tabolizing D4 and PB induction in rats greatly enhances the ability of liver microsomes to metabolize D4 to a variety of metabolites. The use of rCYP450s and inhibitory polyclonal antibodies demonstrated that human CYP2B6 and CYP3A4 are likely involved in the observed metabolism. Supported in part by Silicones Environmental, Health and Safety Council of America

## 88 PRECISION-CUT HUMAN LIVER SLICES AS A MODEL TO EXAMINE CHANGES IN CYTOCHROME P450 EXPRESSION TO PREDICT OR PRECLUDE POTENTIAL DRUG-DRUG INTERACTIONS.

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Precision-cut human liver slices offers a practical alternative to hepatocyte cell cultures to examine cytochrome P450 (CYP) induction by compounds to predict or preclude potential drug-drug interactions. Induction of P450 gene expression was measured in human liver slices by real-time PCR using probes/primers developed to specifically recognize CYP1A1, CYP1A2, CYP3A4, CYP2C8, CYP2C9, or CYP2C18 mRNA. Liver slice viability was monitored by measurement of ATP, GSH, and $K^+$ content. Minimal loss in slice viability occurred between 24 and 48 h (1.2- and 1.7-fold decreases in $K^+$ and ATP content, respectively), whereas, more significant losses of viability occurred by 72 h. CYP1A1 and CYP1A2 gene expression was induced by β-napthoflavone (50 μM), with respect to vehicle control, 99- and 110-fold at 24 h of treatment and 19- and 55-fold at 48 h, respectively, which indicated that maximal induction occurred at or prior to 24 h. Inducers of CYP3A4, carbamazepine (CARB, 50 μM), rifampicin (RIF, 50 μM), phenobarbital (PB, 0.5 mM), and dexamethasone (DEX, 50 μM) induced CYP3A4 gene expression 2.4-, 4.6-, 7.6- and 3.6-fold at 24 h, and 2.9-, 7.1-, 18-, and 10-fold at 48 h, respectively. Known inducers of CYP2C enzymes, RIF, PB, and DEX increased CYP2C9 mRNA expression by 1.9-, 3.1-, and 2.3-fold at 24 h and by 1.9-, 3.7-, and 3.9-fold at 48 h, respectively. RIF, PB, and DEX induced CYP2C8 mRNA expression by 6.0-, 7.0-, and 7.4-fold at 24 h and 2.7-, 5.7-, and 4.6-fold at 48 h, respectively. DEX induced CYP2C18 mRNA by 48 h (2.4-fold), whereas PB and RIF had little effect. The levels of induction of CYP2C and CYP3A4 mRNA were similar to induction levels found recently in human hepatocytes. These studies indicated that induction of P450 gene expression can be measured in human liver slices with a single treatment of inducer within 24 h of slice culturing and up to 48 h with minimal reduction in slice viability.

## 89 DEVELOPING AN *IN VITRO* MODEL SYSTEM TO EVALUATE SWITCH–LIKE BEHAVIORS OF HEPATOCYTES IN RESPONSE TO VARIOUS ENZYME INDUCERS.

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Cytochrome P450 induction in liver by a variety of enzyme inducing chemicals demonstrates switch-like behaviors. As dose increases, more cells become fully induced rather than observing a proportionate induction in all cells in the liver. These inducers interact *via* different receptor systems, indicating that this switch-like response is a generic property of induction with these cells. The molecular mechanisms that control these switches remain unknown. We have developed an *in vitro* model system using cultured rat hepatocytes to study this phenomenon with prototypical inducers. In this study we examined induction of CYP 1A1 protein and CYP 1A1 mRNA by PCB 126, a prototypical aryl hydrocarbon receptor (AhR) agonist. Hepatocytes from male Sprague-Dawley rats were treated for 24 hours after attachment on plates at PCB126 concentrations ranging from $10^{-5}$ to $10^{-10}$ M. Total CYP1A1 protein was evaluated in cell homogenates by densitometry of Western blots and for CYP1A1 mRNA by quantitative PCR. The EC50s for induction of both protein and mRNA were in the range of 2 to 5 nM. These integrated responses of populations of hepatocytes were compared with responses of individual cells by visualization of treated cells on plates. Protein was visualized by CYP 1A1 immunohistochemistry. While induction of individual cells and the Dose-Response for increasing numbers of cells stained with increasing dose was evident by immunohistochemistry, further refinements of imaging techniques are necessary to derive quantitative estimates of numbers of cells induced at each dose level. Nonetheless, the combined evaluation of population and cell level responses of hepatocytes offered by this *in vitro* model permits characterization of the induction switch and provides a system to probe the molecular mechanisms that underlie non-linear dose-response behaviors for these receptor-mediated toxicants. This work was supported by a contract from the American Chemistry Council.

## 90 EVALUATION OF AFLATOXIN B1 AND OTHER CHEMICALS IN GENTEST© CELL LINES: CYP-MEDIATED TOXICITY IS DIMINISHED BY CO-TREATMENT WITH SPECIFIC INHIBITORS.

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Many xenobiotics are more toxic when metabolized by cytochrome P450 enzymes (CYPs). The intent of this study was to test such chemicals in Gentest© cell lines, human lymphoblasts that have undergone stable transfections resulting in expression of human CYPs. Aflatoxin B1 (AB1) was tested in the cell lines h1A2v2, h3A4/OR, h2E1/OR, 2C9-Arg/OR, 2D6-Val, MCL-5 (CYP1A2, 3A4, 2E1, 2A6, and elevated native 1A1), and the control lines cHol and cHyg (no CYP over-expression) for 72 hours alone and concurrently with inhibitors. Inhibitors used were as follows: 5 μM furafylline (1A2), 10 μM ketoconazole (3A4), 10 μM sulfaphenazole (2C9), 10 μM 4-methylpyrazole (2E1), 10 μM quinidine (2D6), and 15 μM aminobenzotriazole (ABT, all). Furafylline inhibited CYP-mediated AB1 toxicity completely in the h1A2v2 cells but not in the MCL-5 cells. Ketoconazole inhibited AB1 toxicity completely in the h3A4/OR cells but not in the MCL-5 cells. Co-treatment with ABT in the MCL-5, h1A2v2, and h3A4/OR cell lines did not inhibit toxicity. AB1 did not demonstrate toxicity in the other cell lines. Aflatoxin B2, B2a, and G2a were not toxic to any of the cell lines at the concentrations tested. Aflatoxin G1 (AG1) and Aflatoxin G2 (AG2) displayed CYP-mediated toxicity similar to that of AB1. Aflatoxin M1 (AM1) was toxic only to the h1A2v2 and MCL-5 cell lines when tested up to 0.5 μM, while Aflatoxin M2 did not exhibit toxicity in any of the cell lines when tested up to 0.25 μM. Colchicine, which is directly toxic, was used as a positive control in these assays. In conclusion, based on results with these cell lines, AB1, AG1, and AG2 toxicities are CYP-generated, and are mediated principally by CYP1A2 and to a lesser extent by CYP3A4.

## 91 COAL DUST IS A MODIFIER OF PULMONARY CYP1A1 INDUCTION IN RATS.

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Cytochrome P4501A1 (CYP1A1) plays an important role in pulmonary carcinogenesis. Pulmonary CYP1A1 metabolizes inhaled exogenous agents, such as cigarette smoke-associated polycyclic aromatic hydrocarbons, into potent carcinogens. We hypothesized that intrapulmonary deposition of particulate might modify susceptibility to inhaled carcinogens by modifying induction and activity of pulmonary CYP1A1. To test this hypothesis, male Sprague-Dawley rats were intratracheally instilled with 0, 2.5, or 10 mg of respirable coal dust or iron oxide. Saline was instilled as a control. Eleven days later, they were treated with the CYP1A1 inducer β-naphthoflavone (NF; 50 mg/kg IP) or vehicle (corn oil). Three days after that, they were sacrificed and the level of functional CYP1A1 in the lungs was measured as ethoxyresorufin-*O*-deethylase (EROD) activity. Lung CYP1A1 protein induction and localization of CYP1A1 protein to alveolar type II cells was assessed by dual immunofluorescence staining for CYP1A1 and cytokeratin 8 (a cytoskeletal protein expressed in type II cells). We found that pulmonary EROD activity was significantly greater in coal dust/NF and iron oxide/NF rats than in saline/NF rats. Increasing coal dust lung burden accentuated the enhancement of EROD induction while increasing iron oxide lung burden diminished the enhancement seen at the lower lung burden. Immunofluorescence demonstrated that within the alveolus, a smaller proportion of CYP1A1 staining was associated with alveolar type II cells in coal dust/NF or iron oxide/NF rats than in saline/NF rats. These findings demonstrate that pulmonary exposure to coal dust modifies the inducibility and localization of CYP1A1 in the lung. These results suggest that respirable coal dust is not a simple co-variable in mixed exposures but a complex modifier of CYP1A1 induction that alters CYP1A1 activity and localization in the lung.

## 92 INDUCTION OF CYTOCHROMES P450 1A1 AND 1B1 BY MOTORCYCLE EXHAUST PARTICULATE IN HUMAN BREAST CANCER MCF-7 CELLS.

T. H. Ueng, H. W. Wang and F. W. Chen. *Institute of Toxicology, College of Medicine, National Taiwan University, Taipei, Taiwan.*

The effects of motorcycle exhaust particulate (MEP) on cytochrome P450 (P450)-dependent monooxygenases were determined using MCF-7 human breast cancer cells treated with organic extracts of MEP. Treatment with MEP extract caused concentration- and time-dependent increases of monooxygenase activity in S9 fractions. Treatment with 50 μg/ml MEP extract for 24 h increased BENZO(A)PYRENE hydroxylase and 7-ethoxycoumarin, 7-ethoxyresorufin and methoxyresorufin O-dealkylases activities in S9. Treatment with 1 or 10 μg/ml MEP extract for 24 h markedly enhanced catabolism of 17 β-estradiol in MCF-7

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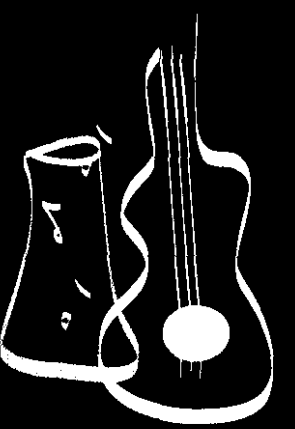

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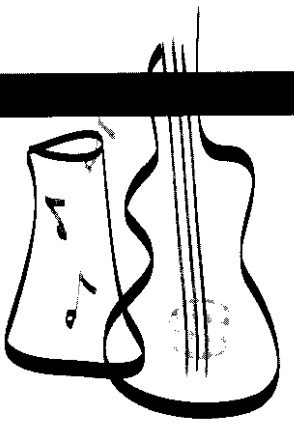

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# *Preface*

**This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, roundtable, and poster sessions of the 41st Annual Meeting of the Society of Toxicology, held at the Opryland Hotel and Convention Center, Nashville, Tennessee, March 17–21, 2002.**

**An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 385.**

**The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 411.**

**The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.**

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