

toluene, ethyl benzene, and styrene can give rise to substantial amounts of soot and other partially combusted products (up to 1% w/w). Working with 1,3-butadiene (a major component of petrochemical VOC mixtures to be flared) as a model, we found that its soot contains a broad and unique array of polycyclic aromatic hydrocarbons (PAHs). These included several known and suspected human carcinogens and high molecular weight PAHs (mass 300->1000 amu) in substantial amounts (e.g., 1000-5000 cigarette equivalents of (benzofluoranthenes+benzopyrenes) + perylene per g substrate burned; 2040 µg benzo(a)pyrene per g soot). When NO_x were present during or after combustion, nitro- and polynitro-phenols, toluenes, and PAHs were found in the mixture in similar amounts. Further, the soot particles were found to have strong solid state free radical character in ESR analyses (i.e., g=2.0024; approx. 4% spin/weight). This free radical was stable for more than a year in the solid state, and extractable into solvents (toluene, DMSO) where it was stable for days. Butadiene soot extract also is electrochemically active at electrodes (+1.2 V vs. SCE) and in solution where it oxidized dissolved ascorbate. These and other data are offered in support of ongoing toxicological studies of these mixtures in normal human cell lines (q.v., sister presentation of C. Kennedy, et al.).

1644 EFFECTS OF PCB MIXTURES AND CONGENERS ON THE BILIARY EXCRETION OF THYROID HORMONE.

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Polychlorinated biphenyl (PCB) mixtures and congeners effectively reduce circulating concentrations of thyroxine (T₄). This is thought to occur because of their ability to induce the UDP-glucuronosyl transferases that conjugate T₄ and the subsequent excretion of the glucuronide into bile. To determine whether there is a good correlation between the ability of PCBs to reduce T₄ and increase the biliary excretion of T₄ glucuronide, PCB congeners 95 (16 mg/kg), 99 (16 mg/kg), 118 (16 mg/kg) and 126 (40 µg/kg); Aroclors 1242 (32 mg/kg) and 1254 (32 mg/kg), and TCDD (3.9 µg/kg) were administered via gavage to male Sprague-Dawley rats for seven days. Twenty-four hours after the last dose, the femoral artery and vein and the common bile duct were cannulated. Following administration of [¹²⁵I]T₄, bile was collected at 30-min intervals for two hrs. Blood was collected at the mid-point of each bile collection period. Urine was also collected at two hrs. The total excretion of T₄ and its metabolites was quantified by gamma spectrometry, followed by HPLC analysis. All seven treatments decreased the serum concentration of T₄. Of the congeners, PCB 99 and 118 produced the largest decreases in serum T₄ concentration, whereas PCB 95, PCB 126 and TCDD had the least effect. None of the seven treatments had a marked effect on the urinary excretion of T₄ and its metabolites. In contrast, biliary excretion of T₄ glucuronide after administration of TCDD, PCB 118 or Aroclor 1254 was increased 6 to 8-fold, PCB 126 produced a 3 to 5-fold increase, whereas PCB 95 and PCB 99 produced less than a doubling. TCDD, one of the treatments that had the least effect on decreasing the concentration of T₄ in serum, increased the biliary excretion the most. In contrast, PCB 99, one of the congeners that decreased the serum concentration of T₄ the most, had the least effect on its biliary excretion. Therefore, there does not appear to be a good correlation between the ability of PCBs to decrease plasma T₄ concentration and increase its biliary excretion. (Supported by NIH grant ES-08156 and ES-05022, and EPA grant R826297.)

1645 ANTIESTROGENICITY OF CLARIFIED SLURRY OIL AND TWO CRUDE OILS IN A HUMAN BREAST-CANCER CELL ASSAY.

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Clarified slurry oil (CSO), a refinery stream produced by processing crude oil, and two crude oil samples, Belridge Heavy Crude Oil (BHC) and Lost Hills Light Crude Oil (LHLCO) were examined for their estrogenic and antiestrogenic properties in a human breast-cancer cell (MCF-7) assay. The MCF-7 focus assay is based on postconfluent cell growth and tissue restructuring measured as the development of multicellular layers or foci. Oil samples were prepared in DMSO resulting in extraction of virtually all of the aromatic hydrocarbons including the 3-7 ring polycyclic aromatic compounds (PACs) comprising 62.2% of the CSO, 9.0% of the BHC, and 2.0% of the LHLCO by total weight. None of the three samples were estrogenic in the MCF-7 focus assay. In contrast, all of the samples were antiestrogenic, i.e. they inhibited the development of foci induced by 1.0 nM 17 β -estradiol (E₂). The poten-

cy of the samples was correlated with the percent of PACs they contained. Radiometric analysis of the catabolism of [³H]E₂ in MCF-7 cell cultures demonstrated that all three samples resulted in an increase in the catabolism of E₂. It is suggested that PACs in the samples induced enzymes in MCF-7 cells that catalyzed the catabolism of E₂, accounting for at least part of the observed antiestrogenicity. (Supported by NIH ES04913 and Mobil Business Resources Corporation.)

1646 CHARACTERIZATION OF ASPHALT FUME GENERATION SYSTEM.

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A dynamic asphalt fume generation system (Heritage Research Group, Indianapolis, IN) was modified to provide consistent test atmospheres emulating road asphalt paving conditions. Characterization of the asphalt fume test atmospheres included (1) identification and characterization of chemical composition of polycyclic aromatic hydrocarbons (PAHs) by gas chromatography-mass spectrometry (GC-MS) of asphalt fume fractions collected by TPF filter (0.5 µm pore size at a flow rate of 1.0 liters/min), XAD-2, charcoal, and cold trap (-190°C, liquid N₂); (2) determination of the asphalt fume generation system uniformity of the aerosol distribution within the generator and exposure chamber; (3) gravimetric analysis (4) use of a photoionization detector (PID) for the vapor phase and a light scattering aerosol monitor for on-line monitoring of chamber concentration; and (5) detection and minimization of interferences from insulation and gasket materials within the system. The asphalt was initially preheated in an oven to 170°C, pumped to a large bitumen kettle, which maintained the asphalt temperature at between 150-170°C and then transferred to the generator (inlet temperature of 145-150°C). The fume was then conducted from the generator to an exposure chamber through a heated transfer line. Positive chemical ionization GC-MS using methane as the reagent gas and a 30 meter, 5% phenyl fused silica capillary column was used to characterize the fume. A total ion chromatography of PAHs was obtained with an initial temperature 50°C increased at 5°C/min to 310°C. The results indicated that asphalt fume chemical group characterization can be achieved using a combination of sampling methods with GC-MS detection. The generator output was both qualitatively and quantitatively very consistent when run 3 to 3.5 hrs/day for 5 consecutive days (CV < 20%). The results demonstrate that reproducible, consistent road paving type asphalt fumes can be generated for inhalation toxicity studies. (This work was supported, in part, by NIEHS/NTP.)

1647 THE AHR AND CYP1B1 AS TARGETS FOR PEPTIDE/TUMOR-SPECIFIC CANCER IMMUNOTHERAPY.

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Recent advances in the definition of requirements for antigen presentation suggest novel strategies for inducing tumor-specific CTL with peptides derived from tumor-associated antigens. The demonstration here of extremely high AhR and CYP1B1 mRNA levels in human myeloma, ovarian carcinoma, and breast carcinoma cell lines indicates that the AhR and the AhR-regulated CYP1B1 enzyme may be two such proteins. Peptide-MHC class I binding algorithms were used to identify human AhR and CYP1B1 peptides predicted to bind the product of the relatively common HLA-A201 allele. Of 3 AhR and 3 CYP1B1 nanomers predicted by the algorithms, 2 derived from each protein bound HLA-A201 with relatively high affinity. To test their immunogenicity, these peptides were loaded onto HLA-A201 dendritic cells (DC) and CD40 ligand (CD40L)-activated B cells and presented to autologous CD8⁺ T cells. CD8⁺ T cell lines were generated following an initial stimulation with peptide-pulsed DC (with IL-7) and repeated stimulations with peptide-pulsed CD40L-activated B cells (with IL-7 and IL-2). These putative CTL lines specifically lysed autologous CD40L-B cells pulsed with cognate but not unrelated peptides. Furthermore, both AhR peptide- and CYP1B1 peptide-specific CTL lines lysed human myeloma and/or carcinoma cell lines expressing both HLA-A201 and high levels of AhR and CYP1B1, respectively, but did not kill tumors expressing little or no AhR/CYP1B1 or tumors expressing a different HLA-A allele. These results encourage the generation of AhR- and CYP1B1-specific CTL for adoptive immunotherapy of AhR^{hi} and CYP1B1^{hi} cancers.



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