

Use of genomic technologies and isotonic dose• response modeling in the development of a biochemical marker of effect for pyrethroid insecticides

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Pyrethroids are pesticides that disrupt nervous system function in both target (insects) and non• target species. These compounds exert their neurotoxic effect through prolongation of sodium current through voltage• sensitive sodium channels (VSSC) present in excitable nerve membranes. The use of pyrethroids has increased as the scientific and regulatory community became increasingly aware of the adverse toxicological outcomes produced by exposure to other groups of pesticides: i.e. carbamates and organophosphates. Risk decisions concerning the latter two groups of compounds were made on based on data from studies measuring a biochemical marker of effect (i.e. acetylcholinesterase inhibition). This endpoint is sensitive, dose• responsive and readily extrapolated to human populations. A pyrethroid specific biochemical marker of effect with these properties is not available. A biochemical marker of effect for pyrethroids with these properties is needed that can be measured in vitro in a high• throughput manner. Developing this type of endpoint will aid the risk analysis process by providing a rapid quantitative assay for use in characterizing the effects of mixtures of pyrethroids in the context of cumulative risk.

The goal of this project is to use genomics technologies, quantitative real• time PCR and protein expression to identify and characterize dose• responsive biochemical markers of effect that are components of biological pathways that are perturbed by the action of pyrethroids on nervous system tissue in vivo. Three cohorts of animals were used in this study. Global gene expression profiles for the first cohort were created using Affymetrix Rat 230 2.0 microarrays hybridized with labeled cRNA derived from the frontal cortex of rats dosed with either permethrin (PERM) or deltamethrin (DLT). Three equipotent doses for each compound were selected based on motor activity data from Wolansky et al. (2006): ED30 (n = 8), NOAEL (n = 8) and sub• NOAEL (n = 8). The sub• NOAEL dose was selected based on the ratio of ED30 / NOAEL values (PERM: 1, 10, 100 mg/kg – DLT: 0.3, 1, 3 mg/kg). A second cohort (n = 7 / group) was treated with the same dosing scheme as the first and were used in follow• up qRT• PCR assays. Samples were collected 6 hours after dosing in each case. Data from the first cohort were used to identify potential dose• responsive biomarkers of effect for confirmation by qRT• PCR in both the first and second cohort of animals. A third cohort is comprised of animals dosed with either 100.0 mg/kg permethrin, 3.0 mg/kg deltamethrin or vehicle (n = 8 / group) and sampled at 1, 3, 6, 9 or 24 hours. Tissues from the third cohort were used to examine the time course of biomarker expression at both the transcriptional (qRT• PCR) and translational level (Western blot).

Identification and prioritization of potential biochemical markers of effect was performed using a novel combination of statistical methods. An isotonic regression statistic (the M• score, Hu et al. 2005) was used to identify transcripts in the microarray study that display significant dose• related alterations in expression for each model compound. This method assumes a monotone dose• response relationship, but otherwise makes no assumptions about the precise form of the dose• response curve. We used this approach in conjunction with permutation calculations of false discovery rates (FDR) as outlined in Storey and Tibshirani (2003) to provide multiple• comparison error control. Potential biomarkers to be examined by qRT• PCR will be

prioritized based on the strength of the dose• response relationship, commonality of the profiles between the two model compounds, and estimated FDR. A subsequent analysis (SAFE: Significance Analysis of Function and Expression, Barry et al., 2005) will be used to determine if any functionally correlated groups of genes are altered by pyrethroid exposure. This permutation• based method interrogates an ordered list of transcripts (sorted by the isotonic regression M• score) to find functional categories (e.g., Gene Ontology) with an overrepresentation of significant genes. Identification of significant functional categories will provide guidance on biological pathways appropriate for the development of in vitro models of pyrethroid toxicity. Preliminary results indicate that a small number of transcripts display similar dose• responsive characteristics for both of the model compounds. These include Ca²⁺/calmodulin dependent protein kinase 1g (Camk1g), corticotrophin releasing hormone (Crh), homer1, Nedd4L and p• glycoprotein (Abcb1a). While alterations in the expression of these transcripts are significantly dose• related the magnitude of the alterations are small in magnitude (< 1.5• fold). For a majority of candidates the dose• response appears to be linear with alterations in expression present even at the NOAEL dose. Several of these targets have been confirmed using Taqman® qRT• PCR gene expression assays in both the first and second cohorts. Results are comparable between technologies and between cohorts concerning both the magnitude of change across doses and shape of the dose• response. Camk1g appears to have the largest and most consistent alteration in expression.

In summary, we have identified several candidate biomarkers for pyrethroid effects based on the in vivo activity of these compounds in the mammalian nervous system. The reliability and specificity of the most robust biomarker will be examined following exposure to a variety of pyrethroid compounds as well as compounds outside the pyrethroid pesticide class, respectively. These studies are the first step in the development of a sensitive, low• cost, high• throughput, in vitro model of pyrethroid toxicity that may be used to provide data for risk assessment decisions concerning the potency of these compounds at the molecular level.