PS 2121 An Experimental Study on the Effects of Low-Level Fluoride Exposure on Memory and Learning Ability of Rats

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Neurotxic effects of fluoride on the ability of learning and memory evaluated by Morris water maze test have been reported for mice in previous studies lately. For rats, there was a study that the effects of fluoride on the ability of learning and memory of the rats were evaluated for high doses of 100 and 200 ppm in the drinking water. In this study, we evaluated the neurotoxic effects of fluoride on the learning ability of rats at low and high doses in the drinking water. Fluoride in the drinking water at 0, 25 or 125 ppm were administered to adult Wistar rats for 2 months. The body weights of the rats were recorded daily. The neurotoxic effects were evaluated by Morris water maze test for 5 days at the end of the exposure of 2 months. The indexes for the evaluation were the search time to find the target on day 1 to day 5 of the session, and the swimming distance before escaping onto the visible platform after the test for the search time on day 5. The mean body weights of the 125 ppm group were significantly lower than those of the control and the 25 ppm-group from 11 days after the beginning of the exposure. The mean search time on day 2 in the 25 ppm or 125 ppm was significantly higher than that in the control. There were no significantly differences among the groups for the swimming distance. Fluoride at 125 ppm in the drinking water decreases the body weight of adult rats. Fluoride at 25 ppm and more in the drinking water may impair the learning ability in the very early days. Further study are required for confirming the effects of fluoride on the ability of learning and memory and evaluating the neurotoxicity of fluoride.

PS 2122 Exposure to Fine Particulate Matter Reduces Glutamate Uptake in Cerebellar Bergmann Glia Cells through a src/MAPK Pathway

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Glutamate, the main excitatory amino acid transmitter triggers a wide variety of signal transduction cascades that regulate protein synthesis at the transcriptional and translational levels. Activity-dependent differential gene expression has been attributed to the activation of both membrane glutamate receptors and transporters. The bulk of glutamate uptake takes place in glia cells. Recent studies have linked exposure to fine particulate matter with toxicity at a neurological level, given that some of its components can reach the brain and instigate damage locally or systemically. Within the cerebellum, Bergmann glia cells are responsible for most of glutamate uptake activity through the Na+-dependent glutamate/aspartate transporter (GLAST/EAAT-1). Taking into consideration the functional role of Bergmann glia, in terms of the recycling of glutamate, the supply to neurons of lactate and the prevention of neurotoxic insults, we decided here to investigate if air pollution particulate matter (PM) target glia cells that surround glutamatergic synapses and by these means alter the major excitatory transmitter system in the brain. To this end, we exposed cultured chick cerebellar Bergmann glia cells to fine PM isolated and concentrated from Mexico City and measured the [3H]- D-Aspartate uptake activity. A time and dose dependent decrease in uptake activity was observed. Furthermore, PM treatment resulted in the activation of a signaling cascade that included Ca2+ influx through the Na+/Ca2+ exchangers, activation of p60src, phosphatidylinositol 3-kinase, protein kinase B and p42/p44 MAPK. These results add a novel mediator of the air pollutants deleterious effects in the CNS: glia cells, strengthening the notion of the critical involvement of these cells in synaptic neurotransmission. (Financement: CONACYT #167778 and Fundación Pandea).

PS 2123 *In Vitro* and *Ex Vivo* Neurotoxicity Assessment of Brominated- and Halogen-Free Flame Retardants

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Many brominated flame retardants (BFRs) are abundant persistent organic pollutants with well-known neurotoxic properties that argue for replacement by halogen-free flame retardants (HFFRs). Since the neurotoxic potential of HFFRs is largely unknown we investigated the *in vitro* neurotoxicity of 13 HFFRs and 3 BFRs. Using PC12 cells we demonstrate that the majority of FRs induced negligible cytotoxicity, except zinc hydroxystannate (ZHS) and zinc stannate (ZS). Single-cell fluorescent Ca^{2+}-imaging revealed that aluminium trihydroxide (ATH), ZHS and ZS increased the basal intracellular calcium concentration ($[Ca^{2+}]_i$). In the low µM range, many FRs, including tetrabromobisphenol A (TBBPA), triphenylphosphate (TPP), ZHS and ZS reduced depolarization-evoked increases in $[Ca^{2+}]_i$ due to inhibition of voltage-gated calcium channels. Next, using Xenopus oocytes expressing nicotinic acetylcholine receptors (nACh-R) we demonstrate that some FRs, including TBBPA, TPP and aluminium diethylphosphinate (Alpi), act as nACh-R antagonists. Based on the *in vitro* derived neurotoxic potential we could identify suitable (e.g. Alpi) and less suitable (e.g. ZS) candidates for replacement. To substantiate this notion, we studied effects of neonatal exposure to TBBPA, Alpi or ZS on synaptic plasticity in mouse hippocampus *ex vivo*. These FRs did not significantly affect long-term potentiation and the expression of postsynaptic proteins. The FRs were absent from the brains, suggesting low bioavailability and/or rapid elimination/metabolism. Our findings demonstrate that several HFFRs could be suitable alternatives for BFRs. However, data on (*in vivo*) toxicity following prolonged (developmental) exposure is yet lacking.

PS 2124 Chronic Glucocorticoid and Nerve Agent DFP Exposures Produce a Neuroinflammatory Model of Gulf War Illness without Neurodegeneration

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Gulf War Illness (GWI) is a persistent, multi-symptom disorder with features characteristic of sickness behavior. Several exposures have been hypothesized as triggers of the recurring/chronic symptoms associated with GWI, including exposure to the nerve agent sarin that resulted from munition detonations at multiple sites. Here, we investigated the effects of exposure to the sarin surrogate, diisopropyl fluorophosphate (DFP), on peripheral and CNS inflammation. Male C57BL/6J mice were pretreated with corticosterone (CORT) in the drinking water for 7 days to mimic high physiological stress, followed 1 day later by DFP (4 mg/kg, i.p.) exposure to model GWI. DFP exposure alone did not change inflammatory markers in serum and liver, however, increases in expression of inflammatory cytokines/chemokines were found in the brain. Further, CORT exposure for 7 days prior to exposure to DFP greatly augmented inflammatory responses in the brain. Pretreatment with anti-inflammatory antibiotic, minocycline, attenuated this neuroinflammatory effect. Subsequent investigation of neurodegeneration as indexed by GFAP protein content revealed no treatment-related increases following exposure to DFP or CORT+DFP. Immunohistochemical data revealed small, region-specific (e.g. hippocampus CA-1) changes in microglia and astrocyte morphology (Iba-1 and GFAP, respectively); and no neurodegeneration was seen with silver or Fluoro-Jade B assessments of damage. These results suggest increases in neuroinflammation, findings consistent with sickness behavior, may be characteristic of GWI, potentially induced by exposure to nerve agents, and can be exacerbated by exposure to chronic stress conditions (e.g., extreme temperatures, daily threat to safety/survival). While exposure to both stress hormones and nerve agents may be enough to produce increases in neuroinflammation, which likely contribute to sickness behavior seen in GWI, these conditions do not produce neural damage. Supported by USAMRMC W81XWH-09-2-0098

PS 2125 Pyridostigmine Bromide Suppresses Neuroinflammation Induced by DFP

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Use of our Gulf War Illness (GWI) mouse model has shown that administration of diisopropyl fluorophosphate (DFP) results in neuroinflammatory responses involving multiple cytokines/chemokines. Paradoxically, pretreatment with the anti-inflammatory stress hormone corticosterone (CORT) exaggerates this response. Neuroinflammation in the CNS results in sickness behavior, a persistent element of GWI. Soldiers in the 1991 Gulf War were instructed to consume the reversible cholinesterase inhibitor pyridostigmine bromide (PB) when under threat of nerve agent exposure. Here, we investigated how exposure to PB alone and combined with our GWI model affected neuroinflammatory responses. Male C57BL6J mice acutely exposed to PB (3.0 mg/kg) or PB following 4 days of CORT (400 mg/L in the drinking water) showed no significant neuroinflammatory changes in most markers (TNF-alpha, OSM, IL-1beta, LIF, IL-6) and no signs of astrogliosis (no increases in GFAP) in the hippocampus and cortex, findings suggestive of a lack of underlying neural damage. Investigation of the effects of chronic PB treatment prior to DFP exposure revealed a general reduction of DFP-induced neuroinflam-

matory markers (IL-6, LIF, TNF-alpha, IL-1 beta, OSM), findings consistent with the expected competitive effects of a reversible inhibitor given prior to an irreversible inhibitor. PB pretreatment prior to chronic CORT and DFP exposure produced little to no suppression of neuroinflammatory responses. These results suggest that, unlike exposure to irreversible AChE inhibitors, exposure to PB, a reversible inhibitor, produces negligible proinflammatory effects on the CNS with no signs of neural injury. Furthermore, PB exposure prior to nerve agents, such as DFP, blunts neuroinflammatory responses, but this dampening effect of PB on neuroinflammation is not present when baseline cortisol levels are heightened. Supported by USAMRMC W81XWH-09-2-0098

PS 2126 Altered Emotional Reactivity and Dopamine Turnover in Juvenile Rats Exposed Developmentally to Chlorpyrifos

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Repeated developmental exposure to the organophosphorus (OP) insecticide chlorpyrifos (CPF) results in the inhibition of fatty acid amide hydrolase (FAAH), the enzyme that metabolizes the endocannabinoid anandamide (AEA), and leads to the accumulation of AEA in the forebrain. At lower dosages, this occurs without measurable inhibition of cholinesterase (ChE), the canonical target of CPF, suggesting that the endocannabinoid system may be an important target in the developmental toxicity of OP insecticides. However, it is not clear if these biochemical changes during development will result in functional effects as the animal ages. The endocannabinoid system regulates emotional reactivity and this study investigated the persistent effects of CPF exposure on emotional reactivity. Following daily oral exposure to either 0.5, 0.75, or 1.0 mg/kg CPF from postnatal day 10-16, emotional reactivity was measured on day 25. The rats were placed into a dark container in a novel open field and the latency to emerge from the container was measured. In this test, rats that stay in the dark for a long time are considered emotionally reactive. All CPF treated groups spent significantly less time in the dark prior to emerging as compared to control suggesting a decreased level of emotional reactivity induced by CPF exposure. Immediately following behavioral testing, the levels of dopamine, serotonin, and their metabolites were measured in the amygdala and hippocampus. The levels of the dopamine metabolites were significantly elevated in the amygdala of all treatment groups suggesting that altered dopamine turnover plays a role in the decreased emotional reactivity.

PS 2127 Neurotoxic Effects of Tri-Cresyl Phosphates (TCPs) and Cresyl Saligenin Phosphate (CBDP) *In Vitro*

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We recently showed that the ortho-isoform of tri-cresyl phosphates (TCPs) ToCP impaired glutamate signaling in mouse primary cortical neurons (pCNs) at levels fare below its cytotoxic concentrations (Hausherr et al. 2014). Since TCPs are commercially used as mixtures of various isoforms and the ortho-isoform is metabolized into cresyl saligenin phosphate (CBDP) we performed additional experiments using TmCP, TpCP, a commercial TCP mixture and CBDP. We evaluated cell viability, neurite outgrowth, and the functionality of neurochemical processes. A 24 h exposure of mouse primary cortical neurons (pCNs) to the non-ortho TCPs and the mixtures yielded EC50 values for cell viability above 100 μM. In contrast, CBDP was cytotoxic at concentrations as low as 10 μM. Using fluorescence-based life-cell Ca2+ imaging, we investigated TCP and CBDP effects on signals evoked by the main excitatory neurotransmitter glutamate which was significantly decreased after 24 h exposure to ToCP in concentrations of 100 nM. Glutamate-evoked signals in pCNs were. None of the other TCP isomers, nor the mixture or the metabolite CBDP decreased the percentage of glutamate-responsive neurons and the mean response amplitudes at concentrations below 10 μM indicating a highly specific effect of the ortho-isoform on glutamate signaling. This specificity was confirmed by the simultaneous application of TOCP (100 μM) together with the glutamate stimulation resulted in a block of glutamate-induced responses by 70 %. Such a reduction was not observed when simultaneously applying CBDP (1 to 100 μM) to the glutamate stimulation. Even though CBDP was more cytotoxic that ToCP (EC50=89 μM) the impairment glutamate signaling seems to be a specific effect of the unmetabolized ortho-isoform of the tri-cresyl phosphates. Further research aims at investigating the mode of action of ToCP on the various glutamate receptors and effects of different ToCP amounts in TCP mixtures.

PS 2128 Characterization of a Rat Model of Acute Diisopropylflurophosphate (DFP) Intoxication

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Exposure to anticholinesterase organophosphates (OPs) can cause severe cholinergic crisis and death. Individuals that survive acute cholinergic crisis often develop persistent behavioral deficits for which effective therapeutics are not currently available. One of the challenges of developing therapeutic approaches to protect against the neurologic sequelae of acute OP intoxication is the limited characterization of OP-specific neurological damage. Using DFP as a model OP, we characterized the long-term behavioral and neurological effects in rats. Adult male Sprague Dawley rats were exposed to a potentially lethal dose of DFP (4 mg/kg, sc) and rescued from death by atropine sulfate (2mg/kg, im) and 2-PAM (25 mg/kg, im). Pyridostigmine bromide (0.1 mg/kg, im) was also given 30 min prior to DFP injection to reduce peripheral cholinergic symptoms. Behavioral assessments followed by immunohistochemical analyses of the brain were performed at 1 and 2 months post-DFP. DFP rats displayed hyperreactivity, aggressiveness and spontaneous recurrent behavioral seizures during this 2-month post-exposure period. In the elevated plus maze, DFP animals spent significantly more time in the open arms at both time points relative to the vehicle control indicating that DFP had a strong anxiolytic effect. Performance of DFP rats in the open field test for locomotor activity and forced swim for depression-like behavior were not significantly different from vehicle controls; however, the DFP group exhibited a significant increase in climbing behavior during the swim test. DFP intoxication significantly impaired performance in contextual fear conditioning indicating cognitive deficits. GFAP immunoreactivity was significantly increased in the hippocampus and cortex at 1 but not 2 months post-DFP. Collectively, these data suggest that acute DFP intoxication causes persistent behavioral deficits that may be mediated by reactive astrogliosis. Supported by the NIH CounterACT program (NS079202) and by an NIH training grant (T32 GM099608).

PS 2129 *In Vitro* Study of the Neuropathic Potential of the Organophosphorus Compounds Fenamiphos and Profenofos: Comparison with Mipafox and Paraoxon

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Organophosphorus-induced delayed neuropathy (OPIDN) is a central-peripheral distal axonopathy that develops 8-14 days after poisoning by a neuropathic organophosphorus compound (OP). Several OPs that caused OPIDN were withdrawn from the agricultural market due to induction of serious delayed effects. Therefore, the development of *in vitro* screenings able to differentiate neuropathic from non-neuropathic OPs is of crucial importance. Thus, the aim of this study was to evaluate the differences in the neurotoxic effects of mipafox (neuropathic OP) and paraoxon (non-neuropathic OP) in SH-SY5Y human neuroblastoma cells, using the inhibition and aging of neuropathy target esterase (NTE), inhibition of acetylcholinesterase (AChE), activation of calpain, neurite outgrowth, cytotoxicity and intracellular calcium as indicators. Additionally, the potential of fenamiphos and profenofos to cause acute and/or delayed effects was also evaluated. Mipafox had the lowest IC50 and induced the highest percentage of aging of NTE among the OPs evaluated. Only mipafox was able to cause calpain activation after 24 hours of incubation. Concentrations of mipafox and fenamiphos which inhibited at least 70% of NTE were also able to reduce neurite outgrowth. Cytotoxicity was higher in non-neuropathic than in neuropathic OPs while the intracellular calcium levels were higher in neuropathic than in non-neuropathic OPs. In conclusion, the SH-SY5Y cellular model was selective to differentiate neuropathic from non-neuropathic OPs; fenamiphos, but not profenofos presented results compatible with the induction of OPIDN.

PS 2130 Chlorpyrifos Oxon (CPFO) and 2, 2', 3, 5', 6-Pentachlorobiphenyl (PCB 95) Modulate Fc-Gamma Receptor Expression in Developing Neurons

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Fcγ receptors (FcγR) bind the constant (Fc) region of immunoglobulin G (IgG), and mediate uptake of IgG and cellular responses triggered by IgG binding in immune cells, including microglia in the central nervous system. We have previously shown that FcγR is expressed in the developing rat brain by cells other than microglia, specifically neurons and astrocytes and that binding of these receptors triggers

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