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Altered Hepatic Cytochrome P450-Mediated Steroid Metabolism in Environmentally-Exposed Seals

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Hepatic microsomal cytochrome P450 isozymes are involved in xenobiotic detoxification and steroid metabolism. Seals are highly exposed to persistent lipophilic organic pollutants, in particular polychlorinated biphenyls (PCBs) due to their position as top predators in the marine food chain. Xenobiotic exposure can result in overall induction of CYP450 which may have concomitant effects on CYP450-mediated steroid metabolism as has been observed in laboratory animals (Porter and Coon, 1991; Colborn and Smolen, 1996). Experiments were conducted to investigate any difference in rates of hepatic progesterone (P) and testosterone (T) metabolism in Harbour Seal pups (*Phoca vitulina*) exposed to different levels of PCB from the environment.

Seals were sampled by RSPCA within 2 h of death (net entanglement victims) and stored in liquid nitrogen until analysis. Methods for preparation of microsomal fractions and determination of CYP450 concentrations is described elsewhere (Troisi and Mason, 1997; Johanessen and DePierre, 1978). For steroid metabolism assays microsomal suspensions ($\sim$ 1.0 nmol P450 in 0.1 M phosphate buffer, pH 7.4) were incubated with 20 μM steroid in 20 μl methanol in final volume of 900 μl and equilibrated for 5 min at room temperature. Reactions were initiated with 100 μl of 10 mM NADPH and incubated at 37°C for 25 min (substrates and co-factor concentrations were saturating). Reactions were terminated with 6 ml dichloromethane and centrifuged for 2 min ($800 \times g$) to obtain the organic phase for analysis. Using a Beckman HPLC system, metabolites were eluted from a 5 μm C_{18} Ultrasphere ODS column (25 cm × 4.6 mm i.d.) with an isocratic gradient (60% methanol:40% water) for 30 min then linear gradient to 100% methanol over 15 min held for 15 min (flow rate 1 ml/min), and analysed with UV detection at 254 nm. Samples were prepared for PCB analysis as described by Allchin et al. (1989). Concentrations of 22 PCB congeners were determined using a Varian 3400 gas chromatograph (GC) with electron capture detector (ECD) fitted with a 50 m BPX5 capillary column.

CYP450 was induced with increasing liver PCB concentrations between 2.29 and 144.33 μg/g lipid weight (Fig. 1). P ($P < 0.005$) and T ($P =$ not significant) metabolism increased with increasing liver PCB concentration (Fig. 2). Major P metabolites were 6β-OH (68.1%) and 16α-OH (12.8%) suggestive of CYP1A-like and CYP2B-like activity respectively. Major T metabolites were 6β-OH (55.8%) and 2β-OH (16%) suggestive of CYP3A-like activity as reported in Ringed Seals (*Phoca. hispida*) (Wolkers et al., 1998). There was no difference in metabolite patterns produced by seals with different exposure. The results suggest PCBs may have a modulating effect on hepatic steroid metabolism at environmental levels of exposure, possibly influencing steroid deactivation and clearance.

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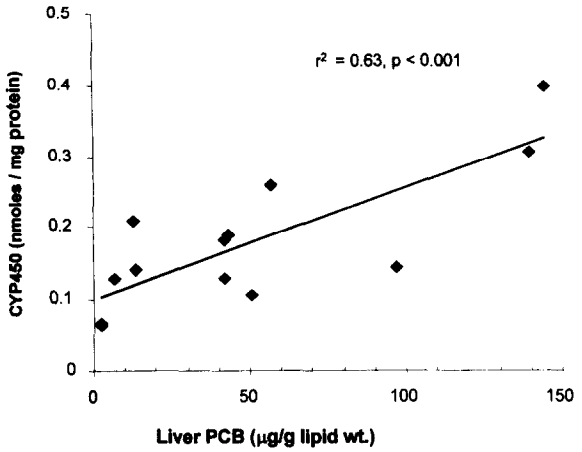

Fig. 1. Change in microsomal CYP450 concentration with increasing liver PCB concentration (µg/g lipid weight) showing induction of P450 protein.

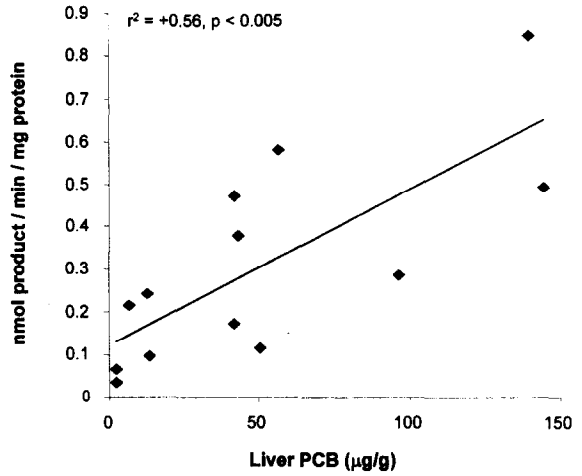

Fig. 2. Rate of progesterone metabolism per mg total protein with increasing liver PCB concentration (µg/g lipid weight).

Poster Abstracts

The Effect of Penicillin, a Prototypic Drug Allergen, on the Expression of Cell Surface Markers in Human Dendritic Cell Cultures

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In order to develop an in vitro system with the capacity to identify potential drug allergens, we have focused on the development of cultures of the dendritic cell (DC), a professional antigen presenting cell, and the study of the way in which they might influence Th1/Th2 lymphocyte development. Using the drug, penicillin,