57 AN IMPROVED, SEMI-AUTOMATED METHOD FOR MEASURING *HPRT*/*HPRT* GENE MUTATIONS IN MOUSE AND HUMAN LYMPHOCYTES. Galbert LA[1], Guerin AT[1], Carmical JR[2], Herring SM[1], Abdel-Rahman SZ[1], Ward JB[1], Wickliffe JK[1]. [1]Department of Preventive Medicine and Community Health, University of Texas Medical Branch, Galveston, TX 77555-1110. [2]Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, TX 77555.

The *Hprt*/*HPRT* cloning assays are used to determine the mutagenicity of environmental agents. The current approach of visually scoring lymphocyte clones is a costly, laborious method that carries the risk of subjective or variable scoring. We refined a technique developed by the National Center for Toxicological Research (NCTR) that incorporates a metabolically activated dye, alamarBlue®, to identify wells containing actively growing lymphocyte clones. To examine the usefulness of this refinement, we treated mice with ethylnitrosourea (ENU), a well-known mutagen that shows a consistent mutagenic response. We have also applied the same approach to the human cloning assay by examining cells from tobacco smokers and matched non-smokers. Plates were scored visually and by fluorescence. Lethally irradiated lymphocytes (IR cells) were plated in a similar manner to the cloning efficiency (CE) plates. Fluorescence values (FV) from IR plates, 5 per assay, were used to obtain an average FV for a negative well. Any FV 3 standard deviations above this average was considered to be a positive CE well. Because of considerable variation in FVs among mice and among human individuals, a different approach was used for thioguanine (TG) plates. We calculated a median FV for each plate and found that wells visually identified as positive exhibited FVs that were usually equal to or greater than 25% of the median value. Applying this 25% rule, we found a 99.1% correlation between visual scores and fluorescence scores. This approach, if validated by other laboratories, will serve to standardize the *Hprt*/*HPRT* cloning assays by eliminating potential researcher bias and interlaboratory variation in results. Supported by: RO1 ES 06015, S11 ES10018, P03 ES 06676, T32 ES07254, and Philip Morris

58 CYTOCHROME P4501A1 (CYP1A1) INDUCTION IS SUPPRESSED BY COAL DUST EXPOSURE IN THE OVINE LUNG. Ghanem M[1,2], Hubbs AF[1,2], Kashon M[1], Weissman D[1], Porter D[1,2], Batelli LA[1,2], Vallyathan V[1], Nath J[2]. [1]NIOSH. [2]Genetics & Developmental Biology, West Virginia University, Morgantown, WV.

Cytochrome P4501A1 (CYP1A1) is associated with polycyclic aromatic hydrocarbon-mediated carcinogenesis. We have previously demonstrated suppression of CYP1A1 induction by coal dust (CD) exposure in a rat model. Since it has been controversial whether rat pulmonary responses to particulate parallel those of humans, we questioned whether CD exposure would also alter CYP1A1 induction in a larger species. We instilled the right apical lobes of Katahdin crossbred lambs with 500 mg CD (<5 microns) or saline using a flexible fiberoptic bronchoscope. 53 or 54 days later, the lambs received 50 mg/kg IP of the CYP1A1 inducer, beta-naphthoflavone (BNF). The lambs were sacrificed on day 56. The principal histological change in CD-instilled lobes was histiocytic bronchinterstitial pneumonia. CD-instilled lung lobes had increased percentages of bronchoalveolar lavage macrophages and neutrophils and decreased percentages of lymphocytes. CYP1A1-dependent 7-ethoxyresorufin-O-deethylase activity in microsomes prepared from lung tissue was significantly reduced in CD-instilled right lobes relative to uninstilled left lobes or the right lobes of sheep receiving BNF alone. Dual immunofluorescence staining of lung sections for CYP1A1 and the alveolar type 2 (AT-II) cell marker, cytokeratins 8/18, showed that expression of CYP1A1 in whole alveolar septum, AT-II cells and non-type II cells were all significantly reduced by CD exposure. These findings show that in addition to causing pulmonary inflammation, intrapulmonary deposition of CD also modifies induction and cellular localization of CYP1A1 protein in the sheep lung.

58A GENOMIC INSTABILITY INDUCED BY LOW DOSES OF GAMMA-RADIATION. Gibbons C[1], Ritter L[1], Grosovsky AJ[1]. [1]University of California, Riverside, CA 92521

Genomic instability, characterized primarily by an increased rate of chromosome aberrations, gene amplification, and mutation, is intrinsic to tumorigenesis and is also observed in cells surviving radiation exposure. The high percentage of irradiated clones that display karyotypic heterogeneity suggests that the phenotype is unlikely to be solely attributable to a mutational mechanism in a specific gene or gene family. This extraordinarily high frequency suggests that genomic instability can be significantly induced at doses that are relevant to environmental, occupational, or medical exposures. We therefore postulate that low-dose induction of genomic instability will deviate from the dose-dependent response expected if mutation alone were responsible for the phenotype. Previous work from our laboratory has suggested that persistent chromosomal instability can reflect the formation of chromosomal rearrangement junction sequences, which may act in *cis* as *de novo* breakage hotspots. This investigation therefore also sought to determine if a similar mechanism could be discerned in chromosomal instability induced by low dose exposure to ^{137}Cs gamma-radiation. TK6 human B-lymphoblastoid cells were exposed to 0, 1, 5, 10, 20, and 200 cGy of gamma radiation. Independently derived clones were analyzed karyotypically approximately 40 generations after radiation exposure. Karyotypic heterogeneity was observed in approximately 30% of clones exposed to doses as low as 1 cGy, which rose to a plateau of approximately 40% in clones receiving doses of 10 – 200 cGy. Since this dose is associated with low levels of DNA double strand breaks (0.4 dsb/cell), these findings make it difficult to attribute radiation-induced KH to the occurrence of dsb. We also observed that persistently unstable clones were usually associated with multiply rearranged chromosomes, which is consistent with our hypothesis that instability frequently reflects the formation of rearrangement junctions that act as chromosomal breakage hotspots.

59 TESTING THE SPECIFICITY OF THE *IN VIVO* RODENT SKIN MICRONUCLEUS ASSAY AS DEVELOPED BY NISHIKAWA ET AL., FOR CHEMICALS NEGATIVE IN DERMAL CARCINOGENESIS ASSAYS. Gibson DP[1], Krsmanovic LS[2], Aardema MJ[1]. [1]Procter & Gamble, Cincinnati, Ohio 45252. [2]BioReliance Inc., Rockville, MD 20850.

It is now well known that the standard *in vivo* rodent micronucleus assay may not be appropriate for testing all classes of chemicals, specifically those with short half-lives or those not readily absorbed and others that do not make it to the bone marrow. Several assays have been developed that have the potential to address this gap in safety testing (site-of-contact genotoxicity). These assays include the comet assay and this *in vivo* skin micronucleus assay. An *in vivo* rodent skin micronucleus assay has been developed by Nishikawa et al., and reported on in MR 444 (1999) 159-166 and in MR 513 (2002) 93-102. They were able to show that the method could detect numerous rodent skin carcinogens (MMC, MMS, ENNG, BaP, MNNG, DMBA and 4 NQO) as positive in an *in vivo* skin micronucleus assay in rats and mice. To test the usefulness of this *in vivo* genotoxicity model in predicting the results of the skin carcinogenesis our lab (P&G) has conducted multiple assays on both known skin carcinogens and known skin non-carcinogens as determined in skin painting or topically applied carcinogenesis assays without the use of initiators. Compounds tested in the assay include the skin carcinogens mitomycin C, cyclophosphamide and vinblastine sulfate along with the several non-carcinogens. The focus of this poster deals specifically with the specificity of the method for three skin non-carcinogens. Results will be presented for 2-ethyl-1,3-hexanediol, trichloroethylene and n-nitrosodiethylamine. Collaborative studies with BioReliance Inc. to evaluate the reproducibility of this assay are on-going and will be included.