function as a metal binding protein. Interestingly, increased brain manganese (Mn) levels have been reported in various prion diseases indicating divalent metals may play a role in the disease process. Recently, our group found that Mn upregulated cellular prion protein levels in neuronal cell culture independent of transcription by significantly altering the turnover and stability of PrPC. To further understand the role of Mn in prion diseases, we examined the prion protein levels in animal models of Mn neurotoxicity. Administration of 10mg/kg of Mn once per day for 10-30 days by oral gavage induced regionally specific increases in PrPC levels, indicating that Mn can influence the level of prion protein in the brain. We also examined the effect of Mn on infectious cell culture models of prion disease. Treatment with 300μM Mn in persistently infected CAD5 cells showed reduced mitochondrial impairment, cytotoxicity, and caspase-3 activation when compared to uninfected cells. Scrapie infected cells also showed significantly reduced Mn uptake as measured by inductively coupled plasma-mass spectrometry (ICP-MS). Together, our data indicate that Mn interacts with prion protein to alter the stability of the protein and suggest that understanding the interaction of metals with disease specific proteins may provide further insights to prion-like propagation of neurodegenerative diseases. (Supported by NIH grant RO1ES19267 and MHRP grant W81XWH-05-10239)

PS 174 CHRONIC NEUROTOXICITY AFTER CHRONIC MOLD EXPOSURE.

R. M. Singer. *Raymond Singer, Ph.D., Professional Association, Santa Fe, NM.*

A 56 year old female with possible mold neurotoxicity was evaluated on 5 occasions from 2003-2008, after having resided in a contaminated, high-end condominium for 1.5 years. The evaluation included diagnostic interviews, neuropsychological tests, and medical/environmental record review. Shortly after moving into the contaminated residence, she had developed flu-like symptoms with nasal congestion, burning eyes, and fatigue. Environmental testing found mold including stachybotrys (SB) (3,424 counts of fungal structure (FS); 91,307 FS/m3, 97% of sample, categorized as massive fungal growth, with black mold in many locations in the apartment, including air samples of 260 particulates SB/M3. SB IgE was 51 ELISA units, above the reference range of 50. Her full scale IQ prior to exposure was estimated to be at the 97 percentile (pct). Post-exposure, she was unemployed since 2003 due to various cognitive malfunctions. MRI showed right temporal lobe abnormality. Results of the Neurotoxicity Screening Survey were elevated (436) and consistent with neurotoxicity, with Symptom Distortion not indicated. Current WAIS-III Working Memory was at the 5pct; WMS-III Working Memory 13pct; Selective Reminding Test 11 pct; Comprehensive Trail-Making Test 9pct; Controlled Oral Word Association Test 5pct, Stroop Color and Word Test 1 pct, indicating significant declines in memory and executive function. Repeated testing over 5 years after being removed from exposure found cognitive function partially improved, but her function did not return expected values.

PS 175 HYPERPHOSPHATEMIA AND HYPOZINCEMIA AFFECT PERIPHERAL NERVOUS SYSTEM IN SHR/NDMCR-CP, A MODEL OF METABOLIC SYNDROME.

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Background: It is known that the patients with type 2 diabetes mellitus affect peripheral nervous system. However, it is unclear whether metabolic syndrome is associated with peripheral neuropathy. Given that patients with diabetic nephropathy are easily suffering from hyperphosphatemia and hypozincemia are clinically described, we assessed the higher phosphorus and lower zinc levels in plasma may affect peripheral nervous system using SHR/NDmcr-cp, a rat model of metabolic syndrome. We also investigated the effects of antioxidant, N-acetyl-L-cysteine (NAC), on peripheral nervous system under such conditions. Methods & Results: Male SHR/NDmcr-cp and control (WKY) rats were divided into 3 groups and were fed control diet (P 0.3% w/w, Zn 0.2% w/w) or a high-phosphorus and zinc-deficient (P 1.2 % w/w, Zn 0.0 % w/w) diet. The latter group was treated with either NAC (1.5 mg/g per day) or vehicle from 8 to 12 weeks of age (n = 6 or 8 for each group). Maximum motor nerve conduction velocity (MCV) and distal latency (DL) of the tail nerve were measured in rats at 12 weeks of age. The level of MCV was significantly smaller in CP control group than that of WKY control group. MCV of the tail nerve deteriorated more significantly by the high-phosphorus and zinc-deficient diet in CP compared with WKY. The treatment with NAC significantly prevented the toxic effects on the peripheral nervous system in both strains. Conclusion: Metabolic syndrome induces deterioration of MCV. Dietary high phosphorus and deficiency of zinc induces further damage to peripheral nerve system that may be protected by the treatment with antioxidant.

PS 176 EFFECTS OF 1-BROMOPROPANE EXPOSURE ON MYELINATION AND ASTROGLIA IN RAT BRAIN.

S. Sheik Mohideen[1], S. Ichihara[2], Z. Huang[1], L. Zhang[1], K. Subramanian[1], J. Kitoh[3] and G. Ichihara[1]. *[1]Department of Occupational & Environmental Health, Nagoya University Graduate School of Medicine, Nagoya, Japan, [2]Mie University Graduate School of Regional Innovation Studies, Tsu, Mie, Japan and [3]Nagoya University, Nagoya, Japan.*

Introduction: 1-Bromopropoane (1BP) is used as an alternative to ozone depleting solvents. 1-BP exhibits neuro-reproductive toxicities in both animals and humans. A human case intoxicated with 1BP showed patchy areas of increased T2 signal in periventricular white matter in magnetic resonance image scan of the brain. We hypothesize that 1-BP might induce demyelination and affect myelination. The present study investigated the effects of 1-BP exposure on myelination through expression levels of genes and proteins related to myelination. Methods: Forty eight F344 rats were randomly divided into four groups of twelve each and exposed to 1BP at 0, 400, 800 and 1000 ppm for 8 hrs/day, 7 days/week for 4 weeks. Total RNA from brain parts was extracted and quantitative real time PCR was conducted to quantify the mRNA levels of Glial fibrillary acidic protein (Gfap), myelin basic protein (Mbp), OL lineage gene 2 (Olig2), myelin OL glycoprotein (Mog), chondroitin sulfate proteoglycan 4 (NG2), interleukin 11 receptor α (Il 11rα), caspase 3 (Casp3) and tumor necrosis factor α (Tnfα). 3 rats from each group was perfused using 4% paraformaldehyde for immunohistochemistry of Gfap, Mbp and oligodendrocyte marker O4. Results: In the cerebellum, mRNA levels of Mbp, Mog, Ng2, Tnfα and Olig2 genes decreased dose-dependently, while the mRNA level of Gfap gene increased dose-dependently. In the hippocampus, mRNA levels of the examined genes did not show any significant difference among groups. The Gfap immunostaining showed clearly higher expression in astrocyte including its glial process in the 1000ppm group when compared to the control. Conclusion: Thus exposure to 1-BP activated astroglia and decreased mRNA expression of myelin related genes in the rat cerebellum. 1BP exposure might disrupt myelination leading to astrocytes activation for CNS repair and remyelination.

PS 177 EXPOSURE TO 1-BROMOPROPANE DEGENERATES NORADRENERGIC AXONS IN THE RAT BRAIN.

G. Ichihara[1], S. Sheik Mohideen[1], S. Ichihara[2] and S. Nakamura[3]. *[1]Department of Occupational & Environmental Health, Nagoya University Graduate School of Medicine, Nagoya, Japan, [2]Mie University, Tsu, Japan and [3]Neuroscience, Yamaguchi University Graduate School of Medicine, Ube, Japan.*

1-bromopropane (1-BP) has been used as an alternative to ozone-depleting solvents such as chlorofluorocarbons, which were used as cleaning agents for metal parts in electronics factories. Previous studies showed that 1-BP exhibits potent neurotoxicity. In humans, exposure to 1-BP caused a variety of neurological and neurobehavioral symptoms or signs including numbness and diminished vibration sense in the lower extremities and disturbance of memory. Moreover, depressed or irritated mood was reported by workers after exposure to 1-BP. However, neurobiological changes underlying depressive symptoms induced by exposure to 1-BP remain to be determined. Axonal degeneration of neurons containing noradrenaline (NA) and serotonin (5-HT) is thought to be associated with the occurrence of depressive symptoms. Based on this hypothesis, the present study examined whether repeated exposure to 1-BP causes the degeneration of 5-HT and NA axons. Exposure to 1-BP induced dose-dependent decreases in the density of NA axons in the rat brain, while the density of 5-HT axons did not appear to be affected by 1-BP. The present study suggests that depressive symptoms that occur after exposure to 1-BP are attributable, at least in part, to the degeneration of NA axons in the brain.

PS 178 CHRONIC EXPOSURE TO GLUCOCORTICOIDS PRIMES THE CNS PROINFLAMMATORY RESPONSE IN METHAMPHETAMINE NEUROTOXICITY.

K. A. Kelly, D. B. Miller and O. P. James. *Health Effects Laboratory Division, CDC-NIOSH, Morgantown, WV.*

Upregulation of proinflammatory cytokines and chemokines in the brain ("neuroinflammation") accompanies brain injury and disease as well as systemic infections. Previously we documented a neuroinflammatory response associated with the neurotoxic effects of the dopaminergic neurotoxicants, MPTP and METH. These elevations in a variety of proinflammatory mediators may serve as modulators or mediators of astroglial and microglial activation, cellular responses associated with all types of brain injury. Activated glia may have neuroprotective roles or may exacerbate neural damage. Our prior genetic and pharmacological interventions have resulted in partial suppression of neuroinflammatory responses associated with exposure to MPTP and METH without affecting neurotoxicity and gliosis. Because glucocorticoids are regarded as potent anti-inflammatory agents, we pretreated

mice with corticosterone (CORT) prior to administration of MPTP or METH and assessed a variety of cytokines/chemokines by qPCR and examined dopaminergic terminal damage and astrogliosis by tyrosine hydroxylase (TH) and GFAP immunoassay, respectively. Acute CORT (20 mg/kg, s.c.) 30 minutes prior to MPTP or METH reduced, but did not completely suppress the expression of LIF, CCL2, IL-1B induced neurotoxicity, whereas the decrease in TH and increase in GFAP remained unaffected. A chronic (1 week) CORT pretreatment in the drinking water was employed to achieve a longer and higher level of anti-inflammatory therapy on METH. Surprisingly, this CORT regimen appeared to prime the neuroinflammatory response to METH as most proinflammatory mediators showed exacerbated responses. In contrast to acute pretreatment with CORT, the effect of METH on TH and GFAP was exacerbated by chronic CORT. As the levels of chronic CORT approached or exceeded those associated with high physiological stress levels, our data suggest chronic CORT therapy or sustained physiological stress sensitizes CNS neuroinflammatory and neurotoxicity responses to METH.

PS 179 GST-PI INHIBITS DOPAMINE NEURON DEGENERATION IN *C. ELEGANS* MODELS OF PARKINSON'S DISEASE AND MANGANISM.

R. Settivari, J. LeVora, N. VanDuyn, S. Zhou, G. Sinclair and R. Nass. *Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN.*

Idiopathic Parkinson's disease (PD) and manganism are oxidative stress-related movement disorders that result in abnormal dopamine (DA) signaling and cell death. Both neurological disorders involve basal ganglia and mitochondria dysfunction, and suggest overlapping epidemiology, yet the origin of the pathogenesis and the molecular determinants common between the diseases are ill-defined. Nrf2/SKN-1 regulates the gene expression of phase II detoxification enzymes, and is upregulated following exposure to oxidative stress in mammals and the nematode Caenorhabditis elegans (*C. elegans*). Glutathione-S-transferases (GSTs) of the class pi (GST-PI) are Phase II detoxification enzymes that conjugate both endogenous and exogenous compounds to glutathione to reduce cellular oxidative stress. GST-PI's have also recently been implicated PD-associated DA neurodegeneration. In this study we asked whether GST-PI expression may modulate DA neuron vulnerability following exposure to manganese (Mn) and rotenone. We demonstrate that a GST-PI homologue modulates DA neuron vulnerability to both of the PD-associated neurotoxicants. We show that SKN-1 and GST-PI are expressed in the *C. elegans* DA neurons, exposure to either toxicant increase reactive oxygen species, reduces mitochondrial membrane potential, induces GST-PI gene and protein expression, and the induction is partially dependent on SKN-1. We also show that GST-PI inhibits toxicant-induced DA neurodegeneration and toxicant-induced movement deficits, and identify cell death pathways involved in the neurodegeneration. Finally we present our initial studies from a reverse genetic screen that identifies modulators of PD-associated DA neuron vulnerability. Our studies provide the first *in vivo* linkage that a reduction in a xenobiotic metabolizing enzyme confers an increase in DA neuron vulnerability in models of PD and manganism. Support contributed by: NIH R011ES014459, NIH R01ES010563 (RN)

PS 180 ROLE OF GLIAL ACTIVATION IN A PROGRESSIVE NEUROINFLAMMATORY MODEL OF PARKINSON'S DISEASE USING MPTP AND PROBENECID.

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Inflammatory activation of glia is implicated in the progressive loss of dopaminergic neurons in Parkinson's Disease (PD). Suppression of neuroinflammation may prove useful in slowing the continued neuronal degeneration. In the present study we set out to investigate the efficacy of novel para-substituted diindolylmethane (cDIM) compounds in attenuating this progressive neuron loss. We first assessed cDIM activity in vitro using qPCR for NOS2, a prototypic neuroinflammatory gene. qPCR revealed attenuation of LPS induced NOS2 expression in astrocytes co-treated with cDIM5. Next, cDIM5 efficacy was assessed in vivo using the parkinsonian neurotoxicant, MPTP (2 x 15 mg/kg, bid 12 hr). Degeneration was determined by quantifying tyrosine hydroxylase loss in the striatum. Co-treatment of cDIM5 (50 mg/kg, daily oral gavage) returned striatal TH intensity to control levels. Lastly, we set out to establish a model of progressive neurodegeneration/neuroinflammation employing MPTP (80 mg/kg, total dose) in conjunction with probenecid (250 mg/kg). Mice were treated every other day for 7 days and monitored for a total of 14 days. To assess loss of dopaminergic neurons, stereological counts of TH positive neurons in the SNpc were determined on day 7 and day 14. Stereological counts revealed a reduction in the total number of dopaminergic neurons on day 7 which progressed to an even greater loss on day 14. These data show that upon cessation of MPTP/probenecid treatment, loss of nigral neurons continues to occur, emulating a progressive lesion as seen in PD.

PS 181 DEVELOPMENT OF A HIGH-THROUGHPUT SCREENING PLATFORM FOR MONOAMINE TOXICITY.

A. I. Bernstein[1], K. A. Stout[1], J. D. de Gastyne[1] and G. W. Miller[1, 2]. *[1]Environmental Health, Emory University, Atlanta, GA and [2]Center for Neurodegenerative Disease, Emory University, Atlanta, GA.*

The vesicular monoamine transporter 2 (VMAT2) is responsible for packaging monoamine neurotransmitters such as dopamine (DA), serotonin, norepinephrine and epinephrine into synaptic vesicles. Levels of these monoamines are highly regulated and their dysregulation plays a role in Parkinson's disease, Huntington's disease, drug addiction, neuropsychiatric disorders and pesticide toxicity. While the primary function of VMAT2 is to sequester these monoamines into vesicles, they can also translocate toxicants away from cytosolic sites of action. Identification of compounds that modify the action of VMAT2 may be useful as possible therapeutic agents for preventing or reversing monoamine-related toxicity. To this end, we are utilizing the fluorescent substrate, 4-(4-dimethylaminostryl)-N-methylpyridinium (ASP+) to develop a high throughput screening assay. ASP+ is transported into cells by the plasma membrane transporters of DA, norepinephrine and serotonin and has been developed into a rapid, high-throughput assay for function of these transporters (Molecular Devices). We demonstrate here that ASP+ is also transported in vesicles by VMAT2 and uptake can be blocked by the VMAT2-specific inhibitor tetrabenazine (TBZ). HEK cell lines stably expressing the dopamine transporter and VMAT2 were generated. Uptake of 3H-DA was measured in parallel to uptake of ASP+. Time course experiments indicated a similar time course of uptake with maximal signal occurring at 20 minutes. Dose response experiments with TBZ demonstrated that uptake of 3H-DA and ASP+ is inhibited by similar concentrations of TBZ. We are currently optimizing this assay into a platform amenable for high-throughput screening for VMAT2 function. Supported by NIEHS P01ES016731.

PS 182 SEROTONERGIC AND NORADRENERGIC SYSTEMS ARE DISRUPTED IN THE VMAT2-DEFICIENT PARKINSON'S DISEASE MOUSE MODEL.

S. P. Alter, T. N. Taylor, K. R. Shepherd and G. W. Miller. *Environmental Health, Emory University, Atlanta, GA.*

Our lab has established that mice with reduced vesicular monoamine transporter 2 (VMAT2; Slc18a2) display motor and nonmotor symptoms of Parkinson's disease (PD). Previously, we demonstrated dopaminergic degeneration occurs in VMAT2-deficient mice, paralleling the pathology of human PD (J Neuroscience, 2007; 2009). Here, we report disruptions in the noradrenergic and serotonergic systems in the VMAT-2 deficient mouse model. It is known that the locus ceruleus (LC) undergoes degeneration prior to clinical presentation of motor symptoms in human PD. Similarly, stereological analysis revealed progressive LC degeneration in the VMAT2-deficient mouse model, beginning at 12 months of age. At 18 months, this degeneration is more drastic than that of the substantia nigra. Consistent with the notion that oxidative damage can drive neurodegeneration, DCF assays show that primary cultured LC neurons from VMAT2-deficient mice are sensitive to oxidative challenge when treated with dopamine and norepinephrine. WT LC neurons are protected from this effect. Serotonergic dysfunction also occurs in PD. Previous work by our lab has shown that VMAT2-deficient mice brains have decreased levels of serotonin and decreased serotonin turnover. Here, we show that VMAT2 loss adversely affects the dorsal raphe, leading to markedly increased cell death as detected by silver staining in 24 month old VMAT-2 deficient mice, in contrast to age-matched WT, and we observed an increase in alpha-synuclein accumulation in 30 month old mice. DCF assays revealed a 60 % increase in serotonergic-induced oxidative stress in cultured postnatal VMAT2-deficient raphe neurons. In tail-suspension tests, VMAT2-deficient mice display greater immobility time than age-matched wildtype, and this is attenuated with fluoxetine treatment. These findings show that the VMAT2-deficient mice experience serotonergic and noradrenergic pathologies that are similar to those in human PD. Supported by 5P01ESO16731.

PS 183 VASCULAR IMPLICATIONS OF NRTI-INDUCED MITOCHONDRIAL TOXICITY.

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Associations between the use of nucleoside analog reverse transcriptase inhibitors (NRTIs) in the treatment of HIV and various cardiovascular side effects have been well-documented; however, the precise mechanisms underlying NRTI-induced cardiovascular pathogenesis have not yet been fully elucidated. Previous work from our

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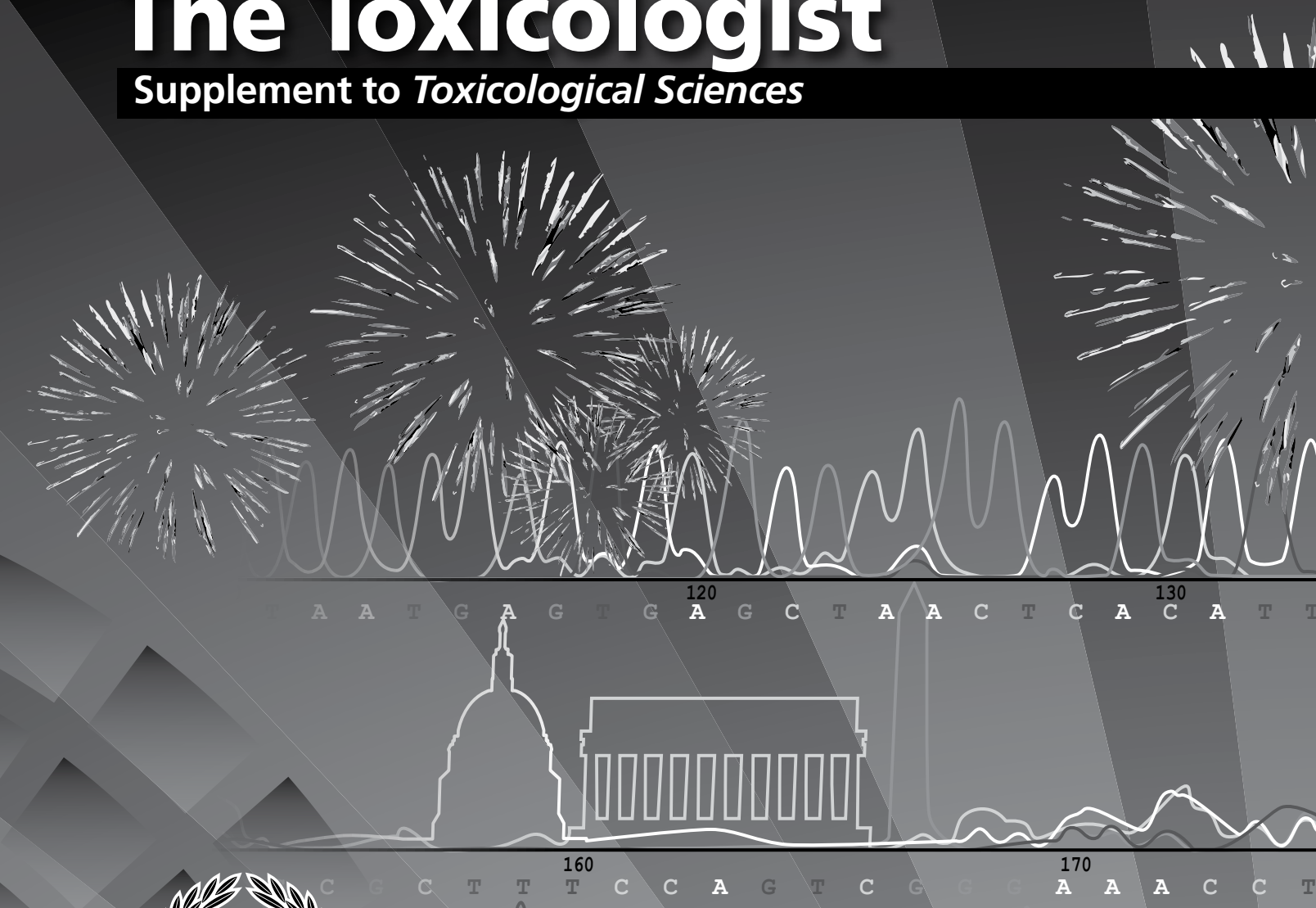

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 50th Annual Meeting of the Society of Toxicology, held at the Walter E. Washington Convention Center, March 6–10, 2011.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 578.

The issue also contains a Key Word Index (by subject or chemical) of all the presentations, beginning on page 606.

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