PS 1519 Rapid Determination of Mercapturic Acids from Acrylamide and Glycidamide in Human Urine

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A sensitive and specific electrospray tandem mass spectrometry method using a column switching unit with trap column and analytical column was established to quantify the mercapturic acid (MAs) of acrylamide (AA) and glycidamide (GA) in human urine. Urine was spiked with deuterated internal standards and injected directly into LC–MS/MS. The Waters Symmetry ShieldTM RP18 and Waters Xbridge-C8 were used for trapping and hydrophilic MAs, respectively. With this method, the limits of quantitation for AAMA and GAMA in urine were 6ng/mL and 0.5ng/mL, respectively; the inter-day and intra-day precision of AAMA and GAMA ranged from 2.7% and 7.3%, and the recoveries ranged from 99.6% to 105.3%. The method was simple, sensitive and accurate. The developed method was also applied to analysis urines of smokers and nonsmokers, results showed that amount of AAMA, GAMA in smokers urine were much higher than that in nonsmokers, indicating that AAMA and GAMA may be effective biomarkers to estimate acrylamide exposure in smoking.

PS 1520 Oxidized Cardiolipins As a Biomarker of Mitochondrial Dysfunction Triggered by Pesticide, Rotenone

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Exposure to a commonly used pesticide, rotenone, has been associated with the development of Parkinson's disease. We demonstrated that treatment of human blood lymphocytes with rotenone resulted in decreased mitochondrial membrane potential, inhibition of mitochondrial respiratory complex I, induction of apoptosis and selective oxidation of mitochondrial phospholipid, cardiolipin (CL). Here, we used rat rotenone-infusion Parkinson disease model to assess possible accumulation of peroxidized phospholipids. Adult male Lewis rats (6 months old) were exposed to rotenone daily (3 mg/kg, I.P.) and sacrificed 1, 5 and 10-14 days thereafter. Substantia nigra was isolated and lipids were extracted. To enhance the sensitivity of LC-MS protocols for the detection of oxidation products, phospholipids were treated either with phospholipase A1 from Thermomyces lanuginosus (10 μl/μmol phospholipids) or phospholipase A2 form porcine pancreas (10U/μmol of phospholipids) to release fatty acids residues from sn-1 and sn-2 position, respectively. Using the combination of lipidomics and oxidative epitope-targeted enzymatic digestion of total phospholipids we found a decrease of polyunsaturated fatty acids (PUFA) esterified into phospholipids on day 1 and 5 after expose. In addition we were able to detect oxygenated species of PUFA that were represented by their hydroxy-derivatives. Moreover, a decrease of CL species containing PUFA and accumulation of its oxygenated molecular species was oserved. We conclude that CL oxygenation products may represent a new biomarker of rotenone-induced mitochondrial dysfunction associated with Parkinson disease. The study was approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh in accordance with the guidelines published by the NIH. Supported by NIH ES020693, U19 AI068021, NIOSH OH008282.

PS 1521 N^6-Formyllysine As a Biomarker of Formaldehyde Exposure: Inhalation Studies in Rats Reveal Formation and Accumulation of N^6-Formyllysine Adducts in Nasal Epithelium

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There is increasing recognition that aberrant protein modifications play an important role in the pathophysiology of many human diseases. N^6-Formyllysine, a chemical homolog of the biologically important N^6-acetyllysine, has recently emerged as a widespread modification of proteins. With formaldehyde as the major source of N^6-formyllysine, we quantified endogenous and exogenous adducts in nasal epithelium of rats exposed by inhalation to 0.7, 2, 5.8, and 9.1 ppm [$^{13}C^2H_2$]-formaldehyde using liquid chromatography-coupled tandem mass spectrometry. Exogenous adducts were detected in nasal epithelium and not in lung, liver, or bone marrow, with concentration-dependent formation in total and fractionated (cytoplasmic, membrane, nuclear) proteins. Endogenous adducts dominated at all exposures, with a 6 hr 9.1 ppm exposure resulting in one-third of the total load of N^6-formyllysine being derived from exogenous sources. We have further examined N^6-formyllysine accumulation in several tissues from rats exposed by inhalation to 2 ppm [$^{13}C^2H_2$]-formaldehyde for 7, 14, 21, and 28 days (6hr/day) and determined loss of N^6-formyllysine over a 7 day post exposure period. Our results showed detection and accumulation of exogenous adducts in nasal epithelium and not in lung, liver, or bone marrow, with exogenous N^6-formyllysine levels showing a 2-fold increase over a 3-week exposure period. The post exposure studies indicated a $t_{1/2}$ of ~50 h for this adduct in total proteins. Formation and accumulation of N^6-formyllysin in proteins, including histones with important epigenetic regulatory roles, could be another mechanism contributing to formaldehyde toxicity and carcinogenicity.

PS 1522 Serial Measurements of BPA and BPS in Texas Mother-Infant Pairs from the 3rd Trimester of Pregnancy through the 4th Month of Lactation

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Relatively little is known about in utero or early infancy exposure to Bisphenol A (BPA) and related bisphenols. Here we report on a formative prospective cohort study to monitor exposure to BPA and a BPA substitute, Bisphenol S (BPS), in mother-infant pairs in a rural Texas county. Participants were recruited from a pool of consented National Children's Study (NCS) mothers as well as volunteers from the same clinics as part of an NCS formative research project. The use of human subjects was approved by the IRB of the University of Texas Health Science Center at Houston and Battelle's IRB. Specimens were collected prospectively from the third trimester of pregnancy through the fourth month of lactation from 10 mother-infant pairs who completed the protocol. BPA and BPS were analyzed in 86 urine, 50 breast milk, and 7 plasma samples utilizing enzymatic hydrolysis followed by high performance liquid chromatography coupled with electrospray triple-quadrupole mass spectrometry. These data document measurement of BPA and BPS in each of these matrices: urine, plasma and milk. Maternal urine specimens here had higher geometric mean (GM) concentrations of BPA (2.04 ng/mL wet wt [ww]) and BPS (0.52 ng/mL ww) than infant urine BPA (1.85 ng/mL ww) and BPS (0.19 ng/mL ww). The breast milk GM concentration of BPA (0.52 ng/mL) was higher than the BPS concentration (0.07 ng/mL). BPA and BPS breast milk GM concentrations increased from one month (0.41 and 0.06 ng/mL respectively) to four months postpartum (0.64 and 0.14 ng/mL respectively). This study documents that a BPA substitute, BPS, is detectable in human urine, plasma and breast milk and suggests the importance of monitoring for potentially toxic chemical substitutes for BPA. This study was funded by NIH.

PS 1523 Measurement of Endogenous and Exogenous 1, N^2-Propano-Deoxyguanosine DNA Adducts by Liquid Chromatography—Tandem Mass Spectrometry

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1,N^2-Propano-dG is a mutagenic DNA adduct formed by the reaction of 2'-deoxyguanosine (dG) with crotonaldehyde, a lipid peroxidation by-product. This lesion can also be formed by the reaction of two acetaldehyde (AA) molecules with dG to form an identical adduct. AA and crotonaldehyde are highly reactive chemicals that form a number of lesions on cellular macromolecules including DNA and proteins. AA is a ubiquitous environmental chemical with a variety of human exposures from industrial/occupational activities, consumer products, lifestyle choices (food/alcohol/cigarette consumption), and environmental sources. AA is also formed following the metabolism of vinyl acetate and ethanol. In addition, AA is endogenously produced as a by-product of cellular respiration and metabolism. Since both compounds are endogenously present and can have exogenous exposures, a sensitive 2-dimensional HPLC-tandem mass spectrometry method was developed to quantitate endogenously and exogenously formed 1,N^2-propano-dG adducts using [$^{13}C_2$]-stable isotope exposures. The method monitored the endogenous and exogenous (+2 and +4 m/z) adducts using selected reaction monitoring allowing for the differentiation between the potential causative agents for the formation of the lesion. The method was validated and had a limit of detection of 1 fmol on column. Reactions of AA and [$^{13}C_2$]-AA with an amino acid catalyst were used to confirm the ability to differentiate between labeled and unlabeled adducts with a 1:2:1 formation ratio between the different isotopic forms. Endogenous 1,N^2-Propano-dG was quantitated in non-exposed rodent lungs.

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