Linking Dose to Effect Research for Pyrethroid Insecticides

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Contributing Organizations: ORD: NHEERL NERL; NCCT; Office of Pesticides and Toxic Substances

LTG 2 Poster 06

Science Questions

The research addresses the following key science questions:

• Can physiologically-based pharmacokinetic models be constructed that link tissue dose to neurological effects for pyrethroid insecticides?

• What are the critical pharmacokinetic parameters needed to build such models and extrapolate between animals and humans?

• What is the appropriate dose-metric for pyrethroid insecticides?

• Can relative potency data from both *in vitro* and *in vivo* endpoints be used to determine the additivity or non-additivity of pyrethroid effects?

• Can this science and tools be used to inform cumulative neurological risks to pyrethroid pesticides?

Research Goals

• To better understand the metabolism and mode of action of pyrethroids

• To identify and characterize dose to effects linkages

• To determine whether or not the effects of pyrethroid mixtures are additive

Findings and Conclusions

Pyrethroids are metabolized by oxidative and hydrolytic processes. However, there are chemical- and species-specific differences in their metabolism. For example, deltamethrin is metabolized in vitro by rat hepatic P450s and esterases. In humans, deltamethrin is metabolized in vitro by hepatic esterases but not hepatic P450s.

Momentary brain concentrations correlate with the behavioral effects of pyrethroids

PBPK models for deltamethrin and permethrin can predict blood and brain concentrations of these pyrethroids in rats and humans

RPF values have been determined for several measures both in vivo and in vitro. These RPFs will inform the risk assessment by providing a basis for testing the additivity of mixtures. The effects of pyrethroids on motor activity in the rat are consistent with dose-additivity. Additional mixture studies using in vitro endpoints are in progress.

Laboratory data on the pharmacokinetics and neurotoxic effects of pyrethroids have been developed that can be used in exposure-dose-response models which will aid OPP in their risk assessment of these pesticides

Pharmacokinetic Modeling

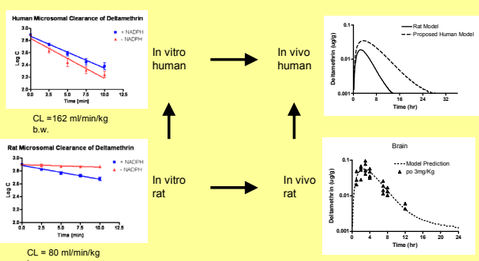
1) Analytical Methods

Using HPLC tandem mass spectrometry, parent pyrethroid can be detected at the part per trillion level in several tissues of treated rats and microsomal incubations following extraction

Chemical	Blood	Brain	Fat	Liver
Permethrin-trans	110	155	58	126
Permethrin-cis	76	91	98	72
Cypermethrin	111	160	128	140
Esfenvalerate	163	233	207	192
Cyfluthrin	171	219	143	287
Deltamethrin	150	210	130	68

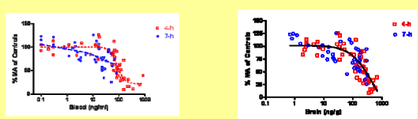
2) Acquisition of pharmacokinetic and dosimetry data for model development and extrapolation

In vitro studies were conducted with rat and human hepatic microsomes to determine clearance values for the pyrethroids. In vivo studies were conducted to determine the tissue time course of orally administered deltamethrin and permethrin as well as the oral bioavailability of deltamethrin in the rat. PBPK models for deltamethrin and permethrin were developed that can predict blood and brain concentrations of these pyrethroids.



3) Correlation of pyrethroid tissue dose to neurotoxic effect

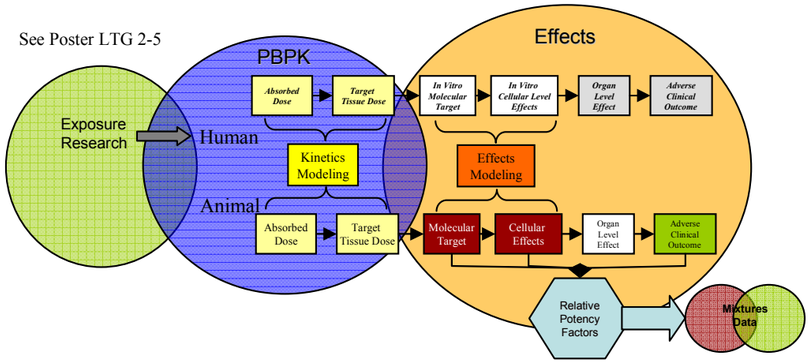
Rats were administered bifenthrin orally. Rat motor activity was assessed at 4 or 7 h and the rats were sacrificed. Blood and brain were removed and analyzed for bifenthrin.



4) Dermal absorption of pyrethroids

In vitro dermal absorption studies (24 hr) with pyrethroids were done with rat and human cadaver skin. In vivo studies (24 hr) were done in rats.

Methods/Approach

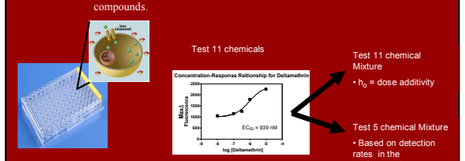


Molecular and Cellular Effects:

Understanding Relative Potencies and Mixtures

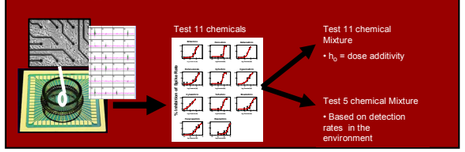
1) Pyrethroid Effects on Sodium Flux into Neurons

Using the sodium sensitive dye and an Ionworks™ device, cellular actions of pyrethroids, and their relative potencies for those effects can be determined. The approach is high-throughput and allows rapid assessment of multiple compounds.



2) Pyrethroid Effects on Firing Rate in Neuronal Networks

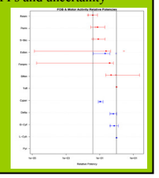
Using the cortical neurons grown on multi-electrode arrays (MEAs), actions of pyrethroids on spontaneous firing rates in networks of interconnect neurons can be determined. We have determined the relative potency of 11 different pyrethroids on firing rate as well as the effects of mixtures



Adverse Outcomes: Determination of RFPs and uncertainty from data submitted by registrants

Principal component analyses on behavioral screening data submitted to the Agency by registrants was used to differentiate pyrethroid effects into the two clusters – T and CS activities.

ORD/NHEERL scientists converted behavioral data into severity scores along these dimensions, providing another means of determining RPFs on the basis of behavioral outcomes. In addition, ORD/NCCT scientists determined the confidence limits surrounding those data.



Relative Potency Data

The table below indicates the relative potencies for pyrethroid effects on in vitro and in vivo endpoints. Deltamethrin is used as the index chemical

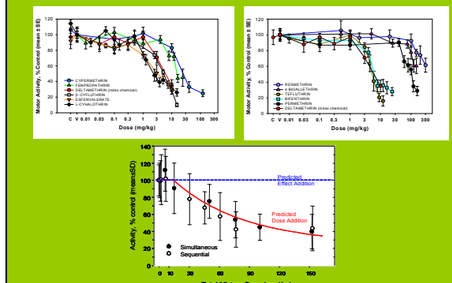
Na Flux (SBFI)	Rel Potency	Firing Rate (MEAs)	Rel Potency	Motor Activity	Rel Potency	FOB	Rel Potency
Deltamethrin	1	Cyhalothrin	15.667	Esfenvalerate	2.090	Cyhalothrin	1.620
Cyhalothrin	0.670	Cypermethrin	1.541	Cyhalothrin	1.900	Tefluthrin	1.350
Esfenvalerate	0.524	Deltamethrin	1.000	Cyfluthrin	1.140	Cyfluthrin	1.150
Cyfluthrin	0.520	Bifenthrin	0.470	Tefluthrin	1.110	Bifenthrin	1.010
Bifenthrin	0.388	Cyfluthrin	0.465	Deltamethrin	1.000	Deltamethrin	1.000
Bioallethrin	0.376	Tefluthrin	0.316	Bifenthrin	0.780	Esfenvalerate	0.358
Tefluthrin	0.329	Esfenvalerate	0.287	Fenpropathrin	0.330	Cypermethrin	0.190
Cypermethrin	0.329	Fenpropathrin	0.189	Cypermethrin	0.230	Bioallethrin	0.108
Fenpropathrin	0.246	Permethrin	0.157	Permethrin	0.060	Permethrin	0.093
Permethrin	NE	Bioallethrin	0.141	Bioallethrin	0.030	Fenpropathrin	0.050
Resmethrin	NE	Resmethrin	0.010	Resmethrin	0.009	Resmethrin	0.049

NE; No Effect on the endpoint

Adverse Outcomes: Neurobehavioral Studies

A series of behavioral studies were conducted that:

1. Characterized extensive dose-response functions for individual chemicals for two endpoints, motor activity (11 chemicals) and thermoregulation (5 chemicals; data not shown) for use in the dose-addition model.
2. Tested the hypothesis of dose-addition for two mixtures using motor activity. The mixing ratios were based on either RPFs (11 chemicals), or environmental occurrence (5 chemicals).



1. Single and multiple compound exposure to pyrethroids produced dose dependent depression of activity.
2. Empirical data from the mixtures studies showed no evident departure from that predicted by the dose-additivity model.
3. Mixtures composed of sub-threshold concentrations of individual pyrethroids were effective at reducing motor activity.

Impact and Outcomes

Impact and Outcomes:
The enhanced science and products of this research will reduce uncertainty in risk assessment/management by:

• Providing OPP with enhanced science knowledge, data, and models that can be used to inform the development of the 2011-2012 pyrethroid CRA
• Providing OPP with ORD evaluated PBPK models for pyrethroids that predict target tissue levels in animals and aid in extrapolation between animals and humans.
• Providing OPP with an improved understanding of the relationship between pyrethroid brain concentrations and behavioral effects, increasing ORD's and OPP's confidence that the PBPK models are appropriately structured to predict the most suitable dose metric.
• Providing OPP relative potency factors for molecular, cellular, and behavioral endpoints that allow for rank potency determinations as well as determinations of additivity informing the pyrethroid CRA
• Providing OPP with data characterizing pyrethroid dose response in vivo and in vitro at whole animal and target sites informing the pyrethroid CRA
• Providing OPP with data that test dose additivity for pyrethroids, a major assumption in CRA.
•Providing the enhanced science and ORD products to the scientific community through peer reviewed publications.

Future Directions

Much of the pyrethroid-specific work for this project has been completed, and research on this class of chemicals will be declining. The following projects are ongoing or planned for the future:

• PBPK models for bifenthrin, cypermethrin and cyfluthrin will be developed.
• Evaluation of human neuroprogenitor cells grown on MEAs for spontaneous firing activity. If models are established, concentration-response curves for a limited set of pyrethroids will be established to aid in animal to human extrapolation at the toxicodynamic level.

This approach will continue to be incorporated into upcoming cumulative risk assessments for pesticides and other classes of compounds.

Researchers Involved

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Cumulative Risk