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

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Abstract: We assessed the hazards of the anticoagulants diphacinone and brodifacoum to salamanders of the family Plethodontidae or lungless salamanders. We completed this research in anticipation of an attempt to eradicate the invasive house mouse (*Mus musculus*) from the Farallon Islands National Wildlife Refuge, California, USA, where the endemic subspecies Farallon arboreal salamander (*Aneides lugubris farallonensis*) occurs. We exposed live-captured salamanders of 3 species (*Aneides lugubris, Ensatina eschscholzii xanthoptica*, and *Batrachoseps attenuatus*) to anticoagulant rodenticides by both oral and dermal exposure routes in laboratories at the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA. The amount of exposure was high, simulating a worst-case scenario. There were some deaths (9 of 37 treated salamanders; 24.3% mortality). We did not observe the sublethal effects of weight loss or reduced food (cricket) consumption that has been observed in studies of other taxa (mammals and birds). Skin sloughing and sores on the undersides of certain salamanders exposed to rodenticides as well as some controls left it unclear whether this effect was caused by the anticoagulant. Following trial completion, we analyzed whole bodies of salamanders for rodenticide residues. Residue concentrations were very low (<1 parts per million) when compared with results from some other studies. We concluded that while anticoagulant rodenticide posed some hazards (both lethal and sublethal) 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.

Key words: *Aneides*, anticoagulants, hazards, invasive rodents, lungless salamanders, non-target species, Plethodontidae, rodenticides

House mice (*Mus musculus*; mice) cause many types of damage, and when introduced to islands, mice can cause significant damage to natural resources, including native flora and fauna (Witmer and Jojola 2006, Howald et al. 2015). For example, on Gough Island in the South Atlantic, mice fed on nestling albatross (*Diomedea exulans*) chicks (Cuthbert and Hilton 2004). Invasive mice are also negatively impacting bird populations on the U.S. Fish and Wildlife Service's (USFWS) Midway Atoll (USFWS 2018). Additionally, Witmer et al. (2012) documented seedling damage by mice in a pen study.

Mice are omnivores, yet their diet is largely dominated by insects (at least on tropical Pacific islands), some of which are likely plant pollinators (Shiels et al. 2013, Shiels and Pitt 2014). Mice diets also vary depending on habitat, environmental conditions, and food availability (Polito et al. 2022). Because of the damage caused by mice on islands, there have been numerous attempts to control or eradicate them.

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). The USFWS proposes to use registered rodenticides to eradicate mice on the Farallon Islands National Wildlife Refuge off the coast of central California, USA (USFWS 2019).

The U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) maintains the registrations for 2 pelleted anticoagulant rodenticide products for invasive rodent eradication. The rodenticides contain the active ingredients diphacinone (0.005% a.i.) and brodifacoum (0.0025% a.i.). In most eradication efforts, these pellets are aerially applied by helicopter at an application rate of 18

Figure 1. *Aneides* salamander in its plastic cage showing the high level of dermal exposure in this study. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA.

kg/ha or less. This results in about 2 rodenticide pellets per m^2. However, in some cases, the project personnel request a higher application rate because of rodenticide pellet consumption by non-target animals, land crabs (Gecarcinidae) in particular. This is to help ensure a successful rodent eradication. The rodenticide labels also allow for a second aerial application to help ensure that all targeted rodents are exposed to a lethal dose of the rodenticide (Witmer 2019).

Rodenticides can pose hazards to non-target animals, so careful considerations and measures must be taken to reduce those risks (Witmer et al. 2007, van den Brink et al. 2018). In the case of salamanders (Caudata), they could be exposed to rodenticides during an eradication project by moving across the material (e.g., dermal exposure) or by consuming invertebrates that have consumed baits (secondary oral exposure). Because salamanders respire through the skin, dermal exposure may be of greater concern than with other vertebrates (Ockleford et al. 2018).

Invasive mice inhabit the USFWS Farallon Islands National Wildlife Refuge (hereafter, refuge). The mice impact the endemic arboreal salamander (*Aneides lugubris farallonensis*; Figure 1) as well as native seabirds, invertebrates, and plants (USFWS 2019, Polito et al. 2022). The USFWS proposes to eradicate mice from the refuge. The USFWS analyses of action alternatives for the mouse eradication included an assessment of the potential hazards of brodifacoum and diphacinone to salamanders (USFWS 2019). They requested that the USDA APHIS Wildlife Services (WS) National Wildlife Research Center (NWRC) located at Fort Collins, Colorado, USA, complete this assessment. The NWRC has extensive animal research facilities and experience in assessing hazards of anticoagulants to reptiles (Mauldin et al. 2020). We conducted this study because of specific concerns about the potential hazards of anticoagulant rodenticides to salamanders. This was especially important because the Farallon arboreal salamander is an endemic subspecies (USFWS 2019). No scientific literature could be located on exposure of salamanders to rodenticides; however, the potential hazards to reptiles has been studied to some extent (Hoare and Hare 2006, Weir et al. 2016, Mauldin et al. 2020).

The objective of this study was to assess the potential hazards of the rodenticides brodifacoum and diphacinone to Farallon arboreal salamanders using conspecifics from other populations of closely related salamanders as surrogates because of the Farallon population's relatively small and endemic status. Ultimately, we used 3 closely related species of Plethodontid salamanders in the study: yellow-eyed ensatina (*Ensatina eschscholzii xanthoptica*), arboreal salamander (*Aneides lugubris*; mainland variety), and California slender salamander (*Batrachoseps attenuatus*). For a description of the phylogenetic relationships of the largest family of salamanders, the Plethodontidae, see Vieites et al. (2011). The salamanders were exposed to rodenticides through oral and direct dermal exposure. We assumed that these would be the main routes of potential exposure in a rodent eradication project. We hypothesized that the rodenticide exposure would cause some mortality, internal or external bleeding, or other sublethal effects (e.g., decline in food consumption and/or loss of weight).

Methods

The salamanders we used in this study were live-captured in California and shipped to NWRC by the herpetology lab of Dr. Vance Vredenburg of San Francisco State University (SFSU). Dr. Vredenburg 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 contract with the USFWS to conduct those activities. The salamanders are not sexually dimorphic, and we did not know the age or sex of the salamanders brought to NWRC.

The salamanders were housed at the NWRC individually in small plastic rodent "shoebox" cages (26.5 cm long, 15.5 cm wide, 20.5 cm high; Figure 1) and fed small crickets (Grylloidea; 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, are easily maintained, and are readily consumed by captive salamanders (V. Vredenburg, SFSU, personal communication). The floor of each cage was lined with wet paper towels to provide needed moisture and a plastic tube for shelter (Figure 1). Paper towels were kept saturated with water at all times.

Cages were cleaned and changed weekly throughout the study unless mildew became obvious, at which time the cage was changed. Salamanders were maintained as per SFSU Standard Operating Procedure on salamander maintenance. Upon arrival, salamanders were quarantined for 2 weeks to help ensure their healthy condition before starting the trials. We presumed that this also allowed the salamanders to stabilize in body mass prior to initiation of the trials.

We tested rodenticides containing the anticoagulants diphacinone and brodifacoum for their potential hazards to salamanders. The specific rodenticides tested were Brodifacoum-25D Conservation and Diphacinone-50 Conservation, which are both registered with the U.S. Environmental Protection Agency for rodent control in island settings. The amount of exposure to the rodenticides was high, simulating a worst-case scenario.

Initially, we planned to have a control and 2 treatment groups for each of these 2 rodenticides, with each providing a different route of exposure (oral exposure and direct dermal exposure). However, because of a shortage of salamanders captured for the study, we had to modify these plans as explained below. Because of their known abundance in the San Francisco Bay area and close relationship with *Aneides*, initially we planned to use *Ensatina* as our main sample species with a smaller sample of the less abundant and harder to obtain *Aneides* for confirmation of results with *Ensatina*. However, when both of these species proved more difficult to obtain than expected, we added the more abundant but somewhat less similar (to *Aneides*) *Batrachoseps* to the study.

Trial 1

We had planned to use 10 salamanders in each group; however, because we did not obtain enough of the first 2 species of salamanders (*Aneides* and *Ensatina*), we combined the 2 routes of exposure and had some of each species in each group (trial 1). The control group had no rodenticide exposure, but was otherwise maintained like the exposure groups (Table 1). Because we had enough *Batrachoseps* salamanders, we were able to have separate groups for each route of exposure along with a control group (trial 2).

Trial 2

In trial 2, we used *Batrachoseps* salamanders for the 2 separate exposure routes (Table 2). The same methods used in trial 1 for the groups of *Aneides* and *Ensatina* salamanders were replicated in trial 2, except that in trial 1 the 2 exposure routes were combined. That is, there was only 1 exposure group for each rodenticide.

Oral exposure procedures

We used 10 *Batrachoseps* for each oral exposure rodenticide procedure. Group size varied somewhat because of the number of salamanders available at the start of the study. Initially, we fed the rodenticides to crickets, but because of high cricket mortality, we then dusted crickets. This high mortality was unexpected because invertebrates have been reported to be unaffected by anticoagulants (Eason and Spurr 1995) and it was later determined that the crickets died from causes other than consumption or exposure to the rodenticide.

The dusting was done by placing crickets in a small plastic container with the powdered rodenticide bait, replacing the cover, and then gently shaking the container. We did not quantify the amount of rodenticide on the crickets but relied on the chemical residue analyses of whole powdered crickets to approximate the burden. Additionally, we presumed that much of the powdered rodenticide bait on the underside of the crickets came off quickly in the salamander cages as they walked around on the wet paper towels.

Table 1. Summary of the *Aneides* and *Ensatina* trial (trial 1). Animals coded QO are *Aneides*; those coded QP are *Ensatina*. BRD = brodifacoum. DPN = diphacinone. ND = not detected. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA.

Treatment	Animal ID	Initial wt. (g)	Final wt. (g)	Weight change (g)	Comments	Whole body residue (ppm)		% Sloughing skin	% Sores	% Mortality
						BRD	DPN			
Brodifacoum /oral and dermal exposure	QO1	9.4	6.1	-3.3	Died	0.103	ND	57.14	14.29	28.57
	QO4	9.0	7.8	-1.2	Euthanized at end of trial	0.0437	ND			
	QO7	9.7	7.5	-2.2	Euthanized at end of trial	0.0906	ND			
	QO10	9.4	6.0	-3.4	Died	0.226	ND			
	QP1	7.7	6.8	-0.9	Euthanized at end of trial	0.0990	ND			
	QP4	7.3	6.9	-0.4	Euthanized at end of trial	0.0860	ND			
	QP7	13.0	10.5	-2.5	Euthanized at end of trial	0.0491	ND			
Diphacinone /oral and dermal exposure	QO2	10.5	7.7	-2.8	Euthanized due to condition	ND	0.174	42.86	28.57	14.29
	QO5	17.3	15.8	-1.5	Euthanized at end of trial	ND	ND			
	QO8	12.9	12.2	-0.7	Euthanized at end of trial	ND	0.011			
	QO11	20.7	17.3	-3.4	Euthanized at end of trial	ND	ND			
	QP2	9.6	8.6	-1.0	Euthanized at end of trial	ND	ND			
	QP5	9.3	8.1	-1.2	Euthanized at end of trial	ND	ND			
	QP8	8.0	6.8	-1.2	Euthanized at end of trial	ND	ND			
Control	QO3	19.4	18.5	-0.9	Euthanized at end of trial	ND	ND	0.00	0.00	0.00
	QO6	10.8	10.4	-0.4	Euthanized at end of trial	ND	ND			
	QO9	20.3	18.2	-2.1	Euthanized at end of trial	ND	ND			
	QO14	10.4	10.0	-0.4	Euthanized at end of trial	ND	ND			
	QP3	6.0	4.8	-1.2	Euthanized at end of trial	ND	ND			
	QP6	15.4	13.3	-2.1	Euthanized at end of trial	ND	ND			

Table 2. Summary of the *Batrachoseps* trial (trial 2). BRD = brodifacoum. DPN = diphacinone. ND = not detected. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, USA.

Treatment	Animal ID	Initial wt. (g)	Final wt. (g)	Weight change (g)	Days until death	Whole body residue (ppm)		% Sloughing skin	% Sores	% Mortality
						BRD	DPN			
Brodifacoum /oral exposure	QS5	0.73	0.73	0.00		0.0852	ND	0.00	0.00	0.00
	QS10	0.45	0.55	0.10		0.0566	ND			
	QS19	0.84	0.94	0.10		0.0513	ND			
	QS27	0.52	N/A*	N/A		N/A	N/A			
	QS35	0.46	0.54	0.08		0.0646	ND			
	QS42	1.17	1.21	0.04		ND	ND			
	QS56	0.78	0.83	0.05		0.0896	ND			
Brodifacoum /dermal exposure	QS6	0.52	0.42	-0.10	2	0.019	ND	0.00	0.00	75.00
	QS11	1.03	0.97	-0.06	9	0.0728	ND			
	QS30	0.81	0.60	-0.21	14	0.0805	ND			
	QS36	0.41	0.34	-0.07	10	0.0342	ND			
	QS38	0.30	0.23	-0.07	10	0.103	ND			
	QS43	0.52	0.52	0.00		0.0339	ND			
	QS51	0.80	0.67	-0.13	10	0.0690	ND			
	QS57	0.58	0.57	-0.01		0.0355	ND			
Diphacinone /oral exposure	QS7	0.50	0.64	0.14		0.011	ND	0.00	0.00	0.00
	QS13	0.69	0.79	0.10		ND	ND			
	QS23	0.56	0.70	0.14		ND	ND			
	QS31	1.15	1.27	0.12		ND	ND			
	QS39	0.30	0.32	0.02		ND	ND			
	QS44	0.89	1.04	0.15		ND	ND			
	QS52	0.29	0.34	0.05		ND	ND			
	QS58	0.56	0.61	0.05		ND	ND			
Diphacinone /dermal exposure	QS8	0.31	0.36	0.05		ND	ND	50.00	0.00	0.00
	QS14	0.39	0.48	0.09		ND	ND			
	QS24	0.88	0.88	0.00		ND	ND			
	QS33	0.88	0.92	0.04		ND	ND			
	QS40	0.83	0.89	0.06		ND	ND			
	QS48	0.86	0.97	0.11		ND	ND			
	QS53	0.82	0.71	-0.11		ND	ND			
	QS55	0.93	0.89	-0.04		ND	ND			
Control	QS9	0.45	0.55	0.10		0.022	ND	20.00	20.00	20.00
	QS17	0.75	0.81	0.06		0.0088	ND			
	QS22	0.54	0.52	-0.02	6	ND	ND			
	QS26	0.90	0.94	0.04		ND	ND			
	QS34	0.38	0.40	0.02		ND	ND			

* N/A = not applicable. This carcass was lost.

Initially, we fed crickets to salamanders twice weekly. However, because many salamanders ate the crickets very quickly, they then went several days without any food (crickets) available. We were concerned about this situation because the salamanders might then start losing body mass, which could be misinterpreted as an anticoagulant effect. To mitigate this concern, we began feeding crickets to the salamanders more frequently to ensure that they always had crickets available in their cages. The treated crickets were fed to the salamanders for 14 days. At the end of the 14-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.

Direct dermal exposure procedures

We used 10 *Batrachoseps* salamanders for each rodenticide assessment. Again, group size varied somewhat because of the number of salamanders available at the start of the study. The salamanders were exposed dermally to powdered/crushed rodenticide pellets sprinkled on the ground cover material (Figure 1) and by spraying the ground cover paper towels with water in which crushed pellets were allowed to dissolve for 7 days.

With this exposure group, there may also have been some direct oral exposure if the salamanders chose to eat some of the crushed pellets, although this was not observed. As in the direct exposure group, the salamanders were exposed to the crushed pellets and treated water for 14 days. At the end of the 14-day exposure period, we placed the salamanders in clean cages and observed for the 14-day post-exposure period. During this entire treatment, the salamanders were fed crickets that had not been exposed to the rodenticide. We maintained control groups with no rodenticide exposure during trials 1 and 2.

Salamanders were fed 5–7 crickets twice weekly, but we made sure that salamanders always had some crickets in their cages. We 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 change in weight occurred. These data provided measures of potential sublethal effects.

Generally, mammals that have consumed enough anticoagulants to exhibit signs of toxicosis will stop feeding and lose weight as the signs of toxicosis advance (Witmer et al. 2016). Birds, however, do not typically show weight loss when fed sublethal doses of anticoagulants, but birds that are severely intoxicated (and perhaps succumbing/dying) stop feeding and lose weight (Rattner et al. 2014).

We examined salamanders twice daily to assess their condition and record mortalities. 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, we and/or the attending veterinarian examined the animal, and it warranted the animal was euthanized. We used signs of severe pain and distress and of a moribund condition as criteria for euthanasia of study animals (Organisation for Economic Cooperation and Development 2000). We also included abnormal vocalization, persistent labored breathing, prolonged impaired ambulation preventing the animal from reaching food or water, persistent convulsions, and significant blood loss as additional criteria. However, only 1 salamander was euthanized because of its condition (Table 1).

Dead salamanders were rinsed in clean water, weighed, and placed in individual, labeled resealable bags and frozen for later rodenticide residue determination by the Chemistry Laboratory Unit (CLU) staff. All surviving salamanders were euthanized at the end of the study by placement in a liquid formulation of tricaine mesylate (which also served to rinse the animals of surface residues) for later submission to CLU staff.

The *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 small size, we did not necropsy the *Batrachoseps* salamanders. Additionally, some unrinsed crickets dusted with rodenticide bait powder and some control crickets were submitted for rodenticide residue analyses along with samples of the water that had been exposed to the powdered bait pellets.

Whole salamanders and crickets were ho-

mogenized and replicate samples (~0.08 g) were analyzed using a previously described liquid chromatography-mass spectrometry (LC-MS) method (Franklin et al. 2018) that was modified to test for only brodifacoum and diphacinone. Water samples were filtered through 0.7-μm glass fiber syringe filters, acidified with HCl, and extracted into 80/20 (v/v) acetonitrile/chloroform. Extracts were then reduced to dryness, reconstituted in mobile phase, and analyzed using the same LC-MS conditions. Rodenticide pellet baits were analyzed for diphacinone and brodifacoum concentration according to the method described in Pitt et al. (2015).

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, comparing group means. We also compared cricket consumption during the rodenticide exposure period to cricket consumption during the post-exposure period. We used a significance level of $P \leq 0.05$. Other ANOVAs included comparisons of starting weights of the groups of salamanders in trial 1 and again in trial 2. Finally, we compared brodifacoum residue levels between salamanders that died during trial 2 versus those that lived. This study was conducted under the NWRC IACUC-approved study protocol QA-2688.

Results

Trial 1

Because of the relatively small number of *Aneides* and *Ensatina* salamanders available for this trial, we combined the 2 exposure routes for each treatment group (Table 1). The starting weights of the 3 groups of salamanders in trial 1 did not differ ($F = 1.87$, $P = 0.18$). In the brodifacoum group, 2 (both *Aneides*) of the 7 salamanders died (28.6% mortality); while 1 of these salamanders had skin sloughing and external bleeding, the other showed none of these symptoms. The 2 salamanders that died appeared to have higher brodifacoum residue levels than the 5 that lived, but these levels did not differ ($F = 5.82$, $P = 0.06$). We noted a sloughing of skin in some animals (4 of 7; 57.1%) and sores, mainly on the underside of animals (1 of 7; 14.3%). It is important to note, however, that skin sloughing is a normal function in amphibians to ensure proper physiological function and to prevent infection (Ohmer et al. 2017). The pellets for both brodifacoum and diphacinone are rather acidic, so this may have been responsible for some skin sloughing and sores.

We observed considerable variation in cricket consumption by the salamanders. During the 14-day 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, overall cricket consumption did not differ between the 2 periods ($F = 3.83$, $P = 0.08$). Additionally, the presence and severity of 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 (0.4–3.4 g) compared to the control group ($F = 4.80$, $P = 0.05$). Upon necropsy of the 2 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 compared to other studies: *Aneides* 0.0437–0.226 parts per million (ppm); *Ensatina* 0.0491–0.0990 ppm.

In the diphacinone group, 1 (*Aneides*) of the 7 salamanders died (14.3% mortality); this individual exhibited sores and external bleeding and was euthanized. We noted a sloughing of skin in 3 of 7 salamanders (42.7%) and sores on 2 of these individuals (mainly on the underside of animals; 28.6%). 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, overall cricket consumption did not differ ($F = 1.40$, $P = 0.26$) between the 2 periods. Additionally, the presence and severity of skin sloughing and sores decreased in the post-exposure period. Over the course of the trial, the change in weight of the salamanders did not differ ($F = 0.50$, $P = 0.49$). 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* not detected

(ND; <0.001 ppm) to 0.174 ppm; 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. However, 1 of the 6 salamanders in the control group showed some internal bleeding upon necropsy. Cricket consumption increased some over the course of the trial in this group ($F = 2.20$, $P = 0.17$). However, the control salamanders ate more crickets than the other 2 groups of salamanders ($F = 4.43$, $P = 0.03$). Over the course of the trial, the weight loss in salamanders did not differ ($F = 0.14$, $P = 0.71$). All salamanders in the 3 groups tended to lose weight ($F = 1.02$, $P = 0.38$).

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, resulting in 4 treatment groups and 1 control group. The starting weights of the salamanders in the 5 groups were similar ($F = 0.41$, $P = 0.80$). One brodifacoum group ($n = 7$) received oral exposure (dusted crickets) only, while the second brodifacoum group ($n = 8$) received dermal exposure. Similarly, 1 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 ($n = 5$) received no rodenticide exposure.

In the brodifacoum oral exposure group, no salamanders died (Table 2). There was no skin sloughing or sores observed. Cricket consumption varied: 13–70 per individual during the exposure period and 4–59 in the post-exposure period ($F = 0.01$, $P = 0.92$). Salamanders mostly maintained the same weight over the duration of the trial; the most substantial change was 0.1 g in 1 individual. Weight changes did not differ over the course of the trial ($F = 0.15$, $P = 0.71$). Brodifacoum residues in the oral exposed salamanders ranged from not detected (ND) to 0.0896 ppm.

In the brodifacoum dermal exposure group, 6 of 8 salamanders died (75.0%). There was no skin sloughing or sores observed in any of the salamanders including those that died. The salamanders that died tended to have higher brodifacoum residue levels than the ones that lived ($F = 0.98$, $P = 0.37$). Cricket consumption was somewhat variable: 9–27 in the exposure period, but increased in the 2 surviving salamanders (44 and 55) in the post-exposure period. Cricket consumption increased between 2 two periods ($F = 20.9$, $P = 0.002$), but it should be noted that this statistic is based on only 2 data points in the post-exposure period. Salamanders mostly lost a small amount of weight from the start to the end of the trial ($F = 0.49$, $P = 0.50$). Brodifacoum residues in the dermal exposed salamanders ranged from 0.019–0.103 ppm, and the salamanders fed dusted crickets tended to have somewhat higher brodifacoum residue levels ($F = 1.02$, $P = 0.33$).

No animals died in the diphacinone oral exposure group. Skin sloughing or 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 4–66) in the post-exposure period ($F = 0.31$, $P = 0.58$). Weight gain in this treatment group ranged from 0.02–0.15 g ($F = 0.39$, $P = 0.54$). There were no diphacinone residues detected in the oral exposed salamanders.

In the diphacinone dermal exposure group, no animals died, but 50% of salamanders had some skin sloughing. Cricket consumption ranged from 6–57 during the exposure period, but stayed about the same (range of 5–59) in the post-exposure period ($F = 1.89$, $P = 0.19$). Salamander weights were mostly stable over the course of the trial, with changes ranging from -0.11 to 0.11 g. The differences between the start and end of the trial did not differ ($F = 0.05$, $P = 0.83$). There were no diphacinone residues detected in the dermal exposed salamanders.

There was 1 death (20% mortality) in the control group. Interestingly, 1 control animal had sloughing skin and sores. Cricket consumption was also variable in the control group, ranging from 18–145 per salamander ($F = 0.56$, $P = 0.47$) during the 2 periods (treatment vs. post-treatment). Overall, there was no difference in the cricket consumption between the 5 groups of salamanders ($F = 0.84$, $P = 0.51$). Control animals also showed only small changes in weights during the study period: -0.02 to 0.43 g ($F = 0.28$, $P = 0.61$). However, weight changes differed in the 5 groups of salamanders ($F = 3.47$, $P = 0.02$) with the brodifacoum salamanders losing the most weight and the control salamanders losing the least amount of weight.

Crickets, water, bait pellets, and other findings

In trials 1 and 2, we fed crickets that had been dusted with rodenticide bait powder to the salamanders. Brodifacoum residue concentrations in crickets dusted with powdered brodifacoum bait ranged from 2.89–3.34 ppm. Diphacinone residues in crickets dusted with powdered diphacinone bait were quite variable, ranging from 1.82–3.98 ppm. The concentration of brodifacoum in water saturated with powdered brodifaoum bait pellets ranged from 0.00575–0.0297 ppm. Diphacinone concentrations in water saturated with powdered diphacinone bait pellets ranged from 0.00342–0.0177 ppm.

We assayed the Brodifacoum-25D Conservation and Diphacinone-50 Conservation baits to confirm potency. The observed concentration for the brodifacoum bait was 26.3 ppm brodifacoum (105% of label claim) and 46.4 ppm diphacinone (92.8% of label claim) for the diphacinone bait, both within the acceptable range of variation.

Discussion

Based on trial 1 results, it appeared that rodenticide exposure poses some risk to salamanders, but that hazard appears to be relatively low in terms of mortality and sublethal effects, especially considering the experimental design optimized for salamander exposure to rodenticides. It also appeared that salamanders can begin recovery after exposure ceases, as suggested by reduced skin sloughing and fewer sores during the post-exposure period. However, because some skin sloughing and sores were also noted in 1 control salamander, it is unclear whether skin damage was caused by anticoagulant exposure. Also, as noted earlier, skin sloughing is a normal function in amphibians to ensure proper physiological function and to prevent infection.

In our trials, we used a very high exposure rate in the treatment groups, which combined oral and dermal exposures. In the brodifacoum group, the high exposure rates were from the feeding of dusted crickets along with the level of dermal exposure, which was much higher than it would be in an eradication project. Hence, this trial was, in essence, a worst-case scenario. In an actual aerially applied rodenticide baiting operation, using the U.S. Environmental Protection Agency's (EPA) label application rate, there is generally only about 2 rodenticide pellets per m^2. 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.

The trial 2 results basically confirmed the results from trial 1. However, trial 2 suggested that the higher hazard to *Batrachoseps* salamanders from anticoagulants was from dermal exposure versus oral exposure based on mortality. We were able to determine this because we had enough *Batrachoseps* salamanders to separate the 2 types of exposure into separate groups. We again caution, however, that we gave very high dermal exposure rates to the salamanders in this study. 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 represents a worst-case scenario.

Our search of the scientific literature revealed no publications concerning the toxicity of anticoagulants to amphibians. Thus, little is known about the risk of anticoagulants to amphibians, but it is generally considered to be low (Eason and Spurr 1995, Chris et al. 2010). The native *Batrachoseps* salamanders on Anacapa Island are thriving 10 years after the invasive rats were eradicated using Brodifacoum-25D (Newton et al. 2016). There is considerable uncertainty regarding the toxicity of rodenticides to amphibians, but based on the fate and transport of the 2 rodenticides in the environment, we would anticipate relatively low risk to Plethodontid salamanders under most island rodent eradication exposure scenarios (Ockleford et al. 2018). Published studies have focused on risks to mammals, birds, invertebrates, and to a lesser extent, on reptiles. These taxonomic groups are thought to be either the most sensitive or the groups most likely to consume either baits (primary exposure) or animals that have consumed baits (secondary exposure). As such, we have little to compare our salamander results to with the exception of the taxonomic groups listed above. This information and residue levels comes from eradication projects with nontarget monitoring before and after rodenticide application and a study with captive reptiles.

Mauldin et al. (2020) assessed the potential

hazards of anticoagulant rodenticides to reptiles and reported concentrations of diphacinone and brodifacoum residues in whole bodies of captive snakes (*Boa constrictor*), turtles (*Rhinoclemmys pulcherrima*), and lizards (*Ameiva ameiva*, *Iguana iguana*) that had been twice orally gavaged with solutions containing those anticoagulants. Body residues ranged from lows of 0.07 ppm to highs of 1.58 ppm. They also noted that 5 of 37 (13%) *Ameiva* lizards died during the study, with 1 lizard 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 Atoll in the tropical 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 normal EPA recommended label rate). Using whole body carcasses found after the baiting operation, they reported concentrations of 0.10–0.76 ppm in birds, 0.34–0.44 ppm in fish, and below the detection level to 0.97 ppm in land crabs. These concentrations are much lower than those found in rats (*Rattus* spp.) that died from brodifacoum exposure: 3.75 ppm. Pitt et al. (2015) also reported that only 1 freshwater sample had a residue concentration (0.05 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 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 fresh carcasses found from various taxonomic groups (rats, birds, lizards, crabs) had detectible residues of brodifacoum. Liver residues were quite variable, but rats had higher levels (8,930–27,700 ng/g [= ppb]) than non-target animals (127–2,780 ng/g). 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 12.2–1,100 ng/g (= ppb). Additionally, some insects and crabs had detectable residue concentrations ranging from 10.3–1,580 ng/g.

Follow-up studies with larger sample sizes of animals per group might help reduce the wide variability observed in our study and would allow for more robust statistical analyses. There is also a need to fill information gaps (e.g., better exposure and robust toxicity data and histopathology data). Further study could also better explain the reason(s) behind skin sloughing and sores in salamanders. Trials with other species of amphibians would also be useful to compare with the results of this study.

Management implications

Our results suggest a relatively low risk to Plethodontid salamanders from anticoagulant rodenticides. Additionally, it does appear that there would not be population-level effects on the salamander population for island invasive mouse eradication projects. We can also surmise that the salamander population would benefit by not being preyed upon by mice as well as their invertebrate food source remaining intact. Because of the low residue levels in salamanders, it also appeared that the hazard to animals preying or scavenging on salamanders would be low. Finally, a small-scale field application of anticoagulant rodenticides in an area containing amphibians might provide better insight to the real risk of these toxicants to amphibians in an invasive rodent eradication.

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