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*)**.

I modified the last sentence of the abstract to read:

We concluded that while anticoagulant rodenticide pose some hazards (both lethal and sub-lethal) to salamanders, the level appears to be relatively low, especially given the very high exposure rates applied in this study compared to the exposure they would encounter in an aerial broadcast of rodenticide baits in an invasive rodent eradication project.

Specific comments follow.

**Title**

Plethodondid is misspelled; should be Plethodontid.

Corrected.

**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).

Added the citation.

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.

Added the citation.

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).

Added two of the citations. Also added “bleeding” to last sentence.

**Methods**

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

The IUACA approval is stated in the acknowledgments section.

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.

This was added.

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?

We did not determine that the weights stabilized, but rather presumed they probably did by the end of the acclimation/quarantine period. Cage size was added to the paragraph. We also added that cage cleaning remained the same throughout the study.

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.

We probably should have done that, but I generally just randomly assign animals to treatment groups in my studies. But we did use the change in weight from the exposure period to the post exposure which pretty much gets around the point of the reviewer. More importantly, we did an ANOVA of the starting weights of each of the 3 groups in Trial 1 which showed no significant difference in starting weights between groups. The same as found for the 5 groups of Trial 2. These analyses have been added to the report.

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?

I clarified this some in the methods section, based on another reviewer’s similar comment. Originally, I explained this more so in the discussion section, but now it’s discussed in both sections.

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.

Good suggestion, I modified the sentence and added one of the Rattner citations as suggested.

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?

As noted in the comment above, we present the necropsy results with Aneides and Ensatina salamanders in the results section. We had mentioned in the methods section that we did not necropsy the Batrachoseps salamanders because of their very small size. No histopathology was conducted.

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.

See previous comment that the startin weights were not significantly different between groups of salamanders.

**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.

We agree with this comment, but didn’t have placebo bait at the start of the study. We did obtain some placebo bait later to test with the crickets after the first batch of crickets we fed “active” bait to died; this was to see if some of the inert ingredients in the bait caused the crickets to die. However, when the “active” baits were later fed to a new batch of crickets, they all survived.

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).

See previous comments on how this was handled.

Any photographs of necropsy observations of dead individuals with hemorrhage?

While we took some photos, we are discouraged from presenting those in reports and publications (presumably because of possible bad PR and public reaction).

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.

We included this analysis for the dead versus living salamanders of the brodifacoum dermal group; no sissgnificant difference was found.

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. This 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.

It was stated in the methods section that Batrachoseps were not necropsied because of their very small size.

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.

I added a sentence to the end of the first paragraph of the discussion to point this out as suggested: Given that this was a worst-case scenario, the low residue concentrations in the salamanders suggests that there would be a relatively low risk to predators or scavengers consuming a salamander.

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

I changed hazard to “risk”.

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

Not sure where this is referring to.

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.

I ended the sentence with…”based on mortality” as suggested.

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

Not sure where this is referring to.

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.

We agree, but have no data to present; it was merely an observation of the survival of a later batch of crickets that were fed the baits.

**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.

I changed this to say on isolated islands and cited that the Hawaiian islands have no native amphibians (Ziegler 2002, p. 235>

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.

I added a concluding paragraph suggesting further lines of investigation.

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 detection limits are very different for diphacinone when assayed by the two different methods because one uses UV detection (method 163A) and the other uses MS/MS.  The UV method was used to quantify diphacinone baits because it has better accuracy at high concentrations (50µg/g), whereas the LCMS method was used to quantify trace levels of diphacinone in the placebo baits (~0.3µg/g).

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

Thank you Barnett for pointing out the suspicious results for samples QO4 and QS56!  I traced it back to a spreadsheet error in the same row and column for both spreadsheets.  A value of zero had inadvertently been typed over an equation which resulted in a false “ND” for the final result.  The correct result for QO4 is 45.6 ng/g brodifacoum, and 86.6ng/g brodifacoum for QS56.  I looked through all the data and found the error on one other set of data, but the original result of “ND” did not change. This has been corrected in the chemistry report at the end of the final report.

Fix formatting of Tables (wrapping of text).

I don’t know how ro modify this ExCel file to correct this.