PROJECT REPORT:

Project Title: Using Liver Microsomes to Screen Anticoagulant Inhibitor Formulations for Meadow Vole Control

Research Agency: National Wildlife Research Center

Principal Investigator: Katherine Horak

Budget: $72,050.00

Background:

This study is designed to assess and/or screen for the increase in efficacy of anticoagulant rodenticides fortified with potential inhibitors to increase the efficacy of rodenticide baits to control meadow vole populations. The synergistic effect between antibiotics such as tetracycline and an anticoagulant rodenticide such as diphacinone or chlorophacinone can produce more effective and safer products for pest rodent control in California. This study is the next step to potentially develop more efficient anticoagulant baits for meadow vole control with inhibitors more effective than tetracycline.

Resistance to anticoagulants such as warfarin and chlorophacinone by rodents has been observed in various locations around the world. This resistance has been linked to enzyme activity which is carried out in the liver microsomes (Lasseur et al, 1999). Initial liver microsomes experiments with meadow voles, Wistar Norway rats, Norway rats, and Bobwhite quail microsomes incubated with chlorophacinone, diphacinone, and warfarin have been completed. Wistar Norway rat microsome experiments with diphacinone have yielded data very similar to live animal exposure trials as can be observed in figures 1 and 2.

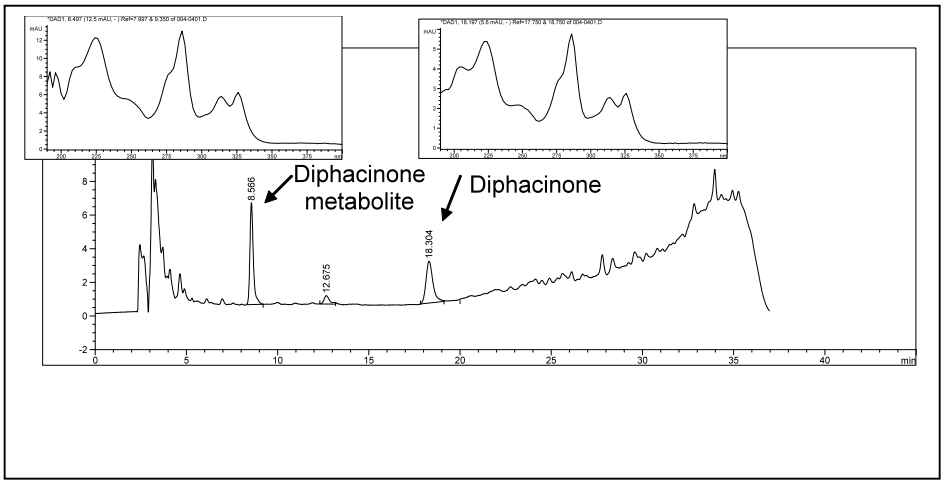

Figure 1. Rat liver microsomes treated with diphacinone and incubated for 3 hours at 37 °C. The UV spectra for the diphacinone and the diphacinone metabolite are virtually identical and verify the presence of the diphacinone metabolite.

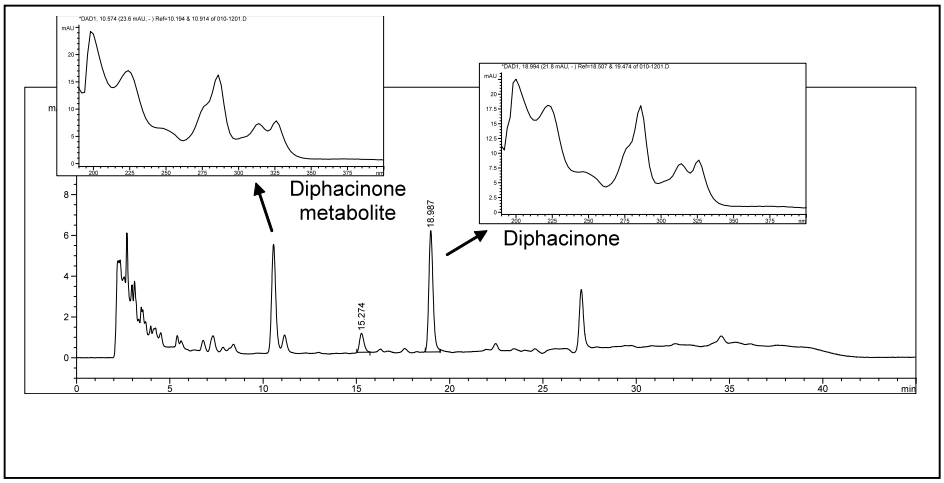

Figure 2. Liver tissue extract from a rat treated with diphacinone oat bait in a laboratory efficacy trial. The UV spectra for the diphacinone and the diphacinone metabolite are virtually identical and verify the presence of the diphacinone metabolite.

We have also demonstrated that *in-vitro* liver microsome experiments can be used to assess anticoagulant resistance and the effects of inhibitors on metabolic activity. The previously funded VPCRAC study (Primus et al., Final Report 2006) demonstrated that combining tetracycline with reduced levels of diphacinone yielded increased efficacy in rodent species while reducing diphacinone residues in the carcasses. Current studies have supported these findings by demonstrating that the addition of tetracycline to incubations with chlorophacinone was observed to inhibit the metabolism of diphacinone to make it more effective against Wistar Norway rats. Additional studies with lower levels of diphacinone combined with tetracycline produced equivalent efficacy with diphacinone residue reductions between 30 to 40% in carcasses (Primus et al., Final Written Report Pending, 2008).

In-vitro experiments can generate data much more efficiently and multiple interactions can be studied much more effectively than with live animal studies (Obach, 1999). By screening inhibitors to the cytochrome p450 enzymes responsible for the majority of toxicant metabolism it was found that cytochrome P450 3A4 is responsible for the primary metabolism of chlorophacinone. The chemicals that act as inhibitors for the metabolism chlorophacinone are ketoconazole and fluvoxamine maleate. Fluvoxamine maleate appears to be more effective than ketoconazole and is water soluble. A more interesting group of inhibitors are the juices from fruits grapefruit and pomegranate. The inhibition is more efficient from both of these juices compared to fluvoxamine maleate. Being natural product these juices may be easier to register than a specific chemical such as fluvoxamine maleate and tetracycline. Additionally, inert products such as citrus meal or citrus pulp may have similar activity and can be added to formulated products with minimal registration requirements (EPA Inert Ingredients Eligible for FIFRA 25b Pesticide Products).

Additionally, it may be possible to prebait with the inhibitor since some inhibitors are not consumed during the enzymatic reaction metabolizing the anticoagulant rodenticide. If this is true for the inhibitors we will be testing, than a prebait with the inhibitor can be followed by the anticoagulant bait with lower levels of anticoagulant without the inhibitor.

Prior to any field efficacy trials to definitively prove efficacy against resistant meadow voles a set of laboratory trials need to be completed. The impact of inhibitors on anticoagulant metabolism will be evaluated with live voles collected in the Castroville region at multiple locations. Once these studies are completed the best combination of anticoagulant and inhibitor will be assessed for registration.

In this study, meadow voles will be trapped and transported to our facility in Fort Collins, Colorado. These voles will be used to assess formulated baits containing the inhibitors in Table 1 with the anticoagulant chlorophacinone.

Table 1. Inhibitors to be Tested.

Fluvoxamine

Grapefruit juice

Pomegranate juice

Orange juice

Citrus pulp

Citrus meal

Citrus pectin

Anticoagulant resistance appears to be increasing with various rodent species within California and around the world. New and innovative tools to evaluate and solve this problem are required. Inhibitors that are water soluble and more easily registered need to be investigated to deal with anticoagulant resistance and reduce potential secondary hazards.

Evaluation of anticoagulant/inhibitor formulations with anticoagulant resistant meadow voles will be completed in order to provide a recommendation for a product that can be field tested within the state of California.

Objectives:

The synergism between anticoagulants and inhibitors can be evaluated to assess their impact on efficacy against meadow voles.

A set of animals will be trapped in the field and transported to Colorado. These animals will be exposed to a formulation containing an anticoagulant and inhibitor to determine if efficacy is comparable to or better than current registered products as well as reducing residues in carcasses.

Progress To Date:

Last Updated:

01/22/2011