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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, and poster sessions of the 45th Annual Meeting of the Society of Toxicology, held at the San Diego Convention Center, San Diego, March 5–9, 2006.

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The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 534.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

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1466 AXONAL DYSTROPHY AND WALLERIAN-LIKE DEGENERATION. TWO MYELINATED NERVE FIBER LESIONS OF MULTI-EXPOSURE ORGANOPHOSPHATE-INDUCED DELAYED NEUROTOXICITY IN RATS

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Axonal dystrophy is a dramatic form of nerve fiber degeneration, manifest by marked enlargement of affected neurites associated with aggregations of tubulovesicular elements, mitochondria and dense bodies (Jellinger, 1973). These lesions are seen in terminal regions of long central nervous system myelinated fibers in conditions such as aging, vitamin E deficiency, genetic disorders and some intoxications (Jellinger 1973; Ohara et al. 1995). The term Wallerian-like degeneration is here used to indicate axonopathy progressing to myelinated nerve fiber breakdown in a non-traumatic neuropathic setting. In our study long-term exposure of rats to organophosphate neurotoxicants elicited both of these nerve fiber lesions. Long-Evans rats were administered two neurotoxic organophosphates in a setting of chronic stress over a 63-day period, with sacrifice on days 63 and 90 (after a 27 day exposure-free interval). The organophosphates were tri-ortho-tolyl phosphate (TOTP) given in 14 gavage doses of 75, 150 or 300 mg/kg and/or chlorpyrifos in two 60 mg/kg subcutaneous exposures. Corticosterone was added to the drinking water at 400 micrograms/ml to model chronic stress. Activity of brain neurotoxic esterase was diminished in a dose-related fashion by TOTP on days 28 and 63 with recovery on day 90. The major neuropathologic change was distal gracile fasciculus and peripheral nerve Wallerian-like myelinated fiber degeneration, with smaller numbers of associated dystrophic axons (in gracile region only). This was time (days 63 and 90)- and TOTP dose (at 300 and 150 mg/kg levels)-related. A dying-back pattern was seen with both types of fiber degeneration. In more proximal levels of the gracile fasciculus the dystrophic fibers often exceeded the Wallerian-like change. This suggests in this neurotoxic model, there is a dichotomous dying-back rate of these two axonal lesions, or that axonal dystrophy has a multifocal occurrence along the fiber. Supported by USAMRMC DAMD17-99-1-9489.

1467 DELAYED NEUROTOXICITY IN CHICKENS: 90-DAY STUDY WITH MOBIL JET OIL 254

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Mobil Jet Oil (MJO) 254 is a pentaerythritol-based synthetic lubricant widely used in a variety of jet engines. Because it contains tricresyl phosphate (TCP) as an anti-wear additive, the current study was conducted to assess the potential to cause organophosphate-induced delayed neurotoxicity (OPIDN) in hens following sub-chronic exposure. MJO 254 was administered to a group of 20 hens by oral gavage at a limit dose of 1 gm/kg, 5 days a week for 13 weeks. Positive and negative control groups were given TOCP or corn oil,respectively. Hens were periodically monitored for signs of OPIDN by means of clinical observations and tests of motor activity, measurement of neurotoxic esterase (NTE) and acetylcholinesterase (AChE)activity, and neuropathological evaluation of brain, spinal cord, and peripheral nerve. Methods were in general accord with EPA and OECD Test Guidelines. Administration of MJO 254 did not affect body weight or cause abnormal clinical signs or impairment of motor activity. No significant differences in activities of NTE or AChE in brain or spinal cord were seen between hens given MJO 254 and those given corn oil. Upon pathological examination, no lesions indicative of OPIDN were visible in perfusion fixed nervous tissue from MJO 254-treated hens. As expected, hens treated with the positive control (TOCP) displayed rapid progression of clinical impairment at time of sacrifice. NTE was markedly inhibited in brain and spinal cord of positive control hens at 48 hours post dosing (values approximately 5-10% of control), and 8/9 developed myelinated fiber degeneration characteristic of OPIDN. It was concluded that MJO 254 will not cause OPIDN or clinical evidence of cholinergic toxicity following repeated daily dosing in a test which followed established guidelines. Oral dosing was used to maximize systemic exposure. As there were no effects under these conditions, the data suggest that occupational exposures by dermal or inhalation routes would not be associated with risk of OPIDN.

1468 TRANSFER AND ACTIVATION OF MALATHION THROUGH AN *IN VITRO* BLOOD-BRAIN BARRIER SYSTEM

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Toxicant-induced effects are dependent on ability to reach the target site. For organophosphate (OP) insecticides, inhibition of neuronal cell acetylcholinesterase (AChE) occurs following transport across a blood-brain or blood-nerve barrier (BBB). Transport and activation of the OP insecticide malathion was examined in an in vitro BBB system consisting of bovine brain microvascular endothelial cells and rat astrocytes. These primary cells were cultured on inserts placed in wells containing human neuroblastoma SH-SY5Y cells. The malathion was placed inside the insert, on the endothelial cells, in concentrations of 10, 1, and 0.01 micromolar. After 16 hours of exposure, electrical resistance of the BBB and activity of AChE in the neuronal cells under the barrier were determined. A dose-related decrease in electrical resistance of the BBB was noted, with 50%, 35%, and 20% reduction in the presence of 10, 1 and 0.01 micromolar malathion. These concentrations did not result in overt cytotoxicity of the co-cultured cells, as measured by LDH leakage. This was less than 20%, even at the highest test concentration. Capability to biotransform the protoxicant malathion to an active toxicant was noted by AChE inhibition greater than 45% in the SH-SY5Y cells underneath inserts exposed to 1 and 10 micromolar malathion. Results suggest that transport as well as biotransformation in the BBB contribute to OP toxicity to neuronal cells.

1469 CHLORPYRIFOS INDUCES BBB DISRUPTION THROUGH UP-REGULATION OF PRO-INFLAMMATORY MEDIATORS IN BRAIN MICROVASCULAR ENDOTHELIAL CELLS

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Pro-inflammatory pathways in the brain microvasculature have been implicated in blood-brain barrier (BBB) disruption and the pathogenesis of neurodegenerative diseases. Our previous studies have demonstrated that exposure to the organophosphorus (OP) insecticide chlorpyrifos induces BBB disruption (Parran et al., 2005). Mechanisms of action underlying this process, however, have not yet been clearly established. Additionally, only limited information is currently available on the direct effects of chlorpyrifos on the cerebrovascular endothelial cell function and BBB integrity. The present study was designed to elucidate the molecular mechanisms of chlorpyrifos-induced BBB disruption. Expression of a variety of inflammatory mediators, such as pro-inflammatory cytokines, chemokines, adhesion molecules and matrix metalloproteinases was analyzed by quantitative real-time RT-PCR. Rat brain microvascular endothelial cells (RBE4) were exposed to increasing concentrations of chlorpyrifos for up to 24 h. A significant and time-dependent increase in mRNA expression of IL-6 was observed with 3.0-, 8.3- and 13.4-fold induction in the presence of 1.0, 10 and 100 nM chlorpyrifos after 24 h exposure. Additionally, 24 h exposure to 1.0, 10 and 100 nM chlorpyrifos markedly up-regulated expression of TNF-alpha (1.4-, 5.2-, 4.6-fold) and MMP-2 (3.0-, 4.9- and 4.6-fold). In contrast, MTT assay showed that the viability of RBE4 cells was not affected by treatment with ≤ 100 nM chlorpyrifos for up to 48 h. These data suggest that pro-inflammatory mechanisms contribute to the disruption of BBB integrity induced by chlorpyrifos. This finding offers the potential to contribute to the development of new therapeutic strategies specifically targeted against pro-inflammatory pathways of environmental agent-induced neurotoxicity. (This work was supported by VMRCVM New Initiative Grants)

1470 EARLY INTRACELLULAR SIGNALING IN SH-SY5Y CELLS AFTER EXPOSURE TO NEUROPATHIC AND NON-NEUROPATHIC ORGANOPHOSPHATES

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The binding of a neurotrophin to its specific high-affinity receptor initiates activation of up to three intracellular pathways: the phospholipase C-γ (PLC-γ) pathway, the phosphoinositol-3 kinase (PI-3K) pathway, and the mitogen-activated protein kinase (MAPK) pathways. While the PLC-γ and PI-3K pathways contribute primarily to cell survival, the MAPK pathway is primarily responsible for neurite outgrowth in vitro. We hypothesize that a neuropathy-inducing organophosphate (OP) compound interferes with the activation specific proteins in these intracellular pathways, specifically PKC-α, Akt, and Mek 1/2, as compared to a non-neuropathic OP compound. As a result, neurite outgrowth of cells exposed to a neuropathic OP compound is inhibited. To test this hypothesis, we exposed SH-SY5Y human neuroblastoma cells to a neuropathic OP compound (phenyl saligenin phosphate [PSP]; 0.01 μM, 0.1 μM, 1.0 μM), a non-neuropathic OP compound (paraoxon; 100 μM), a neuropathic OP compound with nerve growth factor (1.0 μM PSP + 1 ng/ml NGF), and medium only for 4, 8, 24, and 48 hours. We performed Western blots on cell lysates to determine the level of activated PKC-α (pPKC-α), Akt (pAkt), and Mek1/2 (pMek1/2). Our data indicate that cells treated with 100 μM paraoxon have higher levels of pMek1/2 compared to all other treatments. Paraoxon treated cells harvested 4 hours post-treatment had higher levels of pMek1/2 than cells harvested 8 hours post-treatment. Cells treated with 0.1