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-