a CYP3A inhibitor, had no inhibitory effect on progesterone 6β-hydroxylase (PROH) activity catalyzed by P450 LMC5, a CYP3A-like enzyme in trout. Gestodene, another CYP3A inhibitor, strongly inhibited PR-OH activity and, unexpectedly, ethoxyresorufin-O-deethylase (EROD) activity. 17α-Ethynyl-estradiol and 5, 8, 11, 14-eicosatetraynoic acid also were highly inhibitory toward PR-OH. Ketoconazole and miconazole were potent inhibitors of all the measured P450 activities. However, none of the above enzyme activities was selectively inhibited by any of the chemicals used. (Supported by NIH Grants ES00210, ES03850, ES04766 and ES05533).

1412 EFFECT OF XENOESTROGEN EXPOSURE ON THE EXPRESSION OF CYTOCHROME P450 ISOFORMS IN RAINBOW TROUT LIVER

S Intharapanith[1], C L Miranda[2], M C Henderson[2] and D R Buhler[1,2,3]. [1]*Toxicology Program,* [2]*Department of Agricultural Chemistry and* [3]*Marine/ Freshwater Biomedical Sciences Center, Oregon State University, Corvallis, OR*

Injection of juvenile rainbow trout with 17β-estradiol represses expression of several cytochrome P450s and reduces hepatic lauric acid hydroxylase (LA-OH) activity. However, there is no information about the role of environmental estrogens such as pesticides, phytoestrogens and surfactants in the regulation of trout P450s. In this study, therefore, four putative estrogenic chemicals: methoxychlor (20mg/kg), diethylstilbestrol (15 mg/kg), 4-(*tert*-octyl) phenol (25 and 50 mg/kg) and biochanin A (25 and 50 mg/kg) were injected (ip) on days 1, 4 and 7 into one-year-old juvenile trout and the fish then sacrificed on day 9. Plasma vitellogenin was used as a biomarker for estrogenicity and all of the test chemicals caused increases in plasma vitellogenin levels. The xenoestrogens all decreased LA-OH activity and, as shown by Western blots, also markedly repressed expression of hepatic CYP2K1, CYP2M1 and P450 LMC5. However, both doses of biochanin A also induced CYP1A levels and greatly increased hepatic EROD activity. Northern blot results indicated that CYP2K1 mRNA also was repressed in the four trout groups. These findings suggest that methoxychlor, diethylstilbestrol, 4-(*tert*-octyl)phenol and biochanin A are all estrogenic and act like 17β-estradiol in repressing the expression of several P450 isoforms in the trout liver. Biochanin A, however, also was found to induce CYP1A1 in this fish species. (Supported by NIH Grants No. ES000201, ES03850, ES04766, and ES05533).

1413 HETEROLYTIC O-O BOND CLEAVAGE OF HYDROPEROXIDES BY MUTANT CYTOCHROME P450 2F3 EXPRESSED IN E. COLI

H Wang[1], J A Thompson[2], and G S Yost[1]. [1]*Department of Pharmacology, University of Utah, Salt Lake City, UT: and* [2]*Department of Pharmaceutical Science, University of Colorado, Denver, CO*

Cytochrome P450 *2F3* cDNA containing 1473 bp in the transcribing region and encoding a polypeptide of 491 amino acids was cloned from a goat lung cDNA library. The predicted amino acid sequence of 2F3 possessed 82% similarity to human CYP2F1 and mouse Cyp2f-2. Northern blot analysis with *2F3* of goat lung and liver mRNA revealed that a 2.0 kb transcript was detected in a highly tissue-specific manner only in lung but not in liver. This cDNA clone was mutated within the first nine codons, which favors expression in *E. coli*, and placed in pTrc 99A expression vector and then used to transform *E. coli* XL1-Blue cells. The expressed P450 content was 20-91 nmol per liter of culture and the protein was characterized by western immunoblotting using a rabbit anti-mouse 2f-2 polyclonal antibody. Cytochrome P450s have been shown to catalyze the cleavage of organic hydroperoxides by both heterolytic and homolytic processes, and this can be used to characterize the active site of the enzymes. Expressed CYP2F3 was incubated with 2,6-di-*tert*-butyl-4-hydroperoxy-4-methyl-2,5-cyclohexadienone (BHTOOH) or 4-hydroperoxy-2,4,6-trimethyl-2,5-cyclohexadienone (TMPOOH). Ratios of heterolysis to homolysis were determined by HPLC analysis. The expressed enzyme primarily catalyzed the heterolysis of TMPOOH to produce an epoxide, demonstrating unique catalytic preference for epoxidation rather than hydroxylation. However, with the bulkier hydroperoxide, BHTOOH, 2F3-catalyzed homolysis was a principle pathway. Therefore, expressed CYP2F3 is an active enzyme that predominately catalyzes the heterolytic cleavage of TMPOOH to form the epoxide product. (Supported by USPHS grants HL13645 from NHLBI and CA41248 from NCI).

1414 REGULATORY CONTROL OF CYTOCHROME P450 1B1 IN MOUSE EMBRYO FIBROBLASTS

K. Bhattacharyya, L. Zhang, U. Savas and Colin Jefcoate. *Environmental Toxicology Center and Department of Pharmacology, University of Wisconsin - Madison, Madison, WI*

Cytochrome P450 1B1 (CYP1B1) exhibits 40% sequence identity to CYP1A1 but greater differences in mRNA and genomic characteristics. CYP1B1 forms an exceptionally long 5.2 kb mRNA which is transcribed from a very compact gene that has only two introns. CYP1B1 and CYP1A1 are both regulated by the Ah receptor, and are active in the metabolism of polycyclic aromatic hydrocarbons, but with large differences in product formation. CYP1B1 expression is however enhanced by cAMP in steroidogenic tissues and is selective for stromal fibroblasts relative to epithelial cells. Upstream regions of the mouse and rat CYP1B1 genes have been sequenced and various segments have been linked to luciferase expression vector (CYP1B1-LUC). These genes do not have a TATA box but show SP-1 elements in a region immediately upstream of positions reached by primer extension of CYP1B1 mRNA. Several XRE sequences have been identified in this upstream region and further sequence analysis is in progress. Transient transfections of CYP1B1-LUC (exon 1 + 0.9kb. 5' flanking region) into mouse C3H10T1/2 embryo fibroblasts or mouse HEPA 1 hepatoma cells each showed basal expression of luciferase and 8-fold stimulation by TCDD, thus failing to reflect the more than 100-fold preference of CYP1B1 for 10T1/2 cells. An analogous CYP1A1 construct showed a 10-fold preference for TCDD-induction in HEPA cells. While this parallels the selectivity for CYP1A1 expression AhR levels are also far higher in HEPA cells. We conclude that these transiently transfected constructs poorly reproduce cell-selectivity of CYP1B1/ CYP1A1 expression. The effect of full genomic incorporation of CYP1B1-LUC sequences will be reported. Funded by NIH grant CA16265.

1415 CHARACTERIZATION OF CYTOCHROME P450, FORM-SPECIFIC ACTIVITIES AND EXPRESSION IN MALE AND FEMALE ob/ob MICE

IG Howard, ¶A L. Roe, and §J E. Snawder. ¶*Graduate Center for Toxicology and* I*College of Pharmacy, University of Kentucky, Lexington, KY.* §*National Institute for Occupational Safety and Health, Cincinnati, OH*

The cytochrome P450-dependent monooxygenase system is responsible for the majority of oxidation reactions of drugs and other xenobiotics. Variations in these biotransformation activities may alter the bioavailability or efficacy of drugs, provide protection from certain xenobiotic agents, or increase toxicity of others. A mutation in the obese gene in the C57BL/6J mouse results in the mutant obese mouse (*ob/ob*), which displays several altered physiological conditions. The present research reports on the hepatic microsomal cytochrome P450-dependent activities, glutathione transferase activity, and expression in liver of selected P450 mRNAs in the *ob/ob* mouse at 3–4 months (young) and 7–8 months (old) of age. CYP2E1- and CYP3A-dependent activities were measured by p-nitrophenol hydroxylase and erythromycin demethylase, respectively. Activities for CYP1A1/1A2, CYP3A and CYP2B were estimated by determining O-deakylation of ethoxy-, benzoxy- and pentoxy-resorufin, respectively. Female mice, regardless of age, showed an obesity enhanced level of CYP2E1-dependent activity and protein as shown by Western blots. The mRNA level of CYP2E1 in female mice did exhibit age- and obesity-influenced differences in expression. No significant differences in CYP2E1-dependent activity or expression were observed in male mice. CYP3A-dependent activity was significantly higher in young males, regardless of phenotype. Benzoxy-and pentoxy-resorufin dealkylase activities did not differ among any animals; however, ethoxy-resorufin dealkylase activity, while depressed in obese animals of both sexes, were significantly different in old animals. Glutathione transferase activity was lowered in obese male mice, whereas no difference was reported between lean and obese females. **(ALR supported by NIEHS Training Grant 5 T32ES07266).**

1416 REGULATION OF CYTOCHROME P450 (CYP) EXPRESSION BY KETONE BODIES IN PRIMARY CULTURED RAT HEPATOCYTES

R C Zangar and R F Novak. *Institute of Chemical Toxicology, Wayne State University, Detroit, MI*

Fasting, diabetes and high-fat diet have been shown to increase expression of CYP2E1, CYP2B and CYP4A, and these changes have been correlated with increased levels of circulating ketone bodies. We examined the effects of physiological levels of ketone bodies on P450 mRNA levels in primary

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshops, roundtables, and poster sessions of the 35th Annual Meeting of the Society of Toxicology, held at the Anaheim Convention Center, Anaheim, California, March 10-14, 1996.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 351.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 375.

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