Review of Draft Final Report, QA-2688: An assessment of the potential hazards of anticoagulant rodenticides to Plethodontid salamanders

A report to: U.S. Fish and Wildlife Service, Farallon Islands National Wildlife Refuge, Fremont, California through Interagency Agreement No. F16PG00129

This report addresses potential hazards posed to endemic plethodontid salamanders by a proposed mouse eradication project on the Farallon Islands National Wildlife Refuge. The study uses three suitable surrogate salamander species and realistic exposure routes. The results provide useful data on salamander mortality and sublethal effects, despite challenges related to working with difficult-to-obtain species.

Comments below are organized by report section

Introduction

Page 3: APHIS should be defined at first use

Done.

Page 3: It would be helpful to add a brief explanation of how ARs are typically applied (method and rates) in invasive rodent eradications, as well as a more explicit explanation of how salamanders would likely contact ARs under natural conditions.

I added the methods and rates under the APHIS labels. I also added how salamanders might be exposed to the rodenticides.

Methods

Several elements of the study design/methods need additional detail:

The actual number of salamanders of each species exposed in each treatment or control group in Trials 1 and 2 should be specified in a table in the Methods section.

I don’t think a separate table is needed; I already referred readers to tables 1 & 2 which list the number of salamanders and species in each group.

In addition to clarifying the experimental design, creating the table above would allow the information on *Batrachoseps* group sizes to be removed from the “oral exposure” and “dermal exposure” sections.

See previous response.

In oral exposures, what method was used to dust crickets with ARs, and at what rate were they dusted?

This has been clarified in the text and we note that don’t know the exposure rate, but relied on the chemical residue analyses for amount.

In dermal exposures, at what rate were pellets spread on the paper towels? Ideally, this rate should be linked to typical application rates used in the field, or a multiplier thereof (to simulate a worst-case scenario). Because the very high exposure rates are mentioned several times in the interpretation/conclusions of the study, it is critical to know how much higher the exposures might have been than realistic field levels.

In dermal exposures, was the procedure used to powder/crush pellets intended to replicate the size range of pellet pieces that salamanders might encounter in the field, or simply produce a wide range of pellet piece sizes?

I referred to the figures to show the relative amount of rodenticides spread on the wet paper towels. And we note in several places that this a “worst-case” scenario.

How often was water applied to paper towel substrates (in all treatment and control chambers)?

I added the sentence: Paper towels were kept saturated with water at all times.

What was the feeding procedure for crickets? More detail is needed here, because some values of cricket consumption reported in the results appear higher than what would be expected from feeding of 5-7 crickets per salamander twice weekly.

I clarified by adding a sentence that we later decided to make sure the salamanders always had crickets in their cages since some ate the crickets very quickly and then went several days with no food available. That may have resulted in weight loss which might have been attributed to an anticoagulant effect.

Please include a thorough description of all types of samples sent for rodenticide analysis. Appendix A lists many types of samples (particularly for crickets, but also placebo baits, etc.) that are not currently described. In particular, emphasize that crickets fed rodenticides were included in analytical samples for comparison, although they were not used in the trials.

I think this is adequately described in the text, altho I agree that the Analytical Chemistry Units report is a bit daunting!

More detail is needed on the statistical treatment of the data. Was the repeated measures design reflected in the analysis? See additional comment under Results, below.

I added that a number of ANOVAS were performed on the data sets. Several more have been added since the previous version reviewed.

Results

For Trial 1, it would be helpful to report the incidence of skin sloughing and sores by individual (and then summarized by species) to determine if individuals that developed skin problems were the same individuals that later died.

This has been added and interestingly, the salamanders that died (n = 6) in the brodifacoum dermal group had no sores or skin sloughing.

For Trial 1, it would be helpful to report mortality and weight change by species, given potential species-specific differences in rodenticide uptake/ingestion and sensitivity. Table 1 appears to show species-specific differences in weight change in both treatment and control groups.

I stated in the result of Trial 1 that the 3 salamanders that died were all Aneides. I also added an analyses of the starting weights of the 3 groups of salamanders (not significantly different) and an analysis of the change in weights in each group between the treatment and post-treatment periods (not significantly different).

In Trials 1 and 2, reporting cricket consumption by individual salamander (similar to weight change) would allow a better understanding of feeding patterns and potential changes between exposure and post-exposure periods.

Cricket consumption is summarized in the results text.

Related to above, both weight change and cricket consumption should be analyzed with a paired samples t-test to account for the repeated measures design (with *Aneides* and *Ensatina* analyzed separately in Trial 1). This is especially important, given the wide variability among individuals in size and number of crickets consumed. The fact that weight and feeding were tracked for each individual is a strength of the study, and should be reflected in the statistical analysis.

The analyses done show no differences in weights or cricket consumption be Aneides versus Ensatina salamanders

In the results from the control group for Trial 2, is the higher end of the range given for cricket consumption (229 per salamander) correct?

\*\*It turns out we had added two small Ensatinas to the Betrachoseps control group and those two ate many more pin-head crickets. So I eliminated those two salamanders from the data set.

For crickets fed rodenticides, are the residue values listed here from the initial batch of crickets that died shortly after feeding, or from a later batch?

I clarified that a later batch of crickets were fed rodenticides and those crickets all survived.

Discussion

Was the “reduced skin sloughing and fewer sores during the post-exposure period” a qualitative or quantitative observation?

This was a qualitative assessment.

I agree that the rodenticide exposures in the current study clearly represent a worst-case scenario. As mentioned in the Introduction comments, adding information on typical field application rates would help readers understand how much higher exposure rates in the study might have been, compared to potential exposures in the field.

That has been added in the Intro and in the discussion.

When “later batches of crickets [fed rodenticides] survived and were used in the study,” does this refer to being used for analytical samples, or being used in a different way?

I clarified that they were only used for residue analyses.

It would be helpful to add more specific detail regarding the characteristics of 1) salamander physiology and behavior and 2) fate and transport of the two rodenticides that contribute to the determination of relatively low risk.

Because I don’t know much about salamander behavior and physiology, I eliminated reference to that.

Additional points that may warrant mention in the Discussion:

Potential cause for apparent *Batrachoseps* sensitivity to dermal brodifacoum exposure?

Unknown, but maybe because these lungless salamanders presumably get their oxygen thru the skin, maybe that results in quicker and heavier exposure. But this would just be speculation on my part.

Detection of low levels of brodifacoum in a control and a diphacinone-exposed *Batrachoseps* salamander. Also detection of low levels of diphacinone in crickets fed and dusted w/ brodifacoum.

It could be that they quickly eliminate it from their bodies, but again, I would just be speculating here and prefer not to do that.

Table 1

See suggestions for reporting in Results section, above.

Addressed above.

Table 2

Salamander QS27 is missing a final weight value

I clarified that this small salamander was lost down the sink drain.

Two salamanders in the control group are listed as “QO” rather than “QS”

These were two small Ensatinas which have now been eliminated from the data set as discussed earlier.

Appendix A

Given the number of different treatments in Trial 2, it would be helpful to arrange the table of *Batrachoseps* residues by treatment. This would ease comparisons of residue concentrations in animals treated similarly.

I agree, but just want to go with what is described in the text. I do agree that the Analytical Chemistry Units report is a bit daunting, but I choose not to rearrange all of what they gave me! I expect the average reader to not spend time with that elaborate table, but to just go with what the text says.