individually or in combinations, in 110/175 (63%) samples; from HIV positive (64%) and HIV negative (57%), with additional 4 analytes present only in HIV positive samples. AFM1 (10%; mean 0.5, range <LOQ-1.4μg/L) and FB1 (3%; mean 0.6, range 0.5-15μg/L) were detected in the HIV subpopulation whilst low levels (<LOQ) were found in one sample each from HIV negative group. One HIV positive individuals' urine contained 6 metabolites. Levels of these metabolites were generally similar to those reported elsewhere in Africa. For the first time in Africa and elsewhere, this study has reported on 11 mycotoxin biomarkers/bio-measures quantified in human urine. Mycotoxin exposures in HIV individuals may require particular attention. The findings may constitute a major step towards mycotoxin exposure assessment and national mycotoxin regulations in Cameroon.

PS 1884 Development of a New Oxidative Stress Biomarker Dityrosine ELISA.

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Sponsor: K. Rao.

Accumulating evidence indicates that oxidative stress plays an important role in various diseases such as cancer, diabetes and hypertension. Recently it is also reported that oxidative stress is involved in toxicity of chemical substances such as arsenic, asbestos, diesel exhaust micro particles and antineoplastic drugs, and monitoring of oxidative stress inside human body may be informative for toxicological study. Oxidative stress may cause oxidative damages to biomolecules such as nucleic acids, lipids, proteins and enzymes, and oxidized products of such biomolecules have been used for the assessment of oxidative stress in the living bodies. Although protein is one of the most important biomolecules, only limited number of reports about the oxidized proteins has been published. Tyrosine is one of the major targets of protein oxidation, and dityrosine is known to be formed by oxidative stress. In this presentation, development of a new dityrosine ELISA is reported.
A competitive dityrosine ELISA is established using anti-dityrosine monoclonal antibody (clone 1C3) which was developed by Kato et.al. 50 uL of diluted samples or standards are poured into micro plate wells which is pre-coated by dityrosine antigen, and incubated at 4 degree C overnight. After washing by PBS-tween buffer, 100 uL of HRP-conjugated anti mouse antibody is poured, and incubated for 1 hour at room temperature. TMB is used for color development.
Assay range of dityrosine ELISA is 0.05 to 12 umol/L, and shows good linearity and reproducibility. Dityrosine concentration in human urine ranges between 0.12 and 3.95 umol/L (mean 1.44 umol/L), and urinary dityrosine concentration measured by ELISA significantly correlated with that measured by LC-MS/MS. In conclusion, dityrosine ELISA may be useful for the assessment of oxidative stress in the living bodies.

PS 1885 Method Development of Serum Canine Inhibin B Enzyme-Linked Immuosorbent Assay (ELISA).

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Inhibin B (INH-B) is a heterodimeric glycoprotein consisting of an alpha and a beta-B subunit linked by disulphide bridges. INH-B is produced by the testes as well as the ovaries, and is responsible for the selective negative feedback control of follicle stimulating hormone. In males, INH-B is synthesized by the sertoli cells in the testis, and can be used as a marker of sertoli cell function and spermatogenesis in adult males. Hence, it is being considered a biomarker for detecting testicular damage. INH-B has been quantified in humans, rats and non-human primates, but not in canines due to lack of availability of reagents. Here, we report the methods development of the canine INH-B ELISA from Cusabio Biotech Co. (Wuhan, China). Assay standard curve is ranged from 4 to 1000 pg/mL with serum requirement of 50 μL. Assay optimization included modification of the procedure to include sample mixing followed by prolonged primary antibody-antigen incubation time to ensure saturation. Two custom quality controls were prepared at levels that are on the sensitive part of the standard curve. Qualification criteria included assessment of the standard curve, quantification range, reproducibility (precision) and dilutional linearity (% recovery). Standard curve was made more robust by adding more points on the sensitive part of the curve. Lower limit of quantification was qualified to be statistically above the variance of the blank value. Reproducibility was good (%CV≤30%) among assays. Linearity was acceptable for kit standards diluted with castrated dog serum or commercially available serum matrix, also, for intact serum diluted with its respective castrated serum (R2>0.9). This assay can detect >8 fold INH-B difference between intact and castrated canine serum samples. Other parameters like frozen storage, freeze/thaw and lot-to-lot stability are pending. We conclude that this canine INH-B assay can consistently quantify INH-B levels in canine serum under the modified procedures.

PS 1886 Mitigation of Fumonisin Biomarkers by Green Tea Polyphenols.

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Fumonisin B1 (FB1) is a carcinogen and a strong tumor promoter in animal models. Green tea polyphenols (GTP) are highly effective in inhibition of a variety of carcinogen-induced tumorigenic effects in many model systems. In this study we assessed mitigative effects of GTP on FB1-biomarkers in blood and urine samples collected from a randomized, double blinded, and placebo controlled intervention trial, which recruited a total of 124 people aged 20-55 who exposed FB1 via their corn-based diet. These participants were consented, randomly divided into 3 groups, and daily treated with either low-dose (GTP 500 mg, n=42), high-dose (1,000 mg, n=41) or placebo (n=41) for 3 months. Urinary levels of free FB1 at baseline were comparable (medium at 560.73, 574.56, and 559.09 pg/mg creatinine) for all three groups (p=0.162). Levels at urine samples collected at 1-month of the intervention was significantly decreased in the high-dose group (medium: 364.94 pg/mg creatinine; p<0.01) as compared with level in the placebo group (medium: 575.25 pg/mg creatinine). The inhibition rate is 18.95% in low-dose group and 33.62% in high-dose group. Levels of free FB1 at samples collected at 3-month of the intervention showed significant decrease in both low-dose (medium: 319.45pg/mg creatinine; p<0.01) and the high-dose (medium: 215.83pg/mg creatinine; p<0.01) groups as compared with the levels of the placebo group as well as the baseline levels. The inhibition rate is 40.18% in low-dose GTP group and 52.6% in high-dose GTP group. Levels of sphinganine (Sa), sphingosine (So), and their ratio in urine and serum samples were also evaluated in this study. These results demonstrate that supplement of GTP effectively mitigates urinary excretion of free FB1 via to be specified pathways in humans.

PS 1887 Variation of Urinary Creatinine.

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Urinary creatinine has been commonly used for adjusting dilution status of urine species in biological monitoring. However, it can vary according to sex, age, race, BMI, meat intake, etc. The purposes of our study are to investigate the intra- and inter-individual variations of urinary creatinine in a sex, age and race matched subjects, and to study the impact of meat intake on the variations of urinary creatinine. We designed a diet-controlled study among the subjects who were Korean healthy females (N=9, age= 20±4 yrs, BMI 19.7±2.4 kg/m2) and measured urinary creatinine at 5 intervals during 24 hours with and without meat consumption. As results, diverse intra-and inter-variations of creatinine levels were shown in the subjects: When subjects did not take meat, the largest and smallest intra-variations in urinary creatinine ranges were detected in the subject C and G, i.e. 0.34-2.97 (Δ2.63)g/L and 0.93-1.63 (Δ0.7) g/L, respectively. In addition, creatinine levels at 5-intervals were significantly different between the highest and lowest average levels-subjects, i.e. 2.13±0.73 g/L of the subject I and 0.86±0.52 g/L of the subject A (p<0.05), respectively. With intake of meat (charcoal-grilled Korean beef tenderloin), the trend of intra-variation of urinary creatinine in each subject was not different (p=1.00 by Fisher exact test). It suggests that meat intake had little influence on intra- and inter-variation of urinary creatinine. In conclusion, our data re-emphasize that urinary creatinine must be measured in each spot urine even among the subjects who have similar age, sex, race and BMI due to its intra- and inter-variation. In the near future, the causes of intra- and inter-individual variations of urinary creatinine should be further studied.

PS 1888 Cardiolipin As a Biomarker of Mitochondrial Dysfunction Associated with Parkinson's Disease.

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A commonly used pesticide, rotenone, is a mitochondrial respiratory complex I inhibitor capable of selective oxidation of mitochondrial phospholipid, cardiolipin (CL). Given that rotenone exposure is associated with the development of

Parkinson disease (PD), we hypothesized that CL peroxidized molecular species accompanying mitochondrial dysfunction may represent a new biomarker of PD. In this study, we used circulating lymphocytes isolated from human blood and found that rotenone (50-250μM, 12-18h) caused apoptosis (phosphatidylserine externalization, caspase 3/7 activation), reactive oxygen species production (superoxide, H2O2), mitochondrial dysfunction (inactivation of complex I, decrease of mitochondria membrane potential, depletion of ATP) and activation of peroxidase activity of mitochondria. Using an oxidative lipidomics approach, we found that treatment of lymphocytes with rotenone resulted in accumulation of monolyso-CL and oxygenated free fatty acids. In addition we were able to detect oxygenated molecular species of tetra-linoleyl CL, a major CL molecular species in lymphocytes. Notably, molecular species of oxygenated CL formed in human lymphocytes were similar to those formed in cyt c driven reaction in the presence of H2O2 – in line with the known participation of cytochrome as a catalyst of CL peroxidation during apoptosis. Using the combination of lipidomics and oxidative epitope-targeted enzymatic digestion of oxidized tetralinoleoyl-CL we found that its oxygenated LA species were represented by hydroxy- and hydroperoxy-derivatives. Thus, we conclude that CL and its oxygenation products and metabolites may represent a new biomarker of rotenone-induced mitochondrial dysfunction associated with PD. Supported by NIOSH OH008282; NIH ES020693, U19 AI068021, HL70755.

PS 1889 Evaluation of Insulin-Like Growth Factor Acid-Labile Subunit As a Novel Biomarker of Effect to the Mycotoxin Deoxynivalenol.

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Deoxynivalenol (DON) is a trichothecene mycotoxin produced from Fusarium species frequently found in grain products due to its recurrent contamination and resistance to food processing treatments. In growing experimental animals, chronic low-level DON exposure has resulted in anorexia, weight suppression and growth hormone axis perturbations. As a result, children are thought to be especially sensitive to DON. Though a biomarker of exposure exists to measure DON exposure in humans, no biomarker of effect is currently available to predict the adverse negative weight effects of DON, thereby hindering complete risk assessment of this mycotoxin. Two studies were conducted to assess the potential of plasma insulin-like growth factor acid-labile subunit (IGFALS) to be used as an effect biomarker for DON. In the first study, a 9 wk dietary DON exposure was employed in mice to test the hypothesis that depression in plasma IGFALS occurs at toxicologically relevant doses prior to significant weight suppression. Results showed that the 1) NOAEL for depressed plasma IGFALS and weight was 2.5 ppm DON and 2) decreased plasma IGFALS was detectable before significant weight suppression was evident. In the second study, the specificity of reduced plasma IGFALS to DON, rather than DON-induced anorexia, was assessed using a dietary restriction study. Mice were fed ad-lib control diet, restricted control diet or identical amounts of restricted 15 ppm DON diet. Mice fed restricted DON diet exhibited significantly less plasma IGFALS than the restricted control indicating the specificity of plasma IGFALS reductions to DON. Thus, plasma IGFALS might be one suitable biomarker for predicting DON's adverse growth effects in animals and humans.

PS 1890 Validation of a Meso Scale Discovery Immunoassay for KIM-1 Renal Biomarker in Cynomologus Monkey (*Macaca fascicularis*) Urine.

D. Fix and W. J. Reagan. *Drug Safety, Pfizer, Groton, CT.* Sponsor: J. Aubrecht.

The purpose of this study was to validate an immunoassay for detection of Kidney Injury Molecule 1 (KIM-1) in the urine of cynomologus monkeys (NHP). Monkeys were treated with escalating doses of a compound that induced tubular degeneration/regeneration as determined by histopathology. Urine was collected pre-dose, 16 days post dose and 21 days post dose and urine with low, medium and high values of KIM-1 were used to validate the Meso Scale Discovery (MSD) Human KIM-1/TIM-1 (single-plex) immunoassay kit as this kit cross reacts with NHP KIM-1. Additional urine from older colony monkeys as well as normal younger NHPs were also used to establish a preliminary observed range (<0.01ng/mL). We determined intra-assay (7.7% CV) and inter-assay precision (24.5% CV), limit of blank (0.000331ng/mL), limits of quantitation (0.01ng/mL to 10ng/mL), dilutional linearity (not linear when diluted), recovery (84.8% - 122.8%), preliminary quality control range evaluation (0.438ng/mL/ CV 18.7%; 0.615ng/mL/ CV 16.8%), freeze/thaw (F/T) stability out to 4 F/T cycles (95.5% - 112.5%), and sample storage stability out to 10 weeks. There was also a good biologic correlation with time and dose-dependent increases in KIM-1 for the toxicity study samples. All parameters measured showed acceptable immunoassay assay performance except dilutional linearity. Based on this assay performance and the other results obtained, the validated method is robust and can be performed under good laboratory practice conditions to support nonclinical studies to assess for renal toxicity.

PS 1891 Evaluation of a Three-Dimensional Oral Cell Model for the Assessment of Tobacco Products.

W. Fields, K. Fowler and B. R. Bombick. *R. J. Reynolds Tobacco, Company, Winston-Salem, NC.* Sponsor: S. Theophilus.

Oral disease is frequently associated with viral and environmental exposures as well as oral hygiene. The goals of this study were to evaluate the impact of smokeless tobacco extracts (STE) and cigarette total particulate matter (TPM) on cell survival, oxidative stress, inflammatory response and tissue integrity using three-dimensional cultures of human buccal (EpiOral™) cells.
EpiOral™ cells were treated with extracts of 1S2 (reference dry snuff), 2S3 (reference moist snuff) and a smokeless tobacco blend prepared in complete artificial saliva (CAS) as well as with TPM from Kentucky Reference 3R4F cigarette (DMSO-based) for time points through 24 hours. Toxicity was assessed with the lactate dehydrogenase (LDH) assay. Glutathione (GSH) measurement and histological analyses were used to assess oxidative stress and changes in tissue integrity, respectively. Gene expression analyses were also conducted via QRT/PCR and multiplex cytokine testing.
Dose- and time-dependent release of LDH was observed for all test articles. The optimal exposure time appears to be 12 hours where 3R4F TPM elicited up to a 3-fold increase in LDH release; the 1S2 and 2S3 extracts yielded a 2-fold increase while no increase was observed for the smokeless tobacco blend. Tissue integrity was slightly disrupted by TPM exposure, while no impact was observed for the STEs.
Oxidative stress as measured by GSH analysis was not apparent for any of the test articles; however, altered inflammatory response was observed by changes in IL-1α and G-CSF cytokine release and modulations in at least one of the following genes, IL-1α,TNFα or COX-2. The test articles also induced increases in cellular stress and xenobiotic metabolism as determined by changes in HO-1, HSP-70, CYP1A1 and CYP1B1.
Collectively, the data suggest that the EpiOral™ three-dimensional human cell culture model may be useful in evaluating tobacco extracts.

PS 1892 Assessment of Cardiac Biomarkers in Cynomolgus Macaques.

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A number of new cardiac biomarkers have recently been developed for use in rodents; however, there are no validated cardiac biomarkers suitable for use in nonhuman primate (NHP) studies. We previously reported results of cardiac markers in African green monkeys (AGM) and Rhesus macaques (RM). In the current study we have extended these evaluations to Cynomolgus macaques (CM), the most widely used NHP species for toxicity studies. Two CM/sex were given a single subcutaneous (sc) injection of isoproterenol (IPT; 4 mg/kg); 1 CM/sex received sc saline. Cardiac effects of IPT were observed within 1 hr postdose and included hypotension, ventricular premature complexes, ventricular bigeminy, atrial premature complexes, with or without aberrant conduction and ST segment elevation. Blood samples were collected prestudy and at 1, 4, 24, 48 and 72 hr postdose, and evaluated with MSD MIP-1 muscle injury kits (rat: cTnI, cTnT, FABP3, Myl3, sTnI; human: TNI). IPT produced significant increases in the level of most cardiac biomarkers: cTnT, FABP3, Myl3, sTnI and human cTnI were increased over predose levels by 4.2-, 2.5-, 25-, 28- and 23-fold, respectively, with peak times ranging from 4 to 48 hours. Similar results were seen in females, though rat cTnI was not increased in males, but a 4.9-fold increase was seen in females. At 72 hr postdose, there were still elevations in Myl3 and sTnI. IPT plasma levels at 1 hr postdose were higher in males (708 ng/ml) than in females (324 ng/ml) and fell to 324 and 102 ng/ml at 4 hr in males and females, respectively. Heart histopathology 3 days postdose revealed minimal to moderate cardiac myofiber degeneration, myofiber karyomegaly and leukocyte infiltration in all treated animals. These results indicate that the MSD rat and human muscle injury panel provides excellent sensitivity for assessing cardiac effects in CMs, and these data are consistent with the utility of these kits previously reported in RM and AGM. Work supported by NIAID Contract N01-AI-70043.

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