blood concentrations. Metabolic and absorption parameters were fit to the marmoset pharmacokinetic data. The maximum rate of marmoset metabolism of DEHP to MEHP in the gut, VmaxgC (mg/hr/kg$^{0.75}$) was lower than previously estimated for the rat, indicating a species difference in DEHP gut metabolism. Alternatively, the fecal loss rate, loss (1/hr) was higher in the primate model than the rat model, suggesting less complete absorption. The MEHP concentration time courses following repeated exposure were well predicted with parameters estimated from acute exposure time courses. Once validated, a primate PBPK model for DEHP will be able to predict dose metrics for use in human health risk assessment. (Supported by a gift from the American Chemistry Council)

2048 USING *IN VIVO* GAS UPTAKE STUDIES TO ESTIMATE METABOLIC RATE CONSTANTS FOR CCL CHEMICALS: 1, 1-DICHLOROPROPENE AND 2, 2-DICHLOROPROPANE.

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The Safe Drinking Water Act Amendments of 1996 required the USEPA to develop Candidate Contaminant Lists (CCL), to aid in the setting of priorities for the Agency's drinking water research program. 1, 1-Dichloropropene (1, 1-DCPe) and 2, 2-dichloropane (2, 2-DCP) are two of the high priority chemicals identified from the first CCL. Since both are volatile organic compounds, the gas uptake technique was used to estimate rates of metabolism. The gas uptake system is a closed inhalation chamber system in which an initial bolus injection of chemical is made into the chamber, and allowed to decline to obtain a set of decay curves. Individual male F344 rats (200-250g) were exposed to initial chamber concentrations of 50 ppm, 200 ppm, 500 ppm, or 1200 ppm of either 2, 2-DCP or 1, 1-DCP for up to six hours (n=4 rats/exposure level/chemical). Each rat was exposed only once to a single concentration of each chemical. Partition coefficients were estimated using a published quantitative structure-property relationship methodology (Beliveau et al., 2003). These partition coefficients were used with the PBPK model to generate estimates of VmaxC (mg/hr/kg) and KM (mg/L). Optimized VmaxC and KM were 4.9 and 3.17, respectively, for 2, 2-DCP. Optimized VmaxC and KM were 5.76 and 0.31, respectively, for 1, 1-DCPe. The shape of the gas uptake curves for 2, 2-DCP compared to 1, 1-DCPe and the V/K ratios suggest that 2, 2-DCP is more slowly metabolized. These metabolic rate estimates are critical parameters for PBPK models that can ultimately be used for animal to human extrapolation. They are also a vital part of a developing data base comparing *in vivo* to *in vitro* methods for determination of metabolic rate parameters. (This abstract does not reflect EPA policy).

2049 HUMAN MITOCHONDRIAL DNA AMPLIFICATION AND SEQUENCING STANDARD REFERENCE MATERIALS 2392 AND 2392-I.

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The National Institute of Standards and Technology (NIST) supplies industry, academia, and government with over 1300 Standard Reference Materials (SRMs) of the highest quality. These SRMs provide laboratories the quality control and assurance that their results with unknown samples are accurate and compatible with other measurements. NIST has revalidated the human mitochondrial DNA (mtDNA) SRM 2392 and developed the new SRM 2392-I to provide quality control when performing the polymerase chain reaction (PCR) and sequencing of mtDNA for forensic identification, medical diagnosis, or mutation detection. They may also serve as controls when performing PCR and sequencing any DNA. SRM 2392 is certified for the sequences of the entire human mtDNA (16, 569 base pairs) from two lymphoblastoid cell lines (CHR and 9947A) from apparently normal individuals and the cloned HV1 region of CHR containing a C-stretch, following which it is difficult to sequence. SRM 2392-I is certified for the entire mtDNA sequence from HL-60, a promyelocytic cell line from the blood leukocytes from an individual with acute promyelocytic leukemia. The mtDNA sequences (but not the DNA) from two other cell lines (GM03798 and GM10742A) that were amplified and sequenced in their entirety at NIST are provided for information and comparison purposes. The sequences of fifty-eight unique primer sets that allow any area or the entire mtDNA to be amplified and sequenced under the same conditions are also given. Many of the single nucleotide polymorphisms (SNPs) found in these five mtDNA templates did result in amino acid changes when compared with the Cambridge Reference Sequence. Two interlaboratory evaluations for the amplification, sequencing, and data analysis of SRM 2392 and SRM 2392-I were each conducted by three different laboratories and NIST. Corroboration of the results in these SRMs will provide quality assurance that any unknown mtDNA is also being amplified and sequenced correctly.

2050 POLYMORPHISMS IN CYTOCHROME P4503A5 (CYP3A5) MAY BE ASSOCIATED WITH TUMOR SIZE IN BREAST CANCER PATIENTS.

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CYP3A5 is one of the most important members of the cytochrome P450 superfamily. Although this enzyme has been shown to have two polymorphisms (CYP3A5*3 and CYP3A5*6), little is known about the prevalence of these polymorphisms, and whether they are associated with biologic outcomes. Since polymorphisms in other enzymes differ by race, and have been associated with tumor characteristics, the purpose of this work was to test the hypothesis that there are racial/ethnic differences in the odds of having a CYP3A5 polymorphism and that polymorphic status in CYP3A5 affects tumor status in women with breast cancer. To test this hypothesis, 107 women with breast cancer were recruited from a single clinic at the University of Maryland. Each patient completed a survey that obtained information on race and potential confounding factors (age, date at diagnosis, body mass index) and provided a blood sample. Information on tumor characteristics (size, receptor status, nodal involvement) was collected by abstraction of medical records. Blood samples from each patient were subjected to genomic DNA extraction followed by PCR for CYP3A5*3 and CYP3A5*6. The associations between race, polymorphic status, and tumor characteristics were assessed using t-tests and logistic regression models. The data indicate that 40.7% of the women had the CYP3A5*3 polymorphism, and 9.1% had the CYP3A5*6 polymorphism. In addition, white women were 26 times more likely to carry the CYP3A5*3 polymorphism than black women, whereas black women were 9 times more likely to carry the CYP3A5*6 polymorphism than white women. Further, there was significant difference in mean tumor size in women with the CYP3A5*6 polymorphism (3.6cm ±0.98) compared to those without the polymorphism (2.0cm ±0.18) ($p<0.02$). These findings suggest that there are racial/ethnic differences in the prevalence of CYP3A5 polymorphisms, and that polymorphic status may be associated with the size of breast tumors. Supported by DOD grant DAMD 17-00-1-0321 and the University of Maryland, Maryland Statewide Health Network.

2051 GSTP1 A1578G (ILE105VAL) POLYMORPHISM IN BENZIDINE-EXPOSED WORKERS: AN ASSOCIATION WITH CYTOLOGICAL GRADING OF EXFOLIATED UROTHELIAL CELLS.

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A polymorphism at codon 105 (Ile/Val) in the GSTP1 gene has been associated with a higher risk for different cancer types. To assess the role of GSTP1 polymorphisms in the development of benzidine-related bladder cancer, GSTP1 AA, AG and GG alleles were determined in occupationally benzidine-exposed Chinese workers without known disease and benzidine-exposed bladder cancer patients from the same cohort of the Shanghai area. An increased but not significant frequency of GSTP1 AG or GG carriers was observed in the occupationally exposed bladder cancer patient group (OR=1.95, 95% CI 0.70-5.46). The odds ratios for the most important non-genetically determined risk factors for bladder cancer in males were as follows: Age (increase per year): OR 1.05, 95% CI 0.99-1.11, ever smoker: OR 1.31, 95% CI 0.47-3.69, duration of exposure (increase per year): OR 1.19, 95% CI 1.10-1.29, and high exposure: OR 4.50, 95% CI 0.70-5.46. Significant differences were found between all benzidine-exposed workers without known disease with modified exfoliated urothelial cells (grade II and higher) and all workers without known disease with at most minor changes (less than grade II) according to Papanicolaou (OR 1.90, 95% CI 1.13-3.20). These findings show for the first time an association between the GSTP1 AG or GG genotype and higher cytological gradings of exfoliated urothelial cells from formerly benzidine-exposed workers.

2052 GENETIC VARRIATION IN TGF-BETA1 BUT NOT ANTIOXIDANT GENES IS ASSOCIATED WITH PROGRESSIVE MASSIVE FIBROSIS IN COAL WORKERS.

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Progressive massive fibrosis (PMF) is the severe form of coal worker's pneumoconiosis. Although the severity of PMF is mostly dependent on the total amount and duration of dust exposure, genetic factors also play an important role in the devel-

opment of disease and modify the individual susceptibility. Studies have implicated reactive oxygen species (ROS) in the pathogenesis of fibrosis and other lung diseases. Many chemicals and physical agents including mineral dusts are potent generators of ROS. Lung tissue is protected against ROS by a variety of antioxidant mechanisms such as superoxide dismutases (SOD) and glutathione s-transferases (GSTs). Transforming growth factor beta (TGFβ) is a key profibrotic growth factor implicated in fibrosis, including the deposition of extracellular matrix proteins. Antioxidant enzyme and cytokine genes are subject to polymorphisms in their regulatory regions which affect their expression level and thus contribution to disease process. This study was undertaken to examine the association between the functional polymorphisms of TGFβ and antioxidant enzyme genes and progression of PMF as well as possible gene-gene and gene-environment interactions. We genotyped DNA collected from lung autopsy tissues from 270 miners diagnosed with PMF as well as in 270 control miners with no disease using a case-control study design. Polymorphisms in GSTP1, GSTM1, GSTT1, MnSOD and TGFβ were anylised by Taqman® assay to determine each genotype. Our results showed an association of PMF prevalence with TGFβ1 (-509) TT genotype (OR 1.77; 95% CI 1.03-3.09), whereas no statistically significant differences in the allele frequencies of antioxidant genes were observed between the cases and controls. The results suggest that interactions of genetic background with environmental exposure may affect the pathogenesis of PMF.

2053 GENETIC INFLUENCES ON HUMAN ENDOTHELIAL CELL FUNCTION AND SURVIVAL: INFLUENCE OF THE NOS3 EXON7 (GLU298ASP) AND ACE (I/D) POLYMORPHISMS ON GLUCOSE AND FREE 3-NITROTYROSINE INDUCED CELL TOXICITY *IN VITRO*.

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Genetic polymorphisms of NOS3 and ACE have been implicated in diabetes related cardiovascular disease risk in humans. The functional relevance of NOS3 and ACE genetic variations to endothelial cell function is largely unstudied. The objective of this study was to test the functional relevance of NOS3 (Glu298Asp) polymorphism and ACE (I/D) polymorphism to the endothelial cells in-vitro. The central hypothesis was that the presence of these genetic polymorphisms alters endothelial cell sensitivity to glucose and 3-nitrotyrosine, two known diabetes-related vascular toxicants. Primary cell preparations (HUVECs) were initially screened for NOS3 and ACE genotypes. Unstimulated growth characteristics were in initially investigated and cells were incubated with various concentrations of glucose (10, 20, and 30mM), free 3-nitrotyrosine (10nM-5mM), or a combination of these two toxicants. No significant differences in unstimulated growth rates were observed among genotype groups (n=3-6 per group), but significant differences in glucose induced cell death (at 20mM Glucose: 9.27%: 30.35%: 28.69%; Glu/Glu: Glu/Asp: Asp/Asp. $p<0.05$) and free 3-nitrotyrosine induced cell death (LC50 values: 12.94±1.38 μM: 1.55±1.48 μM: 10.78±1.20 μM; Glu/Glu: Glu/Asp: Asp/Asp. $p<0.05$) were observed among the NOS3 genotypes. Combined exposures of glucose/3NT caused increased toxicity among the NOS3 genotypes. In contrast, no differences were observed among the ACE genotypes in terms of their responses to these concentrations of the toxic agents. These data demonstrate that the exon 7 NOS3 genotype may an important predictor of or mechanistically involved in endothelial vulnerability to these two diabetes related toxicants, whereas the ACE I/D genotype is apparently less important. Thus the NOS3 (Glu298Asp) genetic variation may play a specific role in vulnerability to diabetes related vascular complications

2054 PARAOXONASE STATUS IN A MEXICAN POPULATION AND ITS RELATIONSHIP TO THE SUSCEPTIBILITY TO DNA DAMAGE ON CULTURED HUMAN LYMPHOCYTES TREATED WITH METHYL-PARATHION AND PARAOXON.

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Human paraoxonase (PON) is an enzyme that participates in the detoxification of organophosphorus pesticides (OP). PON polymorphisms responsible for different catalytic activities and expression levels have demonstrated the relevance of PON in OP toxicity (mainly acethylcholinesterase inhibition), and the importance of evaluating PON status (genotype/phenotype). No information is available about the role of PON in the susceptibility to DNA damage. The aim of this study was to evaluate the role of PON status in the susceptibility to cytogenetic effects on cultured human lymphocytes treated with methyl-parathion or paraoxon. Serum PON activities were assayed using phenylacetate and paraoxon as substrates. Three PON variants (−108, 55 and 192) were evaluated by PCR-RFLP. Induction of sister-chromatid exchange and mitotic index were determined in cultured lymphocytes added with homologous serum from two individuals with the haplotypes PON $C_{-108}L_{55}R_{192}$ ("resistant") or two individuals with the haplotypes PON $T_{-108}M_{55}Q_{192}$ ("susceptible"). Participants (n=74, 28.6 years old) were individuals of both sexes. PON activity with paraoxon was 632.9 IU/L (range 123.4-1645.6), and with phenylacetate was 199.2 IU/ml (range 85.2-422). Genotype frequencies for PON_{108} were 0.14CC, 0.5CT and 0.36TT; for PON_{55} were 0.09MM, 0.27ML and 0.64LL, and for PON_{192} were 0.24RR, 0.33QR and 0.43QQ. Enzymatic activities were different ($p<0.005$) according to genotype. Paraoxon and methyl-parathion showed cytogenetic effects in cultured lymphocytes but a difference in response according to PON genotype was observed only with paraoxon: lymphocytes from "resistant" individuals had lower cytogenetic effect than the "susceptible" individuals. The difference between paraoxon and parathion effect could be explained by the fact that PON hydrolyzes oxons and not the parent compound. Our results suggest that PON could have an important role in the cytogenetic effects caused by OP exposure.

2055 THE ARYL HYDROCARBON RECEPTOR 1 (AHR1) LOCUS IS HIGHLY POLYMORPHIC IN ATLANTIC KILLIFISH (FUNDULUS HETEROCLITUS): RELATIONSHIP TO DIOXIN RESISTANCE.

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AHR agonists such as 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) and polynuclear aromatic hydrocarbons (PAHs) are highly toxic to most vertebrate animals. To understand the role of the AHR pathway in differential sensitivity to TCDD and PAHs, we are studying populations of the Atlantic killifish (Fundulus heteroclitus) that differ in their sensitivity to the toxic and biochemical effects of these compounds. Killifish inhabiting New Bedford Harbor (MA, USA), a federal Superfund site, have developed heritable resistance to AHR agonists. To investigate the mechanism of resistance and the possible role of AHR genetic variability, we cloned killifish AHR1, AHR2, ARNT2, and AHR repressor. AHR1, but not AHR2, ARNT, or AHRR, is differentially expressed between dioxin-sensitive and -resistant fish. Sequencing of AHR1 cDNAs from multiple individuals from several sites in Massachusetts, New York, and New Jersey revealed extensive polymorphism at the AHR1 locus. We identified 39 single nucleotide polymorphisms, 14 of which were non-synonymous. Alleles were assigned to three groups: AHR1*1, AHR1*2, and AHR1*3. AHR1*1 alleles were under-represented in a New Bedford Harbor fish (dioxin resistant) as compared to fish from Scorton Creek, MA (dioxin-sensitive). Initial functional analysis of the two most divergent variants (AHR1*1A and AHR1*3A) expressed by *in vitro* transcription and translation indicated similar [3H]TCDD-binding affinities. In transient transfection assays using mammalian cells, AHR1*1A and AHR1*3A exhibited similar transactivation potentials in the presence of TCDD. Thus, the killifish AHR1 locus is highly polymorphic and allele distributions differ between some dioxin-sensitive and dioxin-resistant populations. The role of AHR1 polymorphisms in the resistant phenotype remains uncertain. (Superfund Basic Research Program (5P42 ES07381) and the Hudson River Foundation.)

2056 EFFECT OF ALDH2 GENE POLYMORPHISMS ON THE METABOLISM AND TOXICITY OF 2-ETHOXYETHANOL IN THE EXPOSED WORKERS.

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2-Ethoxyethanol (ethylene glycol monoethyl ether) has been known to be toxic to testes and blood system. It is metabolized by alcohol dehydrogenase and CYP P450 2E1 to ethoxyacetic aldehyde, and then transformed by aldehyde dehydrogenase (ALDH) to ethoxyacetic acid, which is excreted in urine. It is not clear which metabolite, the aldehyde or the organic acid, plays the role in the occurrence of the injuries induced by the solvent. ALDH2 is the major enzyme in the metabolism of many short chain alcohols. The single nucleotide polymorphisms at nucleotide 1510 (G/A) of the gene result in a substitution of Glu to Lys at amino acid position 487. Approximately 30% of the Orientals possess ALDH2*2 allele encoding the enzyme without activity. In this study, we investigated the toxic effects of 2-ethoxyethanol exposure among workers, and analyzed whether ALDH2 polymorphisms exerts any effect on the toxicity of the compound. It was found that the quantity and quality of sperm were both decreased in the males exposed to high concentration of 2-ethoxyethanol; the organic solvent seemed not to affect the blood concentrations of the sex related hormones such as testosterone, LH, FSH at

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, workshop, roundtable, platform and poster sessions of the 43rd Annual Meeting of the Society of Toxicology, held at the Baltimore Convention Center, Baltimore, Maryland, March 21-25, 2004.

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