PROJECT REPORT

Project Title: Using Liver Microsomes To Screen Anticoagulant/ Antibiotic Formulations For Ground Squirrels and Pocket Gophers

Research Agency: National Wildlife Research Center

Principal Investigator: Thomas M. Primus

Budget: $55,055.00

Background:

Broadcast application of 0.005% chlorophacinone and 0.005% diphacinone SRO bait for ground squirrel control is essential to repress damage to crops and rangeland grasses in California. One of the main concerns is the potential for secondary hazards to non-target species that may scavenge on the carcasses of target species. In field studies with the application of 0.005% and 0.01% diphacinone baits, average residues in California ground squirrels and pocket gophers were 0.397 and 0.280 ppm, respectively (Salmon et al., 2002; Matschke et al., 1999;). When pocket gophers were fed 0.01% diphacinone bait in a controlled environment, the average diphacinone residue was 0.463 ppm for 11 animals (Primus, 2004). Residues were as high as 1 to 3 ppm diphacinone for all of these studies.

The synergistic affect of antibiotics such as tetracycline and erythromycin with anticoagulant drugs is well documented (Raasch, 1987; Bint and Burtt, 1980). During a study to assess the use of tetracycline as a biomarker in rodent baits on Hawaii, several of the test animals expired (Sugihara, 1998). For example, the control bait containing 1.00% tetracycline and extremely low traces of diphacinone (0.0001%) resulted in the mortality of 3 rats out of 12. Without the addition of tetracycline, no mortality would have been expected from bait with such a low concentration of diphacinone. It appears that tetracycline greatly increased the potency of the diphacinone.

Since ingested tetracycline does not concentrate in tissues and is rapidly excreted in feces, it is unlikely that addition of tetracycline to baits would increase the secondary hazards associated with consumption of carcasses by non-target wildlife (Johnston, 2004). Potential applications for tetracycline (antibiotics) baits include:

- Adding tetracycline to diphacinone and chlorophacinone baits could be used to increase the toxicity of baits for hard to control species such as Belding's ground squirrels. It is unlikely that this tetracycline addition would increase the secondary hazards associated with diphacinone or chlorophacinone baits.

- For rodent control in ecologically sensitive areas, tetracycline could be added to baits containing reduced levels (<0.005%) of diphacinone or chlorophacinone. Secondary hazards could be reduced without sacrificing efficacy towards the target species.

The previously funded VPCRAC study (Primus et al., Final Report 2006) demonstrated that combining tetracycline with reduced levels of diphacinone yielded 100% efficacy in target species while reducing diphacinone residues in the carcasses by 75% In 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 this study, liver microsome *in vitro* experiments for a ground squirrel species and pocket gophers will be used to assess anticoagulant metabolism for these rodents. These studies only require the collection of livers from trapped animals and the experiments can be completed in a fraction of the time compared to live animal studies. The impact of antibiotic synergist on anticoagulant metabolism will also be evaluated with liver microsome *in vitro* experiments. Once these studies are completed the best combination of anticoagulant and antibiotic will be tested with live animals in a controlled laboratory environment with either ground squirrels or pocket gophers.

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 in our laboratory with chlorophacinone, diphacinone, and warfarin in Wistar Norway rats, Norway rats, and Bobwhite quail have been completed. Wistar Norway rat microsome experiments have yielded data very similar to live animal exposure trials as can be observed in figures 1 and 2.

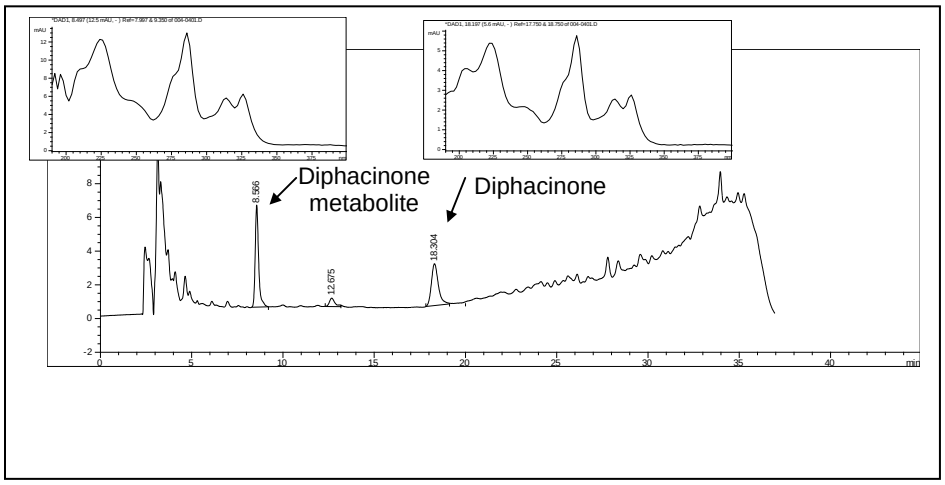

Figure 1. Rat liver microsomes treated with diphacinone and incubated for 3 hours at 37 °C. The UV spectra for the diphacinone and the diphacinone metabolite are virtually identical and verify the presence of the diphacinone metabolite.

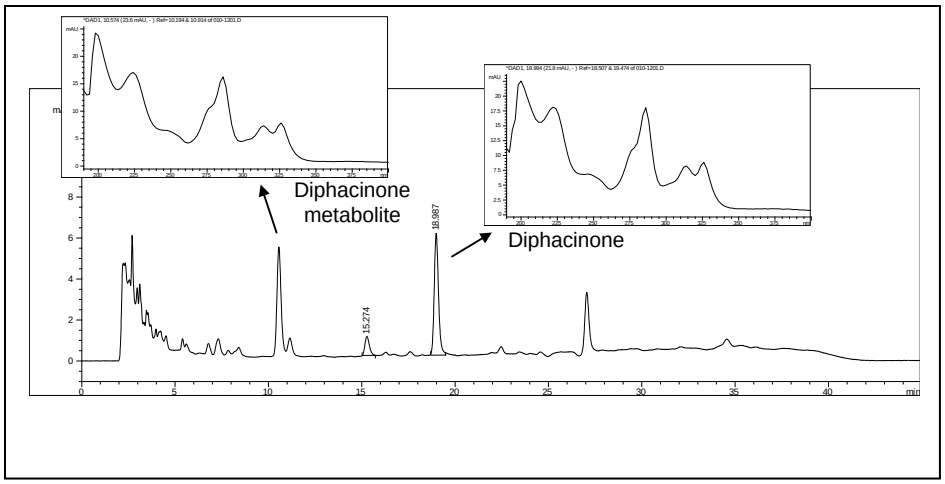

Figure 2. Liver tissue extract from a rat treated with diphacinone oat bait in a laboratory efficacy trial. The UV spectra for the diphacinone and the diphacinone metabolite are virtually identical and verify the presence of the diphacinone metabolite.

We have also demonstrated that *in vitro* liver microsome experiments can be used to assess synergist activity. The addition of antibiotics such as tetracycline have changed metabolism by as much as 4 times at levels observed in Wistar Norway rats dosed with tetracycline. An antibiotic such as tetracycline when combined with an anticoagulant can increase the effectiveness of the anticoagulant. The *in vitro* experiments can generate data much more

efficiently and multiple interactions can be studied much more effectively than with live animal studies.

Objectives:

We propose to trap a problem species of ground squirrels and/or pocket gophers and collect and freeze their livers. The livers will be shipped to facilities in Colorado.

We then will harvest or collect liver microsomes from both sets of rangeland rodents.

The microsomes from both sets of rodents will be used to evaluate the metabolism of both chlorophacinone and diphacinone.

The synergism between anticoagulants and agents such as antibiotics can be evaluated to assess their impact on efficacy. Based on the results of these *in vitro* experiments one of the rodents species will be selected for an *in vivo* study.

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 synergist to determine if efficacy is comparable to or better than current registered products, as well as, reducing residues in carcasses.

Progress To Date:

To Be Updated