1174 ANALYSIS OF URINARY 1, 1, 2, 2-TETRACHLOROETHYLENE (PERC) METABOLITES BY HPLC ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY (ESI-MS/MS) AS POTENTIAL EXPOSURE BIOMARKERS.

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The industrial solvent and dry cleaning agent, PERC (CAS 127-18-4), produces liver tumors in mice and nephrotoxicity and renal tumors in male rats. This toxicity may involve reactive intermediates which could induce similar toxicity in workers. Bioactivation of PERC is reported to occur by oxidation by CYP2E1 and GSH. Trichloroacetic acid (TCA) and dichloroacetic acid (DCA) are reported PERC urinary metabolites and the GSH conjugate is cleaved to S-(1, 2, 2-trichlorovinyl)-L-cysteine (TCVC) and acetylated to N-ac-TCVC. A biomonitoring method was developed to measure urinary levels of TCA, DCA, TCVC and N-ac-TCVC with deuterated TCVC, N-ac-TCVC and DCA as internal standards. Samples were loaded onto SPE columns and PERC metabolites were eluted with acetone, dried and diluted in MeOH for HPLC ESI-MS/MS analysis on a Phenomenex Jupiter C18 column. A 10-min linear gradient (55:45 H2O:MeOH 1% acetic acid to MeOH 1% acetic acid) at 300 µL/min eluted the compounds of interest within 12 min. The mass spectrometer was operated using ESI-MS/MS, initially in the negative ion mode for detection of TCA and DCA, and subsequently in the positive ion mode for TCVC and N-ac-TCVC. Some urine samples from laundry workers with no demonstrated PERC exposure and dry cleaning workers that were potentially exposed to PERC during loading or unloading of garments contained detectable amounts of PERC metabolites in urine collected during pre or post-shift periods. The limit of detection (LOD) was 0.47 pmol for TCVC, 0.8 pmol for N-ac-TCVC, 11.45 pmol for DCA, and 7.17 pmol for TCA. A small number of samples contained amounts of PERC metabolites that exceeded the limit of quantification (LOQ) - 4.68 pmol for TCVC, 8.03 pmol for N-ac-TCVC, 114.5 pmol for DCA, and 71.73 pmol for TCA. The analysis appears to offer significant advantages over typical extraction and derivatization procedures required for GC-MS analysis of these compounds. Thus, PERC internal exposure levels may be quantified by HPLC ESI-MS/MS analysis of these metabolites.

1175 LEVELS OF POLYCYCLIC AROMATIC HYDROCARBONS IN AMNIOTIC FLUID SAMPLES FROM SMOKERS AND NONSMOKERS.

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Previous studies from this laboratory have focused on the characterization of blood protein adducts formed *in utero* as a result of maternal smoking during pregnancy. These biological samples, obtained during the third trimester of pregnancy, at delivery, have clearly shown a correlation between maternal smoking and exposure of the fetus to tobacco smoke carcinogens, including 4-aminobiphenyl and benzo(a)pyrene. In the present study, we examined exposure of the fetus during the first trimester of development to various environmental carcinogens, particularly those found in tobacco smoke. Amniotic fluid samples were obtained from women undergoing routine amniocentesis at between 16 and 20 weeks gestational age. Amniotic fluid, produced by the fetal lungs and kidneys, is an important part of pregnancy and fetal development and this fluid surrounds the fetus throughout pregnancy. In these studies, samples of amniotic fluid were obtained from nonsmokers as well as 0.5 pk/da smokers through >2pk/da smokers. Amniotic fluid samples were extracted and analyzed by HPLC and GC/MS for the presence of polycyclic aromatic hydrocarbons (PAHs). Amniotic fluid levels of PAHs were found in almost all samples analyzed. However, there was a clear correlation between levels of maternal smoking and PAHs in the amniotic fluid. 1-hydroxypyrene levels ranged from 1.54 ± 0.12 µg/ L in nonsmokers to 11.72 ± 0.67 µg/L in women smoking >2 pks/da, indicating approximately a 10X increase over nonsmokers. Similar results were found with more widely established carcinogens, including hydroxylated benzo(a)pyrene derivatives, which ranged from 1.41 ± 0.13 µg/L in nonsmokers to 11.56 ±0.59 µg/L in >2pk/da smokers. These results indicate that exposure to harmful environmental carcinogens can occur during early gestational periods and may place the fetus at a risk of genotoxic as well as teratogenic events.

1176 HYPOURICOSURIA, A BIOMARKER OF INORGANIC ARSENIC EXPOSURE.

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Arsenic (As) is a human carcinogen, affecting million of people worldwide. Most ingested As is excreted in the urine within a few days, making measurements of urine As a good biomarker of recent As exposure. However, other biomarkers for assessing continue As exposure or toxic As effect are necessary. The catabolic steps that generate uric acid from nucleic acids and free purine nucleotides involve degradation through purine nucleoside intermediates to hypoxanthine and xanthine. The latter are ultimately oxidized to uric acid in man using sequential reactions catalyzed by the enzyme xanthine oxidase. In rats, the presence of uricase transforms uric acid to allantoin. Arsenite (AsIII) complexed xanthine oxidase preventing reduction of the enzyme by purines. This effect causes xanthinuria and hypouricosuria. Urine is the preferential material for screening of abnormal metabolism of purines. Three different models of arsenite (AsIII) exposure were evaluated, human or rats exposed chronically and mice exposed to 0, 3, 6 and 10mg/kg daily during 9 days. Urinary uric acid from 97 individuals chronically exposed to water naturally contaminated with As content (129 ppb) were significantly lower (2.4 mg/L) than those found in 28 control people (3.2 mg/L). Urinary levels of uric acid in rats treated daily with 1.2 mg As/kg v.o during six weeks were evaluated. A significant decrease of uric acid concentrations in the urine was observed during the first 3 weeks of As exposure. Significant dose-response decrement of urinary uric acid was observed in mice treated orally during 9 days with arsenite. These results suggest that uric acid may be a useful easy biomarker for assessing continue As exposure.

1178 ANALYSIS OF PLASMA AND URINE FOR METABOLITES FOLLOWING INHALATION EXPOSURE OF FEMALE AND MALE MICE AND RATS TO 1, 3-BUTADIENE OR 1, 2-DIHYDROXY-3-BUTENE.

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1, 2-Dihydroxy-3-butene (ene-diol) is a marker for the flux through the hydrolysis detoxication pathway of butadiene (BD) that leads to the major human urinary metabolite, M1 (diolmercapturate). Ene-diol is also the precursor to 1, 2-dihydroxy-3, 4-epoxybutane (EBD), which is the major source of BD-induced protein and DNA adducts in all species studied. To characterize the impact of ene-diol formation during BD metabolism *in vivo*, we evaluated the levels of ene-diol as well as the urinary metabolites MI and MII (monohydroxy mercapurate) in mice and rats following inhalation exposure to BD or directly to ene-diol. Metabolites were analyzed by gas chromatography equipped with mass selection detection, with both the cis- and trans-isomers measured as total ene-diol. In mice and rats necropsied immediately after nose-only exposure to 0, 62.5, 200, or 625 BD for 6 h, the dose-response curves for ene-diol in plasma had positive curvature (e.g., in control and BD-exposed female mice, the respective plasma levels were 0, 0, 171 ± 6 ng/ml, and 10, 480 ± 3, 320 ng/ml). MI and MII in 24 h urine samples were increased above background after BD exposures, but only MI was increased after exposure of animals to 6 or 18 ppm ene-diol for either 6 h or for 4 weeks (6 h/day, 5 days/wk). Lower levels of ene-diol, compared with MI, were also detected in urine samples from animals exposed to BD or ene-diol (e.g., in female rats exposed to 200 ppm BD, the levels of ene-diol and MI were 41 ± 2 ng/mL plasma and 44 ± 20 mg/mL urine, respectively). Repeated exposures to these levels of ene-diol led to increased frequencies of *Hprt* mutations in mice and rats (see companion abstract by Q. Meng *et al.*). The study indicates that ene-diol is rapidly cleared after low-level (< 200 ppm) BD exposures but accumulates following high-level exposures. Further studies are needed to determine the fraction of ene-diol that is cleared *via* formation of the EBD compared to excretion of ene-diol and M-I.

1179 URINARY (2-METHOXYETHOXY)ACETIC ACID: AN EFFECTIVE GAS CHROMATOGRAPHIC TEST METHOD FOR QUANTIFICATION.

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(2-Methoxyethoxy)acetic acid (MEAA) is a metabolite and biomarker for exposure to 2-(2-methoxyethoxy)ethanol (diethylene glycol monomethyl ether, DEGME, or DiEGME) and bis(2-methoxyethyl) ether (diglyme); both are glycol ethers and are of concern because of the general toxicity of these compounds. Glycol ethers have been frequently reported to damage the male reproductive system, hemaopietic system, and fetal/embryonic development. Occupational exposure by these widely used glycol ethers is likely, since they are readily absorbed through the skin. Specifically, 2-(2-methoxyethoxy)ethanol is used as an anti-icing additive to the military jet fuel JP-8, and bis(2-methoxyethyl) ether is an aprotonic solvent with industrial uses and is a component of some hydraulic fluids including brake fluid. A simple and effective general test method for MEAA in urine samples was developed to monitor any exposed population. Urine specimens were first spiked with deuterated (2-butoxy)acetic acid, which was used as a procedural internal standard. The samples were extracted with ethyl acetate, concentrated, and treated by acid catalyzed esterification to produce the corresponding ethyl esters of MEAA and the internal standard. Subsequently, the ethyl ester derivatives were extracted using meth-

ylene chloride and concentrated to produce the final solution for gas chromatographic analysis. A mass selective detector (MSD) using a 50-m X 0.20-mm (id) HP-1 capillary column and a temperature program of 50 to 230C was used for the gas chromatographic measurement. Ion m/z 59 was monitored for the ethyl ester of MEAA and ion m/z 66 was monitored for the internal standard. A recovery study using 2, 10 and 20 micrograms/mL MEAA spiked urine samples demonstrated good accuracy and precision; recovery varied between 95-103%. The limit of detection (LOD) was found to be approximately 0.1 micrograms/mL (0.8 micromoles/L) for this analysis method.

1180 DETERMINATION OF PLATELET ACTIVATION LEVELS AND TIME COURSE IN CYNOMOLGUS MONKEYS.

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Blood platelets are essential for normal blood clotting; however, excessive platelet activation can contribute to thromboses that can lead to disseminated intravascular coagulation, heart attacks and strokes. Evaluation of platelet activation is an important part of safety assessment of therapeutics and several markers have been described for clinical evaluation of platelet activation. This study examines platelet activation assays for potential use in preclinical safety evaluation studies performed in Cynomolgus monkeys (Macaca fascicularis). Platelet activation levels and kinetics were tested using ELISA and flow cytometry methods for measurement of plasma levels of b-thromboglobulin, P-selectin, platelet factor-4, and cell-surface expression of P-selectin (CD62P) on platelets. Essential to measurement of platelet activation was the minimization of non-specific activation during blood collection using syringes containing CTAD (citrate, theophylline, adenosine, and dipyridamole) anticoagulant. Upon determination of antibody crossreactivity in Cynomologus monkey samples, the degree and time course of platelet activation were examined. The b-thromboglobulin average baseline level in CTAD anticoagulant samples was 13.2 +/- 4.6 IU/mL (SEM, n=4). In comparison, b-thromboglobulin levels in samples obtained using 3.2% w/v sodium citrate anticoagulant had an average b-thromboglobulin level of 179.6 +/- 3.3 IU/mL (n=4). Sodium citrate plasma samples with *in vitro* platelet activation using 20 mM adenosine diphosphate (ADP) had an average b-thromboglobulin level of 175.2 +/- 3.7 IU/mL (n=4). Similar results were seen for CD62P levels detected by flow cytometry. This study should identify reliable sample collection and analytical methods to be used for the determination of platelet activation in the Cynomolgus monkey as part of safety assessment of therapeutics.

1181 SENSITIVITY OF SPECIFIC BIOCHEMICAL MARKERS TO PREDICT CATECHOLAMINE INDUCED CARDIOMYOPATHY IN CYNOMOLGUS MONKEYS.

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In the last 10 years relatively sensitive and specific peripheral biochemical markers of cardiomyopathy have been characterized. These biochemical markers, creatine phosphokinase (CPK) and its isoenzymes, myoglobin, and more recently, the troponin T and troponin I subunits of the troponin protein complex permit identification of cardiac injury. This study evaluated the feasibility of using of CPK and its isoenzymes, myoglobin, and troponins T and I for the detection of myocardial injury in cynomolgus monkeys (*Macaca fascicularis*). Myocardial injury was induced chemically by slow, short-term infusion of norepinephrine bitartrate. Ten animals were implanted with telemetry transmitters configured to provide lead II electrocardiograms (ECG) and measurements of central arterial pressure and body temperature. A two-hour continuous intravenous infusion of norepinephrine (0.0625 mL/kg/min) was used to induce cardiomyopathy. Cardiovascular parameters were collected by radiotelemetry, continuously between prestudy and 7 days post-dose. Blood samples for measurement of cardiac biomarkers were collected predose and at various time points, beginning 2 hours after the end of infusion and continuing until necropsy and gross and histological evaluation, 7 days after treatment. Postmortem examination confirmed cardiac injury ranging in severity from minimal to marked. The severity of myocardial lesions was well correlated with serum troponin levels and ECG abnormalities. This data indicated that, of the biochemical markers evaluated in this study, troponin T and troponin I were the most specific and sensitive indicators of cardiac muscle injury. The results of this study indicate that serum troponin levels rise quickly and persist long after cardiac injury making them a more reliable indicator of cardiac muscle injury by permitting confirmation of cardiac injury well after other biochemical markers cease to be increased. Furthermore with coexistent skeletal muscle injury, troponins were near normal while the other biochemical markers were generally increased.

1182 MECHANISM-BASED URINARY BIOMARKERS OF RENAL PHOSPHOLIPIDOSIS AND INJURY.

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In animals treated with aminoglycoside antibiotics renal phospholipidosis causes nephrotoxicity and limits the dose and length of drug therapy. Therefore, a biomarker that can be used to monitor phospholipidosis before the onset of nephrotoxicity would be extremely valuable for preclinical and clinical safety studies and could be used for candidate screening. Megalin, a glycoprotein receptor, is expressed in numerous tissues (renal proximal tubule (RPT), lung, testis and macrophages)that are sensitive to the development of phospholipidosis. Although the physiologic role of megalin in the RPT brush border membrane is to mediate the uptake of proteins (e.g. albumin) and receptor-vitamin complexes (e.g. retinol binding protein-retinol) from the glomerular filtrate, it also mediates the uptake of polybasic drugs such as gentamicin by the RPT, a process necessary for the development of RPT phospholipidosis, injury and cell death. In theory, toxicants that are substrates for megalin binding should decrease megalin-mediated uptake of endogenous substrates by competing for megalin binding and preventing the recycling of megalin. The result should be an increase in excretion of endogenous substrates (e.g. retinol, albumin, and cubulin) in the urine in an amount that is proportional to degree of competition. In male Sprague-Dawley rats treated with gentamicin we show that urinary retinol and albumin excretion (ca. 2-fold increase) are early and sensitive markers of renal phospholipidosis that precedes both intracellular accumulation of phospholipids (sudan black staining), formation of myeloid bodies (confirmed by electron microscopy), and histologic and serum chemistry evidence of renal injury. This work supports the usefulness of monitoring urinary retinol and albumin excretion for early detection and monitoring of renal phospholipidosis and resultant injury.

1183 ELECTROENCEPHALOGRAPHIC RESPONSE TO ACUTE 3-NITROPROPIONIC ACID (3-NPA) EXPOSURE.

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3-NPA is a suicide inhibitor of succinate dehydrogenase (SDH) and has been implicated in food poisoning in China following ingestion of moldy sugarcane. SDH is the TCA cycle, as well as oxidative phosphorylation Complex II, enzyme. Its inhibition leads to cellular energy deficits. Studies have shown that energy deficit in GABAergic neurons after 3-NPA exposure led to an increase in GABAergic neurotransmission. This effect may be associated with the depression of motor activity and somnolence in the acute stage of 3-NPA neurotoxicity. However, after the acute phase, interference with GABAergic neurotransmission may increase excitability and lower threshold for seizures as well. In order to assess the effect of 3-NPA on electrocerebral activity, the ECoG was recorded in conscious, adult, male Sprague-Dawley rats *via* bipolar, epidural electrodes implanted at the level of the somatosensory cortex. Following baseline recording (saline injection), rats were injected s.c. with 3-NPA at 30 mg/kg or pretreated with L-carnitine (LC), an enhancer of mitochondrial energy metabolism, administered i.p. at 100 mg/kg prior to 3-NPA. The power spectra obtained by use of Fast Fourier Transformations were divided into 1.25-4.50 Hz (delta), 4.75-6.75 Hz (theta), 7.00-9.50 Hz (alpha-1), 9.75-12.50 Hz (alpha-2), 12.75-18.50 Hz (beta-1), and 18.75-35.00 Hz (beta-2). Treatment with 3-NPA was associated with a trend toward a power decrease in the delta frequency band. Following pretreatment with LC prior to 3-NPA, ECoG power in the right hemisphere was elevated relative to the left hemisphere in all frequency bands. Results indicate dominance of inhibitory neurotransmission during the acute stage of 3-NPA-induced neurotoxicity and a stimulatory action of LC on mitochondrial energy metabolism.

1184 A COMPARATIVE RELIABILITY STUDY OF THREE TEST BATTERIES: THE BEHAVIORAL EVALUATION FOR EPIDEMIOLOGY STUDIES (BEES), THE NEUROBEHAVIORAL EVALUATION SYSTEM2 (NES2) AND THE BEHAVIORAL ASSESSMENT AND RESEARCH SYSTEM (BARS).

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The BEES is distinguished from other batteries by its focus on re-engineering traditional tests on a touch-screen. Psychometric properties are compared with those for the NES2 and BARS. The tests include Finger Tapping, Digit, Spatial, and Auditory Span, Pattern Discrimination, Pattern Memory, Simple and Choice