California Department of Food and Agriculture
Integrated Pest Control Branch

VERTEBRATE PEST CONTROL RESEARCH ADVISORY COMMITTEE MEETING

Sponsored by: Fresno County Department of Agriculture
CDFA Beat Curly Top Virus facility
2895 N. Larkin Ave, Fresno, CA 93727
Phone (916) 262-1102 Fax (916) 262-2020
April 24, 2019
9:00 a.m. to 3:00 p.m.
www.vpcrac.org

AGENDA

1) **Welcome from Fresno County Agricultural Commissioner staff**

2) **Bagley-Keene Open Meeting Act and VPCRAC Compliance**

3) **Approval of Minutes – October 18, 2018**

4) **California Department of Food and Agriculture Updates**
   a) Program Updates
   b) Vertebrate Legislation and Regulations
   c) California Department of Pesticide Regulation Update
   d) County Monthly Report Data

5) **Financial Reports**
   a) 2019/2020 Proposed Budget
   b) 2018/2019 Budget, Expenditures, and Revenue Review
   c) 2017/2018 VPCRAC Budget, Fund Condition and Revenue Projections

6) **Committee Membership**
   a) Board Member Requirements
   b) Chairman
   c) Vacancy

7) **2018-2019 Research Proposal Guidelines**

8) **Research Updates**
   a) “Investigation of the interaction between rodenticide secondary exposure and barn owls effective control of vertebrate pest population”
   b) “Efficacy testing of anticoagulant formulation with metabolic inhibitor as additive”
   c) “An assessment of secondary toxicity risk for 0.005% diphacinone treated grain via three application strategies for California ground squirrels”

d) “An assesment of secondary impacts of anticoagulant rodenticides on predators”
e) “Rangeland forage loss from California ground squirrels”

9) Research Proposals

a) “Efficacy and palatability testing of a novel rat specific toxicant”

10) Other Items

a) Yearly Meeting Calendar review
b) Public Comment on Matters not on this Agenda
c) Future Action Items
d) Next Meeting Schedule Location and Date

Adjournment

Contact:
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Note: Action is possible on any item contained in this agenda. Audience members may address the Committee following each agenda item. Each speaker from the audience is limited to three minutes. For information, please contact the Integrated Pest Control Branch, 916-262-1102

Grant Proposal to
CDFA Vertebrate Pest Control Research Advisory Committee

Title: Efficacy and palatability testing of a novel rat specific toxicant

Principal Investigators

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Project Duration
September 1, 2019-December 31, 2021

Total Budget Request $ 111,161

Overall Goal of Proposed Project
The goal of this project is to determine the efficacy and palatability of an analog of the rat specific toxicant norbormide. Norbormide showed promise as a rat specific toxicant, but difficulties with bait acceptance initially slowed development. Analogs of norbormide have been developed and shown efficacy for various rat species. Developing an analog efficacious to black rats (*Rattus rattus*) has continued to be difficult. Also, additional data on efficacy in Norway rats (*Rattus norvegicus*) is needed. Both of these rat species have been deemed extremely damaging to United States agricultural. Therefore, this study will test the efficacy of the most promising norbormide analog, DR8, against black rats from California citrus orchards and Norway rats from dairies and agricultural settings in Colorado. The data generated from this study will inform the decision to move forward with pursuing registration of this product for its use in the United States.

Introduction- Background Information
Norbormide
Norbormide, NRB, is a compound discovered in the early 1960s that is uniquely toxic to rats and relatively harmless to non-target species. When tested in non-target mammals and birds, it was found to be non-lethal at doses 20-200 times the LD50 in rats (1, 2, 3, 4, 5), with little or no toxic, behavioral, or gross abnormalities being observed even at 1000mg/kg orally. Based on these data, NRB has been tested in nearly 50 non-target species, including marsupials, other rodents, fish, a variety of birds, primates including humans, and ungulates.

NRB was found to exert its lethal effect in rats through mechanisms involving the control of blood pressure causing a rapid generalized irreversible vasoconstriction, hypoxia, and death within minutes (2). Vasoconstriction and circulatory changes were not observed in other species, but no further insight into the mechanism of action of this selective toxicant was made at that time. Additional research into NRB started in 1996 when Bova et al. confirmed the mechanism of action and reported that this action did not occur in non-target species tested, such as mice, guinea pigs, and humans. In non-target species, NRB induces only a temporary and non-lethal

vasodilatory action on the microvasculature rather than the extreme lethal vasoconstriction seen in rats (7). Their results suggested that it was the vasospastic effect of NRB on rat microvasculature that determined the species-selective lethal action of NRB in rats. This has subsequently been confirmed and expanded in later publications by various groups (8, 9, 10, 11). Despite these initial positive observations, subsequent research and development of this compound or related analogues did not proceed because rats showed a bait aversion due to a bitter taste or rapid onset of effects, leading to palatability and bait aversion problems culminating in sub-lethal dosing and low field efficacy. Therefore, rat baits based on this active substance were never widely used.

More recently, Rennison et al. (12) and Choi et al. (6) synthesized several series of NRB analogs as a means of developing a species selective toxicant with enhanced performance compared to parent NRB with regard to palatability and efficacy. The analogs that were progressed through to *in-vivo* trials all exhibited vasoconstriction in rat peripheral arteries identical to that of the NRB parent compound.

DR8

DR8 is an analog of NRB and is the compound tested in this proposal. It consists of NRB attached to a long chain fatty acid ester that prevents NRB from binding to the receptor whereby inhibiting NRB's lethal action. This fatty acid chain is critical because it delays the onset of symptoms of the lethal compound (NRB) in the rat whereby solving the palatability and bait aversion problems described earlier. The fatty acid chain includes an enzyme-susceptible site that is cleaved in the blood stream, releasing active NRB from the fatty acid chain and allowing it to bind to its receptor and bring about its lethal response (6) (Figure 1).

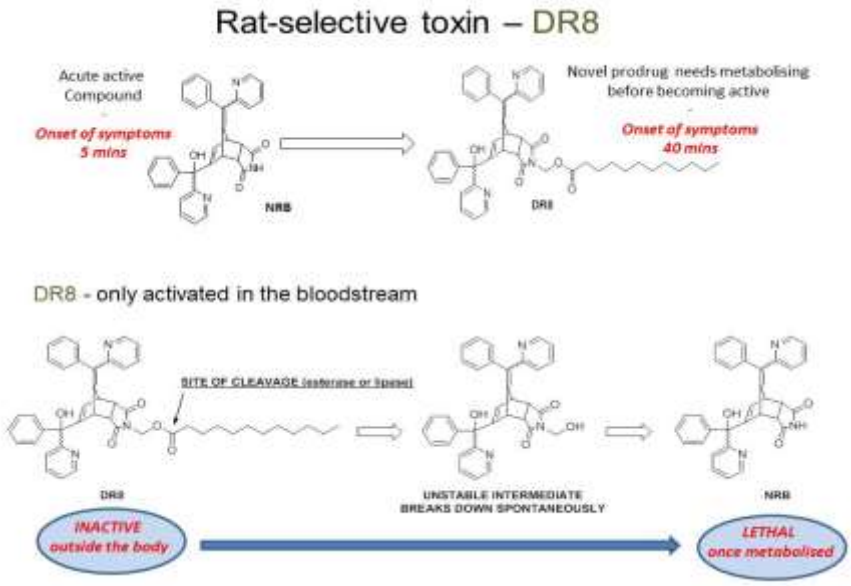

Fig 1: The fatty acid chain is quickly degraded to lauric acid, which is readily absorbed and metabolized.

In summary, the only active agent within DR8 is NRB, so all NRB's attributes regarding activity, species specificity, environmental fate, human health risk, primary and secondary toxicities can be ascribed to DR8. The fatty acid ester analog merely acts as a novel delivery mechanism for NRB, resulting in a delay to onset of symptoms and an increase in palatability and efficacy.

Description and Extent of Problem

Rodent damage to agriculture occurs worldwide, and in the U.S. alone agricultural losses due to invasive rats and mice are >$20 billion/year (13). Anticoagulant rodenticides have been a mainstay in control of rat populations for decades. Although they have been historically effective, anticoagulants are not useful in all situations and often not effective for the control of rat populations in agricultural settings. Moreover, social acceptance of the use of anticoagulants is shifting as more regulations controlling their use are being proposed to reduce non-target risks. To address these concerns, novel toxicants are being researched for the control of rats. DR8 is one such toxicant that is species-specific and shows great promise for use against rats while showing very low non-target risk.

Objectives

1. Determine LD50s of DR8 norbormide analog in black rats and Norway rats
2. Determine efficacy and palatability of DR8 in black rats and Norway rats in no choice and two choice feeding trials

Research Plans and Methodology

Overview

- Rats captured at agricultural settings in both California and Colorado
- Oral gavage of DR8 to determine LD50 for black rats and Norway rats
- No choice and two choice tests of DR8 performed to determine lethality and bait acceptance

Capture and transport

We will capture 50 black rats from citrus farms (e.g., Sun Pacific) in California, and 50 Norway rats from dairy farms and agricultural storage silos in Colorado using baited live-traps (Tomahawk type), and transport them to USDA's National Wildlife Research Center (NWRC) in Fort Collins, Colorado using our climate-controlled animal trailers and vehicles. Upon arrival to NWRC, rats will be individually housed in cages and maintained on a diet of standard rodent chow and apple wedges. Bedding, hides, and water will also be available in each cage. Rats will be treated for ectoparasites and held for 1-2 weeks in quarantine to ensure that they are healthy and fit for trial.

LD50 determination

We will administer known dosages of DR8 in a suitable carrier to test the efficacy via oral gavage to determine the LD50. To minimize the number of animals used, we will assess the LD50 using the OPPTS Harmonized Test Guideline up-and-down method (14). The up-and-down method relies on a single animal being dosed, observing its response for a period of time, and then dosing a second animal with either a higher or lower dose depending on the response of the first animal.

Feeding trials

Two types of captive-feeding trials will occur.

1) No-choice trial, where the DR8 (in a palatable bait matrix, such as peanut butter) will be offered to the 'treatment rats' (n = 12 of each species), and compared to the 'control rats' (n = 12 of each species) that will be offered the palatable bait matrix without DR8. Although water will be available *ad libitum*, there will be no other food present in cages during the trial.

2) Two-choice trial, where the 'treatment rats' (n = 12 of each species) will receive the DR8 mixed in a palatable bait matrix plus an equal amount of standard rodent chow, and the 'control rats' (n = 12 of each species) will receive the palatable bait matrix without DR8 plus an equal amount of rodent chow and water *ad libitum*.

Once rats are placed on trial, they will be monitored each day, for up to 7 days, for the amount of each bait type eaten, health/sickness, and death.

Expected Results

The goal of this project is to test the efficacy and palatability of DR8 in two rat species. We will determine the LD50 of DR8 via oral gavage and then test efficacy in the no-choice feeding trials. Palatability will be tested with the two-choice feeding trials. Data from these trials will directly inform the path forward for the further development, registration, and use of this toxicant for rat management in the United States. Registering a novel toxicant in the United States requires information about efficacy in target species. Much work has been done in New Zealand, Europe, and Asia determining the efficacy in some rat species and numerous non-target animals. However, additional work needs to be done to determine the efficacy for black rats as achieving efficacy in that species has proven challenging. Efficacy in Norway rats captured in the United States also needs to be determined for U.S. registration. Because of the abundance of data and testing about NRB and DR8, we are hopeful for its registration as a rat specific toxicant in the United States.

Previous Research or Outreach Efforts Related to This Proposal

As highlighted in the background section, significant research in the toxicity of NRB and the development of analog DR8 has been done both *in-vitro* and *in-vivo*. This includes, but is not limited to, laboratory studies using excised cardiac and vascular tissues, caged animal studies determining efficacy of NRB and analogs, and current studies on the efficacy of DR8 in wild caught rats in New Zealand and Asia. The research proposed herein will determine the toxicity of DR8 to rats captured in the United States that are causing agricultural damage. This will inform the decisions related to the registration of DR8 for use in rat management in the United States.

Need for Research or Outreach

Though anticoagulants have been critical for the control of rodents in agricultural settings, their future is unclear. They are facing new regulations limiting use and are not effective in a variety of scenarios. To ensure that we have sustainable tools for rodent control, research on new active ingredients must be done.

Benefits to California's Agriculture

Anticoagulant rodenticides have not been effective in controlling rat populations in California citrus orchards. Also, pressure to limit or prohibit the use of anticoagulant rodenticides poses a serious threat to agricultural producers that suffer losses from rat damage both in the fields and to infrastructure. The data generated in this study will inform the future registration efforts for DR8 as a rat specific toxicant. This will benefit California agricultural producers as rat damage causes significant losses in revenue as a result of damage to both crops and infrastructure.

Budget Justification

Please see attached Budget Template

Supplemental or Matching Funds

NWRC will contribute $105,000 in the form of salary and benefits for Drs. Katherine Horak and Aaron Shiels. Drs. Horak and Shiels will develop protocols, perform dosing in experiments, analyze data, and write reports and manuscripts. They will oversee technicians trapping animals, monitoring animals during experiments, and performing logistical tasks related to the experiments.

Literature cited

1. Roszkowski AP. et al. (1964). Selective rat toxicant. Science 144: 412-413
2. Roszkowski AP. (1965). The pharmacological properties of norbormide, a selective rat toxicant. J Pharmacol Exp Ther. 149(2):288-99
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4. Rennison Bd et al. (1968). A comparative trial of norbormide and zinc phosphate against Rattus norvegicus on farms. J. hyg. Camb. 66: 147-158
5. Russell, R. U. (1965) NORBORMIDE--A RATTUS SPECIFIC TOXIC AGENT. J Forensic Sci Soc., 12: 80-3.
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7. Bova S et al. (1996). Vasorelaxant properties of norbormide, a selective vasoconstrictor agent for the rat microvasculature. Br J Pharmacol.117(6):1041-6
8. Bova s et al. (2001). Signaling mechanisms for the selective vasoconstrictor effect of norbormide on the rat small arteries. J Pharmacol Exp Ther. 296(2):458-63
9. Bova s et al. (2001). Norbormide: a calcium entry blocker with selective vasoconstrictor activity in rat peripheral arteries. Cardiovasc Drug Rev. 19(3):226-33
10. Fusi F et al. (2002). Ca(2+) entry blocking and contractility promoting actions of norbormide in single rat caudal artery myocytes. Br J Pharmacol. 137(3):323-8.
11. Bova S et al. (2003). Relaxant and Ca2+ channel blocking properties of norbormide on rat non-vascular smooth muscles. Eur J Pharmacol. 470(3):185-91.
12. Rennison D et al. (2012). Design and synthesis of prodrugs of the rat selective toxicant norbormide. Bioorg Med Chem. 20(13):3997-4011
13. Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol. Econ. 52:273 – 288
14. OPPTS Guideline 870.1100. https://ntp.niehs.nih.gov/iccvam/suppdocs/

Project Title:	Efficacy and palatability testing of a novel rat specific toxicant
Project Leader(s):	Katherine Horak and Aaron Shiels

		2019-2020	2020-2021	2021-2022	Total
A.	**PERSONNEL (name, role, % based on full time salary)**				
	Salary				
	2 NWRC technicians (trapping, animal monitoring, 15%)	$38,602.00	$31,603.00		$70,205.00
	Tyler Cochran, Danike Spock				$0.00
					$0.00
					$0.00
	Salary Total	$38,602.00	$31,603.00	$0.00	$70,205.00
	Benefits				
	NWRC technicians	$9,650.00	$7,901.00		$17,551.00
					$0.00
					$0.00
					$0.00
	Benefits Total	$9,650.00	$7,901.00	$0.00	$17,551.00
	Personnel Cost (A)	$48,252.00	$39,504.00	$0.00	$87,756.00
B.	**OPERATING EXPENSES**				
	Supplies				$0.00
	Equipment				$0.00
	Travel	$12,600.00	$700.00		$13,300.00
	Professional/Consultant Services(Cannot exceed $65/hour)				$0.00
	Other				$0.00
	Operating Cost (B)	$12,600.00	$700.00	$0.00	$13,300.00
	TOTAL Costs (A+B)	$60,852.00	$40,204.00	$0.00	$101,056.00
C.	Indirect Costs (Cannot Exceed 10% of Total Costs (A+B))	$6,085.00	$4,020.00		$10,105.00
	TOTAL CDFA FUNDING REQUESTED (A+B+C)	$66,937.00	$44,224.00	$0.00	$111,161.00
D.	**OTHER FUNDING SOURCES**				
					$0.00
					$0.00
					$0.00
					$0.00
					$0.00
	TOTAL OTHER FUNDING (C)	$0.00	$0.00	$0.00	**$0.00**
	TOTAL PROJECT BUDGET (A+B+C+D)	$66,937.00	$44,224.00	$0.00	$111,161.00