THE INTERACTION OF THE REFERENCE HEPATOTOXINS CARBON TETRACHLORIDE AND ALLYL FORMATE WITH BETA NAPHTHOFLAVONE MEDIATED P-450 INDUCTION IN THE WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS).

Kevin M. Kleinow, Brad F. Droy, Don R. Buhler and David E. Williams
Dept. of Veterinary Pharmacology, School of Veterinary Medicine, Louisiana State University, Baton Rouge; Dept. of Pharmacology, West Virginia Medical Center, Morgantown; and Marine/Freshwater Biomedical Sciences Center, Oregon State University, Corvallis.

Cytochrome P-450 isozymes are involved in the biotransformation of many xenobiotic chemicals in mammals and fish. These enzymes are inducible by a wide variety of environmental contaminants. Numerous studies have demonstrated induction of P-450 dependent monooxygenase (MO) activity in fish with environmental exposure to low levels of contaminants (Payne, et al., Comp. Biochem. Physiol. 86C:233,1987). Recently, the induction of hepatic P_1-450 like" dependent MO activity in fish by polycyclic aromatic hydrocarbon (PAH) type agents has received attention as a biological monitoring tool. The apparent utility of this method lies with the identification and delineation of aquatic environments impacted by pollutants. Exposure of fish to polluted waters, however, not only presents inducing agents to the fish but, in certain areas hepatotoxic agents (Bellar, et al., J. Am. Water Works Assoc. 6:703, 1974). Ahokas, et al. (Bull, Envir. Contam. Toxicol. 16:270, 1976) demonstrated in pike a decline in MO activity relative to the controls with exposure to polluted water. These results were indirectly attributed to the presence of waterborne hepatotoxins. The objectives of this study were (1) to determine if the co-exposure of a hepatotoxin with a MO inducer would attenuate the development of an inductive response and 2) to examine the effect of isozyme specific induction upon the toxicity of select hepatotoxins in the flounder.

Male and female winter flounder, Pseudopleuronectes americanus (160-460 g) were administered inducer and/or hepatotoxin alone or in combination to provide 4 treatment groups of 3 animals each 1) control; 2) inducer; 3) hepatotoxin; and 4) inducer/hepatotoxin in combination. The inducer β-naphthoflavone (BNF) was administered to appropriate groups at 100 mg/kg by IP injection. Carbon tetrachloride (CCl_4) (1 ml/kg) and allyl formate (AF) (75 µg/kg) administered in separate experiments via gavage were utilized as representative mammalian centrilobular and periportal hepatotoxins. The BNF, CCl_4 and AF were administered to appropriate treatment groups 5, 1 and 2 days prior to sample collection respectively.

Hepatotoxicity was evaluated enzymatically and histologically. Blood was collected via the cardinal sinus and serum utilized for SGOT and SGPT determinations. Hepatic tissues were provided for hematoxylin/eosin histological assessment and preparation of hepatic microsomes (Elcombe and Lech, Toxicol. Appl. Pharmacol. 49:437,1979). Following microsomal protein determination 10 µg of microsomal protein for each animal was subjected to SDS polyacrylamide gel electrophoresis with a 3% stacking and 10% resolving gel (Lammeli, Nature 227:680, 1970). The proteins were subsequently transblotted to nitrocellulose and immunologically stained with rabbit polyclonal antibodies against the trout P-450 isozymes LM_4 (BNF inducable form) and LM_2 (constitutive form). The blots were then counter stained with a goat antirabbit

IgG gold conjugate and enhanced with a silver stain. Quantification was accomplished by densitometry.

Carbon tetrachloride when administered alone resulted in a significant decline in LM_4 content relative to the controls. The LM_4 content was increased in both BNF and BNF/CCl_4 treatments. These treatments were not significantly different from each other indicating that BNF was protective against CCl_4 mediated reductions in LM_4 content. Similar correlations were observed with the LM_2 isozyme content.

The SGOT levels demonstrated a 3.8 fold increase with CCl_4 administration as compared to the controls and a 8 fold increase compared to the BNF treatment (Fig 1a). SGOT values near the control levels were evident with BNF/CCL_4 co-administration. SGPT levels following CCl_4 administration reflected 5.5 and 6.9 fold increases over control and BNF treatments respectively (Fig 1b). The BNF/CCl_4 administration resulted in a 30% reduction in SGPT from that demonstrated with CCl_4 alone. The AF and AF/BNF treatments results in SGOT and SGPT levels which were not significantly different from the respective controls.

Although biochemical alterations were demonstrated on several levels there were no indications of overt necrosis for either hepatotoxin in any of the treatment groups. Evidence of limited cellular damage for the CCl_4 group was demonstrated as diminished cellular definition, and in some cases a slightly vacuolated appearance.

These findings indicate that inducer-hepatotoxin interactions do occur in the flounder for select agents under the conditions described. The significant findings include: the protective effect of BNF against CCl_4 mediated toxicity, the reductions of both LM_4 and LM_2 P-450 isozymes with CCl_4 administration and the evidence of enzymatic damage in the face of limited observable cellular damage. Further studies are necessary to assess the environmental significance of these interactions. (Support: Center for Membrane Toxicity Studies and L. P. Markey Trust.)

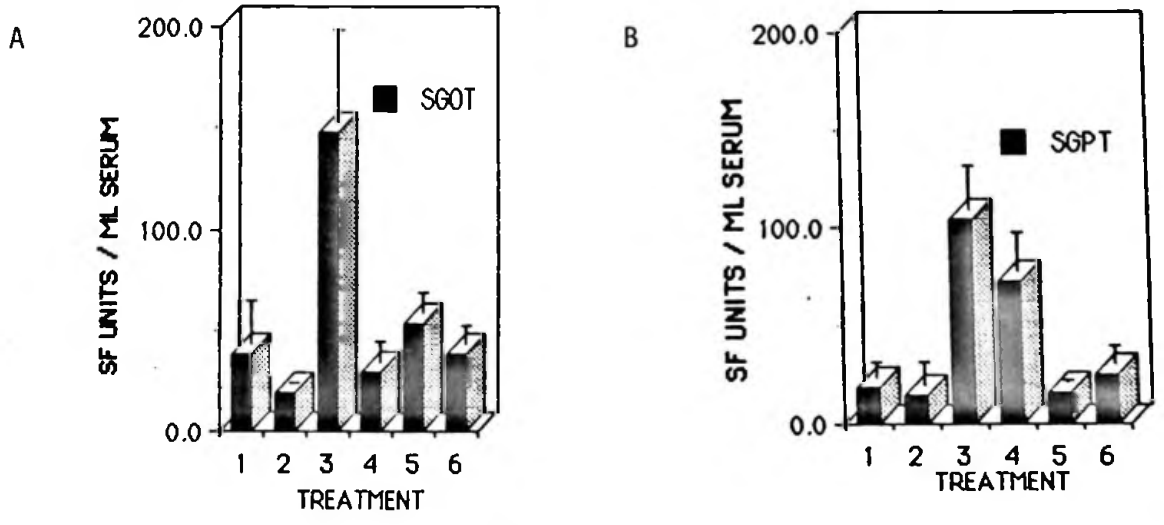

Figure 1a. SGOT, 1b SGPT; Treatments: 1) Control 2) BNF 3) CCl_4 4) CCl_4/BNF 5) AF and 6) AF/BNF