17-B		9/28/2017	ND	97.1
S170602-17-C		9/28/2017	ND	89.3
S170602-18-A	QO10 (Brodifacoum, Dermal + Cricket)	9/28/2017	ND	239
S170602-18-B		9/28/2017	ND	214
S170602-18-C		9/28/2017	ND	224

S170602-22-A		9/28/2017	182	ND
S170602-22-B	QO2 (Diphacinone, Dermal + Cricket)	9/28/2017	176	ND
S170602-22-C		9/28/2017	165	ND
S170602-23-A		9/28/2017	ND	ND
S170602-23-B	QO5 (Diphacinone, Dermal + Cricket)	9/28/2017	ND	ND
S170602-23-C		9/28/2017	ND	ND
S170602-24-A		9/28/2017	9.0 *	ND
S170602-24-B	QO8 (Diphacinone, Dermal + Cricket)	9/28/2017	13.7 *	ND
S170602-24-C		9/28/2017	9.8 *	ND
S170602-25-A		9/28/2017	ND	ND
S170602-25-B	QO11 (Diphacinone, Dermal + Cricket)	9/28/2017	ND	ND
S170602-25-C		9/28/2017	ND	ND
		DL (ng/g) =	7.5	8.6
		QL (ng/g) =	25.1	28.6

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

***Batrachoseps* salamanders (whole body)**

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170602-30-A	QS22 (control)	9/19/2017	ND	ND
S170602-30-B		9/19/2017	ND	ND
S170602-30-C		9/19/2017	ND	ND
S170711-04-A	QS9 (Control)	9/19/2017	ND	22.0 *
S170711-04-B		9/19/2017	ND	22.6 *
S170711-04-C		9/19/2017	ND	21.2 *
S170711-08-A	QS17 (Control)	9/19/2017	ND	ND
S170711-08-B		9/19/2017	ND	8.8 *
S170711-08-C		9/19/2017	ND	ND
S170711-12-A	QS26 (Control)	9/19/2017	ND	ND
S170711-12-B		9/19/2017	ND	ND
S170711-12-C		9/19/2017	ND	ND
S170711-17-A	QS34 (Control)	9/19/2017	ND	ND
S170711-17-B		9/19/2017	ND	ND
S170711-17-C		9/19/2017	ND	ND
S170602-31-A	QS6 (Brodifacoum, Dermal)	9/19/2017	ND	22.8 *
S170602-31-B		9/19/2017	ND	16.5 *
S170602-31-C		9/19/2017	ND	18.2 *
S170602-32-A	QS11 (Brodifacoum, Dermal)	9/19/2017	ND	82.1
S170602-32-B		9/19/2017	ND	61.9

S170602-32-C		9/19/2017	ND	74.4
S170602-33-A	QS36 (Brodifacoum, Dermal)	9/19/2017	ND	29.8
S170602-33-B		9/19/2017	ND	38.5
S170602-34-A	QS38 (Brodifacoum, Dermal)	9/19/2017	ND	103
S170602-35-A	QS51 (Brodifacoum, Dermal)	9/19/2017	ND	64.4
S170602-35-B		9/19/2017	ND	71.4
S170602-35-C		9/19/2017	ND	71.3
S170711-01-A	QS5 (Brodifacoum, Cricket)	9/19/2017	ND	87.9
S170711-01-B		9/19/2017	ND	72.5
S170711-01-C		9/19/2017	ND	95.1
S170711-02-A	QS7 (Diphacinone, Cricket)	9/19/2017	ND	10.1 *
S170711-02-B		9/19/2017	ND	12.7 *
S170711-02-C		9/19/2017	ND	9.3 *
S170711-03-A	QS8 (Diphacinone, Dermal)	9/25/2017	ND	ND
S170711-03-B		9/25/2017	ND	ND
		DL (ng/g) =	8.9	8.9
		QL (ng/g) =	29.8	29.7

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

***Batrachoseps* salamanders (whole body)**

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170711-05-A		9/25/2017	ND	54.7
S170711-05-B	QS10 (Brodifacoum, Cricket)	9/25/2017	ND	54.6
S170711-05-C		9/25/2017	ND	60.4
S170711-06-A		9/25/2017	ND	ND
S170711-06-B	QS13 (Diphacinone, Cricket)	9/25/2017	ND	ND
S170711-06-C		9/25/2017	ND	ND
S170711-07-A		9/25/2017	ND	ND
S170711-07-B	QS14 (Diphacinone, Dermal)	9/25/2017	ND	ND
S170711-07-C		9/25/2017	ND	ND
S170711-09-A		9/25/2017	ND	48.0
S170711-09-B	QS19 (Brodifacoum, Cricket)	9/25/2017	ND	55.9
S170711-09-C		9/25/2017	ND	49.9
S170711-10-A		9/25/2017	ND	ND
S170711-10-B	QS23 (Diphacinone, Cricket)	9/25/2017	ND	ND
S170711-10-C		9/25/2017	ND	ND
S170711-11-A		9/25/2017	ND	ND
S170711-11-B	QS24 (Diphacinone, Dermal)	9/25/2017	ND	ND
S170711-11-C		9/25/2017	ND	ND
S170711-13[a]	QS27 (Brodifacoum, Cricket)	N/A	N/A	N/A
S170711-14-A		9/25/2017	ND	73.5

S170711-14-B	QS30 (Brodifacoum, Dermal)	9/25/2017	ND	84.4
S170711-14-C		9/25/2017	ND	83.7
S170711-15-A		9/25/2017	ND	ND
S170711-15-B	QS31 (Diphacinone, Cricket)	9/25/2017	ND	ND
S170711-15-C		9/25/2017	ND	ND
S170711-16-A		9/25/2017	ND	ND
S170711-16-B	QS33 (Diphacinone, Dermal)	9/25/2017	ND	ND
S170711-16-C		9/25/2017	ND	ND
S170711-18-A		9/25/2017	ND	64.1
S170711-18-B	QS35 (Brodifacoum, Cricket)	9/25/2017	ND	65.6
S170711-18-C		9/25/2017	ND	64.0
S170711-19-A		9/25/2017	ND	ND
S170711-19-B	QS39 (Diphacinone, Cricket)	9/25/2017	ND	ND
S170711-19-C		9/25/2017	ND	ND
		DL (ng/g) =	8.9	8.9
		QL (ng/g) =	29.8	29.7

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

[a] No sample available.

***Batrachoseps* salamanders (whole body)**

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170711-20-A		9/27/2017	ND	ND
S170711-20-B	QS40 (Diphacinone, Dermal)	9/27/2017	ND	ND
S170711-20-C		9/27/2017	ND	ND
S170711-21-A		9/27/2017	ND	ND
S170711-21-B	QS42 (Brodifacoum, Cricket)	9/27/2017	ND	ND
S170711-21-C		9/27/2017	ND	ND
S170711-22-A		9/27/2017	ND	33.0
S170711-22-B	QS43 (Brodifacoum, Dermal)	9/27/2017	ND	34.1
S170711-22-C		9/27/2017	ND	34.7
S170711-23-A		9/27/2017	ND	ND
S170711-23-B	QS44 (Diphacinone, Cricket)	9/27/2017	ND	ND
S170711-23-C		9/27/2017	ND	ND
S170711-24-A		9/27/2017	ND	ND
S170711-24-B	QS48 (Diphacinone, Dermal)	9/27/2017	ND	ND
S170711-24-C		9/27/2017	ND	ND
S170711-25-A		9/27/2017	ND	ND
S170711-25-B	QS52 (Diphacinone, Cricket)	9/27/2017	ND	ND
S170711-25-C		9/27/2017	ND	ND
S170711-26-A		9/27/2017	ND	ND
S170711-26-B	QS53 (Diphacinone, Dermal)	9/27/2017	ND	ND

S170711-26-C		9/27/2017	ND	ND
S170711-27-A	QS55 (Diphacinone, Dermal)	9/27/2017	ND	ND
S170711-27-B		9/27/2017	ND	ND
S170711-27-C		9/27/2017	ND	ND
S170711-28-A		9/27/2017	ND	90.8
S170711-28-B	QS56 (Brodifacoum, Cricket)	9/27/2017	ND	91.4
S170711-28-C		9/27/2017	ND	86.6
S170711-29-A		9/27/2017	ND	37.3
S170711-29-B	QS57 (Brodifacoum, Dermal)	9/27/2017	ND	35.0
S170711-29-C		9/27/2017	ND	34.2
S170711-30-A		9/27/2017	ND	ND
S170711-30-B	QS58 (Diphacinone, Cricket)	9/27/2017	ND	ND
S170711-30-C		9/27/2017	ND	ND
		DL (ng/g) =	8.9	8.9
		QL (ng/g) =	29.8	29.7

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

Crickets

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170711-51	Control Tissue 1/2"	9/13/2017	ND	ND
S170711-52	Control Tissue Pinheads	9/12/2017	ND	ND
S170711-45	Placebo Diphacinone + no potato (PDFC1), n=20	9/13/2017	31.5	ND
S170711-45-A		9/12/2017	31.2	ND
S170711-46	Placebo Diphacinone + no potato (PDFC2), n=21	9/13/2017	18.8	ND
S170711-46-A		9/12/2017	15.8 *	ND
S170711-47	Placebo Diphacinone + no potato (PDFC3), n=24	9/13/2017	19.5	ND
S170711-47-A		9/12/2017	14.6 *	ND
S170711-48	Placebo Brodifacoum + no potato (PBFC1), n=22	9/13/2017	ND	ND
S170711-49	Placebo Brodifacoum + no potato (PBFC2), n=23	9/13/2017	ND	ND
S170711-50	Placebo Brodifacoum + no potato (PBFC3), n=21	9/13/2017	ND	ND
S170602-36-A	Brodifacoum + potato (BFC1), n=15	9/13/2017	ND	296
S170602-36-B		9/13/2017	ND	282
S170602-36-C		9/13/2017	ND	309
S170602-37-A	Brodifacoum + potato (BFC2), n=14	9/13/2017	ND	589
S170602-37-B		9/13/2017	ND	687
S170602-38-A	Brodifacoum + potato (BFC3), n=13	9/13/2017	ND	538
S170602-38-B		9/13/2017	ND	672
S170602-38-C		9/13/2017	ND	528
S170602-39-A	Diphacinone + potato (DFC1), n=11	9/13/2017	1490	ND
S170602-		9/13/2017	1600	ND

39-B				
S170602-40-A	Diphacinone + potato (DFC2), n=15	9/13/2017	3130	ND
S170602-40-B		9/13/2017	3040	ND
S170602-40-C		9/13/2017	2620	ND
S170602-41-A	Diphacinone + potato (DFC3), n=14	9/13/2017	1140	ND
S170602-41-B		9/13/2017	1260	ND
S170711-33-A	Brodifacoum + no potato (BFC4), n=24	9/13/2017	ND	495
S170711-33-B		9/13/2017	ND	519
S170711-33-C		9/13/2017	ND	530
S170711-34-A	Brodifacoum + no potato (BFC5), n=23	9/13/2017	ND	423
S170711-34-B		9/13/2017	ND	420
		DL (ng/g) =	4.9	5.9
		QL (ng/g) =	16.2	19.7

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

Crickets

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170711-35-A	Brodifacoum + no potato (BFC6), n=23	9/13/2017	ND	560
S170711-35-B		9/13/2017	ND	638
S170711-35-C		9/13/2017	ND	490
S170711-36-A	Diphacinone + no potato (DFC4), n=27	9/12/2017	1060	ND
S170711-36-B		9/12/2017	950	ND
S170711-36-C		9/12/2017	943	ND
S170711-37-A	Diphacinone + no potato (DFC5), n=27	9/12/2017	907	ND
S170711-37-B		9/12/2017	1140	ND
S170711-37-C		9/12/2017	1050	ND
S170711-38-A	Diphacinone + no potato (DFC6), n=21	9/12/2017	2040	ND
S170711-38-B		9/12/2017	2350	ND
S170711-38-C		9/12/2017	1720	ND
S170711-39-A	Diphacinone + dusted (DD1), n=23	9/12/2017	1740	ND
S170711-39-B		9/12/2017	1950	ND
S170711-39-C		9/12/2017	1780	ND
S170711-40-A	Diphacinone + dusted (DD2), n=25	9/12/2017	3090	ND
S170711-40-B		9/12/2017	3490	ND
S170711-40-C		9/12/2017	3410	ND
S170711-41-A	Diphacinone + dusted (DD3), n=18	9/12/2017	4200	ND
S170711-41-B		9/12/2017	4280	ND

S170711-41-C		9/12/2017	3460	ND
S170711-42-A	Brodifacoum + dusted (BD1), n=16	9/12/2017	9.9 *	3320
S170711-42-B		9/12/2017	7.8 *	3080
S170711-42-C		9/12/2017	9.5 *	3260
S170711-43-A		9/12/2017	9.7 *	3620
S170711-43-B	Brodifacoum + dusted (BD2), n=23	9/12/2017	7.1 *	3220
S170711-43-C		9/12/2017	6.2 *	3180
S170711-44-A		9/12/2017	7.1 *	2670
S170711-44-B	Brodifacoum + dusted (BD3), n=18	9/12/2017	7.5 *	3160
S170711-44-C		9/12/2017	6.0 *	2830
		DL (ng/g) =	4.9	5.9
		QL (ng/g) =	16.2	19.7

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

Water (saturated with ground bait)

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)
S170602-03-A		10/13/2017	6.31	ND
S170602-03-B	Water/Diphacinone #1	10/13/2017	6.44	ND
S170602-03-C		10/13/2017	6.15	ND
S170602-04-A		10/13/2017	9.02	ND
S170602-04-B	Water/Diphacinone #2	10/13/2017	9.63	ND
S170602-04-C		10/13/2017	8.74	ND
S170602-05-A		10/13/2017	17.6	ND
S170602-05-B	Water/Diphacinone #3	10/13/2017	18.0	ND
S170602-05-C		10/13/2017	17.6	ND
S170606-01-A		10/13/2017	3.52	ND
S170606-01-B	Water/Diphacinone #4	10/13/2017	3.34	ND
S170606-01-C		10/13/2017	3.39	ND
S170606-02-A		10/13/2017	4.84	ND
S170606-02-B	Water/Diphacinone #5	10/13/2017	4.89	ND
S170606-02-C		10/13/2017	4.77	ND
S170606-03-A		10/13/2017	3.89	ND
S170606-03-B	Water/Diphacinone #6	10/13/2017	3.57	ND
S170606-03-C		10/13/2017	3.36	ND
S170602-06-A		10/13/2017	ND	5.78
S170602-06-B	Water/Brodifacoum #1	10/13/2017	0.080 *	5.78
S170602-		10/13/2017	ND	5.69

06-C				
S170602-07-A	Water/Brodifacoum #2	10/13/2017	0.125 *	29.3
S170602-07-B		10/13/2017	0.147 *	29.6
S170602-07-C		10/13/2017	0.133 *	29.5
S170602-08-A		10/13/2017	0.131 *	29.9
S170602-08-B	Water/Brodifacoum #3	10/13/2017	0.110 *	28.6
S170602-08-C		10/13/2017	0.127 *	30.7
S170606-04-A		10/13/2017	0.134 *	26.5
S170606-04-B	Water/Brodifacoum #4	10/13/2017	0.109 *	24.7
S170606-04-C		10/13/2017	0.127 *	25.2
S170606-05-A		10/13/2017	0.121 *	18.5
S170606-05-B	Water/Brodifacoum #5	10/13/2017	0.140 *	19.4
S170606-05-C		10/13/2017	0.123 *	19.5
S170606-06-A		10/13/2017	0.100 *	18.9
S170606-06-B	Water/Brodifacoum #6	10/13/2017	0.171 *	18.8
S170606-06-C		10/13/2017	0.119 *	18.4
		DL (ng/mL) =	0.080	0.13
		QL (ng/mL) =	0.267	0.419

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

Baits (Method 163A)

NWRC ID	Sample Description	Analysis Date	Observed Diphacinone Concentration (µg/g)	Observed Brodifacoum Concentration (µg/g)
S170925-01-D	Placebo Diphacinone Bait	10/27/2017	0.424 [a]	ND
S170925-01-E		10/27/2017	0.266 [a]	ND
S170925-01-F		10/27/2017	0.278 [a]	ND
S170925-02	Placebo Brodifacoum Bait	10/27/2017	ND	ND
S170925-03-D	Diphacinone Conservation 50 (0.0050%) Bait	10/27/2017	46.8	ND
S170925-03-E		10/27/2017	46.3	ND
S170925-03-F		10/27/2017	46.1	ND
S170925-04-D	Brodifacoum Conservation 25 (0.0025%) Bait	10/27/2017	ND	26.0
S170925-04-E		10/27/2017	ND	27.2
S170925-04-F		10/27/2017	ND	25.8
		DL (µg/g) =	2.8	0.043
		QL (µg/g) =	9.40	0.142

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

* Results reported with an asterisk denote concentrations less than the Quantitation Limit (QL).

[a] Method 163A is not sufficiently sensitive to detect diphacinone concentrations less than 2.8 µg/g. To better assess trace level contamination in the baits extracts were also tested by a more sensitive LCMS method with detection limits of 0.0072 µg/g for diphacinone and 0.0081 µg/g for brodifacoum. The placebo diphacinone bait (S170925-01) had diphacinone concentrations of 0.278 – 0.424 µg/g. None of the other baits had detectable contamination.

QC Results:

QC Recoveries – *Ensatina* Salamander (whole body, S170602-13)

ID	Analysis Date	Theoretical Diphacinone Concentration (ng/g)	Observed Diphacinone Concentration (ng/g)	% Recovery	Theoretical Brodifacoum Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)	% Recovery
QC-41	9/14/2017	0	ND	N/A	0	ND	N/A
QC-42	9/14/2017	0	ND	N/A	0	ND	N/A
QC-43	9/14/2017	0	ND	N/A	0	ND	N/A
QC-44	9/14/2017	52.9	53.4	101%	52.7	61.3	116%
QC-45	9/14/2017	53.5	54.8	102%	53.3	66.7	125%
QC-46	9/14/2017	52.0	51.1	98.3%	51.8	64.6	125%
QC-47	9/14/2017	427	400	93.7%	425	508	120%
QC-48	9/14/2017	393	364	92.6%	391	472	121%
QC-49	9/14/2017	400	364	91.0%	398	448	113%
QC-50	9/14/2017	4400	4240	96.4%	4380	4750	108%
QC-51	9/14/2017	4360	4250	97.5%	4340	4720	109%
QC-52	9/14/2017	4380	4200	95.9%	4370	4850	111%
		DL (ng/g) =	5.9		DL (ng/g) =	6.6	
		QL (ng/g) =	19.6		QL (ng/g) =	21.9	

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

QC Recoveries – *Aneides* Salamanders (whole body, S170711-31)

ID	Analysis Date	Theoretical Diphacinone Concentration (ng/g)	Observed Diphacinone Concentration (ng/g)	% Recovery	Theoretical Brodifacoum Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)	% Recovery
QC-29	9/28/2017	0	ND	N/A	0	ND	N/A
QC-30	9/28/2017	0	ND	N/A	0	ND	N/A
QC-31	9/28/2017	0	ND	N/A	0	ND	N/A
QC-32	9/28/2017	53.3	64.1	120%	53.1	62.1	117%
QC-33	9/28/2017	52.6	48.3	91.8%	52.4	60.1	115%
QC-34	9/28/2017	51.5	49.4	95.9%	51.3	50.3	98.1%
QC-35	9/28/2017	407	389	95.6%	405	428	106%
QC-36	9/28/2017	401	382	95.3%	400	400	100%
QC-37	9/28/2017	409	406	99.3%	407	428	105%
QC-38	9/28/2017	4110	4010	97.6%	4090	4140	101%
QC-39	9/28/2017	4410	4310	97.7%	4390	4570	104%
QC-40	9/28/2017	4340	4330	99.8%	4320	4400	102%
		DL (ng/g) =	7.5		DL (ng/g) =	8.6	
		QL (ng/g) =	25.1		QL (ng/g) =	28.6	

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

QC Recoveries – *Batrachoseps* Salamanders (whole body, S170602-29)

ID	Analysis Date	Theoretical Diphacinone Concentration (ng/g)	Observed Diphacinone Concentration (ng/g)	% Recovery	Theoretical Brodifacoum Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)	% Recovery
QC-53	9/19/2017	0	ND	N/A	0	ND	N/A
QC-54	9/19/2017	0	ND	N/A	0	ND	N/A
QC-55	9/19/2017	0	ND	N/A	0	ND	N/A
QC-65	9/25/2017	0	ND	N/A	0	ND	N/A
QC-66	9/25/2017	0	ND	N/A	0	ND	N/A
QC-67	9/25/2017	0	ND	N/A	0	ND	N/A
QC-77	9/27/2017	0	ND	N/A	0	ND	N/A
QC-78	9/27/2017	0	ND	N/A	0	ND	N/A
QC-79	9/27/2017	0	ND	N/A	0	ND	N/A
QC-56	9/19/2017	53.9	47.7	88.5%	53.7	56.3	105%
QC-57	9/19/2017	51.7	46.5	89.9%	51.5	57.0	111%
QC-58	9/19/2017	52.2	52.8	101%	51.9	55.0	106%
QC-68	9/25/2017	53.9	51.9	96.3%	53.7	64.1	119%
QC-69	9/25/2017	52.9	56.3	106%	52.7	57.9	110%
QC-70	9/25/2017	54.8	55.7	102%	54.5	69.5	128%
QC-80	9/27/2017	53.2	56.7	107%	53.0	57.2	108%
QC-81	9/27/2017	52.2	48.4	92.7%	51.9	61.2	118%
QC-82	9/27/2017	52.7	59.3	113%	52.5	63.0	120%
QC-59	9/19/201	398	371	93.2%	396	346	87.4%

	7						
QC-60	9/19/2017	389	384	98.7%	388	363	93.6%
QC-61	9/19/2017	393	376	95.7%	392	381	97.2%
QC-71	9/25/2017	404	412	102%	402	462	115%
QC-72	9/25/2017	412	395	95.9%	410	483	118%
QC-73	9/25/2017	415	423	102%	413	471	114%
QC-83	9/27/2017	472	483	102%	470	527	112%
QC-84	9/27/2017	468	462	98.7%	466	426	91.4%
QC-85	9/27/2017	469	446	95.1%	467	543	116%
QC-62	9/19/2017	4330	4210	97.2%	4320	4040	93.5%
QC-63	9/19/2017	4410	4200	95.2%	4390	3880	88.4%
QC-64	9/19/2017	4210	4110	97.6%	4200	3640	86.7%
QC-74	9/25/2017	4140	4080	98.6%	4120	4190	102%
QC-75	9/25/2017	4250	4240	99.8%	4230	4330	102%
QC-76	9/25/2017	4320	4320	100%	4300	4380	102%
QC-86	9/27/2017	3570	3490	97.8%	3560	3980	112%
QC-87	9/27/2017	3720	3540	95.2%	3700	4150	112%
QC-88	9/27/2017	3670	3540	96.5%	3650	4060	111%
		DL (ng/g) =	8.9		DL (ng/g) =	8.9	
		QL (ng/g) =	29.8		QL (ng/g) =	29.7	

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

QC Recoveries – Crickets (S170711-52)

ID	Analysis Date	Theoretical Diphacinone Concentration (ng/g)	Observed Diphacinone Concentration (ng/g)	% Recovery	Theoretical Brodifacoum Concentration (ng/g)	Observed Brodifacoum Concentration (ng/g)	% Recovery
QC-1	9/13/2017	0	ND	N/A	0	ND	N/A
QC-2	9/13/2017	0	ND	N/A	0	ND	N/A
QC-3	9/13/2017	0	ND	N/A	0	ND	N/A
QC-13	9/12/2017	0	ND	N/A	0	ND	N/A
QC-14	9/12/2017	0	ND	N/A	0	ND	N/A
QC-15	9/12/2017	0	ND	N/A	0	ND	N/A
QC-4	9/13/2017	54.3	54.2	99.8%	54.1	61.4	113%
QC-5	9/13/2017	54.3	50.4	92.8%	54.0	63.3	117%
QC-6	9/13/2017	57.7	50.8	88.0%	57.5	60.5	105%
QC-16	9/12/2017	54.8	51.1	93.2%	54.6	65.1	119%
QC-17	9/12/2017	53.3	59.5	112%	53.1	59.0	111%
QC-18	9/12/2017	56.5	53.7	95.0%	56.3	62.1	110%
QC-7	9/13/2017	390	349	89.5%	389	447	115%
QC-8	9/13/2017	426	387	90.8%	425	436	103%
QC-9	9/13/2017	399	376	94.2%	397	452	114%
QC-19	9/12/2017	421	408	96.9%	420	464	110%
QC-20	9/12/2017	430	400	93.0%	428	465	109%
QC-21	9/12/2017	404	382	94.6%	403	450	112%

QC-10	9/13/2017	4620	4390	95.0%	4600	4870	106%
QC-11	9/13/2017	4480	4250	94.9%	4460	4780	107%
QC-12	9/13/2017	4480	4150	92.6%	4470	4620	103%
QC-22	9/12/2017	4560	4420	96.9%	4540	4720	104%
QC-23	9/12/2017	4280	4130	96.5%	4270	4310	101%
QC-24	9/12/2017	4610	4440	96.3%	4590	4660	102%
		DL (ng/g) =	4.9		DL (ng/g) =	5.9	
		QL (ng/g) =	16.2		QL (ng/g) =	19.7	

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

QC Recoveries – Water (saturated with ground placebo brodifacoum bait (S170925-02))

ID	Analysis Date	Theoretical Diphacinone Concentration (ng/mL)	Observed Diphacinone Concentration (ng/mL)	% Recovery	Theoretical Brodifacoum Concentration (ng/mL)	Observed Brodifacoum Concentration (ng/mL)	% Recovery
QC-113	10/13/2017	0	ND	N/A	0	ND	N/A
QC-114	10/13/2017	0	ND	N/A	0	ND	N/A
QC-115	10/13/2017	0	ND	N/A	0	ND	N/A
QC-116	10/13/2017	0.924	1.06	115%	0.920	1.04	113%
QC-117	10/13/2017	0.924	1.12	121%	0.920	1.11	121%
QC-118	10/13/2017	0.924	1.03	111%	0.920	1.01	110%
QC-119	10/13/2017	10.4	11.0	106%	10.3	11.0	107%
QC-120	10/13/2017	10.4	11.1	107%	10.3	11.0	107%
QC-121	10/13/2017	10.4	11.0	106%	10.3	10.8	105%
QC-122	10/13/2017	74.8	79.0	106%	74.5	64.7	86.8%
QC-123	10/13/2017	74.8	79.0	106%	74.5	67.0	89.9%
QC-124	10/13/2017	74.8	78.7	105%	74.5	66.6	89.4%
		DL (ng/mL) = QL (ng/mL) =	0.080 0.267		DL (ng/mL) = QL (ng/mL) =	0.13 0.419	

ND Not Detected. This was reported when no response was detected or when the result was less than the Detection Limit (DL).

QC Recoveries – Baits (Method 163A, S170925-02)

ID	Analysis Date	Theoretical Diphacinone Concentration (µg/g)	Observed Diphacinone Concentration (µg/g)	% Recovery	Theoretical Brodifacoum Concentration (µg/g)	Observed Brodifacoum Concentration (µg/g)	% Recovery
QC-137	10/27/2017	0	ND	N/A	0	ND	N/A
QC-138	10/27/2017	0	ND	N/A	0	ND	N/A
QC-139	10/27/2017	0	ND	N/A	0	ND	N/A
QC-140	10/27/2017	52.5	51.6	98.3%	27.1	25.9	95.6%
QC-141	10/27/2017	51.8	53.4	103%	26.7	26.3	98.5%
QC-142	10/27/2017	52.5	52.2	99.4%	27.1	26.6	98.2%
		DL (µg/g) =	2.8		DL (µg/g) =	0.043	
		QL (µg/g) =	9.40		QL (µg/g) =	0.142	

QC Recoveries – Baits (LCMS Method, S170925-02)

ID	Analysis Date	Theoretical Diphacinone Concentration (µg/g)	Observed Diphacinone Concentration (µg/g)	% Recovery	Theoretical Brodifacoum Concentration (µg/g)	Observed Brodifacoum Concentration (µg/g)	% Recovery
QC-137	10/27/2017	0	ND	N/A	0	ND	N/A
QC-138	10/27/2017	0	ND	N/A	0	ND	N/A
QC-139	10/27/2017	0	ND	N/A	0	ND	N/A
QC-140	10/27/2017	52.5	64.7	123%	27.1	18.0	66.4%
QC-141	10/27/2017	51.8	64.6	125%	26.7	17.7	66.3%
QC-	10/27/20	52.5	64.7	123%	27.1	17.3	63.8%

142	17				
		DL (µg/g) =	0.0072	DL (µg/g) =	0.0081
		QL (µg/g) =	0.0241	QL (µg/g) =	0.0270