Comments on “Draft Final Report - An assessment of the potential hazards of anticoagulant rodenticides to salamanders”

This is an interesting initial effort to examine the “toxicity” of anticoagulant rodenticides (ARs) to salamanders. Based upon the study design, measurement endpoints, residues, and their qualitative analysis (but no statistical analysis), it is difficult to draw definitive conclusions other than brodifacoum and diphacinone can be toxic to salamanders (i.e., no traditional quantitative measurements or estimates such as LD50, lowest lethal dose, threshold of toxicity, etc.). Surprisingly, the term “toxicity” is rarely used in this report.

A distinction should made between hazard (something that can cause harm/toxicity) and risk (potential/probability of a hazardous substance causing harm). If there is no exposure to an AR, then the risk of a highly toxic compound to salamanders would be low. This point is raised because the concluding sentence of the Abstract (page 2) is not justified from the data that are presented. Specifically, “We conclude that while anticoagulant rodenticide pose some hazards to salamander” **(i.e., *YES, demonstrated AR toxicity in Table 1*)**….”the levels appear to be relatively low” **(*levels of what? and compared to what?*)**…”especially given the very high exposure rates applied in this study” **(*true, but no data or modeling or risk assessment provided based on exposure or potential exposure in a field setting*)**.

Specific comments follow.

**Title**

Plethodondid is misspelled; should be Plethodontid.

**Introduction**

Para 1, sentence 1 – Besides Witmer and Jojola 2007, cite Chapter 19 (Howald et al. Rodent control and island conservation) in 2015 reference text Rodent Pests and their Control by Buckle and Smith 2015, 2nd edition).

Para 2. Last sentence – non-target effects – cite reference text “Anticoagulant rodenticides and wildlife” (van den Brink et al. 2018; peer-reviewed) rather than/in addition to Proceedings paper.

Para 4 – Point out and cite that data that are available in reptiles (Hoare, Hare NZ J Ecol 30:157, Weir et al. Environ Toxicol Chem 34:1778 and NZ J Ecol 40:342). Final sentence – surprised that you did not hypothesis that ARs “might cause bleeding or coagulopathy” (principal mechanism of action).

**Methods**

Page 4 - State that the study was approved by the Institutional Animal Care and Use Committee (right?).

Para 1 – State whether or not these species are sexually dimorphic. State whether the gender and approximate age of the individuals was known or unknown.

Para 2 – state or provide evidence that salamander weight stabilized over the course of the acclimation period. Provide dimension of each cage and more detail on husbandry (cage cleaning, exchange of wet paper towel, etc.). Was the cleaning protocol the same during the 10-day exposure and 14-day recovery periods?

Para 3 and Table 1 – Weight change/loss was an endpoint used in the study. Why wasn’t initial body weight balanced more evenly among the species/exposure in trial 1? For example, on average the *Aneides* in the brodifacoum/oral & dermal exposure group weighed 35% less than individuals of this species in the diphacinone/ oral & dermal exposure and control groups (controls weighed the most). Similarly, in trial 2 differences in average initial body weight varied by as much as 26%, with the controls weighing the most.

Page 5, para 1 – Title should be “Oral & Dermal Exposure” as in Table 1. Elaborate on death of crickets fed rodenticide bait (number etc.). Did “control” crickets succumb when feed the material the bait block was composed of (without rodenticide present) or was this not done? What killed the crickets…rodenticide in the bait or just the excipient material?

Para 3 – Incorrect citation and statement – In my experience, birds orally dosed or ingesting anticoagulant rodenticides at sublethal do not exhibit weight loss (Rattner et al. 2011, 2012, 2014 and 2015). In such studies, birds that are severely intoxicated (and perhaps succumbing/dying) stop feeding and lose weight. Perhaps cite the Rattner et al. owl study published in Ecotoxicology 21:832-846, 2012.

Para 5 on Pages 5 and 6, – Were all salamanders necropsied? Signs of intoxication and sores/skin sloughing are mentioned in Tables 1 and 2, but additional details that could have been easily obtained by a quick/superficial necropsy (evidence of hemorrhage) are not mentioned (lost opportunity?). Necropsy of dead salamanders is mentioned on page 6. Was any histopathology done on the conducted on the dermal sores or other tissues?

Page 6, statistics – Body weight was measured at the beginning and end of each trial (or when animals died or were euthanized). Body weight change [(Final BWt-Initial BWt)/Initial BWt x 100)] (and may need to be scale, add 100, so don’t have to deal with negative values in stats) “OR” percent change should have been used to normalize weight change (e.g., compare loss per 100 grams body weight) for individuals to overcome disparate initial weights, and then compare groups by ANOVA and multiple comparison test. This should be done for both trials 1 and 2.

**Results**

Page 6, para 3, Trial 1 – Clearly, “controls” should have been provided bait without rodenticide in it (i.e., excipient). This would have permitted getting a better understanding if the sloughing of skin was due to the chemical (brodifacoum) or the bait (less rodenticide). In most toxicity studies, a “vehicle control” group is included to help better interpret the cause such observations.

Page 6, paras 3 and 4, and Page 7 para 2 - Weight loss needs to be re-evaluated based on percent weight change as described above (by inspection of the data, doesn’t look like much is going on, but need to do stats).

Any photographs of necropsy observations of dead individuals with hemorrhage?

Page 6, paras 3 and 4 – How did residues in animals that died or were euthanized before the end of the trial compare to residues in animals that survived the entire trial and were then euthanized? This should be described for trials 1 and 2.

Page 7 – Trial 2 - even though the ARs seemed to be far more toxicology potent when absorbed through skin compared to ingestion, some effort should be undertaken to estimate the quantity of brodifacoum and diphacinone that was ingested. The could be done by averaging concentrations on crickets (on a per cricket basis), and then multiplying that value by the number of crickets ingested by each salamander. This would provide some information of the quantity of AR ingested that didn’t cause mortality in *Batrachoseps*.

Were necropsies done of the *Batrachoseps* that died in trial 2 or were they too small? State in report.

Comparing results from Trials 1 and 2, might there be an inter-specific difference in sensitivity to ARs?

Page 7 – The low residues of ARs in salamanders is potentially good news as they would pose little in the way of hazard if ingested by a predator. This should be mentioned in the Discussion.

Page 8 – Discussion, para 1, lines 1 and 2 – believe you might mean “risk” rather than “hazard”.

Page 9, para 1, sentence 1 – delete “very”

Page 9, para 2 – Sentence 2 needs to clarify that in *Batrachoseps* brodifacoum was more potent dermally compared to the oral route based on mortality. The difference in route of exposure for diphacinone is less clear; there was only skin sloughing (not mortality), and some sloughing was even observed in the control group. Temper sentence 2 of this paragraph.

Page 9, para 4, line 1 – change “residues” to “residue”.

Page 9, para 4 “However, when we later fed rodenticides to crickets, all the crickets survived.” Surprising, where are the data for this statement? Unfortunate event in overall design of the study.

**Discussion**

Page 9, last paragraph., final sentence – Provide a citation documenting that “few native amphibians occur on Islands and many Islands don’t have any.” Not sure this is true on a North American or global scale.

Page 10 - Need to include and cite what is known about AR toxicity from controlled exposure studies (e.g., Weir et al. Environ Toxicol Chem 34:1778 and NZ J Ecol 40:342).

A “**Conclusion Section**” on page 10 is warranted. Besides briefly summarizing findings, it should address “uncertainty”, information gaps (better exposure and robust toxicity data) and research needs.

Page 24 – Is there an error in the quantitation limit (Baits Method 163A) for diphacinone in bait (more than an order of magnitude poorer than brodifacoum)?

The analytical results are acceptable (generally great). Any explanation for a couple of the replicate determinations for brodifacoum (QO4, QS56) that exhibited poor precision?

Fix formatting of Tables (wrapping of text).