1691 DECREASED CYP1A1-DEPENDENT ENZYME ACTIVITY AND PROTEIN LEVELS IN HEPG2 CELLS EXPOSED TO BENZO(A)PYRENE IN THE PRESENCE OF 1-NITROPYRENE.

S. Cherng[1, 2], S. Hsu[3], J. Yang[4] and H. Lee[1]. [1]*Institute of Toxicology, Chung Shan Medical University, Taichung, Taiwan,* [2]*Food Science and Nutrition, Hung Kuang University, Taichung, Taiwan,* [3]*Department of Education & Research, Taichung Veterans General Hospital, Taichung, Taiwan and* [4]*Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan.* Sponsor: P. Howard.

The genotoxicity of polycyclic aromatic hydrocarbons (PAHs) and nitrated PAHs may be influenced by interaction of the compounds at the level of enzyme activity. Limited information was available concerning the effect of 1-nitropyrene (1-NP) on benzo[a]pyrene (BaP)-induced genotoxicity in mammalian cells. In this study, the human hepatoma cell line HepG2 was simultaneously treated with 1-NP and BaP to investigate the effects of chemical interaction on the genotoxicity of this binary mixture. The BaP-DNA adduct levels, evaluated by 32P-postlabeling, decreased in the presence of added 1-NP in a dose-dependent manner. Our results showed that the inhibition of BaP-DNA adduct formation by 1-NP was due to decreased cytochrome P450 1A1 (CYP1A1)-linked aryl hydrocarbon hydroxylase activity. Furthermore, northern blot analysis showed that 1-NP slightly attenuated BaP-induced CYP1A1 mRNA expression, but western blot analysis revealed a large decrease in the level of CYP1A1 protein. Analysis of *in vitro* proteolysis demonstrated that the addition of 1-NP enhanced CYP1A1 protein degradation. The proteolysis of CYP1A1 protein was inhibited by the addition of an antioxidant, dithiothreitol. These findings suggested that a posttranslational mechanism is involved in loss of CYP1A1 protein, causing the decrease of BaP-DNA adduct levels in the presence of binary mixtures of 1-NP and BaP.

1692 ROLE OF MOUSE CYP2E1 IN THE O-HYDROXYLATION OF P-NITROPHENOL: COMPARISON OF ACTIVITIES IN *CYP2E1* (-/-) AND WILDTYPE MICE.

K. K. Wolf[1], S. G. Wood[3], J. L. Bement[3], P. R. Sinclair[3, 2, 1], S. A. Wrighton[4], E. Jeffery[5], F. J. Gonzalez[6] and J. F. Sinclair[3, 2, 1]. [1]*Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH,* [2]*Biochemistry, Dartmouth Medical School, Hanover, NH,* [3]*VA Medical Center, White River Junction, VT,* [4]*Lilly Research Laboratories, Indianapolis, IN,* [5]*Food Science and Human Nutrition, University of Illinois, Urbana, IL and* [6]*Laboratory of Drug Metabolism/ Disposition, National Cancer Institute, Bethesda, MD.*

Selective enzymatic activities are used to identify the role of an individual form of CYP in a particular biotransformation. p-Nitrophenol O-hydroxylation (PNPH) has been widely used as a measure of CYP2E1. However, rat and human forms of CYP3A have also been shown to catalyze this activity. In mice, the contribution of CYP3As and CYP2E1 to PNPH is not known. Here we compared hepatic microsomes from *Cyp2e1* (-/-) and wild type mice to investigate the contribution of constitutive and alcohol-induced levels of murine CYP2E1 and CYP3A to PNPH. In untreated mice, hepatic levels of PNPH were much greater in wildtype mice compared to *Cyp2e1* (-/-) mice, suggesting a major role of CYP2E1 in catalyzing PNPH. Hepatic levels of PNPH were not significantly different between males and females, even though females have dramatically higher levels of CYP3A. Treatment with ethanol in combination with isopentanol resulted in induction of CYP3A proteins in wildtype and knockout mice, and CYP2E1 protein in wildtype mice. The alcohol treatment increased PNPH in hepatic microsomes from wildtype mice but not from knockout mice. Our findings suggest that constitutive and alcohol-induced forms of mouse CYP3A have little to no role in PNPH. Therefore, in untreated and alcohol-treated mice, PNPH can be used as an unambiguous assay for CYP2E1.

1693 STUDY OF METABOLIC INTERACTIONS OF FIPRONIL AND SOME CYP3A4 SUBSTRATES.

J. Tang, A. Usmani, E. Hodgson and R. L. Rose. *Environmental and Molecular Toxicology, North Carolina State University, Raleigh, NC.*

Fipronil, a highly active and broad spectrum insecticide from the phenyl pyrazole family, is a potent disruptor of the ?-aminobutyric acid (GABA)-gated chloride channel in the insect central nervous system. It owes its insect selectivity to the fact that it is less potent in binding the GABA receptor in mammals. Since its discovery about a decade ago, fipronil has been used against a variety of agricultural, domestic and veterinary insect pests. Despite its wide range of use, however, mammalian metabolism of fipronil has not been extensively investigated. The human metabolism of fipronil has been studied in our laboratory using human liver microsomes (HLM) and cytochrome P450 (CYP) isoforms. CYP3A4 has been identified as the major enzyme catalyzing fipronil metabolism in human. Because of broad substrate specificity of CYP3A4, fipronil may interact with many other CYP3A4 substrates. This study investigated metabolic interactions of fipronil with testosterone or diazepam, two other CYP3A4 substrates. Fipronil was incubated with testosterone or diazepam in HLM and metabolites were analyzed using HPLC. When 1.25, 5 or 20 ?M fipronil incubated with a serial concentrations of testosterone (0-200 ?M), activation of fipronil S-oxidation was observed using HLM as the enzyme source. Inhibition of testosterone 6?-hydroxylation was observed in HLM when incubating testosterone (4, 20, or 100 ?M) with fipronil (0-160 ?M). There were no significant effects of diazepam (0-400 ?M) on metabolism of fipronil (5, 20 or 80 ?M). However, when 6.25, 25 or 100 ?M concentrations of diazepam were incubated with fipronil (0-160 ?M), diazepam hydroxylation was not significantly affected although demethylation of diazepam was inhibited. These results demonstrate that fipronil has the potential to interact with other CYP3A4 substrates (Supported by NIOSH Grant OH07551-ECU).

1694 N-DEALKYLATION OF N-ETHYL-N-(2-HYDROXYETHYL)PERFLUOROOCTANESULFONAMIDE (N-ETFOSE) BY RAT LIVER MICROSOMES AND BY EXPRESSED RAT AND HUMAN CYTOCHROMES P450 (CYPS).

L. Xu[1], A. S. Seacat[2], J. L. Butenhoff[2] and M. W. Anders[1]. [1]*Pharmacology and Physiology, University of Rochester, Rochester, NY and* [2]*3M Co., St. Paul, MN.*

N-EtFOSE is a N-substituted perfluorooctanesulfonamide that is degraded to PFOS. Male rat hepatic microsomal fractions catalyze the NADPH-dependent dealkylation of N-EtFOSE to N-(2-hydroxyethyl)perfluorooctanesulfonamide (FOSE alcohol), which is dealkylated to perfluorooctanesulfonamide (FOSA). The nonselective CYP inhibitors 1-octylamine and 1-benzylimidazole and the NADPH-cytochrome P450 reductase inhibitor diphenyliodium chloride blocked the biotransformation of N-EtFOSE to FOSE alcohol, indicating a role for the CYPs. The objective of this study was to identify the major rat and human CYPs that catalyze the N-dealkylation of N-EtFOSE. Inhibition of N-dealkylase activity by several CYP isoform-selective inhibitors was first examined in rat hepatic microsomal fractions. The N-deethylation of N-EtFOSE was inhibited 90% by disulfiram (CYP2E1), 70% by furafylline (CYP1A2), orphenadrine and diphenhydramine (CYP2B1), and cimetidine (CYP2C11), and 55% by clotrimazole (rat CYP3A2). Studies with expressed rat CYPs showed that CYP3A2, CYP2C11, and CYP2B1 catalyzed the N-deethylation of N-EtFOSE at rates of 15.6, 14.7, and 6.6 pmol/min/nmol P450, respectively. Studies with expressed human CYPs showed that hCYP2C19, hCYP3A4, and hCYP3A5 catalyzed the N-deethylation of N-EtFOSE at rates of 267, 23.6, and 204 pmol/min/nmol P450, respectively. Incubation of FOSE alcohol with the same series of expressed rat and human CYPs showed that rat CYP2C11 and hCYP2C19 catalyzed the N-dealkylation of FOSE alcohol at rates of 0.69 and 1.55 nmol/min/nmol P450, respectively. Rat CYP3A2, CYP1A2, and CYP2B1 catalyzed the N-dealkylation of FOSE alcohol at rates of 98, 39, and 30 pmol/min/nmol P450, respectively. These results establish a role for rat CYP3A2, CYP2C11, and CYP2B1 and human hCYP2C19 hCYP3A4/5 in the biotransformation of N-EtFOSE and FOSE alcohol. (Supported in part by the 3M Co.)

1695 COMPARATIVE HEPATIC MICROSOMAL ENZYME STUDIES IN COMMERCIALLY RAISED GAMEBIRDS.

K. A. Cortright and A. L. Craigmill. *Environmental Toxicology, University of California-Davis, Davis, CA.*

Species differences in drug metabolism can affect therapeutic efficacy and residues in food animals. For example, Webb et al. (2000) has shown that it is safe to treat bobwhite quail with fenbendazole but the therapeutic index in this species is much lower than for other gamebird species. Using hepatic microsomes, we have characterized the kinetics of hepatic cytochrome P450 (CYP)1A and 3A-like activities in chicken, pheasant and quail, using the fluorescent substrates ethoxyresorufin and 7-benzyloxy-(trifluoromethyl)-coumarin (BFC). Inhibition studies were also conducted with prototypical inhibitors of CYP1A2, furafylline, and CYP3A, ketoconazole and erythromycin. Pheasant had the highest ethoxyresorufin-O-deethylase (EROD) activity compared to chicken and quail, 418 versus 46.8 and 15.08 pmoles product/mg x min (quail pooled), respectively. The calculated IC50's for furafylline toward EROD activity were 39uM, 40uM and >100uM for bobwhite quail, chicken, and pheasant, respectively. Kinetic parameters for oxidation of BFC (a CYP3A-selective substrate) were estimated. The apparent Vmax in chicken, pheasant and bobwhite quail was 1628, 7915, and 28347 pmoles product/mg x min, respectively. The apparent Km was 5.0, 4.2 and 34.3uM in chicken, pheasant and bobwhite quail, respectively. The IC50's for ketoconazole inhibition of BFC (20uM) metabolism were 98uM, 100uM and >400uM for chicken, bobwhite quail and pheasant, respectively. Chicken and quail were similar to rats and sheep in their sensitivity to ketoconazole inhibition (IC50's 130 and 106uM, respectively), while pheasant was again much less sensitive. Erythromycin showed very minor inhibi-

Society of Toxicology
43rd Annual Meeting
Baltimore, Maryland

THE TOXICOLOGIST

a supplement to
TOXICOLOGICAL SCIENCES

An Official Journal of the Society of Toxicology

Volume 78, Number S-1, March 2004

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, workshop, roundtable, platform and poster sessions of the 43rd Annual Meeting of the Society of Toxicology, held at the Baltimore Convention Center, Baltimore, Maryland, March 21-25, 2004.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 443.

The document also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 473.

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

Copies of *Program* and CD-ROM with the Itinerary Planner are available at $45 each plus $5 postage and handling (U.S. Funds) from:

Society of Toxicology
1821 Michael Faraday Drive, Suite 300
Reston, VA 20190

http://www.toxicology.org