1146 PERSISTENT CHANGES IN CHOLINERGIC SYNAPTIC MARKERS AFTER NEONATAL CHLORPYRIFOS EXPOSURE.

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Despite recent restrictions on home use, chlorpyrifos (cpf) remains one of the most widely used pesticides. Recent studies indicate that cpf elicits developmental neurotoxicity through mechanisms that are unique to replicating and differentiating brain cells. Presumably, if disruption of cellular development affects CNS function, then there should be corresponding, long-term changes in synaptic proliferation and/or activity. In the current study, neonatal rats were exposed to cpf during periods of terminal cell differentiation, axonogenesis and synaptogenesis using treatments, 1 mg/kg/day on postnatal days (PN) 1-4, or 5 mg/kg daily on PN11-14, that evoked neither overt systemic toxicity nor growth inhibition. We then assessed two markers of cholinergic synaptic development: choline acetyltransferase (ChAT), the enzyme that synthesizes acetylcholine, a constitutive biomarker for cholinergic nerve terminals; and hemicholinium-3 (HC-3) binding to the presynaptic transporter, which increases in response to neural impulse activity. In males given the late treatment regimen, cpf elicited deficits in ChAT in regions with major cholinergic projections, an effect which disappeared by PN60. The ChAT deficits were not accompanied by compensatory upregulation of neural activity as monitored with HC-3 binding. In females, deficits emerged later and persisted through PN60, again with a lack of compensatory changes in HC-3 binding. These data indicate that neonatal cpf exposure elicits long-term, gender-dependent reductions in cholinergic synaptic projections and neural activity. These effects may account for the late emergence of sexually dimorphic behavioral alterations. (Supported by USPHS ES10387 and ES10356)

1147 AGE-RELATED EFFECTS OF CHLORPYRIFOS AND PARATHION ON ACETYLCHOLINE SYNTHESIS IN RAT BRAIN.

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Organophosphorus insecticides (OPs) elicit acute toxicity through inhibition of acetylcholinesterase (AChE). Excessive AChE inhibition leads to synaptic accumulation of the neurotransmitter acetylcholine (ACh) and consequent toxic signs including autonomic dysfunction and involuntary movements. Earlier work from our laboratory demonstrated that the OPs chlorpyrifos (CPF) and parathion (PS) exhibit differential effects on presynaptic cholinergic processes including high affinity choline uptake and ACh release. In the present study, we examined the in vivo effects of CPF and PS on AChE activity and ACh synthesis in striatum from neonatal (7day), juvenile (21 day) and adult (90 day) rats. AChE activity and ACh synthesis were measured in crude synaptosomal preparations by radiometric methods. Rats were treated (sc) with OPs (0, 0.3 or 1.0 x acute maximum tolerated dosage-MTD) and biochemical endpoints were measured at 4, 24 and 96 hr after treatment. Following MTD exposure to either OP, relatively similar maximal degrees of AChE inhibition were noted among the age groups. Times to peak inhibition varied among the age groups, however, *i.e.*, maximal AChE reduction occurred at earlier time points in neonates (PS, 4 hr; CPF, 24 hr) but later in juveniles (PS, 24 hr; CPF, 96 hr) and adults (96 hr after both). In neonates, CPF had no effect while PS (MTD) reduced ACh synthesis (27% at 24 hr). In contrast, CPF (MTD) increased ACh synthesis (20%) 24 hr after exposure in juveniles. PS (MTD) also increased ACh synthesis in juvenile tissues at 4 hr (20%) and 24 hr (33%) after treatment. In adults, CPF (0.3MTD) decreased synthesis at 4 hr (15%) while PS increased synthesis at 4 hr (both dosages, 13-20%) and 24 hr (MTD, 23%) after exposure. The results suggest that both CPF and PS may alter ACh synthesis in an age-, time- and OP-dependent manner. Such changes in neurotransmitter synthesis could contribute to the differential toxicity of these OPs. (Supported by NIEHS R01-ES09119.)

1148 ACETYLCHOLINESTERASE INHIBITION, MUSCARINIC RECEPTOR DOWNREGULATION AND PHOSPHOINOSITIDE HYDROLYSIS IN NEONATAL, JUVENILE AND ADULT RAT BRAIN FOLLOWING CHLORPYRIFOS OR PARATHION EXPOSURE.

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Chlorpyrifos (CPF) and parathion (PS) elicit acute toxicity primarily through inhibition of acetylcholinesterase (AChE). Marked age-related differences in acute sensitivity exist with both CPF and PS. Previous studies have reported that persistent AChE inhibition can alter muscarinic receptor (MR)density and MR-mediated phosphoinositide (PI) hydrolysis. We compared AChE inhibition, MR downregulation and MR-mediated PI hydrolysis in cortex from neonatal (7 day), juvenile (21 day) and adult (90 day) rats at 4, 24 and 96 hr after 0, 0.3 or 1 x maximum tolerated dosage (MTD) of either PS or CPF (MTDs: PS=2.1, 4.8, 18 mg/kg; CPF=45, 127, 279 mg/kg). Carbachol (CARB)-stimulated (1 mM) PI hydrolysis was measured by the accumulation of [3H]inositol phosphates in the presence of lithium chloride. Peak AChE inhibition following MTD exposure in neonates, juveniles and adults was 70-86%, 79-92%, and 91-93%, respectively, with maximal reduction occurring earlier in neonates and juveniles (4-24 hr after exposure) than in adults (96 hr after exposure). MR downregulation (quinuclidinyl benzilate [QNB]) binding was not significantly reduced in neonates but was significantly lower (16-22%) in both juveniles and adults 96 hr after PS (MTD). Similarly, QNB binding was significantly reduced 96 hr after CPF in juveniles (13 and 28%; 0.3MTD, MTD) and adults (16%, MTD) but not in neonates. No significant differences in CARB-stimulated hydrolysis were found among any dosages, age groups or time points following exposure to either OP. We conclude that while high dosages of CPF and PS can elicit changes in AChE activity and MR binding in an age-related manner, these neurochemical changes have little influence on MR-mediated PI signaling. (Supported by RO1 ES09119 from NIEHS)

1149 AGE-RELATED EFFECTS OF CHLORPYRIFOS ON HIGH AFFINITY CHOLINE UPTAKE IN RAT BRAIN.

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Organophosphorus (OP) insecticides elicit toxicity through inhibition of acetylcholinesterase (AChE), allowing accumulation of acetylcholine (ACh) and consequent cholinergic toxicity. High affinity choline uptake (HACU), the rate-limiting step in ACh synthesis, is modulated in adult rat brain in vivo by some OP toxicants. Young rats are generally more sensitive than adults to the acute toxicity of OP pesticides, but little is known regarding age-related effects of OP toxicants on HACU. AChE inhibition and changes in HACU in cortex of neonatal (7 days), juvenile (21 days) and adult (90 days) rats (n=8-15/treatment group) were evaluated at 4, 24 or 96 hr after oral exposure to chlorpyrifos (0, 0.5 or 1 x LD10: 15, 47 and 136 mg/kg). LD10 dosages generally elicited similar levels of maximal AChE inhibition in all age groups, but inhibition was highest at 4 hr in neonates and juveniles but not until 24 hr in adults. In general, HACU was reduced in a time-dependent manner by LD10 exposure, *i.e.*, uptake was maximally reduced (43%) in neonates at 4 hr, moderately reduced (22%) in juveniles at 24 hr and least affected (18% reduction) in adults at 96 hr. No significant changes in HACU were noted with the lower dosages at any timepoint in either age group. An early reduction in HACU following high-dose exposure could potentially modulate the toxic effects of AChE inhibition by limiting the amount of ACh released into the synapse, whereas later changes in HACU noted in adults may be an adaptive response to altered cholinergic neurotransmission. A reduction in HACU, in particular in a system having relatively little acetylcholine synthesis and release (neonatal brain), could also lead to neurotoxic consequences on its own. Changes in HACU may therefore differentially contribute to age-related neurotoxicity following AChE inhibition. (Supported by GR825811 from U.S. EPA)

1150 NEUROCHEMICAL CORRELATES OF COGNITIVE DEFICITS IN WEANLING RATS EXPOSED TO CHLORPYRIFOS.

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Previous studies have shown that moderate to high levels of chlorpyrifos (CPF) alter cognitive function in adult and immature rats, probably related to the inhibition of acetylcholinesterase (AChE). We have previously reported on an alteration of cAMP signaling by CPF in rats exposed to moderate doses, and PC12 cells exposed to CPF at doses lower than those required to inhibit AChE. In the present study, we tested the hypothesis that exposure to CPF during brain development causes deficits in cognitive function of weanling rats, and we determined the relationship between cognitive effects, AChE activity, muscarinic receptor binding, and the expression of the phosphorylated form of Ca2+/cAMP-dependent response element binding protein (pCREB). Rat pups were administered 0, 0.3, or 7.0 mg/kg CPF by subcutaneous injection on postnatal day (PD) 7, 11, and 15, and tested in the Morris swim task immediately after weaning on PD24. CPF did not cause signs of overt cholinergic intoxication or impaired growth, nor did the exposures cause significant inhibition of AChE or reduction in muscarinic receptor binding sites identified with [3H]-N-methylscopolamine ([3H]-NMS) in the cerebral cortex, hippocampus, or cerebellum. However, spatial learning was impaired after 5 days of

training in the group of weanling rats administered 7.0 mg/kg CPF. Additionally, the expression of pCREB was significantly reduced in the brains of rats treated with 7.0 mg/kg CPF that were sacrificed by focused microwave irradiation. These data indicate that exposure to 7.0 mg/kg CPF caused deficits in cognitive function in weanling rats, and these effects did not appear to be mediated by AChE inhibition. Although, the relevant molecular pathways important in neural and cognitive development that may be impacted by CPF have yet to be identified, these results point to an alternative mechanism that involves events leading to the expression of pCREB. Supported by ES03819 and ES10161.

1151 CHLORPYRIFOS INDUCES AIRWAY HYPERREACTIVITY *VIA* LOSS OF NEURONAL M2 MUSCARINIC RECEPTOR FUNCTION AND *VIA* DECREASED ACETYLCHOLINESTERASE.

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In the lungs, the vagal parasympathetic nerves provide control of airway smooth muscle tone. Stimulation of these nerves releases acetylcholine (ACh) onto M3 muscarinic receptors. Release of acetylcholine is limited by neuronal M2 muscarinic receptors (nM2Rs). In asthmatics the nM2R are dysfunctional. In animal models of asthma, loss of nM2R function leads to increased ACh release and to hyperreactivity. It is possible that the alteration of cholinergic function in the lung by CPF could contribute to the prevalence of asthma, especially in inner-city children where increases in the number of asthma cases has been a recent concern., and CPF has been used for pest control in low-income housing. We tested the hypothesis that CPF causes airway hyperreactivity and does so *via* nM2R dysfunction in guinea pigs. Guinea pigs were treated with CPF (390 mg/kg s.c.). Vagally-induced bronchoconstriction was significantly increased in CPF treated animals compared to controls. By stimulating neuronal M2 receptors, the muscarinic agonist pilocarpine (0.1-100ug/kg, iv) inhibited vagally-induced bronchoconstriction in controls. In contrast, in CPF treated guinea pigs, pilocarpine did not inhibit vagally-induced bronchoconstriction, indicating that nM2Rs were dysfunctional in CPF-treated animals. Bronchoconstriction induced by i.v. ACh was also potentiated by CPF although bronchoconstriction induced by i.v. methacholine (metabolically-stable analog of ACh) was not, suggesting that the function of M3 receptors on airway smooth muscle was not altered by CPF. AChE activity was inhibited in the brain by 27%, and in the lung by 47%. These data show that CPF induces airway hyperreactivity and does so by 1) inhibiting nM2R function resulting in increased ACh release from the parasympathetic nerves and 2) by inhibiting AChE, thus further enhancing the ability of ACh to induce bronchoconstriction. CPF may contribute to the hyperreactivity associated with asthma.

1152 DIFFERENTIAL EFFECTS OF ORGANOPHOSPHATES ON ACETYLCHOLINE (ACH) RELEASE AND MUSCARINIC AUTORECEPTORS.

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Chlorpyrifos oxon (CPO), paraoxon (PO) and methyl paraoxon (MPO) are the active metabolites of the organophosphorus (OP) insecticides chlorpyrifos, parathion and methyl parathion. These agents primarily elicit toxicity through acetylcholinesterase (AChE) inhibition, but they may have additional actions. ACh release and its modulation by muscarinic autoreceptors can be differentially affected by in vivo OP insecticide exposures. We evaluated the effects of CPO, PO and MPO *in vitro* on ACh release by loading brain slices with [3H]choline, stimulating with high-potassium (20 mM) buffer containing hemicholinium-3 (10 uM) and analyzing fractions for tritium release. Muscarinic agonists (*e.g.*, carbachol) and anticholinesterases (*e.g.*, physostigmine, PHY) reduce ACh release in an atropine (ATR)-sensitive manner directly by activation of autoreceptors or indirectly through inhibition of AChE and elevation of endogenous ACh, respectively. Some anticholinesterases may also interact directly with muscarinic autoreceptors, but any direct action would be potentially masked by their indirect action. We therefore studied the effects of CPO, PO and MPO on release under two conditions, *i.e.*, 1) in the absence of PHY and ATR or 2) in the presence of both. In the absence of PHY and ATR, all OP toxicants reduced release in a concentration-dependent manner (24-38% maximal reduction at 10 uM). In the presence of PHY (20 mM), CPO, PO and MPO had little effect on release. When both PHY and ATR (100 nM) were added, the relative potencies of all OP anticholinesterases were reduced and both PO and MPO decreased release as before. In contrast, CPO increased release. We conclude that all three oxons have direct and indirect effects on muscarinic autoreceptors. Qualitative and quantitative differences in direct actions at muscarinic autoreceptors could contribute to differential toxicity with some OP insecticides. (Supported by GR825811 from U.S. EPA).

1153 IDENTIFICATION AND CHARACTERIZATION OF MOLECULAR MARKERS OF SH-SY5Y CELL LINE AFFECTED BY ORGANOPHOSPHORUS COMPOUNDS.

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Organophosphorus (OP) compounds are broadly used as insecticides throughout the world today. OP compounds also comprise of major portion of the U.S. military stockpile of chemical nerve agents. Although the immediate, acute neurotoxic action of OPs is the inhibition of acetylcholinestease (AChE), some OPs also produce a neurodegenerative disorder known as organophosphate-induced delayed neuropathy (OPIDN), which is characterized by a delayed onset of neuropathological symptoms. Our lab has previously reported that mipafox, an OPIDN inducing agent, induces the degeneration of neurite extension in differentiated SH-SY5Y cell line, while paraoxon, an OPIDN non-inducing agent, fails. Based on these observations, some protein markers affected by OPIND-inducing agent have been identified with SH-SY5Y cell line, which was grown in medium containing human serum. With Western blot analysis, NGF produces an increase on the expression of neurofilament 200 (NF200) protein and microtubule associated protein (MAP) 2ab by about 2 and 1.3 fold, respectively. Mipafox (40 μM) down-regulated the expression of NF200 about 3 fold ($p \leq 0.01$), compared to NGF control, while paraoxon (40 μM) failed. However, both OP compounds failed to produce a significant difference in expression of MAP 2ab, compared to NGF control. However, mipafox produced a different pattern for the expression of NF200 in FBS containing medium system. The highest dose (30 μM) of mipafox upregulated the expression of NF200 by about 3 fold with a dose-dependent manner. Although mipafox did not produce significant change in the expression of MAP2ab, the expression showed a pattern of reduction dose-dependently. These differences in the protein expression between two cell growing environments are considered mainly due to cytotoxic effect of mipafox and/or degree of cell differentiation.

1154 CYTOTOXICITY OF PARAOXON FOR NG108-15 CELLS.

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Neuroblastoma X glioma NG108-15 cells, widely used to study acute and chronic drug actions, have muscarinic receptors and high acetylcholine transferase activity. These cells were used in the current study and revealed a growth inhibitory effect of paraoxon. The cells were grown at 37° C in an incubator with 5% CO_2/95% humidified air in DMEM fortified with 7% fetal bovine serum plus 50 units/ml of penicillin and 50 mg of streptomycin. The cells were routinely passaged 2 times per week and allowed to grow for at least 24 hours before adding paraoxon to give the final concentrations indicated below. Following incubation, cytotoxicity was assessed by crystal violet staining of the adherent cells. The media was removed and the attached cells rinsed with phosphate buffered saline, fixed with 4% formaldehyde for 1 hour and stained with 0.1% crystal violet. The stained cells were washed with water, air-dried and the crystal violet extracted with 5% SDS and the intensities quantitated spectrophotometrically at 595 nm. The viability of paraoxon treated cells was compared to that of control cells. During static phases of cell growth, paraoxon had no effect on the cell number as reflected by crystal violet optical densities. However, in growing cell cultures, the paraoxon caused a dose dependent decrease in cell number. For example, following 24 hours of incubation the paraoxon treated cells had densities of 0.83, 0.79 and 0.68 of control values for concentrations of 0.5, 1.0 and 2.0 mM paraoxon, respectively. Similar results were obtained following 48 hours of incubation with paraoxon. In a control group of NG108-15 cells the level of acetylcholinesterase activity (determined by the Ellman method and a reported turnover number of 8333 pm/second) was 2.99 pM and 0.88 pM in the presence of 2 pM paraoxon. These observations suggest that acetylcholinesterase may have a role in neuronal NG108-15 cell growth.

1155 EFFECTS OF SUBCHRONIC EXPOSURES OF CHLORPYRIFOS OR PERMETHRIN ON BEHAVIOR AND STRIATAL CHOLINERGIC BIOMARKERS IN C57BL/6 MICE.

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The C57BL/6 mouse given 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is an established animal model of Parkinson's disease (PD). Because cholinergic systems interact with the neural substrates of PD, behavorial and neuro-

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, roundtable, and poster sessions of the 40th Annual Meeting of the Society of Toxicology, held at the Moscone Convention Center, San Francisco, California, March 25–29, 2001.

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