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-

tories use the colorimetric Ellman assay based on hydrolysis of acetylthiocholine. CHPPM (US Army Center for Health Promotion and Preventive Medicine) uses a slower method, the delta pH based on that of Michel to monitor approximately 25, 000 DOD personnel annually. One of the goals of this project is to establish a conversion factor between the pH and colorimetric assays applicable to monitoring studies and field tests. Blood drawn under the appropriate regulations by CHPPM was centrifuged and detergent-lysed before being subjected to the delta pH assay with acetylcholine as substrate. Duplicate RBC samples were sent to UC Davis to be assayed with acetylthiocholine by the Ellman method. Samples were lysed, diluted with buffer and run with and without quinidine to separate activities due to acetylcholinesterase and non-specific cholinesterases. For example, slopes of delta pH vs Ellman for three of five sets of samples yielded r^2 correlations of 0.74 to 0.8. Comparisons continue to establish critical assay conditions and Ellman equivalents of the delta pH assay. Supported by DOD (DAMD17-01-1-0772), NIOSH (#CDC U07/CCU906162-06) and NIEHS (#ES05707).

1490 DIFFERENTIAL PROFILES OF CHOLINESTERASE INHIBITION AND NEUROBEHAVIORAL EFFECTS IN RATS EXPOSED TO FENAMIPHOS AND PROFENOPHOS.

K. L. McDaniel, P. M. Phillips and V. C. Moser. *NHEERL, USEPA, Research Triangle Park, NC.*

The relationship between cholinesterase (ChE) inhibition and neurobehavioral changes was examined using two ChE-inhibiting organophosphorus pesticides, fenamiphos and profenophos. Both pesticides inhibit blood ChE, yet brain ChE is relatively spared (little to no inhibition up to lethal doses). Interestingly, pronounced neurobehavioral signs were observed following fenamiphos but not profenophos. A direct comparison was then undertaken to evaluate the influence of brain ChE on the behavioral signs observed. After a single oral dose, both pesticides greatly inhibited blood ChE (87-98% inhibition), yet whole brain ChE was only inhibited by 9-14% at the highest doses. Fenamiphos produced dose-dependent lacrimation, salivation, tremors, gait abnormalities, and decreased motor activity and tail pinch response. Despite the similar ChE inhibition profile, profenophos produced no changes in any of these measures. Thus, the neurobehavioral effects of fenamiphos could not be explained based on brain ChE inhibition alone. Pretreatment with anticholinergic drugs was used to evaluate the contribution of peripheral vs central ChE inhibition. Scopolamine (SCO) and methylscopolamine (MSC) were used as central/peripheral and peripheral-only cholinergic receptor blockers, respectively, in combination with fenamiphos. Neither drug altered fenamiphos effects on ChE. Some effects of fenamiphos were blocked or attenuated only by SCO, whereas other effects were blocked by both drugs. These data indicate that some of the pronounced neurobehavioral changes observed following fenamiphos may be centrally mediated (blocked by SCO only), despite the relative sparing of brain ChE. Regionally specific ChE inhibition or direct cholinergic receptor activation may be responsible for these effects. Other behavioral changes may be mediated more peripherally (blocked by both MSC and SCO), yet the contrast between profenophos and fenamiphos indicates that these neurobehavioral effects cannot be predicted on the basis of blood ChE. This abstract does not necessarily reflect EPA policy

1491 CHRONIC DIETARY EXPOSURE WITH INTERMITTENT SPIKE DOSES OF CHLORPYRIFOS FAILS TO ALTER BRAINSTEM AUDITORY EVOKED RESPONSES (BAERS) IN RATS.

J. E. Graff, D. W. Herr, R. S. Marshall and D. L. Hunter. *Neurotoxicology, USEPA, Durham, NC.*

Human exposure to pesticides is often characterized by chronic low level exposure with intermittent spiked higher exposures. Cholinergic transmission is involved in auditory structures in the periphery and the brainstem and is altered following chlorpyrifos exposure. This study examined the effects of chronic (1 year) dietary exposure (0, 1, or 5 mg/kg/day) to chlorpyrifos in male Long Evans rats (100-110 days old at study initiation) on BAERs. The chlorpyrifos doses were chosen to produce minimal and approximately 50% inhibition of brain cholinesterase activity, respectively. In addition to dietary exposure, half of the animals received an oral bolus of 45 mg/kg chlorpyrifos (in corn oil) every other month (n = 16-18 rats/treatment). Subjects were weight maintained at 350g throughout the study. After the final spiked exposure, the animals were allowed to recover for about 2.5 months, so only irreversible effects would be examined. Subjects were surgically implanted with screw electrodes over the cerebellum and allowed to recover for one week. Unanesthetized animals were placed in a restrainer and presented with the following auditory stimuli (presented at 5.6 hz): rarefaction click, 4 and 16 kHz pure tone pips presented at 50, 65, and 80 dB SPL, and 64 kHz pure tone pip presented at 65, 70 and 80 dB SPL. Dietary exposure to chlorpyrifos (alone or in combination with oral spike doses) did not produce changes in brainstem auditory evoked responses. The evoked responses showed the expected intensity and frequency-dependent changes, indicating that the animals responses were under stimulus control. Thus, chronic exposure to chlorpyrifos did not appear to alter auditory responses at the level of the brainstem in adult animals. *This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.*

1492 COMPARATIVE EFFECTS OF METHYL PARATHION (MPS) AND ITS METABOLITE METHYL PARAOXON (MPO) ON ACETYLCHOLINE (ACH) RELEASE AND MUSCARINIC AUTORECEPTORS IN JUVENILE AND ADULT RATS.

G. Wang[1,2], J. Liu[1], H. Zhang[1,3] and C. N. Pope[1]. *[1]Oklahoma State University, Stillwater, OK, [2]Baylor College of Medicine, Houston, TX and [3]University of Rochester, Rochester, NY.*

Previous studies have demonstrated that young rats are more sensitive than adults to acute toxicity of high dosage of MPS. The present study investigated the relationship between the toxicity of MPS and ACh release and its muscarinic receptor mediated regulation in juvenile and adult rat brain. AChE activity and QNB binding from the same rats and effects of MPS and MPO *in vitro* on muscarinic receptors were also evaluated. The results showed that in absence of physostigmine (PHY) and atropine (ATR) in buffers, MPO *in vitro* reduced ACh release in a concentration dependent manner (20-40% in 21 day rats, 10-40% in adult rats); in presence of PHY and in absence of ATR in buffers, MPO had no effect on ACh release in both age groups; and PHY was always included in the perfusion buffers and ATR was added 10 min before the second stimulus, MPO also decreased release as before; but MPS *in vitro* did not show any effect on the ACh release. The ChE activity in juvenile rats showed a quicker recovery by 24h and 96h than that in adult rats. Different quantitative reductions in QNB binding were noted in both age groups 24h and 96h after exposures to both dosages of MPS, by 4 hours, however, a significant reduction of QNB binding was noted in juvenile rats only. DSAR or S1 was significantly reduced 24h after LD10 exposure in juvenile striatum, but was no remarkably affected at any other timepoint or with lower dosage. Different quantitative reductions in S2/S1 release ratios were noted in both age groups 96h after exposures to both dosages of MPS, but no significant reduction was noted at other timepoints. The results suggested that MPO may have potential direct effects on muscarinic receptor function and effects of MPS on ACh release and its muscarinic receptor-mediated regulation during maturation as a possible contributing factor to age-related differences in sensitivity.

1493 PYRIDOSTIGMINE BLOCKS PARAOXON-INDUCED BLOOD-BRAIN BARRIER LEAKAGE.

X. Song[1], C. N. Pope[1], R. Murthy[2], J. Shaikh[1] and J. Bressler[2]. *[1]Physiological Sciences, Oklahoma State University, Stillwater, OK and [2]Kennedy Krieger Institute, Johns Hopkins School of Public Health, Baltimore, MD.*

Increased entry of pyridostigmine (PYR) into the brain has been hypothesized as a contributing factor to unexplained Gulf War Illnesses. Some organophosphorus (OP) cholinesterase (ChE) inhibitors can compromise blood-brain barrier (BBB) integrity, generally through elicitation of convulsions. Preliminary results suggested, however, that low-level paraoxon exposure (0.1 mg/kg, im, causing roughly 50% ChE inhibition) could increase BBB leakage as evidenced by the enzymatic marker horseradish peroxidase (HRP) in 25-30 day old Long Evans rats. We evaluated the interaction between acute paraoxon and acute PYR on HRP accumulation and brain ChE activity. PYR (30 mg/kg, po, or saline) was administered 50 minutes prior to paraoxon (or vehicle, 0.4% DMSO in saline). Rats (n=3/trt) were anesthetized (diethyl ether) 7.5 minutes later and heart was exposed for intracardiac HRP injection (40 mg/ml in 2% Evans Blue, 200 ml/rat) through the left ventricle, beginning exactly 10 min after paraoxon. One minute after HRP injection, rats were sacrificed, whole brain was dissected and placed in 2.5 % glutaraldehyde for 48 hours. Cortical (temporal and frontal) regions were sectioned at 70 mm on a vibrotome and then histochemically processed and counted for number of BBB leaks per section as evaluated by HRP staining. Brain ChE activity was measured under the same treatments except rats were not injected with HRP. Paraoxon caused a significant >4.5 fold increase in number of BBB leaks in the temporal cortex. Rats treated with PYR alone or PYR prior to paraoxon exhibited minimal leaks, similar to controls. Little brain ChE inhibition was noted after PYR exposure while paraoxon alone caused 55-58% inhibition. Interestingly, brain ChE inhibition was somewhat lower (40-42% inhibition) in rats exposed to both PYR and paraoxon. The results suggest that paraoxon-induced BBB leakage does not facilitate PYR entry, but that PYR may inhibit BBB leakage by paraoxon. (Supported by grant DAMD17-00-1-0070 from US Army)