(bioallethrin, cypermethrin, deltamethrin, fenvalerate, permethrin, sumithrin, and tetramethrin) were tested with 17b-estradiol as a positive control. Among the pyrethroid compounds tested, only sumithrin increased the MCF-7 BUS cell proliferation in a dose-dependent manner, maximum induction of cell proliferation was observed at dose of 10-5M. In ER expression, 17b-estradiol (10-10M) decreased the level of cytosolic ERa and ERb protein expression compared with the vehicle control, and sumithrin significantly decreased the expression of ERa and ERb protein at high concentrations, 10-7 ~ 10-5M, in a dose-dependent manner. Similarly to 17b-estradiol, sumithrin dose-dependently increased pS2 mRNA expression. The other six test compounds used in the present study did not show any estrogenic effect at all concentrations (from 10-11 to 10-5M). These findings suggest that sumithrin could be considered to induce weak estrogenic activity *via* ER related pathways. Supported by NITR/Korea FDA Grant ED 2001-19.

440 INTESTINAL METABOLISM OF ORGANOPHOSPHATE INSECTICIDES: POTENTIAL FIRST-PASS METABOLISM.

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Chlorpyrifos (CPF) and diazinon (DZN) are organophosphate (OP) insecticides, and their toxicity is mediated through CYP450 metabolism to CPF-oxon and DZN-oxon, respectively. Detoxification of CPF and DZN is also mediated by CYP450 and A-esterase (A-EST) metabolism of CPF- and DZN-oxon, resulting in the formation of trichloropyridinol (TCP) and 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP), respectively. This study evaluated the potential role that intestinal metabolism (CYP450 and A-EST) may play in the first-pass clearance of OPs. Microsomes prepared from whole liver or isolated intestinal enterocytes demonstrated similar CYP450 and A-EST metabolic profiles. CYP450 content, as measured by reduced CO spectra, was ~10-fold lower in enterocyte than hepatocyte microsomes. For enterocyte CYP450 metabolism, the overall metabolic efficiency for the conversion to their active oxon metabolites was ~5-fold greater for CPF than DZN. When metabolism was compared per nmol P450 (nmol/min/nmol P450), the Vmax was ~3 and ~2 times higher in enterocytes than liver microsomes for CPF-oxon and TCP, respectively. The affinity (Km) for the metabolism of CPF to CPF-oxon was the same in liver and enterocyte microsomes, however the Km for TCP production was lower in enterocytes. Due to the smaller volume of intestine, the lower amount of CYP450, and the higher Km for TCP in the enterocyte microsomes, the catalytic efficiency was lower in intestine than liver for CPF-oxon, DZN-oxon and TCP. Enterocytes also demonstrated A-EST metabolism of CPF- and DZN-oxon. Although the Km for the substrates were comparable in hepatic and enterocyte microsomes, the Vmax was significantly faster, 69- to 255-fold in liver. These results suggest that intestinal metabolism may impact first-pass metabolism of OPs following low-dose oral exposures that would be expected from residues on foods. (Sponsored by CDC/NIOSH Grant R01 OH03629-01A2 and EPA grant R828608).

441 HPLC ANALYSIS OF VINCLOZOLIN AND ITS METABOLITES IN SERUM.

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The fungicide vinclozolin (V) is used predominantly for treatment of grains, fruits, ornamental plants and turfgrass. V administered to rats is hydrolyzed to 2-[[(3,5-dichlorophenyl)-carbamoyl]oxy]-2-methyl-3-butenoic acid (M1), 3'5'-dichloro-2-hydroxy-2-methylbut-3-enanilide (M2), and 3,5-dichloroaniline (M3). V, M1 and M2 have antiandrogenic properties both in vivo and in vitro by interacting with the androgen receptor. However, data on V and its metabolites in biological samples is limited. The aims of this study were to optimize a method for the analysis of V and its metabolites by HPLC and to evaluate the stability of V in rat serum. A gradient program with a mobile phase consisting of 60-75% methanol:acetonitrile (70:30) and 0.05 M phosphate buffer (PB) pH 3.3 at 1 ml/min, C-18 column and wavelength of 212 nm were used. The method was validated using spiked serum samples (pH 1.0) in a concentration range of 2-10 µg/ml. All analytes were extracted with acetonitrile (pH 2.5) from 100 µl aliquots. Retention times for M3, M1, M2 and V were 10.3, 12.8, 15.4 and 18.2 min, respectively. Detection limits for analytes ranged from 8.3 to 36.2 ng/ml. The recoveries were from 60 to 105% and the coefficient of variation was lower than 10% for all analytes. The relative concentration of V in PB pH 7.4 and serum (37°C) decreased over 4 h. V was more stable in PB than in serum. M3, M2 and M1 were observed in both media and at 48 h M3 had the highest relative concentration (50-60%). High serum levels of V, M1 and M3 in rats orally exposed to V (100 mg/kg) during 24 h after dosing were observed. These results suggest that the metabolism of V is both enzymatic and non-enzymatic. A better understanding of the biotransformation and pharmacokinetics of V will clarify the relationship between toxicity and tissue dose of V and its metabolites. (Funded in part by cooperative agreement CR 828790 with the NRC. This abstract does not represent USEPA policy).

442 CHLOROPYRIFOS OXON AND CARBARYL INHIBITION OF TRANS-PERMETHRIN HYDROLYSIS IN HUMAN LIVER FRACTIONS.

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Permethrin is a pyrethroid insecticide used in the Gulf War with other deployment related chemicals such as chlorpyrifos, pyridostigmine bromide and N, N-diethyl-m-toluamide. The hydrolysis of permethrin by esterases is a major detoxification process. Chemical interactions among deployment related chemicals have been suggested as a potential cause of Gulf War Related Illnesses. In this study the inhibitory effects of chlorpyrifos on trans-permethrin hydrolysis in humans is investigated using human liver fractions. Chlorpyrifos is an organophosphorus insectide and chlorpyrifos oxon (CFO), an active metabolite, is a potent inhibitor of t-PMT hydrolysis. The parent chlorpyrifos must be metabolically activated to the oxon by cytochrome P450 (CYP) to exert inhibitory effects. Pyridostigmine bromide and N, N-diethyl-m-toluamide did not inhibit trans-permethrin hydrolysis. Complete inhibition by CFO indicated that the esterases involved in trans-permethrin hydrolysis are B-esterases, which are inhibited by organophosphorus pesticides. Ki values for CFO inhibition of trans-permethrin hydrolysis are 20 nM in the cytosolic fraction and 100 nM in the microsomal fraction. The pattern of inhibition kinetics was non-competitive irreversible reflecting the known mechanism of organophosphorus pesticides, covalent modification of the active site of esterases. Carbaryl, another anticholinesterase agent with a similar inhibition mechanism, also showed the same inhibition kinetics but with higher Ki values, which was expected from the fact that carbaryl is reversible and a less persistent esterase inhibitor compared to organophosphorus compounds. Different from CFO, carbaryl does not completely inhibit trans-permethrin hydrolysis even at high concentrations. This indicates that two or more esterases are involved in trans-permethrin hydrolysis in human liver fractions, all sensitive to organophosphate but variably sensitive to carbaryl.

443 DEFINING MULTIGENE DOSE-RESPONSE RELATIONSHIPS BY MICROARRAY ANALYSIS.

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Microarray studies often focus on increasing replicates to maximize statistical power. As a result, conducting complete dose response microarray studies can be cost prohibitive. However, in drug safety assessment, margin of safety (MOS) calculations are based on the difference between efficacy and toxicity. If genes are to serve as biomarkers to refine MOS calculations, then understanding their dose response relationships is critical. The purpose of this investigation was to develop methods to generate ED50 and maximum response (Rmax) values from microarray data. Peroxisome proliferator-activated receptor (PPAR) agonists are ideal for this type of investigation since their effects are transcriptionally-regulated. We determined PPAR-induced global liver gene expression profiles in rats exposed to either Fenofibrate (Feno), a weak, but specific PPARα agonist, or another potent PPAR agonist (PPARag). Eight doses were chosen spanning the ED50s for efficacy parameters (lowered triglyceride and/or glucose) with gene expression data collected on pooled liver RNA samples from control and treated rats. Overall, Feno induced more gene changes than PPARag. Both agonists induced fatty acid (FA) and drug metabolism-related gene expression. Comparing ED50 values for FA genes affected by Feno and PPARag yielded gene-based potency information. Consistent with the affinities for PPARα, Feno ED50s were right-shifted compared to PPARag. However, ED50 values for genes induced by either Feno or PPARag vary between genes suggesting gene selective responses. FA gene changes and gene-based potencies correlate with induction of *in vitro* and *in vivo* liver peroxisomal β-oxidation by Feno and PPARag. Feno, while less potent, produced higher Rmax values for FA gene expression compared to PPARag. Based on these studies, a dose response-based microarray data analysis design is proposed that is both cost effective and maximizes the value of microarrays by yielding ED50 and Rmax values that allow for compound comparisons.

444 GENOMIC AND PROTEOMIC PROFILING IN A PARKINSONIAN MODEL OF NEURODEGENERATION.

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The pathogenic mechanisms underlying Parkinson's disease (PD) remain enigmatic. In an effort to identify early molecular events associated with PD, we profiled genomic and proteomic changes in the MPTP mouse model of PD. cDNA and antibody microarray analysis revealed time-dependent (1h-48h) changes in striatal gene expression following dopaminergic neurotoxicity and associated reactive gliosis. A medley of genes exhibiting altered expression is tabulated. Other genes expressed included cytokines & chemokines, growth factors, transcription factors, protein kinases and genes related to stress, cell cycle and apoptosis. Further,