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**An assessment of the potential hazards of anticoagulant rodenticides to Plethotondid salamanders**

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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 mice (*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 attenuateus*) 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 could be expected in a rodent eradication project. We did not note the sub-lethal effects of weight loss or reduced food (cricket) consumption, however, skin sloughing and sores on the undersides of animals were noted in some cases. However, some skin sloughing and sores were noted in some control salamanders. 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 to salamanders, the level appears to be relatively low, especially given the very high exposure rates applied in this study.

**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). For example, on Gough Island in the South Atlantic, house mice fed on nestling albatross chicks (Cuthbert and Hilton, 2004). 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, 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 mice 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). APHIS maintains the registrations for two rodenticide active ingredients for invasive rodent eradication: diphacinone and brodifacoum. However, rodenticides can pose hazards to non-target animals so careful considerations and measures must be taken to reduce those risks (Witmer et al. 2007).

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, and may be dispersing weed seeds. 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. 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*), and California slender salamander (*Batrachoseps attenuateus*); 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 external 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 or other sub-lethal effects (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 Vredenberg of San Francisco State University (SFSU). Dr. Vredenberg 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.

Salamanders were housed individually in plastic mouse shoebox cages 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. Vredenberg, 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). 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. This also allowed all 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. The two USEPA registered products, Brodifacoum-25D Conservation and Diphacinone-50 Conservation, were used in the study. There was 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). 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.

**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. Consequently, we amended the study protocol so that the powdered rodenticide was sprinkled on the crickets just before putting them in with the salamanders (see Discussion). Some crickets were fed to salamanders twice weekly. The treated crickets were fed to the salamanders for 10 days. At the end of the 10-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 us with measures of sub-lethal effects. Generally, birds and mammals that have consumed anticoagulants will stop feeding and lose weight as the signs of toxicosis advance (e.g., Rattner et al. 2014: Witmer 2011).

Salamanders were examined twice daily by the Study Director (the report author) and/or study 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, they 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 reclosable 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 some 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 < .05.

**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. In the brodifacoum group, two (both *Aneides*) of the seven salamanders died (28.6% mortality). 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, the cricket consumption was not significantly (F = 3.83, P =0.08) different between the two periods. 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 = 0.049). 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 salamander was bleeding externally and was euthanized. We noted a sloughing of skin 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, 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, there was some loss of weight in the salamanders (0.7-3.4g), but this 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. 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). Most salamanders in this group lost a little weight (-0.4 to -2.1 g) over the course of the trial, but this was not significant (F = 0.14, P = 0.71). One of the six salamanders in the control group showed some internal bleeding upon necropsy.

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. 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 animals 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). 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. 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.

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 animals 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). Again, there were no diphacinone residues detected in the dermal exposed salamanders.

There was one death (14.3% mortality) in the control group. Interestingly, 14.3% of the control animals had sloughing skin and sores. Cricket consumption was also variable in the control group, ranging from 18-229 per salamander, but these differences were not significant (F = 0.56, P = 0.47) during the two periods. Control animals also showed only small changes in weights: -0.02-0.43g and these differences were not significant (F = 0.28, P = 0.61).

Analyses common to both trials

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 were substantially different between the two exposure groups of crickets with brodifacoum residue concentrations fed brodifacoum pellets (ranging from 296-688 ppb) were much lower than the residue concentrations in crickets dusted with powdered brodifacoum (2887-3340 ppb).

Diphacinone residues in crickets fed diphacinone pellets were quite variable (954-2930 ppb), as were crickets dusted with powdered diphacinone (1823-3980 ppb).

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 varied from 5.75-29.7 ppb. Diphacinone residues were similar among water samples and varied from 0.08-17.7 ppb.

Because of the low 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.

All the residue analyses results are presented in Appendix A.

**Discussion**

From our Trial 1 results, it appears that rodenticide exposure poses some hazard to salamanders, but that hazard appears to be relatively low, considering the experimental design optimized salamander exposure to rodenticides. It also appeared that salamanders can begin recovery after exposure ceases, as evidenced by reduced skin sloughing and fewer sores during the post-exposure period. 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. 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.

The Trial 2 results basically confirm 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. This could be determined because we had enough slender 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 residues 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 crickets, all the crickets survived. We now surmise that we got a bad batch of crickets early on in the study. Later batches of crickets survived and were used in the study without problems. 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).

A search of the scientific literature revealed no publications concerning the toxicity of anticoagulants in amphibians. As stated in some reviews, little is known about the risk of anticoagulants to amphibians, but it is generally considered to be low (Eason, 1995; Chris et al., 2010). There is considerable uncertainty regarding the toxicity of rodenticides to amphibians, but based on salamander physiology, behavior, etc., and 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. Studies have focused on risks to mammals, birds, invertebrates, and to a much lesser focus, on reptiles. These taxonomic groups are thought to be either the most sensitive or the groups most likely to consume baits (primary exposure) or animals that have consumed baits (secondary exposure). Additionally, relatively few species of native amphibians occur on islands and many islands don’t have any.

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 Island in the 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 carcasses found from various taxonomic groups had detectible residues of brodifacoum. 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.

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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 |  | -0.52 |  |
| 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 |  | 14.29% | 14.29% | 14.29% |
| 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 |  |
| QO12 | 1.41 | 1.83 | 0.42 |  |
| QO13 | 1.43 | 1.86 | 0.43 |  |

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



|  |  |
| --- | --- |
| To:  Subject:  Methods:  Analysis Dates:  Notebook Reference:  QC Notebook Reference:  Analyst: | Dr. Gary Witmer  Research Wildlife Biologist  NWRC  Determination of Diphacinone and Brodifacoum in Salamanders, Crickets, Water, and Baits (QA-2688); Invoice #17-019, Nov. 6, 2017  Non-GLP (salamanders, crickets, water); Method 163A (baits)  9/12, 9/13, 9/14, 9/19, 9/25, 9/27, 9/28, 10/13, 10/27, and 10/30/2017  AC-161, pp. 86-109  QC-33, p. 137; AC-162, p. 4  Steve Volker |

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**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 D4-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 MgSO4 (150 mg), C18 sorbent (25 mg), and primary-secondary amine (PSA) sorbent (25 mg). The extract was exposed to the sorbents and MgSO4 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 |  | | |  | Time (min) | %A | %B |
| Column temp. | 60°C |  | | |  | 0.00 | 90% | 10% |
| Injection volume | 7.5 µL |  | | |  | 0.50 | 90% | 10% |
| Run time | 4.0 min |  | | |  | 3.00 | 20% | 80% |
|  |  | |  |  |  | 3.01 | 0% | 100% |
| Source | AJS ESI, negative mode | | | |  | 3.50 | 0% | 100% |
| Gas temp. | 300°C |  | | |  | 3.51 | 90% | 10% |
| Gas flow | 5 L/min |  | | |  |  |  |  |
| Nebulizer | 45 psi |  | | | Precursor | Product | Fragmentor | Collision |
| Sheath gas | 250°C, 7 L/min | Analyte | | | Ion (m/z) | Ion (m/z) | (V) | Energy (V) |
| Capillary | -4500 V | Diphacinone | | | 339.1 | **167.1** | 100 | 23 |
| Nozzle | -500 V | 145 | 18 |
|  |  | D4-Diphacinone | | | 343.1 | 167.1 | 120 | 23 |
|  |  | Chlordifacoum | | | 477.1 | 135.1 | 61 | 37 |
|  |  | Brodifacoum | | | 522.9 | 135.0 | 165 | 44 |
|  |  | **80.9** | 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 |  | |  | Time (min) | %A | %B |
| Column temp. | 60°C |  | |  | 0.00 | 85% | 15% |
| Injection volume | 10 µL |  | |  | 1.00 | 85% | 15% |
| Run time | 26 min |  | |  | 17.00 | 45% | 55% |
|  |  |  |  |  | 17.01 | 0% | 100% |
| Detector | UV (DAD); 325 nm | | |  | 23.00 | 0% | 100% |
|  |  |  | |  | 23.01 | 85% | 15% |
| Detector | Fluorescence (FLD) | | |  |  |  |  |
| Excitation | 310 nm |  | |  |  |  |  |
| Emission | 390 nm | | |  |  |  |  |

**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)** | | | | |
|  | | | Observed Diphacinone  Concentration (ng/g) | Observed Brodifacoum  Concentration (ng/g) |
| NWRC ID | Sample Description | Analysis Date |
| S170602-13 | QP3 (Control) | 9/14/2017 | ND | ND |
| S170602-14 | QP6 (Control) | 9/14/2017 | ND | ND |
| S170602-19-A |  | 9/14/2017 | ND | 101 |
| S170602-19-B | QP1 (Brodifacoum, Dermal + Cricket) | 9/14/2017 | ND | 95.9 |
| S170602-19-C |  | 9/14/2017 | ND | 100 |
| S170602-20-A |  | 9/14/2017 | ND | 86.9 |
| S170602-20-B | QP4 (Brodifacoum, Dermal + Cricket) | 9/14/2017 | ND | 85.7 |
| S170602-20-C |  | 9/14/2017 | ND | 85.5 |
| S170602-21-A |  | 9/14/2017 | ND | 50.1 |
| S170602-21-B | QP7 (Brodifacoum, Dermal + Cricket) | 9/14/2017 | ND | 50.7 |
| S170602-21-C |  | 9/14/2017 | ND | 48.3 |
| 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)** | | | | |
|  | | | Observed Diphacinone  Concentration (ng/g) | Observed Brodifacoum Concentration (ng/g) |
| NWRC ID | Sample Description | Analysis Date |
| 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 |  | 9/28/2017 | ND | ND |
| S170711-31-B | QO13 (Control) | 9/28/2017 | ND | ND |
| S170711-31-C |  | 9/28/2017 | ND | ND |
| S170711-32-A |  | 9/28/2017 | ND | ND |
| S170711-32-B | QO12 (Control) | 9/28/2017 | ND | ND |
| S170711-32-C |  | 9/28/2017 | ND | ND |
| S170602-15-A |  | 9/28/2017 | ND | 108 |
| S170602-15-B | QO1 (Brodifacoum, Dermal + Cricket) | 9/28/2017 | ND | 98.0 |
| S170602-15-C |  | 9/28/2017 | ND | 103 |
| S170602-16-A |  | 9/28/2017 | ND | ND |
| S170602-16-B | QO4 (Brodifacoum, Dermal + Cricket) | 9/28/2017 | ND | 46.6 |
| S170602-16-C |  | 9/28/2017 | ND | 38.8 |
| S170602-17-A |  | 9/28/2017 | ND | 85.5 |
| S170602-17-B | QO7 (Brodifacoum, Dermal + Cricket) | 9/28/2017 | ND | 97.1 |
| S170602-17-C |  | 9/28/2017 | ND | 89.3 |
| S170602-18-A |  | 9/28/2017 | ND | 239 |
| S170602-18-B | QO10 (Brodifacoum, Dermal + Cricket) | 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)** | | | | |
|  | | | Observed Diphacinone  Concentration (ng/g) | Observed Brodifacoum Concentration (ng/g) |
| NWRC ID | Sample Description | Analysis Date |
| S170602-30-A |  | 9/19/2017 | ND | ND |
| S170602-30-B | QS22 (control) | 9/19/2017 | ND | ND |
| S170602-30-C |  | 9/19/2017 | ND | ND |
| S170711-04-A |  | 9/19/2017 | ND | 22.0 \* |
| S170711-04-B | QS9 (Control) | 9/19/2017 | ND | 22.6 \* |
| S170711-04-C |  | 9/19/2017 | ND | 21.2 \* |
| S170711-08-A |  | 9/19/2017 | ND | ND |
| S170711-08-B | QS17 (Control) | 9/19/2017 | ND | 8.8 \* |
| S170711-08-C |  | 9/19/2017 | ND | ND |
| S170711-12-A |  | 9/19/2017 | ND | ND |
| S170711-12-B | QS26 (Control) | 9/19/2017 | ND | ND |
| S170711-12-C |  | 9/19/2017 | ND | ND |
| S170711-17-A |  | 9/19/2017 | ND | ND |
| S170711-17-B | QS34 (Control) | 9/19/2017 | ND | ND |
| S170711-17-C |  | 9/19/2017 | ND | ND |
| S170602-31-A |  | 9/19/2017 | ND | 22.8 \* |
| S170602-31-B | QS6 (Brodifacoum, Dermal) | 9/19/2017 | ND | 16.5 \* |
| S170602-31-C |  | 9/19/2017 | ND | 18.2 \* |
| S170602-32-A |  | 9/19/2017 | ND | 82.1 |
| S170602-32-B | QS11 (Brodifacoum, Dermal) | 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 |  | 9/19/2017 | ND | 64.4 |
| S170602-35-B | QS51 (Brodifacoum, Dermal) | 9/19/2017 | ND | 71.4 |
| S170602-35-C |  | 9/19/2017 | ND | 71.3 |
| S170711-01-A |  | 9/19/2017 | ND | 87.9 |
| S170711-01-B | QS5 (Brodifacoum, Cricket) | 9/19/2017 | ND | 72.5 |
| S170711-01-C |  | 9/19/2017 | ND | 95.1 |
| S170711-02-A |  | 9/19/2017 | ND | 10.1 \* |
| S170711-02-B | QS7 (Diphacinone, Cricket) | 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)** | | | | |
|  | | | Observed Diphacinone  Concentration (ng/g) | Observed Brodifacoum Concentration (ng/g) |
| NWRC ID | Sample Description | Analysis Date |
| 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)** | | | | |
|  | | | Observed Diphacinone  Concentration (ng/g) | Observed Brodifacoum Concentration (ng/g) |
| NWRC ID | Sample Description | Analysis Date |
| 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 | ND |
| 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** | | | | |
|  | | | Observed Diphacinone  Concentration (ng/g) | Observed Brodifacoum Concentration (ng/g) |
| NWRC ID | Sample Description | Analysis Date |
| 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 |  | 9/13/2017 | ND | 296 |
| S170602-36-B | Brodifacoum + potato (BFC1), n=15 | 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 |  | 9/13/2017 | ND | 538 |
| S170602-38-B | Brodifacoum + potato (BFC3), n=13 | 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 |
| S170602-39-B | 9/13/2017 | 1600 | ND |
| 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 |  | 9/13/2017 | ND | 495 |
| S170711-33-B | Brodifacoum + no potato (BFC4), n=24 | 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** | | | | |
|  | | | Observed Diphacinone  Concentration (ng/g) | Observed Brodifacoum Concentration (ng/g) |
| NWRC ID | Sample Description | Analysis Date |
| S170711-35-A |  | 9/13/2017 | ND | 560 |
| S170711-35-B | Brodifacoum + no potato (BFC6), n=23 | 9/13/2017 | ND | 638 |
| S170711-35-C |  | 9/13/2017 | ND | 490 |
| S170711-36-A |  | 9/12/2017 | 1060 | ND |
| S170711-36-B | Diphacinone + no potato (DFC4), n=27 | 9/12/2017 | 950 | ND |
| S170711-36-C |  | 9/12/2017 | 943 | ND |
| S170711-37-A |  | 9/12/2017 | 907 | ND |
| S170711-37-B | Diphacinone + no potato (DFC5), n=27 | 9/12/2017 | 1140 | ND |
| S170711-37-C |  | 9/12/2017 | 1050 | ND |
| S170711-38-A |  | 9/12/2017 | 2040 | ND |
| S170711-38-B | Diphacinone + no potato (DFC6), n=21 | 9/12/2017 | 2350 | ND |
| S170711-38-C |  | 9/12/2017 | 1720 | ND |
| S170711-39-A |  | 9/12/2017 | 1740 | ND |
| S170711-39-B | Diphacinone + dusted (DD1), n=23 | 9/12/2017 | 1950 | ND |
| S170711-39-C |  | 9/12/2017 | 1780 | ND |
| S170711-40-A |  | 9/12/2017 | 3090 | ND |
| S170711-40-B | Diphacinone + dusted (DD2), n=25 | 9/12/2017 | 3490 | ND |
| S170711-40-C |  | 9/12/2017 | 3410 | ND |
| S170711-41-A |  | 9/12/2017 | 4200 | ND |
| S170711-41-B | Diphacinone + dusted (DD3), n=18 | 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)** | | | | |
|  | | | Observed Diphacinone  Concentration (ng/g) | Observed Brodifacoum Concentration (ng/g) |
| NWRC ID | Sample Description | Analysis Date |
| 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 |
| S170602-06-C |  | 10/13/2017 | ND | 5.69 |
| 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)** | | | | |
|  | | | Observed Diphacinone  Concentration (µg/g) | Observed Brodifacoum Concentration (µg/g) |
| NWRC ID | Sample Description | Analysis Date |
| S170925-01-D |  | 10/27/2017 | 0.424 a | ND |
| S170925-01-E | Placebo Diphacinone Bait | 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 |  | 10/27/2017 | 46.8 | ND |
| S170925-03-E | Diphacinone Conservation 50 (0.0050%) Bait | 10/27/2017 | 46.3 | ND |
| S170925-03-F |  | 10/27/2017 | 46.1 | ND |
| S170925-04-D |  | 10/27/2017 | ND | 26.0 |
| S170925-04-E | Brodifacoum Conservation 25 (0.0025%) Bait | 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).

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| **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 | 9/19/2017 | 398 | 371 | 93.2% |  | 396 | 346 | 87.4% |
| 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).

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| **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).

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| **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) = | | 0.080 |  | DL (ng/mL) = | | 0.13 |  |
|  | QL (ng/mL) = | | 0.267 |  | QL (ng/mL) = | | 0.419 |  |

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

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| **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 |  |

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| **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-142 | 10/27/2017 | 52.5 | 64.7 | 123% |  | 27.1 | 17.3 | 63.8% |
|  |  |  |  |  |  |  |  |  |
|  | DL (µg/g) = | | 0.0072 |  | DL (µg/g) = | | 0.0081 |  |
|  | QL (µg/g) = | | 0.0241 |  | QL (µg/g) = | | 0.0270 |  |