and glucagon, which activate signaling through specific membrane receptors, and to NaF, which activates the G-proteins that couple the receptors to AC. Few immediate effects on AC were apparent when CPF doses remained below the threshold for systemic toxicity. Nevertheless, CPF exposures on GD9-12, GD17-20 or PN1-4 elicited sex-selective effects that emerged by adulthood (PN60), whereas later exposure (PN11-14) elicited smaller, nonsignificant effects, indicative of closure of the window of vulnerability. Most of the effects were heterologous, involving signaling elements downstream from the receptors, and thus were shared by multiple inputs; superimposed on this basic pattern, there were also selective alterations in receptor-mediated responses. These results suggest that the developmental toxicity of CPF extends beyond the nervous system, to include cell signaling cascades that are vital to cardiac and hepatic homeostasis. Future work needs to address the potential implications of these effects for cardiovascular and metabolic disorders that may emerge long after the end of CPF exposure. Support: USPHS ES10387 and ES10356.

2062 INHIBITION OF DIAZINON METABOLISM BY CHLORPYRIFOS IN RAT LIVER MICROSOMES.

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Chlorpyrifos (CPF) and diazinon (DZN) are major organophosphorus pesticides that are structurally and mechanistically similar, but have very different rates of metabolism. Their toxicity is mediated *via* CYP450 metabolism to oxons. Detoxification of the oxons is mediated by CYP450 and A-esterases, resulting in the formation of trichloropyridinol from CPF and 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP) from DZN. Since exposures often involve more than one pesticide, this study was conducted to assess the effect of co-incubation with both CPF and DZN on the metabolism of DZN. Substrate concentrations of DZN ranging from 20 to 575 μM were incubated with 0, 100, 300, or 750 μM CPF for 15 min and bioactivation to DZN-oxon and deactivation to IMHP quantified. Data was analyzed using Lineweaver-Burke (1/v; 1/s) plots, by fitting the Michaelis-Menten equation to the data with non-linear regression using SlideWrite Plus®, and by fitting the data to a mathematical model written in Simusolv®, which employed Michaelis-Menten equations including inhibition constants to describe metabolism. The 1/v; 1/s plots were used to estimate the types of inhibition. Metabolism to IMHP appeared to be competitively inhibited whereas metabolism to the oxon was uncompetitive (or suicide). Fitting the data with non-linear regression, apparent Km concentrations vary for the production of IMHP, but not the apparent Vmax; whereas, the apparent Vmax rates change for the production of oxon, confirming the types of inhibition. Inhibitory rate constants (Ki) for oxon and IMHP metabolism were ~250 μM and ~50 μM, respectively. Using the different methods to predict Vmax and Km, apparent Vmax and Km with inhibition, and Ki resulted in similar estimations. The mathematical model has the advantage of providing a methodology by which all parameters are considered at once. The relatively high Ki values indicate the importance of metabolic interactions on kinetics of *in vivo* co-exposures to DZN and CPF may be limited to high acute dose exposures. (Sponsored by CDC/NIOSH Grant R01 OH03629-01A2 and EPA grant R828608).

2063 COMPARISON OF CHLORPYRIFOS-OXON AND PARAOXON ACETYLCHOLINESTERASE INHIBITION DYNAMICS: POTENTIAL ROLE OF A PERIPHERAL BINDING SITE.

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The primary mechanism of action for organophosphorus (OP) insecticides involves the inhibition of acetylcholinesterase (AChE) by active oxon metabolites. This inhibition has been attributed to the phosphorylation of the serine hydroxyl group in the active site of AChE. Inhibition is described by the bimolecular rate constant (Ki), which is used to assess OP inhibitory capacity. It has been reported that the Ki for inhibition of AChE by certain oxons changes as a function of oxon concentration, and that this phenomenon could be explained by the presence of a peripheral binding site. When such a putative site would be occupied, the capacity of additional oxon molecules to phosphorylate the active site is reduced. Therefore, the objective of the current study was to evaluate the *in vitro* inhibition of AChE by a wide range of chlorpyrifos oxon (CPO) and paraoxon (PO) concentrations (0.5 pM -100 nM) using the Ellman assay combined with a dynamic model. The results indicated that the Ki is inversely changed in proportion to the oxon concentrations. The Ki determined using high oxon concentrations, were 238 and 22 μM-1h-1 and the spontaneous reactivation rates were 0.087 and 0.078 h-1 for CPO and PO, respectively. While the estimated Ki using high oxon concentrations were similar to those reported previously, the Ki estimates following 1 pM oxon, which have not been reported previously, were >1000-fold higher. Surprisingly, the Ki estimated using 1 pM CPO was similar to the Ki estimate using an equivalent PO concentration (0.18 and 0.25 pM-1h-1, respectively). This implies that both oxons exhibit similar inhibitory potency at the lower concentration, in contrast to the marked difference following higher concentrations, which was explained by considering a peripheral binding site. The potential of a peripheral site should be considered when modeling AChE kinetics particularly at low environmentally relevant concentrations. (Sponsored by CDC/NIOSH Grant R01 OH03629-01A2 and EPA grant R828608)

2064 PHARMACOKINETIC & PHARMACODYNAMIC INTERACTIONS OF A BINARY MIXTURE OF CHLORPYRIFOS AND DIAZINON IN THE RAT.

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Chlorpyrifos (CPF) and diazinon (DZN) are organophosphorus (OP) insecticides, with the potential for concurrent exposures. Their oxon metabolites are potent inhibitors of cholinesterases (ChE). This study evaluated the impact of an acute binary OP exposure on dosimetry and ChE inhibition in rats. Groups (3-4/time point) of male S-D rats were orally administered CPF, DZN or a CPF/DZN mixture (0, 15, or 60 mg/kg) and blood and brain were collected at 0, 3, 6, 12 and 24 hr post-dosing. CPF, DZN and their respective metabolites trichloropyridinol (TCP) and 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP) were quantified in blood and/or urine using gas chromatography. A modified Ellman method was used to measure ChE inhibition in blood and brain. Co-exposure at 15 mg/kg, did not appreciably alter the pharmacokinetics of CPF, DZN or TCP. The total amount of urinary TCP was decreased ~50% for the co-exposed group, but the amount of urinary IMHP was not altered. Co-exposure to 60 mg/kg delayed and increased the Cmax for both CPF (1.6x) and DZN (7.2x), and resulted in the respective blood AUCs increasing 145 and 422%. The AUC for TCP decreased to 82%, but was slightly increased (107%) for IMHP. For the co-exposed group the total amount of urinary TCP was decreased ~60%, but did not appreciably alter the amount of IMHP excreted relative to the single exposure. These results suggest that both CPF and DZN are capable of inhibiting each other's metabolism, but the effect is observed at higher doses (>15 mg/kg). A dose-dependent inhibition of ChE activity was also noted in plasma, RBC and brain homogenates for both the single and co-exposures to CPF and DZN. In all tissues, CPF exposure resulted in greater ChE inhibition than DZN, however the overall ChE response appeared to be additive for the co-exposures. These results characterize both the pharmacokinetic and pharmacodynamic interactions of CPF and DZN in the rat and will be used to further develop a binary kinetic/dynamic model for OPs. (Sponsored by CDC/NIOSH R01OH03629-01A2)

2065 CONTINUOUS SYSTEMS MODELING OF THE INTERACTIONS OF PARAOXON WITH HUMAN RECOMBINANT ACETYLCHOLINESTERASE.

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Previous studies from this laboratory have reported that the inhibitory constant k_i for the inhibition of acetylcholinesterase by the organophosphate paraoxon (*O, O*-diethyl *p*-nitrophenyl phosphate) changes as a function paraoxon concentration. The k_i values were calculated by the application of continuous systems modeling to experimentally determined residual acetylcholinesterase activity following the addition of various paraoxon concentrations. The current study used the fluorescent compound N-methylacridinium to ascertain an independent measure of active site concentration for human recombinant acetylcholinesterase, which was then used in the model for a precise determination of k_i over a range of oxon concentrations at both 24°C and 37°C. Incubations at 24°C used oxon concentrations ranging from 78 pM-750 nM with active sites concentrations ranging from 3.01-4.87 pM. The k_i for 750 nM paraoxon was 0.111 $nM^{-1}h^{-1}$ whereas the k_i for 78 pM paraoxon was 0.410 $nM^{-1}h^{-1}$. Incubations at 37°C used oxon concentrations ranging from 15 pM-100 nM with active sites concentrations ranging from 4.36-5.60 pM. The k_i for 100 nM paraoxon was 0.146 $nM^{-1}h^{-1}$ whereas the k_i for 78 pM paraoxon was 4.21 $nM^{-1}h^{-1}$. These data suggest that individual paraoxon molecules at the lower concentration have a greater capacity to inhibit acetylcholinesterase than individual paraoxon molecules at the higher concentration (consistent with previous data from our lab). At 37°C the difference from the highest k_i to the lowest was approximately 30 fold whereas at 24°C the difference from the highest k_i to the lowest was approximately 4 fold. These data indicate that the kinetic scheme utilized for the derivation of the inhibitory constant k_i is inadequate to explain the interaction of human recombinant acetylcholinesterase and paraoxon.

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