(CYP4A) activities. Of these, CYP3A-selective testosterone hydroxylase activity was consistently increased in samples from the second surgery as compared to corresponding samples from the first (obese) surgery 215 ± 40%, P<0.05). Small increases in acetanilide and lauric acid hydroxylases were also observed (+52 ± 28%, +35 ± 12% respectively), whereas chlorzoxazone hydroxylase activity was slightly decreased (-18 ± 11%). This suggests that obesity does influence the expression of CYP3A and possibly other CYP isoforms in human liver.

356 BIPHENYL PROPARGYL ETHERS AS INHIBITORS OF CYP 1A1, CYP 1A2, AND CYP 2B1.

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Cytochrome P450 enzymes are a superfamily of hemoproteins involved in the metabolism of endogenous and exogenous compounds including many drugs and environmental chemicals. Certain aromatic acetylenes have been shown to be inhibitors of these enzymes. Size of the ring system, the location of the acetylenic moiety, and the distance of the triple bond from the ring influence the specificity, efficiency and mechanism of inhibition. Previously we found that 4-(1-propynyl)-biphenyl is a potent inhibitor of CYP 1A1 and 1A2, but 4-(1-butynyl)-biphenyl is only a moderate inhibitor. In an attempt to identify potent inhibitors of CYP 1A and 2B enzymes, we synthesized a series of mono- and di-substituted biphenyl propargyl ethers and mono-substituted biphenyl methyl propargyl ethers, including 2-biphenyl propargyl ether(2BPhPE), 4-biphenyl propargyl ether(4-BPhPE), 2, 2′-biphenyl dipropargyl ether(2, 2BPhDPE), 4, 4′-biphenyl dipropargyl ether(4, 4BPhDPE), 2- biphenyl methyl propargyl ether(2BPhMPE), and 4-biphenyl methyl propargyl ether(4BPhMPE). The presence of an oxygen atom on the substituent changes the polarity and orientation of the triple bond. The compounds were synthesized by coupling the appropriate biphenyl alcohol with propargyl bromide in the presence of sodium hydride. These compounds have been tested as inhibitors of CYP 1A1, 1A2, and 2B1 with methoxyresorufin (1A2), ethoxyresorufin (1A1), or benzyloxyresorfin (2B1) as the substrate. The preliminary results indicate that 2BPhPE is a potent inhibitor of 1A2 and 2B1 but only a moderate inhibitor of 1A1. 4-BPhPE and 2BPhMPE are potent inhibitors of 1A2 but only moderate inhibitors of 1A1 and 2B1. 4, 4BPhDPE is a moderate inhibitor of 1A1 and 1A2 but a poor inhibitor of 2B1. 2, 2BPhDPE is a moderate inhibitor of all the CYP enzymes tested. 4BPhMPE acts as a potent inhibitor of all the CYP forms tested, but may be a tight binding alternate substrate for 1A1. Experiments continue to determine the mechanism of inhibition as well as the inhibitory parameters.

357 USE OF A HUMAN HEPATOCYTE-DERIVED CELL LINE TO SIMULTANEOUSLY ASSESS CYP3A4 INDUCTION AND TIME-DEPENDENT INHIBITION.

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Cytochrome P4503A4 (CYP3A4) is the major drug-metabolizing enzyme in human liver and can be subject to both induction and inhibition. Changes in CYP3A4 expression and activity are frequently associated with drug-drug interactions. We report the use of a human hepatocyte-derived cell line to simultaneously assess CYP3A4 induction and time-dependent inhibition. Fa2N-4 cells were plated and cultured for two days, then exposed to varying concentrations of test compound in cell culture medium. After 48 hours, cells were washed, then incubated with medium containing a CYP3A4 probe substrate (midazolam) in order to assess catalytic activity. Cells were then harvested, RNA isolated, and CYP3A4 mRNA measured. Replicate sets of plated cells were treated in parallel, and viability of cells assessed using a mitochondrial dehydrogenase activity assay. Treatments that affected cell viability were not used in subsequent mRNA and activity determinations. The prototypical inducers rifampicin (30 uM) and dexamethasone (50 uM) produced 15- and 2-fold increases in CYP3A4 mRNA in these cells after 48 hours of treatment, and 9- and 2-fold increases in enzymatic activity, respectively. In contrast, compounds that were both inducers and time-dependent inhibitors of CYP3A4, produced increases in CYP3A4 mRNA, but decreased CYP3A4 catalytic activity in these cells. For example, ethinyl estradiol (10 uM) and ritonavir (1 uM) each produced approximately 4-fold increases in CYP3A4 mRNA, but reduced midazolam 1-hydroxylase activity relative to control by 75% and 50%, respectively. Since compounds were washed from the cells prior to incubation with probe substrate, the decrease in CYP3A4 catalytic activity was not likely a result of competitive inhibition, but was likely due to time-dependent inhibition. These results indicate that this assay can be used for early identification of CYP3A4 inducers as well as time-dependent inhibitors of CYP3A4 in drug discovery.

358 HUMAN NAPHTHALENE METABOLISM.

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The polycyclic aromatic hydrocarbon naphthalene is an environmental pollutant, a component of jet fuel and since 2000 reclassified as a potential human carcinogen. Few studies of *in vitro* human metabolism of naphthalene are available, and these studies are mostly focused on lung metabolism. Therefore, the current studies were performed to characterize the metabolism of naphthalene mediated by human cytochromes P450 (CYP) using pooled human liver microsomes (pHLM) and CYP isoforms. The major metabolites from naphthalene mediated by pHLM were trans-1, 2-dihydro-1, 2-naphthalenediol (dihydrodiol), 1-naphthol (1-ol), and 2-naphthol (2-ol). Their kinetic parameters showed Km values of 23, 40, and 115 µM, and Vmax values of 2860, 268, and 22 pmol/mg protein/min., respectively. Through human CYP isoform screening of naphthalene metabolism, CYP1A2 was identified as the most efficient isoform among those tested for producing dihydrodiol and 1-ol while CYP3A4 was the most effective for 2-ol production. Only CYP1A2 generated 1, 4-naphthoquinone from naphthalene. Metabolism studies of primary metabolites of naphthalene were also performed in order to identify secondary metabolites. While 2-ol was readily metabolized by pHLM to produce 2, 6- and 1, 7-dihydroxynaphthalene, dihydrodiol and 1-ol were inefficient substrates for pHLM. To further explore the metabolism of dihydrodiol and 1-ol, a series of human CYP isoforms was applied. 1, 4-naphthoquinone and four unknown metabolites from 1-ol were observed, and 1A2 and 2D6*[1] were identified as the most active isoforms for the production of 1, 4-naphthoquinone. Dihydrodiol was metabolized by CYP isoforms to three unidentified metabolites with CYP2A6 having the greatest ability toward this substrate among those tested. The metabolism of dihydrodiol by CYP isoforms was lower than that of 1-ol. These studies identify both primary and secondary metabolites of naphthalene mediated by pHLM and CYP isoforms. The dihydrodiol appears to be a relatively stable biomarker of human exposure to naphthalene.

359 ROLE OF PULMONARY CYTOCHROME P450 3A1 IN 1-NITRONAPHTHALENE BIOACTIVATION AND INJURY IN ADULT AND POSTNATAL RATS.

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Cytochrome P450 (CYP) monooxygenases play an important role in the bioactivation of hazardous air pollutants such as 1-nitronaphthalene (1NN). Despite similar rates of 1NN metabolism in postnatal and adult rat lungs, postnatal rats are more susceptible to 1NN pulmonary injury than adults. The exact isozyme responsible for 1NN metabolism is unknown. The purpose of this study was to determine if CYP3A1, a major constituent of human liver and lung, is involved in the bioactivation of 1NN and if the amount of CYP3A1 explains the difference in susceptibility between postnatal and adult rats. We evaluated airway and liver microsomes for CYP3A1 activity using the substrate Midazolam. Seven-day old rats have lower rates of baseline Midazolam metabolism in lung and liver compared to adults. Ketoconazole, a CYP3A inhibitor, decreased total Midazolam metabolism in lung and liver microsomes from 7-day old and adult rats by at least 50%, *in vitro. In vivo*, ketoconazole inhibited total Midazolam metabolism by 51% in adult rat lungs, 26% in adult liver, 35% in 7-day old lungs, and 55% in 7-day old liver. To determine if ketoconazole prevents 1NN pulmonary injury, rats received an IP injection of carrier or ketoconazole prior to administration of 50mg/kg 1NN. Lungs were evaluated by high resolution light microscopy 24-hrs later. Extensive areas of vacuolated and exfoliated cells and bare basement membrane were detected in the bronchi of adult rats treated with carrier+1NN, whereas only a few vacuolated cells were detected in the bronchi of adult rats treated with ketoconazole+1NN. Ketoconazole did not significantly alter 1NN pulmonary injury in 7-day old rats as observed in adults. We conclude that pulmonary CYP3A1 is involved in the bioactivation of 1NN. We hypothesize the different injury patterns observed between adult and 7-day old rats could potentially be due to inhibition of first pass 1NN metabolism in 7-day rat liver allowing more 1NN to reach the lung. NIH ES06700, USEPA (R827442010) ES 004311 NIH Training Grant HL007013

360 MODIFICATION OF PULMONARY CYP2B1 AND INDUCED CYP1A1 ACTIVITIES BY INTRAVENOUS INJECTION OF IRON DEXTRAN.

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Cytochrome P450s (CYPs) are membrane-bound heme-containing proteins (hemeproteins) that catalyze oxidation of xenobiotics producing less lipophilic substances thereby facilitating their excretion. The heme moiety of this protein is iron-

protoporphyrin IX, which plays the major role in the catalytic activity of the enzyme. We hypothesized that the bioavailability of iron modifies the activity of inducible (CYP1A1) and constitutional (CYP2B1) CYP isozymes in rat lungs. Therefore, male Sprague-Dawley rats were injected i.v. with 50 or 200 mg/kg BW iron as iron dextran. Saline was injected as a control. Eleven days later, rats were injected intraperitoneally with the classic CYP1A1 inducer, beta-naphthoflavone (BNF; 50 mg/kg BW), to induce CYP1A1. Three days after BNF injection, rats were sacrificed and the lungs were perfused by phosphate buffer saline (PBS) through the pulmonary artery to remove blood. Immediately after washing, the microsomes of the right lung lobes were freshly prepared to measure the CYP1A1-dependant enzymatic activity [ethoxyresorufin-*O*-deethylase (EROD)] and the CYP2B1-dependent enzymatic activity [pentoxyresorufin-*O*-deethylase (PROD)]. Before separation of microsomes by differential centrifugation, an equal portion of lung homogenate was freeze dried for measurement of lung iron. The results showed that pulmonary iron concentration was increased in rats receiving 50 mg/kg (310 + 31.45 μg/gm) and 200 mg/kg (796 + 37.8 μg/gm) iron compared to control (180 + 13.33 μg/gm). The activities of PROD and induced EROD were significantly decreased by injection of 200 mg/kg iron. These findings suggest that increasing rat lung iron content modifies xenobiotic metabolism in lung by decreasing the activity of CYP2B1 and inducible CYP1A1.

361 ROLE OF CYP2E1 IN THE OXIDATION OF ACRYLAMIDE (AA) TO GLYCIDAMIDE (GA) AND FORMATION OF DNA AND HEMOGLOBIN ADDUCTS.

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AA is a known animal carcinogen, neurotoxin, and reproductive toxin. AA is formed in baked and fried carbohydrate-rich foods such as french fries. Our earlier studies showed that CYP2E1 is the primary enzyme responsible for AA oxidation to GA. Using CYP2E1-/- (KO) mice, subsequent studies in this laboratory showed that AA metabolism to GA is required for the induction of male germ cell mutagenicity. To assess the role of CYP2E1 in the epoxidation of AA and the formation of GA-DNA adducts as well as AA and GA hemoglobin (HGB) adducts, AA was administered to KO or wild-type (WT) mice at 50 mg/kg IP. Six hours later, mice were euthanized and blood and tissues were collected. Using LC-ES/MS/MS, AA, and GA, as well as DNA and HGB adducts were measured. While the plasma levels of AA and GA were 115.0±14.0 and 1.7±0.31uM in KO mice, respectively, they were 0.84±0.80 and 33.0±6.3uM in the plasma of AA-treated WT mice, respectively. Dosing of AA to WT mice caused a large increase in N7-GA-Gua and N3-GA-Ade adducts in the liver, lung, and testes. Further, while traces of N7-GA-Gua adducts were measured in all three tissues from KO mice treated with AA, the levels were significantly lower than in WT mice (52- to 66-fold). Significant elevation of both AAVal and GAVal adducts was seen in WT mice treated with AA. In AA-treated KO mice, the levels of AAVal were roughly twice as high as those in WT mice. The amount of GAVal in AA-treated KO mice was low but was significantly higher than in vehicle-treated KO mice, and were ≅ 33-fold lower than that found in AA-treated WT mice. In conclusion, these results demonstrated that CYP2E1 is the primary enzyme responsible for the epoxidation of AA to GA, which leads to DNA adduct formation. Oxidative pathways other than CYP2E1 can lead to formation of GA and GA-DNA adducts, but the contribution of these pathways is negligible by comparison.

362 GENE EXPRESSION ALTERATIONS IN IMMUNE SYSTEM PATHWAYS FOLLOWING EXPOSURE TO IMMUNOSUPPRESSIVE CHEMICALS.

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Exposure to xenobiotics can affect a number of adverse immunological outcomes, including infectious disease, hypersensitivity, and autoimmunity. It has been proposed that related disorders may share causative genetic alterations. Diethystilbesterol (DES), Dexamethasone (DEX), Cyclophosphamide (CPS), and 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) are immunosuppressive chemicals that can induce similar pathophysiological endpoints, however the mechanism of toxicity is different for each compound. The primary goals of these studies are to correlate changes in gene expression with alterations in functional immune endpoints, study the mechanisms of action, and evaluate the commonalities among the test compounds. Female B6C3F1 mice were treated with one of the four chemicals daily for five days. The dosages, routes and vehicles selected had been previously optimized for each compound. Splenocyte RNA was analyzed using Illumina Sentrix™ arrays and AnEx software. A dose-dependent trend was evident in differential gene expression for each of the chemicals as compared to controls. DES and TCDD induced the greatest and least number of changes in gene expression, respectively. Many of the differentially expressed genes are known to play a role in apoptosis, host defense, and cell growth, differentiation, and adhesion. The majority of gene alterations were unique to a single compound, however a number of genes were similarly altered across compounds. Alterations common to three different chemicals include upregulation of IL-18, lymphotoxin B receptor, and colony stimulating factor receptor, and downregulation of RANTES and histocompatibility antigens. These findings are consistent with observed alterations in immune function. Genomic analysis revealed several gene expression changes that may be commonly associated with xenobiotic-induced immune system perturbations. However, distinct gene profiles were also found in association with chemicals that target similar immune parameters.

363 THE ROLE OF CYCLIN DEPENDENT KINASE INHIBITOR P21 IN TCDD-INDUCED THYMIC ATROPHY.

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Thymic atrophy is a hallmark of exposure to dioxins, of which 2, 3, 7, 8-tetrachloro dibenzo-p-dioxin (TCDD) is the most potent congener. TCDD-induced thymic atrophy may be regulated by multiple pathways that are triggered following activation of the aryl hydrocarbon receptor (AhR). In the current study, we tested the hypothesis that TCDD exposure results in p21-mediated cell-cycle arrest in the thymus, and that TCDD-induced thymic atrophy results in part from a blockade of thymocyte proliferation. To test our hypothesis, p21 KO mice and age-matched wild-type mice were exposed to 10 g/kg TCDD for 48-72 hrs. We found that TCDD induced G0/G1 cell cycle arrest in thymocytes of wild-type but not in p21 KO mice. In addition, p21 KO mice were resistant to TCDD induced thymic atrophy. We also noted that lack of p21 rendered mice resistant to TCDD-induced thymocyte apoptosis. Finally, we analyzed the effect of TCDD on CD4-/CD8- (DN) thymocyte subsets, defined by expression of CD44 and CD25, which is comprised of immature progenitors that proliferate to become CD4+/CD8+ (DP) thymocytes. TCDD exposure resulted in a blockade of thymocyte maturation at the highly proliferative DN2 stage (CD44+/CD25+), as indicated by the significant increase in the proportion of DN2 thymocytes, and concomitant decrease in DN3 (CD44-/CD25+) thymocytes. In addition, the increase in DN2 thymocytes was accompanied by G0/G1 cell cycle arrest of thymocytes in the DN compartment. However, thymocytes from TCDD-exposed p21 KO mice showed no alteration in the distribution of thymocytes within the DN compartment. Taken together, these results suggest that TCDD exposure results in p21 mediated cell cycle arrest of thymocytes and that the resulting blockade of thymocyte proliferation contributes, at least in part, to apoptosis and thymic atrophy.

364 TCDD SUPPRESSES ANTIGEN-SPECIFIC INTERACTIONS BETWEEN OTII CD4 T CELLS AND OVA-LOADED DENDRITIC CELLS.

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Exposure to the environmental contaminant TCDD (dioxin) causes suppressive effects on the immune system. The mechanism(s) of action at the cellular and molecular levels underlying the potent suppression of T cell-mediated immune responses is not understood. We hypothesize that TCDD exposure impedes activation of CD4 T cells due to a lack of persistent interactions with dendritic cells (DC). To test this hypothesis, C57Bl/6 recipient mice were gavaged with vehicle or TCDD (15 μg/kg), injected iv with OT-II ovalbumin (OVA)-specific naïve T cells, and immunized with OVA-loaded DC. The fate of both the DC and antigen-specific T helper cells in the draining lymph nodes (LN) was then evaluated on multiple days after immunization. On days 3, 4 and 6, TCDD-treated mice had significantly decreased numbers of donor DC, and on day 6 a greater frequency of these cells were PI-positive suggesting their loss was due to increased cell death. Following re-stimulation with OVA, LN cells from TCDD-treated mice produced significantly lower amounts of IL-12. Furthermore, the ability of the OVA-loaded DC to induce clonal expansion of the OT-II CD4 T cells in TCDD-treated mice was significantly suppressed on days 4 and 6 post-immunization when compared to the vehicle-treated controls. OVA-specific T cells in mice treated with dioxin were also less activated based on lower expression of CD11a and CD44, and suppressed production of IL-2 and IFN-γ following *ex vivo* re-stimulation. Additional studies utilizing OVA-loaded DC from AhR wildtype (AhR /) and knockout (AhR-/-) mice demonstrated that the TCDD-induced loss of DC was mediated exclusively via an AhR-dependent process. However, suppression of the OVA-specific CD4 T cell

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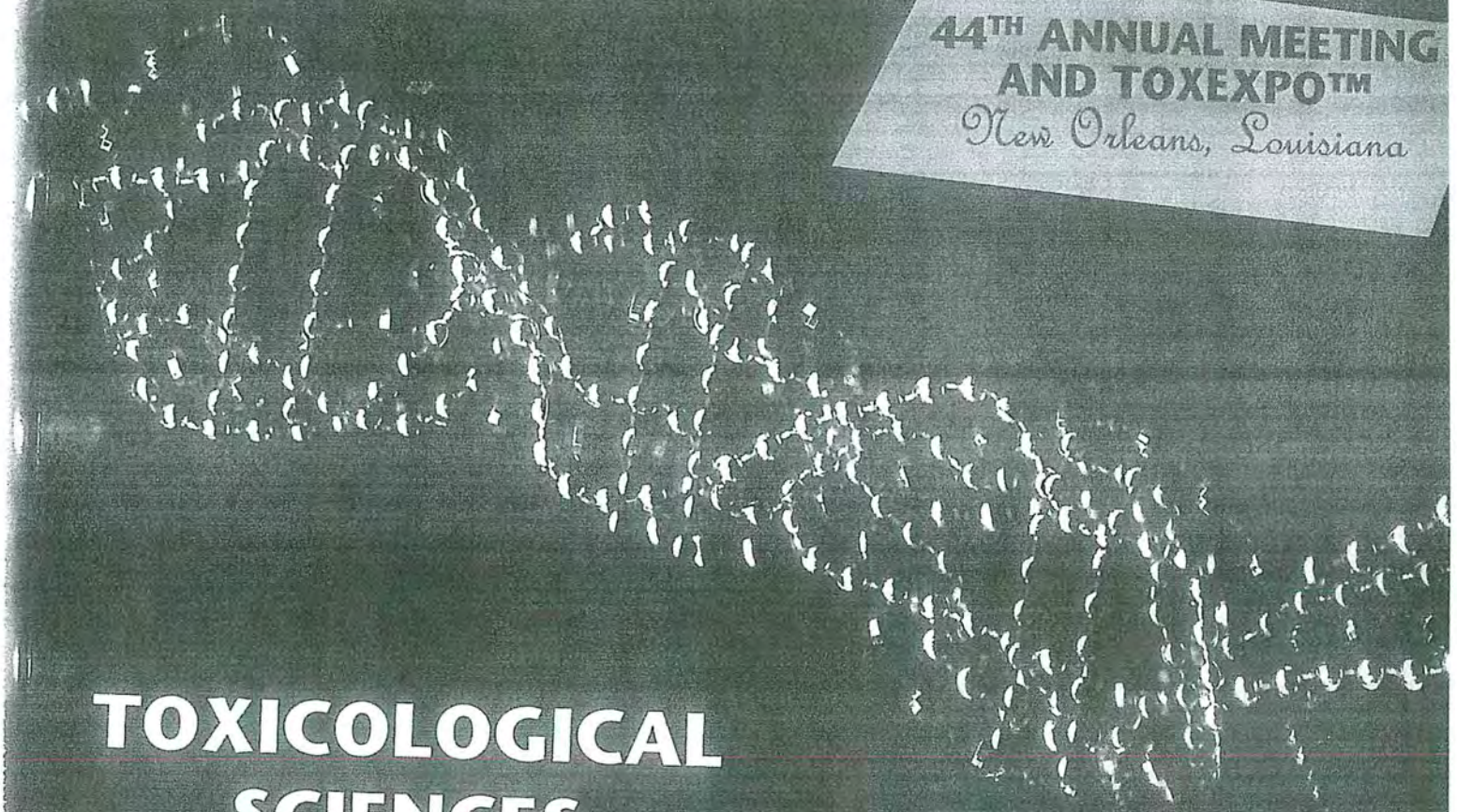

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