compare the disposition of MTBE after different routes of exposure, its biotransformation was studied in humans after oral administration in water. Following approval by an Institutional Human Subjects Review Board, human volunteers (3 males and 3 females) were exposed to 5 and 15 mg 13C-MTBE dissolved in 100 ml of water. Urine samples from volunteers were collected for 96 h after administration in 6 h intervals and blood samples were taken in intervals for 24 h. In urine, MTBE and the MTBE-metabolites tert-butanol (t-butanol), 2-methyl-1,2-propane diol and 2-hydroxyisobutyrate were quantified, MTBE and t-butanol were determined in blood samples and in exhaled air. MTBE blood concentrations were 0.69 ± 0.25 µM after 15 mg MTBE and 0.10 ± 0.03 µM after 5 mg MTBE. MTBE was rapidly cleared from blood with terminal half-lives of 3.7 ± 0.9 h (15 mg MTBE) and 8.1 ± 3.0 h (5 mg MTBE). The blood concentrations of t-butanol were 1.82 ± 0.63 µM after 15 mg MTBE and 0.45 ± 0.13 µM after 5 mg MTBE. A significant part (30 %) of the MTBE administered was cleared by exhalation. MTBE exhalation was rapid and maximal MTBE concentrations (100 nmol/L) in exhaled air were achieved within 10 – 20 minutes after oral administration. t-Butanol was cleared from human blood with half-lives of 8.5 ± 2.4 h (15 mg MTBE) and 8.1 ± 1.6 h (5 mg MTBE). In urine samples, 2-hydroxyisobutyrate was recovered as major excretory product, t-butanol and 2-methyl-1,2-propane diol were minor metabolites. Elimination half-lives for the different metabolites of MTBE were between 7.7 and 17.8 h. Approximately 50 % of the administered MTBE was recovered in urine of the volunteers after both exposures. The results indicate that MTBE-biotransformation and excretion after oral exposure is similar to inhalation exposure and suggest the absence of a significant first-pass effect after oral administration.

449 REACTIVE NITRIC OXYGEN SPECIES CAN INFLUENCE THE CARCINOGENICITY OF AROMATIC AMINES.

T. V. Zenser[1], V. J. Lakshmi[1], F. Hsu[2], P. DeHaven[1] and B. B. Davis[1]. *[1]VA Medical Center/Saint Louis University, St. Louis, MO and [2]Washington University, St. Louis, MO.*

There is a strong association between chronic infection/inflammation and several forms of cancer caused by aromatic amines. Reactive nitric oxygen species (RNOS) are components of the inflammatory response and are thought to contribute to the deleterious effects of inflammation. The possible transformation of the aromatic amine N-acetylbenzidine (ABZ) by RNOS was assessed. RNOS were generated by various conditions to react with ABZ, and samples were evaluated by HPLC. Conditions which generate nitrogen dioxide radical, NO_2^- + myeloperoxidase + H_2O_2; $ONOO^-$; NO_2^- + HOCl, produced 3'-nitro-N-acetylbenzidine. Myeloperoxidase catalyzed reaction with 0.3 mM NO_2^- was completely inhibited by 1 mM cyanide, but not effected by 100 mM chloride with or without 1 mM taurine. In contrast, conditions that generate N_2O_3, such as spermine-NONO, produced 4'-OH-4-acetylaminobiphenyl and 4-acetylaminobiphenyl. ^{1}H NMR and mass spectrometry verified the structure of these compounds. Human polymorphonuclear neutrophils incubated with ^{3}H-ABZ and stimulated with β-phorbol 12-myristate 13-acetate produced 3'-nitro-N-acetylbenzidine in the presence of NO_2^- (0.1 to 1 mM). In RAW264.7 cells incubated with 0.2 mM ABZ ± LPS and γ-INF for 24 hours, NO_2^-/NO_3^- increased from 45 to 295 nmoles per plate with LPS + γ-INF and 4-acetylaminobiphenyl increased from 6 to 35 ng per plate. The results demonstrate ABZ forms unique products with nitrosating and nitrating RNOS, which could influence the carcinogenic process and serve as biomarkers.

450 DETERMINATION OF ALACHLOR AND ITS METABOLITES IN RAT PLASMA AND URINE USING SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/ELECTROSPRAY IONIZATION-MASS SPECTROMETRY.

L. Y. Zang and G. L. Qiao. *NIOSH, EAB/HELD, Morgantown, WV.*

A method based on liquid chromatography (LC) with electrospray ionization tandem mass spectrometry (MS) for the analysis of Alachlor and its metabolites, 2-chloro-N-(2,6-diethylphenyl) acetamide (CDEPA) and 2,6-diethylaniline (DEA) in rat plasma and urine has been developed. The ^{13}C-labeled Alachlor and its metabolites were used as the internal standards for quantitation. The mass-spectrometer was set at ESI MS-SIM mode with a programming procedure. The LC flow rate was constant at 0.3 mL/min. The mobile phase gradient program started at a ratio of 30% acetonitrile and 70% water containing 1% acetic acid for 3 min and then increased to 100% acetonitrile within the next 4 min. An initial sample volume of 500 µl was loaded onto a 96-well Waters Oasis HLB solid phase extraction plate and concentrated into 50 µl acetonitrile, of which a 5-µl aliquot was injected into the LC-MS system. The retention times for Alachlor, CDEPA and DEA on the Xterra MS C8 column (2.5 µm, 2.1 x 50 mm) were 1.61, 2.72 and 3.98 min, respectively with complete peak separation. The sensitivities of the MS detection for Alachlor, CDEPA and DEA were found to be 1 ng, 0.5 ng and 0.2 ng (per-injection), respectively with a signal/noise threshold of 2. The detection linearity range of at least 40 pg to 25 ng was observed with the three target compounds. Reproducibility of the sample handling and LC-MS analysis was good with a CV% of ≤ 10%. Based on six duplicate samples per concentration within the concentration range specified, the average recoveries for Alachlor, CDEPA and DEA in rat plasma were found to be 80, 87 and 90%, respectively. Similar preliminary results were also obtained with rat urine. Therefore, this sensitive, specific, reproducible and cost-effective LC-MS method may be used to identify and quantitate Alachlor and its major metabolites in bio-samples obtained from in vivo studies. (supported by NIOSH/CDC).

451 PHARMACOKINETICS AND METABOLISM OF 3-CHLOROALLYL ALCOHOL AND 3-CHLOROACRYLIC ACID IN THE MALE FISCHER 344 RAT.

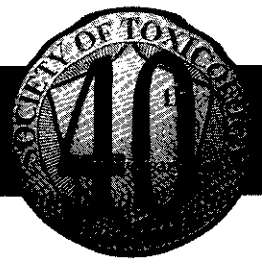
S. C. Hansen, M. J. Bartels and A. L. Mendrala. *The Dow Chemical Company, Toxicology & Environmental Research and Consulting, Midland, MI.* Sponsor: W. Stott.

The pharmacokinetics and metabolism of 3-chloroallyl alcohol and 3-chloroacrylic acid were examined in the male Fischer 344 rat. Following oral administration of a low dose of 5 mg/kg or a high dose of 65 or 75 mg/kg, both test materials were found to be highly absorbed and rapidly eliminated. The major route of metabolism for both compounds was as expired CO2, with 50-52% and 60-68% of the 3-chloroallyl alcohol and 3-chloroacrylic acid, respectively, eliminated *via* this pathway. Urinary excretion accounted for 21% and 16-17% of the administered 3-chloroallyl alcohol and 3-chloroacrylic acid, respectively. The estimated half-life for elimination of radioactivity was quite comparable between the test materials, ranging from 3.1-4.3 hr for expired CO2, and 3.9-8.1 hr *via* urinary excretion. Fecal elimination was higher following administration of 3-chloroallyl alcohol (13.0%), than from 3-chloroacrylic acid (3.3-3.9%). No significant dose-dependence was observed in the overall absorption or metabolism of either test material. Both test materials were highly metabolized, primarily to CO2. Urinary metabolite profiles were comparable for both test materials, indicating a common metabolic pathway. The major common urinary metabolite was tentatively identified as 3,3-bis-S-(N-acetylcysteinyl)-1-propanol, which is proposed to arise from dual conjugation of either test material with glutathione. Moderate levels of radioactivity were found in the tissues/carcass at 168 hr (5.3-7.0%) from either test material, which may reflect incorporation of radiolabel into endogenous metabolic pathways.

452 DISTINCT MECHANISMS OF TOXICITY FOR LINOLEIC ACID MONOEPOXIDES AND DIOLS IN SF-21 CELLS.

L. A. Mitchell, J. H. Moran, T. Mon and D. F. Grant. *University of Arkansas for Medical Sciences, Pharmacology & Toxicology, Little Rock, AR.*

Recent studies have associated linoleic acid (LA) and oxidized metabolites of LA with many pathological conditions. However, there remains controversy over the identity of the reactive intermediates. To investigate discrepancies in these studies, we tested the toxicity of LA-metabolites in *Spodoptera frugiporda* (Sf-21) cells using culturing conditions and toxic end points that directly compare with previous mammalian studies. LA, LA-monoepoxides (9,10-EOA/12,13-EOA) and LA-diols (9,10-DHOA/12,13-DHOA) were all toxic to Sf-21 mitochondria in a time- and dose-dependent manner. LA (100 µM), 9,10-EOA (100 µM), 12,13-EOA (100 µM), and 9,10-DHOA (100 µM) increased oligomycin-insensitive (OI) respiration approximately 2-fold after a 1 min exposure. Concomitant with this effect was irreversible decreases in basal, oligomycin-sensitive (OS), OI, and uncoupled respiration after a 90 min exposure. However, in the presence of FBS supplemented media, these effects were reversible within 45 min. 12,13-DHOA (100 µM) appeared to have a distinct mechanism of toxicity and decreased basal, uncoupled, OI, and OS respiration by approximately 60%, 60%, 40%, and 100%, respectively, after a 1 min exposure. These effects were not reversible in FBS supplemented or non-supplemented media up to 6 hrs. In the presence of BSA (Bovine serum albumin, 500 µM), Sf-21 mitochondria were fully protected against 12,13-DHOA (100 µM) mediated toxicity. In summary, LA, LA-monoepoxides, and LA-diols are all mitochondrial toxins in Sf-21 cells. The primary mechanism of toxicity for LA, 9,10-EOA, 9,10-DHOA, and 12,13-EOA is uncoupling of oxidative phosphorylation; whereas, 12,13-DHOA specifically inhibits the electron transport chain in Sf-21 cells. BSA at physiological concentrations (500 µM) protects against the toxicity



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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, roundtable, and poster sessions of the 40th Annual Meeting of the Society of Toxicology, held at the Moscone Convention Center, San Francisco, California, March 25–29, 2001.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 451.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 479.

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