(Oxy), significantly lowered glutathione (GSH) levels in liver cell cultures. These results indicate that oxidative injury may play a role in AAS toxicity. In order to determine if oxidative damage is involved in the injury process, elevations in malondialdehyde (MDA) (an indicator of oxidative damage), protection due to increased GSH, and cellular morphology were determined in cell cultures established from adult male Sprague-Dawley rats. Cultures were treated with 100 μM Met or Oxy and evaluated for time periods ranging from 10 min to 13 hr. Lactate dehydrogenase (LDH) release was measured to correlate cell death with MDA production. MDA production was measured using a thiobarbituric acid reactive substances (TBARS) assay to evaluate AAS-induced lipid peroxidation. Elevations in MDA formation were observed after treatment with both AASs. Elevations in MDA occurred prior to significant LDH release, indicating that MDA formation was not merely due to cell death. To evaluate the possible protective effects of increased GSH, cultured cells were incubated with 700 μM N-acetylcysteine (N-ace) for 3 hr then treated with 100 μM Met or Oxy. Cultured cells incubated with N-ace had significantly higher levels of GSH than control and afforded protection against Met only. At 4 hr, both the Oxy-alone and N-ace-pretreated Oxy group had significantly higher LDH release than control. Morphologically, blebs, which may be indicative of oxidative damage, were evident after treatment with Oxy for 3 and 4 hr. The increase in MDA formation, the partial protection provided by N-ace supplementation, and the morphological observations indicate that oxidative damage may be a mechanism in AAS-induced cell death to liver cells in culture.

1509 REDUCED LEVELS OF CATALASE ACTIVITY POTENTIATE MPP^+ TOXICITY: COMPARISON BETWEEN MN9D CELLS AND CHO CELLS.

S Hussain[1], B Hass[2], W Slikker Jr.[1] and S F Ali[1]. [1]*Neurochemistry Laboratory, Divisions of [1]Neurotoxicology and [2]Nutritional Toxicology, National Center for Toxicological Research/FDA, Jefferson, AR.*

MPTP has been shown to be toxic by inducing oxygen free radicals in the mammalian nervous system, especially, in the nigrostriatal dopaminergic system. The present study was designed to compare the toxic effects of MPP^+, the major metabolite of MPTP in dopaminergic MN9D cells and CHO cells. The survival of the MN9D cells in the presence of 250 μM of MPP^+ was less than 10%, whereas CHO cells exhibited 90% survival at the same concentration of MPP^+. The IC_{50} values in MN9D and CHO cell lines were 75 and 600 μM, respectively. Thus, there is a 9 fold increase of sensitivity to MPP^+ in MN9D cells compared to CHO cells. The catalase activity was 2 Units per mg of protein in MN9D cells whereas it was 30 Units per mg of protein in CHO cells. The catalase activity in CHO cells increased with increasing MPP^+ concentrations from 100-500 μM and thereafter it tended to decrease with increasing concentrations of MPP^+ up to 1 mM. In MN9D cells the catalase activity was not altered by varying concentrations of MPP^+. When CHO cells were pre-treated with 10 or 25 mM 3-amino-triazole (AT) to inhibit the catalase activity, and then exposed to MPP^+ at various concentrations, the inhibition of catalase increased the susceptibility of cells to MPP^+. It is evident from these results that the toxicity of MPP^+ in these two cell lines differs possibly due to difference in catalase activity. In conclusion, these data demonstrate that catalase may be an important antioxidant enzyme in MPTP induced neurotoxicity.
[The dopaminergic MN9D cell line was obtained from Dr. A. Heller, of the University of Chicago.]

1510 OXIDATIVE STRESS BY CADMIUM INDUCES S-THIOLATION OF α-TUBULIN, ACTIN AND CONNEXIN 43 PROTEIN IN RAT LIVER EPITHELIAL CELLS.

S H Jeong[1,2], J H Cho[1], J C Rhee[1] and M H Cho[2]. [1]*National Veterinary Research Institute, RDA, Korea; [2]College of Veterinary Medicine, Seoul National University, Korea.*

The oxidative stress by cadmium ($CdCl_2$) in WB-F344 rat liver epithelial cells was analyzed by gel electrophoresis and autoradiography after radiolabeling of the intracellular glutathione pool with ^{35}S. Cd^{2+} (200μM) caused rapid (20 min) and irreversible S-thiolation of specific proteins especially with molecular masses of 42 and 22 KDa. These proteins were identified mainly as α-tubulin by immunoblot analysis. Gap junction protein (connexin 43) and actin were also involved in S-thiolation by Cd^{2+}. Fluorescence microscopy revealed that Cd^{2+} caused a severe loss of microtubule, apparent cell retraction and loss of thick cables of actin filaments. Gap junctions immunostained with anti-connexin monoclonal antibody also decreased by Cd^{2+} treatment. These morphological changes were stayed for at least 4 hrs. After 30 min of treatment, cellular GSH was decreased to 78 % of control and maintained for 24 hrs. Four hours later, the products of lipid peroxidation (malondialdehyde) was increased significantly, however, protein-thiol depletion was not observed. These data indicate that Cd^{2+} induced oxidative stresses such as irreversible S-thiolation of α-tubulin, actin and connexin 43. These oxidative stresses may be related with GSH depletion, morphological changes of α-tubulin and actin, and gap junction number decrease.

1511 EXPERIMENTAL VERIFICATION OF THE MATHEMATICAL MODEL OF TRICHLOROETHYLENE-INDUCED LIPID PEROXIDATION IN MOUSE LIVER SLICES.*

S R Channel[a], J Z Byczkowski[a], W J Schmidt[b] and C R Miller[c]. [a]*ManTech Environmental Technology, Inc., [b]Toxicology Division, Armstrong Laboratory, [c]U.S. Army Medical Research Detachment, Wright-Patterson AFB, OH.*

A biologically based pharmacodynamic (BBPD) model was developed to simulate chemically induced lipid peroxidation in precision cut mouse liver slices *in vitro* (Byczkowski et al. Computer Meth.Prog.Biomed. 1996;50,73). The BBPD model simulated formation of lipid hydroperoxides and thiobarbituric acid reactive substances (TBARS) over time as a function of the remaining amount of cytochrome P450 (CYP)-activated chemical inducer, and remaining amount of antioxidants. The rate of peroxidation was controlled by lipid peroxidizability, destruction of CYP, autoxidation, and activity of glutathione peroxidase. The BBPD model was initially calibrated with the literature data for TBARS formed during lipid peroxidation in rat liver slices and in precision-cut mouse liver slices treated with tert-butyl hydroperoxide. Then, the biochemical parameters were optimized to reflect physiology of the mouse liver and the BBPD model was used to simulate TBARS formation during lipid peroxidation in mouse liver slices induced with trichloroethylene (TCE). Two basic algorithms for production of free radicals from TCE were tested, linear and square root. Predictions by the BBPD model which related free radical concentration to the square root of the initial TCE concentration, were in agreement with the experimental data employing TBARS as an end point.
*This research was supported in part by the Department of the Air Force Contract No. F41624-96-C-9010.

1512 ENHANCEMENT OF MENADIONE-INDUCED CYTOTOXICITY TO PLATELETS BY THE PRESENCE OF BLOOD PLASMA.

S H Chung, J Y Lee, M J Kim and J H Chung. *College of Pharmacy, Seoul National University, Seoul, Korea.*

Blood plasma provides important transport functions to major tissues, while being readily exposed to various drugs or chemicals, including endogenous and exogenous quinones. Quinones have been reported to undergo nonenzymatic reaction with thiols to generate reactive oxygens. It is therefore possible that the nonenzymatic reaction of quinones with thiols in plasma could lead to potentiated cellular toxicity or disease. This study addressed the issue by showing that a representative quinone compound, menadione (MEN), reacts nonenzymatically with plasma thiols, thereby generating reactive oxygens. The experimental evidences supporting plasma thiol involvement in enhanced MEN cytotoxicity consisted of the following: 1) MEN depleted plasma thiol levels in a dose-dependent manner; 2) pretreatment of thiol-depleting agent completely blocked MEN-induced oxygen consumption in plasma, and prevented cytotoxicity induced by MEN in platelet rich plasma system; 3) the presence of plasma increased the MEN-induced oxygen consumption in platelet systems; 4) the presence of plasma increased MEN-induced toxicity to platelets, one of MEN's target cells which is readily exposed to plasma *in vivo*. These results suggested that, in the presence of plasma, the nonenzymatic reaction of MEN with thiols following the generation of reactive oxygens could be an important process in MEN-induced cellular toxicity and disease.

1513 PRO-OXIDANT RESPONSES OF RESPIRATORY CELLS TO ORGANIC DUST EXTRACTS.

G Cosma, A Martinez, D Ufferfilge, and V Vallyathan[1]. *Dept. Env. Health, Colorado State Univ, Ft. Collins, CO;* [1]NIOSH, Morgantown, WV.

We have developed a two-compartment cell model to explore the pathways of pro-oxidant injury in respiratory epithelial and alveolar macrophage (AM) cell lines exposed to organic dust extracts. The purpose of these studies is

two-fold: 1) identify major toxic components of these complex dusts; and 2) define the underlying mechanisms of pro-inflammatory cell injury and adaptation in lung. The following responses were examined: 1) AM activation-respiratory burst; 2) cytokine production; 3) oxidative DNA damage; and 4) stress protein induction. Our evidence suggests that lung cells respond in a qualitatively, but not quantitatively, similar fashion following exposures to grain dust extracts vs. purified endotoxin, suggesting the participation of other agents in toxic responses of lung to organic dusts. AM cells demonstrated a rapid pro-oxidant response to dust extracts, including production of hydrogen peroxide and interleukin-1 as detected by immunoassay, which was followed 24 hr later by single-stranded DNA damage as measured by gel electrophoresis and immunoassay. This initial DNA damage resolved 48 hr later. Antioxidants such as catalase (500U/ml) and N-acetylcysteine (20 mM) provided only partial protection against these pro-oxidant responses. Epithelial cells lacked the pro-oxidant responses, but did display DNA damage after prolonged 3-day exposures. AM cells, but not epithelial, demonstrated a rapid induction of metallothionein (MT) and heme oxygenase (HO) gene expression following exposures to dust extracts as measured by mRNA blot analyses. These data support a role for oxidative injury to respiratory cells following exposures to particulates, and potential protective responses by stress proteins. Reactive oxygen species-induced activation of DNA binding protein NF-kappaB and antisense MT/HO studies are ongoing. (NIOSH U07/CCU807121).

1514 INCREASED LEVELS OF HEPTACHLOR EPOXIDE (HE) AND OXYCHLORDANE (OC) IN NON-SURVIVING TRAUMA PATIENTS: ROLE OF NITRIC OXIDE IN CELL DEATH.

R A Cassidy, A Delgado, D Kohler, D G Burleson. *U S Army Institute of Surgical Research, FT Sam Houston, TX.* Sponsor: W H Benson.

HE and OC are epoxides of the insecticide chlordane. These epoxides are estrogenic and affect physiologic function at levels often found in the blood of United States populace. Serum from 20 burn patients were collected and analyzed 7 times during an 18 day period following injury for HE, OC, and DDE, a metabolite of DDT, by gas chromatography. Ten non-surviving patients were matched with 10 surviving patients for burn size, age, and sex. HE and OC levels in non-survivors were significantly greater than in the surviving groups with epoxide levels peaking on post burn day 5. Serum levels of cholesterol and triglycerides decreased during the 18-day period and did not correlate with HE and OC levels. To determine a possible mechanism for HE and OC effects in burn patients, PMNs from volunteers were treated with HE for 40 min at levels found in burn patients' serum. These levels of HE resulted in increased levels of nitric oxide production and DNA strand breaks and decreased in ATP and cell membrane integrity in PMNs as measured by flow cytometry and luminescence. Furthermore, when lymphocytes were presented with HE-treated PMNs, lymphocyte cell membrane integrity also decreased. These data suggest that increased body burdens of these epoxides following trauma activate PMNs, which result in increased cellular pathology.

1515 METHYCYCLOPENTADIENYL MANGANESE TRICARBONYL-INDUCED REACTIVE SPECIES IN DIFFERENT RAT BRAIN REGIONS: AN *IN VITRO* STUDY.

C J Williams[1], H M Duhart[2], M R I Soliman[1], A Weaver[1], W Slikker, Jr[2], and S F Ali[2]. [1]*College of Pharmacy, Florida A&M University, Tallahassee, FL;* [2]*Division of Neurotoxicology, NCTR/FDA, Jefferson, AR.*

Methycyclopendadienyl manganese tricarbonyl (MMT) is the organic manganese containing compound used as an octane enhancer in unleaded gasoline and as a smoke depressant in diesel engines. Little information is available on the potential neurotoxicity of MMT. Recently we have reported that $MnCl_2$ (Mn^{+2}) and MnOac (Mn^{+3}) induce oxidative stress by generating reactive oxygen species (ROS). The present investigation was designed to investigate the effect of MMT on the generation of ROS in various rat brain regions. Adult male Sprague-Dawley rats (NCTR colony) were used in this study. Control rat brains were quickly removed, dissected into different regions and P2 fractions were prepared. ROS were measured by the molecular probe 2,7 - dichlorofluorescein diacetate (DCFH-DA). In vitro exposure of P2 fractions of rat brain regions to MMT (3.16 – 316 μM) resulted in a concentration-dependent increases of ROS in caudate nucleus, frontal cortex, hippocampus, brain stem and cerebellum. The protective effects of ascorbic acid against MMT-induced increases in ROS were also evaluated. Results obtained indicate that 2.5 μM ascorbic acid produced a 60% decrease in ROS generated following exposure to MMT. These findings suggest that ROS increases may be involved in MMT-induced neurotoxicity and that some degree of protection against these effects may be afforded by antioxidants.

1516 EFFECT OF ACRYLAMIDE TREATMENT AND GLUTATHIONE MODULATION ON AP-1 AND NFkB IN RAT GLIAL CELLS.

J Bunting, J K Kaster, M A Friedman and J E Klaunig. *Division of Toxicology, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN and Cytec Industries, Inc., W. Paterson, NJ.*

Chronic toxicity and oncogenicity studies with acrylamide (ACM) in rats have shown a dose and time dependent increase in neurotoxicity. The mechanism by which ACM produces these effects has not been established. Biotransformation of ACM is mediated by glutathione (GSH) conjugation in liver and brain and i.v. administration of ACM to rats rapidly decreases tissue GSH levels. This depletion of GSH, an important cellular antioxidant, may lead to a diminished capacity to eliminate harmful reactive oxygen species. Increased oxidative stress modulates the activity of AP-1 and NFkB, transcription factors which are involved in the expression of a variety of important genes. This study examined the effects of ACM treatment and modulation of GSH on activation of transcription in rat glial cells transfected with a luciferase reporter gene driven by either AP-1 or NFkB binding. Data showed time dependent activation of both AP-1 and NFkB with ACM treatment. Co-treatment of ACM with N-acetylcysteine (a precursor for GSH synthesis) appeared to attenuate the transcriptional activation seen with ACM alone. In contrast, treatment with an inhibitor of GSH synthesis (BSO) alone served to activate NFkB. These results suggest that ACM can induce transcription factors, AP-1 and NFkB, possibly through oxidative stress brought on by GSH depletion.

1517 PRO-OXIDANT ACTIVITIES OF MORIN AND NARINGENIN.

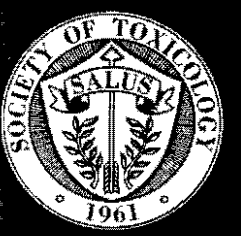
S C Sahu, and G C Gray. *Food and Drug Administration, Washington, DC.*

Morin and naringenin belong to a class of phenolic compounds known as flavonols, which are present in many edible plants. Of the 16 flavonols tested, 13 are positive in the Ames test, quercetin being the most potent. However, the flavonols are mutagenic only under aerobic conditions, which suggests the involvement of active oxygen. In order to determine if morin and naringenin have the potential to produce oxidative stress, an aerobic model system of isolated rat liver nuclei was used to determine what effects, if any, these flavonols might have on nuclear glutathione (GSH), glutathione S-transferase, membrane lipids and DNA. Both compounds induced a concentration-dependent decrease of nuclear GSH content and GST activity, and an increase in membrane lipid peroxidation and DNA strand breaks. The results demonstrate the pro-oxidant activities of morin and naringenin. Their pro-oxidant activities provide an indirect measure of active oxygen, which can be produced by their autoxidation and subsequent redox cycling under aerobic conditions. The unrepaired and/or misrepaired oxidative DNA damage thus produced may be responsible for flavonol mutagenicity.

1518 PARTICIPATION OF ACTIVE OXYGEN SPECIES IN DAPSONE HYDROXYLAMINE-INDUCED HEMOLYTIC ANEMIA.

D C McMillan and D J Jollow. *Dept. of Pharmacology, Medical University of South Carolina, Charleston, SC.*

Dapsone hydroxylamine (DDS-NOH) is a direct-acting hemolytic metabolite responsible for dapsone-induced hemolytic anemia in the rat. Hemolytic activity is known to be a result of oxidative stress within the red cell. However, the identity of the reactive oxygen species and their role(s) in the hemolytic process remain unclear. To examine this process, EPR spectroscopy was used to detect oxygen radicals in rat red cells treated with DDS-NOH. In red cells containing DDS-NOH and DMPO (spin trap), a 1:2:2:1 EPR spectrum was observed with splitting constants consistent with a hydroxyl radical adduct of DMPO. Additional studies were performed to determine whether the DMPO adduct could have been formed by hydroxyl radical attack on the spin trap or by reaction of the spin trap with ferryl heme, which is formed by reaction of hydrogen peroxide with oxyhemoglobin. Both 2, 3- and 3, 5-dihydroxybenzoic acid were detected by HPLC-EC in red cells that contained salicylate and DDS-NOH, which indicated the presence of hydroxyl radicals.



The Toxicologist

Volume 36, No. 1, Part 2,
March 97

AP

The Toxicologist

An Official Publication of the Society of Toxicology

and

Abstract Issues of

Fundamental and Applied Toxicology

An Official Journal of the Society of Toxicology

Published by Academic Press, Inc.

Abstracts of the
36th Annual Meeting
Volume 36, No. 1, Part 2,
March 97

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshops, roundtables, and poster sessions of the 36th Annual Meeting of the Society of Toxicology, held at the Cincinnati Convention Center, Cincinnati, Ohio, March 9-13, 1997.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 371.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 395.

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

Copies of *The Toxicologist* are available at $45 each plus $5 postage and handling (U.S. funds) from:

Society of Toxicology
1767 Business Center Drive, Suite 302
Reston, VA 20190-5332

SOCIETY OF TOXICOLOGY

1767 Business Center Drive
Suite 302
Reston, VA 20190-5332

Phone: (703) 438-3115
Fax: (703) 438-3113
E-mail: sothq@toxicology.org