**Draft Final Report, QA-2688:**

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

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The USDA/APHIS National Wildlife Research Center conducted an assessment of the hazards of anticoagulants (diphacinone and brodifacoum) to salamanders. This was done in anticipation of an attempt to eradicate the invasive house mice from the Farallon Islands National Wildlife Refuge. Live-captured salamanders of three species (*Aneides lugubris*, *Ensatina eschscholzii xanthoptica*, and *Batrachoseps attenuateus*) were exposed to the anticoagulant rodenticides by both oral and dermal exposure routes. There were some deaths (9 of 37 treated salamanders; 24.3% mortality) and it appeared that dermal exposure posed the greatest hazard. 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. 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. 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 Farallon Islands National Wildlife Refuge is conducting plans for an eradication of the house mice on the Refuge (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 (NWR) 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 U.S. Fish and Wildlife Service (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 diphacine 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, using conspecifics from another population of closely related salamanders as surrogates because of the Farallon population’s relatively small and endemic status. For a description of the phylogenetic relationships of the largest family of salamanders, the *Plethodontidae*, see Vieites et al. (2011). Ultimately, three species of 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. Salamanders were exposed to rodenticides through two routes: 1) secondary oral exposure by allowing the salamanders to consume crickets that had been dusted with powdered brodifacoum or diphacinone bait, and 2) direct external exposure by allowing salamanders to be exposed to crushed pellets and water that had been used to soak anticoagulant pellets and then sprayed on the paper towels on the bottom of each plastic cage thus allowing dermal absorption. It was assumed that these would be the main routes of exposure in a rodent eradication project. We hypothesized that the rodenticide exposure will 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 agreement 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 and easily maintained. The cages contained wet paper towels on the floor of cages and a plastic tube for shelter. Salamanders were maintained as per the university-approved SOP 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 (secondary oral exposure and direct dermal exposure). 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 of the third species of salamander (*Batrachoseps*), 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; secondary oral exposure.** Ten salamanders of the third species (*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 again amended the study protocol to state that we would sprinkle powdered rodenticide 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; dermal exposure.** Ten *Batrachosep*s 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 from powdered/crushed pellets being 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 clean 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 decline 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.

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 difficult 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 zip-lock bags and frozen for later rodenticide residue determination by the Analytical Chemistry Unit (ACU) staff. 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, 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 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 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, 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 orparts 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 in three of seven salamanders (42.7%) and sores on two of these individuals (mainly on the underside of animals; 28.6%). There was a considerable difference in cricket consumption by the salamanders. 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, 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 gained a little weight 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 among five 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 study; 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 noted. 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. 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 and 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 to 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 was 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) during the post-exposure period. These differences were not significant (F = 1.89, P = 0.19). Salamander weights were mostly stable when comparing the exposure to recovery period, with changes ranging from -0.11-0.11g. The differences in the two periods 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).

In Trial 2, we did not necropsy any of the *Batrachoseps* salamanders because of their very small size (see Figure 3).

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 in crickets fed brodifacoum pellets (ranging from 296-688 ppb) being much lower thanresidue 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 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 ug/g (= ppm) which is 93% of the label concentration of 50 µg/g. For the brodifacoum pellets, it was 26.3 ug/g (= ppm) which is 105% of the label concentration of 25 µg/g.

The residue report of the Analytical Chemistry Unit will be sent to the USFWS as a separate document.

**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 salamanderscan begin recovery after exposure ceases, as evidenced byreduced 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 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 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.

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 and behaviorand 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 as these are thought to be either the most sensitive taxonomic groups or they are the groups most likely to consume baits (primary exposure) or animals that have consumed baits (secondary exposure). Additionally, relatively few 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 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.



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



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

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| **Treatment** | **ID #** | **Initial Weight (g)** | **Final Weight (g)** | **Weight Change (g)** | **Comments** | **% Sloughing Skin** | **% Sores** | **% Mortality** |
| Brodifacoum  /crickets & 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 | rickets | 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  /crickets & 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. Results of individual Batrachoseps from 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 |  |