“Developing Molecular Biomarkers to Assess Chlorantraniliprole and Imidacloprid Impacts in Aquatic Species”

Mid-Term Status Report for

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For the California Department of Pesticide Regulation

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Executive Summary

Chlorantraniliprole (CHL) and imidacloprid (IMI) are emerging insecticides in California with novel mode of actions, and unknown sublethal impacts on aquatic invertebrates and fish. In this project, the overall **goal** is to develop novel molecular biomarkers that can evaluate the impact of environmentally relevant CHL and IMI exposure in aquatic invertebrates (*Daphnia magna, Chironomus dilutus*, and *Hyalella azteca*) and one fish species (fathead minnow). A secondary goal is to determine whether changes in subcellular molecular pathways correlate to insecticide activity at the corresponding RyR and nAChR receptors.

Here we report about the status of the four tasks of this project to date:

1) Assessment of the subcellular impact of a range of environmentally relevant concentrations, from low to high concentrations, using specific molecular biomarkers in all four species (Organismal Exposures and Development of biomarkers):

Organismal exposures were completed in March 2017. Data is currently being analyzed and will be presented in the next report. Surviving organisms were snap-frozen in liquid Nitrogen and stored at -80°C before isolation of RNA. Total RNA was extracted from homogenates using the Qiagen RNAeasy Plus Kit according to manufacturer's guidelines. RNA concentrations were determined using a NanoDrop ND1000 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE). Complementary DNA (cDNA) was synthesized and stored at -20°C. For following qPCR, gene-specific primers are currently being designed and will be used to measure each gene for each species to quantitatively compare the effects of exposure to CHL and IMI on the expression of a suite of CHL- and IMI responsive genes. In a next step, differential expression will be analyzed using ANOVA and multivariate analyses, along with unsupervised clustering approaches (e.g., hierarchical clustering and principal components analysis). Genes with altered expression will be used as biomarkers for environmentally relevant chemical exposures.

2) Validation of biomarkers using ambient water samples:

This exposure is scheduled to be conducted in mid-August after coordination with DPR staff. Surviving organisms will be processed as described in task 1 to validate the developed biomarkers. Analytical water chemistry of the ambient water samples will be provided by the Department of Pesticide Regulation which will analyze the water samples for a suite of pesticides including IMI and CHL.

3) In-Vitro Assessments:

Receptor based assessments addressing CHL activity toward the ryanodine receptor of *Daphnia, Chironomid. Hyallela* and *Pimephales* have been completed using [^{3}H]Ryanodine binding assays. The species specific concentration response curves are currently being developed and from preliminary evaluations *Chironomid* appear to be the most sensitive to CHL activity, where levels as low as 10 nM (4.8 ppb) CHL cause a 250% over-activation of the ryanodine receptor. Due to these evaluations, lower concentrations of CHL, into the pM (ppt) range, are currently being assessed to fully establish the concentration that lacks activity in *Chironomid.* Tissues preparations needed to assess IMI activity toward the nicotinic acetylcholine receptor of each species are currently underway. After completed, [^{3}H]IMI

assay conditions will be developed for each species because ideal buffer solutions (e.g. pH) or running conditions (e.g. temperature) will likely vary slightly by species. The [^{3}H]IMI assays will then be conducted to evaluate a full concentration response curve, in two separate protein preparations, for each species. Response curves, developed using non-linear regression, will be used to establish effect concentrations able to cause a 50% (EC50) change in receptor activity, as well as, the lowest concentration that lacks activity toward the receptor of each species. Findings will be combined with organismal exposure data to fully define species sensitivity to CHL or IMI.

4) Dissemination of information and outreach/education:

A final report and a presentation on our results are expected to be submitted to DPR by January 31, 2018.