PS 1260 MANEB ENHANCES MPP+-INDUCED CYTOTOXICITY THROUGH ACTIVATION OF NF-KAPPA B IN PC12 CELLS.

S. Cheng, J. Quinones and A. Maynard. *Science, John Jay College of Criminal Justice, New York, NY.*

Environmental factors have been associated with the pathogenesis of neurodegeneration. Exposure to maneb (MB), manganese ethylene-bis-dithiocarbamate, has been linked to the development of parkinsonian-like symptoms in agricultureal workers (Ferraz et al 1988; Meco et al 1994). Barlow et al. (2005) suggested MB has the ability to disrupt the antioxidant systems of dopaminergic cells. MB also can increase nitric oxide (NO) production by mediating inducible nitric oxide synthase (iNOS) activity. The production of excessive NO can generate reactive nitrogen species which in turn increase oxidative stress. NO can act as second messenger molecule to control important cellular processes by regulation of expression/activity of certain proteins such as NF-kappaB. NF-kappaB induction and the activation of nitric oxide synthase through ROS represent a proximate mechanism for Mn-induced neurotoxicity. Since MB is the Mn-containing compound, it is interesting to know whether MB can activate NF-kappa B, thereby enhancing MPP+ toxicity. In this study, PC12 cells were treated with PBS and MB (20 uM) for 1 h prior MPP+ (500 uM) treatment. After 16 h MPP+ treatment, cell viability was assayed by trypan blue exclusion. MB pretreated groups showed 47 % cell death after MPP+ treatment (MB treated groups as 100%) and the PBS pretreated group showed only 30 % cell death after MPP+ treatment (PBS treated groups as 100%). The result demonstrated that MB enhanced MPP+ induced cell death. Western blot analysis was performed to evaluate the NF-kappa B activation. Western blot data showed MB reduced the cytosolic NF-kappa B p65 level (15% reduction) after 3 h MB treatment. This implicated that MB activated the NF-kappa B and caused the nuclear translocation of NF-kappa B. This activation could be responsible for the MB synergistic effect on MPP+ induced cytotoxicity. However, further studies are needed to demonstrate whether NF-kappa B activation induced by MB is directly involved in the synergic effect of MB on MPP+ induced cell death.

PS 1261 ANALYSIS OF C57BL/6 MICE AT 8 AND 16 MONTHS AFTER REPEATED DOSING OF PARAQUAT AND MANEB.

L. J. McIntosh[1], S. A. Benkovic[2], D. B. Miller[2], J. P. O'Callaghan[2], R. Patten[3], M. J. Collier[3], C. Willoughby[3], D. Myers[3], R. C. Switzer[4] and A. A. Li[1].
[1]Exponent, Inc., Menlo Park, CA, [2]Neurotoxicology Laboratories, Centers for Disease Control, Morgantown, WV, [3]Reproductive and Behavioural Studies Groups, Huntingdon Life Sciences, Suffolk, United Kingdom and [4]NeuroScience Associates, Knoxville, TN.

Combined exposure to paraquat (PQ) and maneb (MB) in C57Bl/6 mice has been hypothesized to be an animal model for Parkinson's Disease. This study examines three doses and two timepoints to assess effects of repeated PQ+MB injections on a functional observational battery (FOB) with 12 open field arena observations, motor activity, and neurohistological endpoints. Male and female mice were dosed on PND 5-19 with 0.3/1.0 mg/kg, 0.06/0.18 mg/kg, or 0.0007/0.0021 mg/kg PQ+MB respectively, and/or as adults twice weekly during weeks 27 to 31 of age with 10/30 mg/kg, 0.6/1.8 mg/kg, or 0.0007/0.0021 mg/kg PQ+MB. All animals were tested at 32 weeks, then sets dosed preweaning, as adult, or at both times were sacrificed at 33 weeks. A second set dosed at both times was held to 62 weeks and reassessed by the same FOB and motor activity tests, then sacrificed at 70 weeks. Brains of all animals were serially sectioned and every sixth section examined for cell degeneration and loss using GFAP, amino cupric silver (ACS) staining, and stereology of the substantia nigra pars compacta (SNpc). Gender effects were seen in the FOB, with high dose males showing increased incidence of tremor at 32 but not 62 weeks. High dose females showed increased horizontal activity and rearing at 62 weeks only. The high dose caused body weight loss in preweaning and adult males, and was lethal in males treated only as adults. Females did not show body weight-related effects at any dose, although brain weights were slightly reduced in high dose females. No treatment-related effects were seen across the whole brain using ACS and GFAP staining. Stereology is ongoing using StereoInvestigator v9.0 software to give a non-biased number of TH+ cells in the SNpc using the optical fractionator method.

PS 1262 LOCOMOTIVE ACTIVITY AS AN INDICATOR OF ACUTE CHLORPYRIFOS TOXICITY IN *C. ELEGANS*.

M. J. Pomeroy-Black. *Biology, LaGrange College, LaGrange, GA.*

Chlorpyrifos is a commonly used, broad-spectrum organophosphate insecticide that disrupts the nervous system. Symptoms of chlorpyrifos toxicity include muscle tremors, twitching, and in severe cases, paralysis and death. Neurotoxic effects of several organophosphates have been documented in the soil nematode Caenorhabditis elegans. We evaluated the locomotive activity of C. elegans after exposure to chlorpyrifos as a measure of neurotoxicity in these organisms. We hypothesized that there would be a dose-dependent decrease in locomotive activity in C. elegans exposed to chlorpyrifos. To test this hypothesis, we exposed worms to K-medium with or without chlorpyrifos (0.01 mM, 0.005 mM, or 0.001 mM) for 4 hours. We counted the number of body bends of worms as a measure of the rate of locomotion. The data indicate that nematodes exposed to 0.01 mM chlorpyrifos demonstrated fewer body bends than nematodes exposed to 0.001 mM after only 4 hours exposure, supporting our hypothesis. These results support the use of C. elegans as a model for early acute neurotoxicity testing.

PS 1263 STRAIN AND DOSE-RELATED EFFECTS OF SUBCHRONIC CHLORPYRIFOS (CPF) EXPOSURE ON BIOMARKERS OF EXPOSURE AND OXIDATIVE STRESS IN RATS.

J. R. Olson[1], M. Lasarev[2], D. Bruun[3], D. Milatovic[4], M. Aschner[4] and P. J. Lein[3, 2]. *[1]Pharmacology & Toxicology, University at Buffalo, Buffalo, NY, [2]CROET, Oregon Health & Science University, Portland, OR, [3]Molecular Biosciences, University of California Davis, Davis, CA and [4]Pediatric Toxicology, Vanderbilt University, Nashville, TN.*

Organophosphorus pesticide (OP)-induced neurotoxicity remains a significant public health concern, management of which is complicated by the lack of biomarkers that reliably identify at-risk individuals. We are developing a rat model of OP-induced neurotoxicity based on occupational exposures of Egyptian pesticide applicators to CPF to compare the predictive reliability of currently used biomarkers of exposure versus biomarkers of oxidative stress. Long Evans (LE) is the rat strain used almost exclusively for behavioral studies; however, much of the data describing CPF metabolism in rats were obtained using Sprague Dawley (SD) rats. In this study, therefore, we compared CPF metabolism in these strains by measuring trichloro-2-pyridinol (TCPy), a CPF-specific metabolite, in daily urine and cholinesterase (ChE) in blood collected every 3 days and brain harvested at the end of a 7-day exposure to 0, 3 or 10 mg CPF/kg/d. Oxidative stress was assessed by measuring F2-isoprostanes in urine and brain. Our major findings are: 1) there are no effects of strain on dose-related responses; however, LE rats had increased urinary TCPy levels and decreased brain ChE activity relative to SD rats; 2) peripheral measures of ChE activity and isoprostanes are predictive of CPF effects on these endpoints in the brain; 3) urinary TCPy does not predict blood ChE activity; 4) CPF exposure increases urinary and brain levels of F2-isoprostanes but correlations between F2-isoprostanes and either urinary TCPy or ChE activity were not evident. These data support the use of LE rats for biomarker studies and emphasize the need to evaluate the relationship between these biomarkers and neurobehavioral deficits in future studies. Supported by NIH grant #ES16308 (Anger and Lein, MPI)

PS 1264 IN-COMMUNITY STUDY OF LONG TERM LOW-LEVEL EXPOSURE TO ORGANOPHOSPHATE PESTICIDES (OP) – ARE THERE NEUROBEHAVIORAL EFFECTS AFTER THREE DECADES?

Y. Finkelstein[1], M. Aschner[2], A. Ophir[3], U. Wormser[3] and E. D. Richter[4].
[1]Neurology and Toxicology, Shaare Zedek Med Ctr, Jerusalem, Israel, [2]Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN, [3]Institute of Drug Research, School of Pharmacy, Faculty of Medicine, Hebrew University, Jerusalem, Israel and [4]Department of Occupational and Environmental Medicine, Faculty of Medicine, Hebrew University, Jerusalem, Israel.

Hula Valley in Israel has been extensively cultivated since 1957. Our studies in 1987- 1991 assessed neurological effects of low-level long-term exposure to OP pesticides in 200 workers and residents in the valley. We are currently carrying on the work which started on the original cohorts to evaluate the extended outcome of their daily OP exposure. This is the first study with such a long follow-up. Cognitive tests, nerve conduction and blood studies are now under way; including PON-1 (a parameter of genetic polymorphism), NTE (a biomarker for OP-induced neuropathy); and urine analysis for OP metabolites. The original cohorts showed neurobehavioral dysfunction and mildly impaired Nerve conduction parameters. Results from these studies establish impaired performance of cognitive executive tasks. Trail Making Tests A and B for screening, attention and graphomotor ability were significantly poorer than those of the general population. Digit span test (forward and backward) for auditory and visual memory involving attention and similarities for verbal abstraction was poor. At least one of the children of 24 out of 60 cohort participants (40%) had been reported to be diagnosed with Attention Deficit/Hyperactivity Disorder (ADHD) vs. expected 5%-7%. This finding raises concern, as current theory suggests that a genetic factor is primarily responsible for the pathogenesis of ADHD. The strong database of the cohorts may show persistent late neurotoxic effects of lengthy daily OP exposure. (Supported by