PROJECT REPORT

Project Title: Using Liver Microsomes to assess Resistance of Chlorophacinone and Diphacinone in Meadow Voles

Research Agency: National Wildlife Research Center

Principal Investigator: Thomas M. Primus

Budget: $36,630.00

Background:

Chlorophacinone has been used for meadow vole control in central California artichoke fields for over two decades and meadow voles are showing resistance towards chlorophacinone. Genetic resistance is believed to be the major mechanism for this resistance. *In vitro* metabolism data has become an important tool for understanding pharmacokinetic data in the development of drugs for humans (Obach, 1999). Similarly, metabolic studies with meadow vole liver microsomes can be used to study this resistance using *in vitro* experiments.

Figure 1 represents the addition of diphacinone to male rat liver microsomes. After 3 hours of incubation at 37 °C, a significant amount of a diphacinone metabolite is produced. This same metabolite is produced in rat livers during *in vivo* experiments as shown in Figure 2.

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 in a rodent bait as a biomarker for rats on Hawaii several of the test animals expired. For example, the control bait containing 1.00% tetracycline resulted in the mortality of 3 rats out of 12. The Hacco bait was supposed to be a control bait containing no toxicant. This bait was analyzed by the Analytical Chemistry Project of the NWRC. The result of this analysis showed a concentration with a mean value of 1.0 ppm diphacinone in the bait. Although certainly not highly effective the 1 ppm diphacinone with 1% tetracycline did produce 25% mortality.

This study is a cooperative agreement with the California Vertebrate Pest Control Research Advisory Committee (CVPCRAC - through the CDFA) and is designed to assess and/or screen for the increase in efficacy of an antibiotic fortified rodenticide and evaluate the potential for new methods of control. This information will be used to potentially develop more efficient anticoagulant bait.

Additionally, *in vitro* liver microsome experiments can produce data from multiple species to provide pharmacokinetic data that can be used to develop models for safe and effective management tools in lieu of live animal studies.

Meadow voles are more resistant to chlorophacinone than when anticoagulant baits were first used to control their numbers over twenty years ago. It is believed that this resistance is genetic due to the pattern of use.

Meadow voles in central California have had their blood clotting ratios (BCR) measured to confirm their resistance to chlorophacinone. Liver microsomes experiments can yield data that may help to solve the problem.

Resistance of meadow voles to anticoagulants via liver microsome experiments to prove that monitoring metabolism can be a useful tool in predicting resistance to anticoagulants in other areas or species and possibly solving the problem.

Objectives:

We propose to trap meadow voles from resistant and non-resistant populations and transport them to facilities in Colorado.

We then will harvest or collect liver microsomes from both sets of meadow voles.

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

This resistance can be evaluated and the synergism between anticoagulants and agents such as antibiotics can be evaluated to assess their impact on resistance.

Additionally, *in vitro* liver microsome experiments can produce data from multiple species to provide pharmacokinetic data that can be used to develop models for safe and effective management tools in lieu of live animal studies.

Progress To Date:

To Be Updated.