These results confirm the elevation of thalamic GABA levels in a typical US occupational setting. The significant correlations between increased GABA levels and recent exposure levels, as well as with brain Mn accumulation in the substantia nigra, suggest that GABA-edited MRS in conjunction with quantitative T1 relaxation MRI may serve as a biomarker of Mn exposure. (Supported by NIEHS R01 ES020529 and CDC/NIOSH T03 OH008615)

PS 1368a Potential Prevention Strategies to Reduce the Risk of Neurotoxicity Associated with Manganese-Containing Welding Fumes

K. Sriram, A. M. Jefferson, G. X. Lin, A. Afshari, S. Stone, W. McKinney, M. Jackson, M. J. Keane, B. T. Chen, D. G. Frazer and J. M. Antonini. *CDC-NIOSH, Morgantown, WV.*

Welding generates complex metal aerosols, inhalation of which is thought to cause Parkinson's disease (PD)-like neurotoxicity, due to the presence of manganese (Mn) in the welding electrodes. As neurological disorders are generally progressive in nature, with latency between insult and appearance of clinical symptoms, a logical approach for workplace safety and health is to prevent adverse exposures. For welding, this can be achieved by minimizing welding fume (WF) generation rate and/or suitably modifying existing welding practices to reduce toxic exposures. Here, we show that by specifically modulating welding voltage, keeping current and shielding gas constant, the fume composition and neurotoxicological properties of WF can be significantly altered. Rats were exposed by whole-body inhalation to filtered air or WF particulates generated by gas-metal arc-stainless steel welding (GMA-SS; 40 mg/m3; 3h/d x 10d) either at 25V (standard/low; LVSS) or at 30V (high; HVSS) voltage. Both conditions produced good weld quality and similar particulate morphology, although aerosols from HVSS welding comprised of a larger fraction of ultrafine particulates that are characteristically considered to be more toxic than their fine counterparts. Exposure to particulates from LVSS welding caused neuroinflammation (increased Ccl2, Tnfa, Nos2; 1.5 - 3.9 fold; $P<0.05$) and decreased PD-related proteins (Th, Park5, Park7; 18 - 47%; $P<0.05$) in the dopaminergic brain areas, striatum and midbrain. Paradoxically, exposure to particulates from HVSS welding did not elicit any dopaminergic neurotoxicity. We determined that the lack of neurotoxicity may be a consequence of the reduced solubility of manganese in HVSS fumes. Our findings show promise for modified welding practices as a potential prevention strategy for Mn-related neurotoxicity during welding; however, it warrants additional investigations to determine if such modifications can be suitably adapted at the workplace to avert or reduce neurological risks.

PS 1368b The Role of the Transient Receptor Potential Ankyrin 1 (TRPA1) Channel in Methylmercury (MeHg)-Induced Ca^{2+} Dysregulation

H. Hannon and W. D. Atchison. *Pharmacology/Toxicology, East Lansing, MI.*

MeHg biphasically elevates $[Ca^{2+}]_i$, with the first (P1) and second (P2) phases attributed to Ca^{2+} release from, in part, IP_3-sensitive intracellular stores and extracellular Ca^{2+} influx through Ca^{2+}-permeable ion channels, respectively. MeHg-induced $[Ca^{2+}]_i$ dysregulations have been correlated with reduced viability of susceptible neuronal populations *in vitro*. Because paresthesia is the preeminent sign of MeHg intoxication, and the size and number of neurons of the dorsal root ganglia (DRG) are reduced in poisoned individuals, molecular entities unique to DRG may heighten sensitivity to MeHg. Heterologous expression was used in this study to investigate the contribution of TRPA1, a Ca^{2+}-permeable ion channel highly expressed in DRG, in mediating Ca^{2+} dysregulation throughout acute MeHg exposure. HEK 293 cells were transfected with human TRPA1 72 hrs prior to single-cell microfluorimetry studies. Fura-2 AM, a ratiometric Ca^{2+} fluorophore, was used to measure relative changes in $[Ca^{2+}]_i$ in transfected cells throughout exposure to MeHg (1, 2 μM). Time-to-onset of P1 was hastened in TRPA1-HEK exposed to 1 μM MeHg, as compared to untransfected HEK (UT) (5.9±0.8 min and 11.1±0.5 min, respectively; mean ± SEM). This suggests that TRPA1 provides a more kinetically favorable route of entry for low [MeHg]. Expression of TRPA1 did not alter the time-to-onset of P2 between TRPA1-HEK (39.7±2.2 min) and UT (37.4±1.5 min) exposed to 1 μM MeHg. Similarly, TRPA1 expression had no effect on the times-to-onset of neither P1 nor P2 in TRPA1-HEK (4.4±0.7 and 26.1±2.7 min, respectively) and UT (5.1±0.2 and 24.3±0.9 min, respectively) exposed to 2 μM MeHg. These results suggest Ca^{2+} entry through TRPA1 does not significantly contribute to overall $[Ca^{2+}]_i$ dysregulation following MeHg exposure. This, combined with the fact that P1 is unaffected by TRPA1 expression at higher [MeHg], suggests that TRPA1 may be a sensitive target for time- and concentration-dependent block by MeHg. Supported by NIH grant R01ES03299.

PS 1369 Methylmercury Induces Apoptosis in Neuronal Cells through ROS-Mediated Endoplasmic Reticulum Stress-Regulated Signaling Pathway

Y. Chung[1], F. Tang[2], C. Su[3], T. Lu[4], C. Wu[5], K. Chen[7], D. Hung[8], Y. Chen[4], C. Yen[1] and C. Huang[6]. *[1]Department of Occupational Safety and Health, Chung Shan Medical University, Taichung, Taiwan, [2]Department of Occupational Medicine, Changhua Christian Hospital, Changhua, Taiwan, [3]Department of Otorhinolaryngology, Head and Neck Surgery, Changhua Christian Hospital, Changhua, Taiwan, [4]Department of Physiology and Graduate Institute of Basic Medical Science, China Medical University, Taichung, Taiwan, [5]Department of Public Health, China Medical University, Taichung, Taiwan, [6]School of Chinese Medicine, China Medical University, Taichung, Taiwan, [7]Department of Urology, China Medical University Hospital, Taichung, Taiwan and [8]Division of Toxicology, Trauma & Emergency Center, China Medical University Hospital, Taichung, Taiwan.*

Objectives: Methylmercury (MeHg) is a well-known environmental pollution. The human beings expose to methylmercury through ingestion, which can cause irreversible nervous system dysfunction and injuries. However, effects and possible mechanisms of MeHg-induced neurotoxicity remained unclear. In this study, we attempted to investigate the important roles of endoplasmic reticulum (ER) stress-regulated pathway in MeHg-induced neurotoxicity.
Results: MeHg significantly decreased cell viability in a dose-dependent manner (1-5 μM) and induced the increase in caspase-3 activity, annexin V-FITC binding, and the protein expressions of caspase cascades in Neuro-2a cells, indicating that MeHg could induce neuronal cell apoptosis. Moreover, exposure of Neuro-2a cells to MeHg could trigger ER-stress as indicated by several key markers (GRP-78, GRP-94, CHOP, and XBP-1) and upstream molecules (the phosphorylation of PERK, eIF2-α, and IRE-1), and caspase-12 cleavage. Pretreatment with antioxidant NAC and transfection with specific si-RNA (GRP-78, GRP-94, CHOP, and XBP-1, respectively) could effectively attenuate MeHg-induced cytotoxicity, apoptotic events and ER stress-related signals.
Conclusions: These results indicate that the ROS production triggered ER-stress signaling pathway involves in MeHg-induced neuronal cell apoptosis.

PS 1370 Effect of Methylmercury (MeHg) on RNA Expression of Voltage-Gated Calcium Channels (VGCCs) in Naïve and Differentiated F11 Cells

K. Z. Perez-Vale, H. Hannon and W. D. Atchison. *Pharmacol./Toxicol., Mich State Univ, E. Lansing, MI.*

MeHg is an environmental contaminant that elevates $[Ca^{2+}]_i$ in two kinetically-distinct phases. Phase one (P1) consists of Ca^{2+} release from intracellular storage organelles, whereas phase two (P2) results from an influx of Ca^{2+} through Ca^{2+}-permeable ion channels. MeHg-induced elevations in $[Ca^{2+}]_i$ have been correlated with increased cell death. This study surveyed VGCC RNA expression in MeHg-exposed F11 cells, an immortalized cell line derived from dorsal root ganglia. Changes in RNA expression of the pore-forming subunit of the L-, N-, P/Q-, R- and T-type VGCC isoforms (*cacna1c*, *1b*, *1a*, *1e*, and *1h*, respectively) following MeHg exposure was examined by polymerase chain reaction (PCR). F11 cells were cultured and differentiated (2 μM retinoic acid) for 24-72 hrs prior to 1 hr MeHg exposure (1, 2 and 5 μM). 24 hrs after MeHg exposure, RNA was isolated and converted to cDNA. PCR was performed on cDNAs and relative changes in RNA were determined by $2^{-\Delta\Delta Ct}$. Differentiation alone increases the RNA expression of *cacna1c* and *cacna1a* in F11 cells at 72 hours (2.55±0.05 and 2.51±0.05, respectively; mean fold-change ± SEM). Differentiated F11 cells also express low levels of RNA for *cacna1b* (1.01±0.04), *cacna1e* (0.45±0.04), and *cacna1h* (1.12±0.03); exposure to MeHg reduces RNA expression of these genes in a concentration-dependent manner, with 5 μM MeHg reducing *cacna1a*, *cacna1c*, and *cacna1h* RNA most markedly (0.45±0.06, 1.06±0.09, and 0.63±0.04, respectively). Downregulation of RNA expression was also dependent upon the differentiation time point; cells differentiated for longer periods displayed a greater reduction of *cacna1* expression universally. Changes in RNA expression may reflect a concomitant alteration in protein expression, thus these data may indicate distinct VGCC isoforms as critical contributors to Ca^{2+} influx in P2. Because RNA expression of *cacna1c* and *cacna1a* is most abundant following acute MeHg exposure, the L- and P/Q-type VGCCs may be significantly involved in cytotoxic mechanisms. Supported by NIH grant R01ES03299 and R25NS54467.

The Toxicologist

Supplement to *Toxicological Sciences*

53rd Annual Meeting and ToxExpo™

March 23–27, 2014 • Phoenix, Arizona

An Official Journal of the Society of Toxicology

SOT | Society of Toxicology

Creating a Safer and Healthier World by Advancing the Science of Toxicology

www.toxicology.org

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 138, Issue 1
March 2014

www.toxsci.oxfordjournals.org