Dose-response Modeling of Microarray Data to Identify a Biochemical Marker of Effect for Pyrethroid Insecticides after Acute Exposure In Vivo: Specific Upregulation of CAMK1g1

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Keywords: dose response, pyrethroids, microarray, quantitative regression, isotonic regression

Pyrethroid insecticides produce neurotoxicity in mammals by interacting with a number membrane bound ion channels in excitable nerve membranes. Pyrethroid use has increased as the use of other pesticide compounds has declined. There is a dearth of information concerning the intracellular response of neurons following the interaction of pyrethroids with these molecular targets. Nor is a sensitive, specific, dose-responsive biomarker of effect available that correlates with pyrethroid-induced disruption of nervous system function. This study used a combination of statistical regression methods to identify dose-responsive transcripts from a microarray data set. The goal is to identify and characterize potential biochemical markers of pyrethroid effects. Long-Evans rats (n = 8-12/group) were acutely exposed via oral gavage to corn-oil vehicle, permethrin (1, 10, 100 mg/kg), or deltamethrin (0.3, 1.0, 3.0 mg/kg) and cerebrocortical tissue was collected at 6 hours post-exposure. Affymetrix Rat 230 2.0 GeneChip® microarrays were used to obtain transcriptional profiles. Two methods for identifying dose-responsive transcripts were compared: a quantitative regression method (Significance Analysis of Microarrays), and an isotonic regression model that assumes a monotonic dose-response relationship, but otherwise makes no assumptions about the precise form of the dose-response curve (M-score, Hu et al. 2005). Permutation-based calculations of false discovery rates were used in both cases to provide multiple comparison error control. Quantitative regression provided a superior estimate of false discovery rates compared to isotonic regression. These analyses revealed several candidate transcripts that respond in a dose-related fashion for both compounds. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) confirmed a dose-responsive increase in the expression of Ca+2/calmodulin dependent protein kinase 1-gamma (Camk1g), and decreases in the expression of prominin 1 and dopa decarboxylase (Ddc), for both deltamethrin and permethrin. Use of Camk1g splice variant specific probes demonstrated an effect restricted to the Camk1g1. A qRT-PCR time course study of Camk1g and Ddc expression (1, 3, 6, and 9 hours) confirmed the previous findings and demonstrated peak times of effect between 3 and 6 hours post-exposure. This work has identified several potential biomarkers of effect for pyrethroids in the mammalian central nervous system.

This abstract does not necessarily reflect the policy of the U.S. Environmental Protection Agency.

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