**QA-2688: An assessment of the potential hazards of anticoagulant rodenticides to salamanders**

**Overview of preliminary results, Gary Witmer, Study Director, Oct. 2017**

There were 2 trials conducted. The first used Aneides (n= 12) and Ensatina (n= 8) salamanders. These were divided into 3 groups: brodifacoum exposure group (n= 7), diphacinone exposure group (n= 7), and a control group (no rodenticide exposure (n= 6) and each group contained some of both species, Animals were kept individually in plastic cages. Cage bottoms had wet paper towels and there was a plastic hide in each cage.

Both routes of exposure to the rodenticides were used with the 2 treatment groups: oral exposure (fed crickets dusted with powdered rodenticide) and dermal exposure (paper towels in the cage wetted with water that had been soaked with crushed/powdered rodenticide pellets and then sprinkled with powered and crushed rodenticide pellets). There was a 14 day exposure period, followed by a 14 day post-exposure period with no rodenticide exposure in the latter period.

In the brodifacoum group, 2 (both Aneides) of the 7 salamanders died (28.6% mortality). We noted a sloughing of skin in some animals (57.1%) and sores (mainly on the underside of animals; 14.3%). Our chemist noted that the pellets for both brodifacoum and diphacinone are rather acidic so this may been responsible for much of the skin sloughing andsores. There were no deaths in the control group and we did not note any sloughing of skin or sores. There was a considerable difference in cricket consumption by the salamanders in all 3 groups. During the brodifacoum exposure period, salmanders consumed 3-14 crickets, while in the post exposure period they consumed 1-32 crickets. There was an increase in cricket consumption in the post-exposure period in 3 of 4 salamanders. Additionally, skin sloughing and sores seemed to decrease in the post-exposure period. Over the course of the study, there was a small loss of weight in the salmanders (0.4-3.4g). Brodifacoum residues in salamanders were quite variable, but low: Aneides 42.7-226 ppb (parts per billion); Ensatina 48.3-101 ppb.

In the diphacinone group, 1 (Aneides) of the 7 salamanders died (14.3% mortality). We noted a sloughing of skin in some animals (42.7%) and sores (mainly on the underside of animals; 28.6%). There were no deaths in the control group and we did not note any sloughing of skin or sores. There was a considerable difference in cricket consumption by the salamanders in all 3 groups. During the diphacinone exposure period, salmanders 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. Additionally, skin sloughing and sores seemed to decrease in the post-exposure period. Over the course of the study, there was a small loss of weight in the salmanders (0.7-3.4g). 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.

From the trial 1 results, it appears that rodenticide exposure poses relatively low hazard to salamanders and they can begin recovery after some exposure. One should also realize that there was a relatively high exposure rate in this trial.

In trial 2, we used Batrochoseps salamanders. Because we has considerably more salamanders in trial 2 than in trial 1, we were able to divide the exposure routes. One brodifacoum group (n= 7) received oral exposure (dusted crickets) only, while the second brodifacoum group (n= 8) received dermal exposure ((paper towels in the cage wetted with water that had been soaked with crushed/powdered rodenticide pellets and then sprinkled with powered and crushed rodenticide pellets) only. Similarly, one diphacinone group (n= 8) received oral exposure only, while the second diphacinone group (n= 8) received dermal exposure. This was done to assess which exposure route caused more deaths/problems if there was a difference. The control group (n= 7) received no rodenticide exposure.

In the brodifacoum oral exposure group, no animals died. There was no skin sloughing or sores noted. Salamanders mostly maintained the same weight with the most change only 0.1g. There was one death (14.3% mortality) in the control group, and interesting, 14.3% of the control animals had sloughing skin and sores. Again, cricket consumption was quite variable: 13-70 in the exposure period and 4-59 in the post-exposure period. Cricket consumption was also variable in the control group: 18-229. Control animals also showed only a small change in weights: -0.02-0.43g. Brodifacoum residues in the oral exposed salamanders were variable: 51.3-91.1 ppb.

In the brodifacoum dermal exposure group, 5 of 8 animals died (62.5%). There was no skin sloughing or sores noted. Salamanders mostly lost a small amount of weight: -0.21-0.0g. Again, cricket consumption was somewhat variable: 9-27 in the exposure period, but icreased in the two surviving crickets (44-55). The results of the control group are the same as presented in the previous paragraph. Brodifacoum residues in the dermal exposed salamanders were quite variable: 16.5-95.1 ppb.

In the diphacinone oral exposure group, no animals died. There was no skin sloughing or sores noted. Salamanders mostly maintained weight: 0.02-0.15g. Again, cricket consumption was somewhat variable: 6-68 in the exposure period, but stayed about the same in the post-exposure period: 4-66. The results of the control group are the same as presented in a previous paragraph. Interestingly, 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. Salamander weights were mostly stable: -0.11-0.11g. Again, cricket consumption was variable: 6-57 in the exposure period, but stayed about the same in the post-exposure period: 5-59. The results of the control group are the same as presented in a previous paragraph. Again, there were no diphacinone residues detected in the dermal exposed salamanders.

Brodifacoum residues in crickets fed brodifacoum pellets were quite variable (296-688 ppb), while crickets dusted with powdered brodifacoum were much higher and less variable (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).

With regard to the residues levels in crickets fed rodenticides, we need to clarify an early assumption that we made. When we first tried to feed 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 the early result, for the study we chose to dust crickets with powdered anticoagulants before feeding them to the salamanders. However, when we later fed rodenticides to crickets, the crickets survived (see previous two paragraphs), all survived. We now surmise that we got a bad batch of crickets early on in the study. Later batches of crickets did just fine and were used in the study without problems.

Residues in water used to soak crushed and powder rodenticide pellets were very low probably because of the low solubility of anticoagulants. Brodifacoum residues varied from 5.75-29.7 ppb. Diphacinone residues were similar and varied from 0.08-17.7 ppb.

Because of the low residue levels in the salamanders (i.e., ppb), we tested the brodifacoum and diphacinone pellets for rodenticide concentrations. These were very close to the label concentrations. For the diphacinone pellets, it was 46.4 ug/g (= ppm) which is 93% of the desired 50 ug/g. For the brodifacoum pellets, it was 26.3 ug/g (= ppm) which is 105% of the desired 25 ug/g

The trial 2 results basically confirm the results from trial 1. However, trial 2 seems to suggest that the higher hazard to salamanders from anticoagulants if from dermal exposure versus oral exposure. It is cautioned, however, that we gave very high exposure rates to the salamanders in this study. In an aerial broadcast baiting in an invasive rodent eradication project would result in much lower dermal exposure to all animals.