were quantified in blood, whereas TCP was detected in saliva and only at higher doses. Although the saliva TCP concentrations were significantly less than the plasma concentrations, the TCP pharmacokinetics were comparable (i.e. similar half-life). These results suggest that saliva may be a useful biological matrix for monitoring CPF exposure and response either through measuring the metabolite levels or the degree of ChE inhibition. These data will be used for further validation of an already constructed pharmacokinetic/pharmacodynamic model for CPF. (Sponsors EPA grant R828608 and CDC/NIOSH R01OH03629-01A2).

1485 DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC AND PHARMACODYNAMIC (PBPK/PD) MODEL FOR THE ORGANOPHOSPHATE PESTICIDE, DIAZINON.

T. S. Poet, A. Kousba, H. Wu, S. L. Dennison and C. Timchalk. *Molecular Biosciences, Pacific Northwest National Laboratory, Richland, WA.*

Organophosphate (OP) pesticides, like diazinon (DZN), constitute a large class of insecticides that are widely utilized, and the potential exists for significant exposures from multiple routes. The objective was to develop a PBPK/PD model capable of predicting the relationships between exposure, bioactivation, detoxification, and the inhibition of target esterases (EST). In this model, CYP450 metabolism of DZN to the oxon and detoxification to 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP) are both mediated by CYP450s in the liver. Hydrolysis of the oxon *via* A-EST occurs in the liver and blood and interactions with target B-EST (acetyl-, butyryl- and carboxyl-) were modeled as second order processes occurring in the liver, blood, diaphragm and brain. Metabolic rate constants for the CYP450- and A-esterase-mediated metabolism were measured *in vitro*. B-EST inhibition and regeneration rates have been determined *in vitro* and model optimization against cholinesterase (ChE) inhibition data. To facilitate model development, single oral-dose pharmacokinetic studies were conducted in rats (1 - 100 mg/kg) and the kinetics of DZN and IMHP as well as the extent of plasma ChE and RBC and brain acetylcholinesterase (AChE) inhibition were determined. In blood, the concentration of IMHP was greater than DZN and the kinetic time-course was linear over the dose-range and reasonably simulated by the model. Peak ChE inhibition occurred at ~6 hr post-dosing and the model accurately simulated the dose-dependent inhibition of plasma ChE, RBC AChE and brain AChE. This DZN PBPK/PD model quantitatively estimates target tissue dosimetry and ChE inhibition and will be integral to risk assessments for DZN and OP mixture exposures under a variety of scenarios. (Sponsored by CDC/NIOSH Grant R01 OH03629-01A2).

1486 MASS SPECTRAL EVIDENCE THAT MIPAFOX-INHIBITED NEUROPATHY TARGET ESTERASE (NTE) DOES NOT UNDERGO DEALKYLATION.

T. J. Kropp[1], P. Glynn[2] and R. J. Richardson[1]. *[1]Environmental Health Sciences, University of Michigan, Ann Arbor, MI and [2]MRC Toxicology Unit, University of Leicester, Leicester, United Kingdom.*

Organophosphorus compound-induced delayed neurotoxicity (OPIDN) is thought to be initiated by inhibition and aging of neural NTE. Aging of phosphylated NTE and other serine esterases involves progressive resistance to reactivation, attributed to anion formation *via* time dependent dealkylation of the active site OP adduct. Because N, N? diisopropylphosphorodiamidofluoridate (mipafox, MIP) inhibits NTE and produces OPIDN, it has been assumed that MIP inhibited NTE undergoes aging *via* dealkylation. Recent work, however, showed that MIP-inhibited NTE could be reactivated at low pH after allowing time for aging. In contrast, diisopropylphosphorofluoridate (DFP) inhibited NTE and DFP- or MIP inhibited butyrylcholinesterase (BChE) could not be reactivated after allowing time for aging. These observations suggest the hypothesis that DFP- or MIP-inhibited BChE and DFP-inhibited NTE undergo aging *via* dealkylation, whereas MIP-inhibited NTE does not. This hypothesis was tested by inhibiting horse serum BChE or human recombinant NTE esterase domain (NEST) with MIP or DFP. Using peptide mass mapping with surface enhanced laser desorption/ionization mass spectrometry, *m/z* peaks corresponding to active site peptides and their intact or dealkylated adducts were examined in control and treated samples at 0, 1, 2, 12, 24, and 36 h after inhibition. Time-dependent mass shifts representing a change from intact to dealkylated active site adducts were found for MIP- and DFP-inhibited BChE. Moreover, a peak corresponding to dealkylated active site adduct was found at all times for DPF inhibited NEST. In contrast, a peak representing intact active site adduct was found at all times for MIP-inhibited NEST, showing that dealkylation did not occur. The results suggest that MIP produces OPIDN through a mechanism other than dealkylation of MIP-inhibited NTE. If an anionic active site adduct is required, it is possible that this arises from removal of the acidic phosphoramido proton. (Supported by DAAD19-02-1-0388).

1487 TENTATIVE MODELS FOR THE THREE-DIMENSIONAL STRUCTURE OF THE NTE ESTERASE DOMAIN (NEST): PREDICTIONS FROM THREADING AND DOCKING.

J. Wang and R. J. Richardson. *Environmental Health Sciences, University of Michigan, Ann Arbor, MI.*

Neuropathy target esterase (NTE), the primary target for initiation of organophosphorus compound-induced delayed neuropathy, is a 1327-amino acid integral membrane protein, whose three-dimensional structure is intractable to experimental determination. Moreover, sequence analysis shows that NTE is a member of a novel protein family, so that its theoretical model cannot currently be obtained by homology modeling. The NTE esterase domain (NEST) corresponds to NTE residues 727-1216 and is the minimum NTE construct with full esterase activity. The threading program PROSPECT was employed to conduct fold recognition and sequence structure alignments for NEST. Based on the alignments obtained from threading, atomic structures of NEST were generated using the program MODELLER. Resultant models were refined in the CHARMm module of InsightII 2000. Finally, candidate structures were evaluated by docking the neuropathic compounds diisopropylphosphorofluoridate and ethyl 4-nitrophenyl phenylphosphonate as ligands into the NEST model using the Affinity module of InsightII 2000. This strategy yielded three putative structures of NEST for further study. The models were consistent with experimental data from ligand binding and site-directed mutagenesis. Namely, they predicted Ser^{966} as the active-site serine, Asp^{1086} and Asp^{960} as possible critical residues for catalysis, and Asp^{1044} or Asp^{1004} as possible acceptor residues for the intramolecular transfer of an alkyl group during aging of phosphylated enzyme. These models for NEST provide a starting point for gaining atomic-level insight into interactions of NTE with small molecules and could be further refined and validated through interative modeling and experimental validation. (Supported in part by DAAD19-02-1-0388).

1488 DECREASE OF 5-HT LEVELS AFTER PYRETHROID TREATMENT.

A. Anadon, M. A. Martínez, M. Martínez, M. J. Díaz, M. T. Frejo, V. J. Castellano and M. R. Martínez-Larranaga. *Department of Toxicology and Pharmacology, Faculty of Veterinary Medicine, Complutense University, Madrid, Spain.*

Deltamethrin, cyfluthrin and cyhalothrin, Type II pyretroid insecticides, are used topically for the control of ectoparasites. Type II pyrethroids when injected peripherally to rat produced a severe syndrome characterized by salivation and choreoathetosis. Because of a variety of putative biochemical and physiological target sites may contribute to pyrethroid toxicity, the objective of the present study was to investigate neurochemical effects following the administration of deltamethrin (40 mg/kg, i.p. for 6 days), cyfluthrin (14 mg/kg i.p. for 6 days) and cyhalothrin (8 mg/kg, per os for 6 days) in male Wistar rats(n =6/group). Animals were sacrificed 24 hours following pyrethroid administration and their brains were rapidly removed. The frontal cortex, hippocampus, midbrain and striatum were dissected and analyzed for content of 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxy-3-indole acetic acid (5-HIAA) using a HPLC method with electrochemical detection. A serotonin depleting effect was produced by these pyrethroids. Deltamethrin decreased 5-HT and 5-HIAA levels in midbrain (38%; $P<0.05$; 17%, $P<0.05$) and striatum (46%, $P<0.001$; 21%, $P<0.05$) and decreased 5-HIAA levels in frontal cortex (62%, $P<0.001$) and hippocampus (48%, $P<0.001$) respect to corn oil controls. Cyfluthrin decreased 5-HT and 5-HIAA levels in frontal cortex (25%, $P<0.05$; 30%, $P<0.01$), hippocampus (20%, $P<0.05$; 19%, $P<0.05$) and striatum (31%, $P<0.01$; 36% $P<0.01$) respect to corn oil controls. Cyhalothrin decreased 5-HT levels in frontal cortex (35%, $P<0.001$), hipoccampus (26%, $P<0.05$), midbrain (28%, $P<0.05$) and striatum (24%, $P>0.001$) and decreased HIAA levels in frontal cortex (36%, $P<0.01$) and midbrain (27%, $P<0.05$). The data presented herein suggests that a lower activity of serotonergic system exists in the action of Type II pyrethroids. This work has been supported by projects No. PB9701236, (DIGICYT), No. 08.8/0002/98 (CAM) & No. 99/0936 (FIS), Spain.

1489 CONVERSION OF DELTA PH AND ELLMAN VALUES FOR CHOLINESTERASES.

B. W. Wilson[1], J. D. Henderson[1], D. E. Arrieta[1], S. A. McCurdy[2] and R. Reitstetter[3]. *[1]Environmental Toxicology, University of California, Santa Cruz, CA, [2]Epidemiology and Preventive Medicine, University of California, Davis, CA and [3]Clinical Investigation, Brooke Army Medical Center, San Antonio, TX.*

Intensive use of anticholinergic pesticides such as organophosphate esters and threat of chemical warfare establish the need for rapid, high throughput, reliable and standardizable determinations of blood cholinesterase levels to provide early warning of exposures to neuroactive chemicals. Many clinical and research labora-