PL 1383 INTESTINAL PERMEABILITY AND DISPOSITION OF CHLORPYRIFOS AND CHLORPYRIFOS-OXON IN THE SINGLE-PASS INTESTINAL PERFUSION IN THE RAT.

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Chlorpyrifos (CPF), a common organothiophosphate pesticide, is metabolized in mammals to the active metabolite chlorpyrifos-oxon (CPF-O), which is primarily responsible for the observed toxicity. As part of our efforts for more accurate exposure modeling, we evaluated the intestinal permeabilities of CPF and CPF-O at environmental levels using the single-pass intestinal perfusion technique with mesenteric vein sampling in rats. Tritiated CPF (25, 250 and 2500 ng/ml) and CPF-O (25 and 2500 ng/mL) were perfused through the rat ileum for 30 min, after which the perfusate was switched to blank buffer. Outlet perfusion samples and mesenteric blood samples were collected over the entire perfusion period of 60 min. Radioactivity was determined by liquid scintillation counting. The lumenal, effective permeabilities (P_e), based on perfusate concentrations, and blood permeabilities (P_{blood}), based on mesenteric blood concentrations, were calculated. CPF and CPF-O exhibited P_e values > 10^{-4} cm/s, which are correlated to high fractions of dose absorbed (i.e., > 90%) in humans. P_{blood} values were approximately 10% of P_e values. After switching the perfusate to the blank buffer system, CPF and CPF-O unexpectedly continued to appear in the blood, which suggests that the intestine was acting as a reservoir for the compounds under these experimental conditions. The high lumenal and blood permeability values and the apparent sequestering of CPF and CPF-O have implications for exposure modeling and risk assessment. Financial support (TJC): Dow AgroSciences and NIH R03CA105465, R03CA121403.

PL 1384 EARLY-LIFE ORGANOPHOSPHATE EXPOSURE DISRUPTS LIPID METABOLISM IN ADULTHOOD.

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Organophosphate pesticides are developmental neurotoxicants but new findings point to lasting effects on metabolism, abnormal weight gain and prediabetes. We gave parathion to rats on postnatal days 1-4, using doses straddling the threshold for barely-detectable cholinesterase inhibition and the first signs of systemic toxicity (0.1 or 0.2 mg/kg). In adulthood, we evaluated serum adipokines, leptin and adiponectin, as well as standard lipid markers. Parathion decreased adiponectin and β-hydroxybutyrate in males, while decreasing cholesterol, nonesterified fatty acids and β-hydroxybutyrate in females. When animals were fed a high-fat (HF) diet for 7 weeks, further abnormalities emerged. In controls, there were increases in adiponectin, leptin and all serum lipid markers, verifying the metabolic effect of the HF diet. Males who had received the low neonatal dose of parathion showed an exaggerated increase in adiponectin and leptin on the HF diet, whereas females in the high dose group displayed a decrease in leptin relative to controls fed the HF diet. The sex differences were also apparent in the weight gain elicited by the dietary change: parathion-exposed males gained the same additional weight as controls on the HF diet, whereas females showed enhanced weight gain in the low dose group and suppressed weight gain in the high dose group. These results are consistent with long-lasting perturbations of lipid metabolism even at neonatal parathion exposures that produce minimal signs of exposure. Decreased adiponectin is associated with increased insulin resistance, diabetes, and cardiovascular disease. Further, changes in leptin and adiponectin are likely to play key roles in the weight gain caused by increased dietary fat intake, as these signals are responsible for communicating metabolic status to brain areas that control diet and metabolism. Our findings thus provide further evidence that early-life toxicant exposures may play a contributory role in the explosive increase in the incidence of metabolic dysfunction, obesity, and diabetes. (NIH ES10356, Leon Golberg Fellowship)

PL 1385 METABOLISM AND CHOLINESTERASE INHIBITION IN LUNGS OF RATS FOLLOWING *IN VITRO* CHLORPYRIFOS EXPOSURE.

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Chlorpyfios (CPF) is an organophosphorous insecticide that is routinely applied to crops as an aerosol spray, hence there is potential for workers and bystanders to be exposed via inhalation. Though inhalation represents a primary occupational route of exposure, little is known about lung metabolism or cholinesterase (ChE) response following a CPF exposure. Hence, this study evaluated the in vitro metabolism and ChE response following exposure to CPF or its active metabolite CPF-oxon in rat lung tissue. Rat microsomes were prepared from naïve S-D rat lungs and liver. The comparative metabolism of CPF to the major metabolite trichloropyridinol (TCPy) was evaluated over a range of CPF concentrations (50 – 500 μM), and the amount of TCPy generated was analyzed by GC/MS. The maximum velocity (Vmax) of lung metabolism was ~44% of the liver (0.72 vs. 1.62 μg/min/mg protein) and the predicted Km for the lung and liver microsomes were 5.9 and 21 μM. For in vitro ChE determination, whole lung from naïve S-D rats were homogenized and optimally diluted for the Ellman assay. Substrates for the assay included acetylthiocholine (ATC) and butyrylthiocholine (BTC). The lung homogenates were then incubated with a broad range of CPF-oxon concentration (1 – 1E4 nM). Overall, the BTC substrate was ~43% of the ATC response, suggesting that butyrylcholinesterase (BuChE) represents ~40% of the lung ChE activity. The estimated IC50 for CPF-oxon ChE inhibition in lung for ATC and BTC were 76.5 and 52.7 nM, respectively. These in vitro studies confirm that the rat lung has the capacity to metabolize CPF, and CPF-oxon can readily inhibit lung ChE activity. Future in vivo studies will be conducted to quantify the lung pharmacokinetic and pharmacodynamic dose-response. Finally, these data will then be utilized to further refine the CPF PBPK/PD model to accommodate inhalation exposure. (Funded by CDC/NIOSH grants R01 OH008173 & OH003629)

PL 1386 SEX SPECIFIC CIRCADIAN VARIATION IN PHYSIOLOGICAL AND MOLECULAR RESPONSES TO PESTICIDES.

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Circadian clocks synchronize internal biochemical and physiological rhythms to daily light/dark cycles. Many genes, including many involved in xenobiotic metabolism, reveal circadian expression rhythms. Many of the same genes exhibit sex-dependent induction after xenobiotic exposure. Utilizing Drosophila melanogaster as a model organism, we describe crosstalk between the circadian clock, sex, and xenobiotic metabolism. Flies were maintained in 12h:12h light/dark conditions, or moved to constant darkness or light prior to testing. Flies were acutely exposed to a series of doses of propoxur, malathion, deltamethrin, or fipronil by moving them to scintillation vials coated on the interior with chemical for one hour. Exposures or collections were every four hours for 24 hours. Mortality was scored two days later. Collected flies were processed for qRT-PCR, or to assay enzyme activity. Comparing dose responses at different times of day, we have established sex-specific daily susceptibility profiles. Females were generally less sensitive than males, and in the case of propoxur, LC50 was 100 fold that of males at ZT4. Similar rhythms were found in enzyme activities associated with xenobiotic metabolism, including ECOD, esterase, and GST activity. Mortality and enzyme activity rhythms continue in constant darkness and are lost in constant light, as has been observed in other clock-controlled rhythms. Using qRT-PCR, we are currently comparing daily expression profiles of clock genes, nuclear receptors, and xenobiotic metabolizing genes in males and females. Our previous work has indicated that defects in the central clock genes Pdp1 and CLK/CYC lead to increased susceptibility to pyrethroids in male flies. Our future work includes comparing daily susceptibility, expression, and enzyme activity profiles of these flies to wild-type males and females. This work will detail how the circadian clock modulates sex differences in xenobiotic metabolism in Drosophila. This will in turn lead to further understanding of how circadian disruption affects health in humans.

PL 1387 STRUCTURAL INSIGHT INTO INHIBITION/AGING OF NEUROPATHY TARGET ESTERASE (NTE) FROM X-RAY CRYSTAL STUDIES OF ITS CATALYTIC DOMAIN HOMOLOGUE, PATATIN-17 (PAT17).

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NTE is a neuronal protein of unknown structure whose inhibition/aging by organophosphorus compounds (OPs) is linked to OP-induced delayed neurotoxicity, OPIDN. We hypothesized that NTE inhibition/aging results in toxic gain of function via conformational change. To test this hypothesis, we solved the crystal structure of the NTE catalytic domain homologue, pat17, inhibited/aged with diisopropylphosphorofluoridate (DFP). We purified his-tagged pat17 via affinity and size-exclusion chromatography and determined the 20-min I50 of DFP against its phenyl valerate hydrolase activity to be 172μM. We grew cubic crystals of DFP-inhibited pat17 within one week via vapor-diffusion at 4°C in 0.1M Na-acetate (pH

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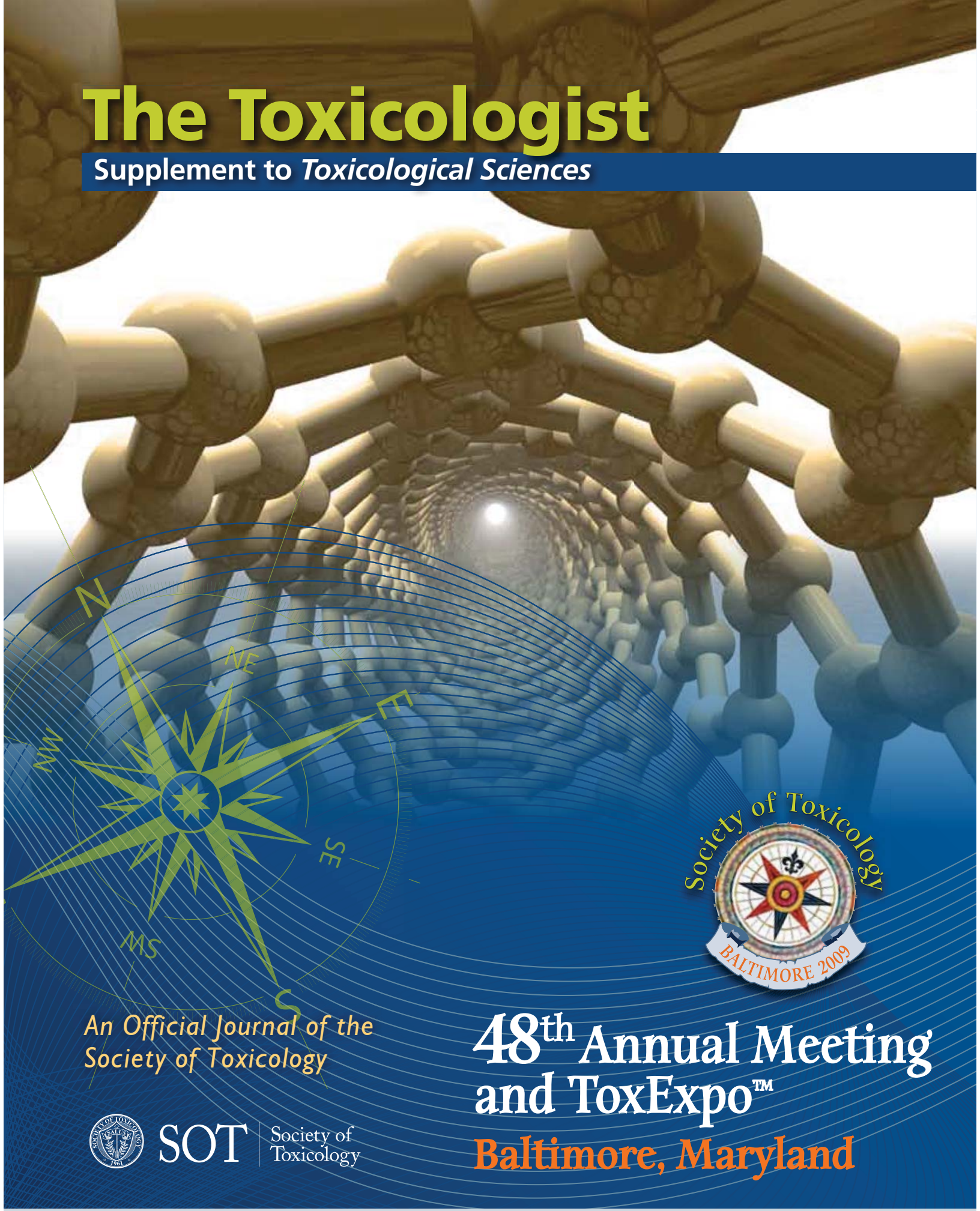

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