

1105 COMPARISON OF CYP1B1 AND CYP1A1 RNA LEVELS IN DIOXIN-TREATED HUMAN LYMPHOCYTES.

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Tissue-specific alteration in expression of dioxin-responsive genes is one possible mechanism for the broad spectrum of biological responses associated with dioxin exposure. Comparative analysis of the expression of these genes may therefore address the uncertainties in risk assessment for human populations exposed to dioxin. In this study, we used a quantitative RT-PCR assay to examine the expression of two dioxin-inducible cytochrome P450 genes, CYP1B1 and CYP1A1, in human peripheral blood lymphocytes obtained from North Carolina volunteers. Treatment of mitogen-stimulated lymphocytes with 10 nM TCDD induced RNA levels of both CYP1B1 and CYP1A1, as well as CYP1A1 enzyme activity, in a time-dependent manner. Significant inter-individual variations in the levels of both CYP1B1 and CYP1A1 RNA were observed in resting, unstimulated, lymphocytes. Inter-individual variation was also observed in CYP1B1 and CYP1A1 RNA levels in mitogen-stimulated lymphocytes treated with 10 nM TCDD for 3 days. Relative inducibility of CYP1B1 and CYP1A1 RNA varied between individuals. These observations indicate that CYP1B1 and CYP1A1 are inducible by *in vitro* exposure to TCDD in human lymphocytes, and that the magnitude of induction varies within the population. These studies also indicate that CYP1B1 may be a useful biomarker in epidemiological studies of human populations exposed to dioxin.

1106 ROLE OF PROTEIN KINASE C IN TCDD-INDUCED CYP1A1 EXPRESSION IN LYMPHOCYTES.

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While the induction of cytochrome P450 1A1 (CYP1A1) has recently been used in several studies as a sensitive biomarker for assessing individual exposure to persistent environmental contaminants, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the mechanisms that underlie the cell specific expression of this protein are not completely understood. The present study investigates the expression of CYP1A1 in rat thymocytes and splenocytes and human peripheral blood mononuclear cells (PBMCs) and its modulation by TCDD under various conditions. Resting rat thymocytes in culture had detectable levels of CYP1A1 activity (ethoxyresorufin-o-deethylase, EROD) and CYP1A1 mRNA expression which increased following *in vitro* exposure to ≥ 0.1 nM TCDD for 24 or 48 hours. Interestingly, concomitant *in vitro* exposure of rat thymocytes to TCDD and the mitogen concanavalin A (con A) inhibited the induction of EROD activity. Resting rat splenocytes in culture had no detectable EROD activity and CYP1A1 activity could not be induced by TCDD treatment with or without mitogen (con A). Human PBMCs treated with ≥ 5.0 nM TCDD also demonstrated increased EROD activity but only in the presence of con A. TCDD-induced CYP1A1 expression in cultured rat thymocytes and human PBMCs was inhibited by the concomitant addition of calphostin C, a specific protein kinase C (PKC) inhibitor, suggesting a role for PKC as a second messenger in the CYP1A1 induction pathway. Thus, mitogen mediation of CYP1A1 is dependent on the species and/or the cell type/maturity and appears to be under the regulation of PKC (Supported in part by ES06556).

1107 EXCRETION OF POLYCHLORINATED DIBENZO-P-DIOXINS AND DIBENZOFURANS INTO MILK OF COWS.

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Pentachlorophenol-treated wood was administered to four cows to characterize the diet to milk transfer coefficients of all 2,3,7,8- substituted dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs). The cows were dosed with 3 g/day ground wood for 56 days. Concentrations of congeners in the milk were measured at 0, 28 and 56 days. Steady state was reached by Day 28 because concentrations of all congeners in milk did not change significantly from that time to Day 56. Variations among cows in concentrations of individual congeners in milk and milk fat were small and were not related to body weight (543 to 784 kg), daily dry matter consumption (22 to 41 g/kg body weight), or daily production of milk (9.3 to 33.5 kg) and milk fat

(0.40 to 1.24 kg). The PCDFs that were unsubstituted in both the 4 and 6 positions (2,3,7,8-, 1,2,3,7,8- and 1,2,3,7,8,9-CDF) were not accumulated in significant amounts. The remaining PCDFs and the PCDDs had dose-adjusted concentrations inversely related to the number of chlorines ($r^2 = 0.78$), and less closely to the octanol-water partition coefficient ($r^2 = 0.63$). The comparable biological behavior of compounds within an isomer group, such as the hexa-CCDs, suggest that selected isomer ratios can be an aid in identifying sources. The biotransfer factors derived from these data are more extensive and more representative than those available in the literature.

1108 EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON MOLECULAR, CELLULAR, AND HISTOLOGIC MARKERS OF AVIAN CARDIAC MORPHOGENESIS.

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A hallmark sign of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity in avian embryos is edema: pericardial, peritoneal, and subcutaneous. Since edema is frequently associated with chronic cardiac insufficiency, we investigated the effects of TCDD on cardiac morphogenesis. Chicken eggs were injected with control (triolein) or 1.0 pmol TCDD/g egg prior to incubation and collected on day 5 or 10 when cardiac morphogenesis is in progress or is complete, respectively. Embryo hearts were dissected and fixed for immunohistochemistry or homogenized for protein or RNA analysis. On day 5, TCDD decreased cardiac expression of transforming growth factor (TGF)- β 3 mRNA and reduced expression of collagen I in the developing septa. On day 10, TCDD increased heart wet weight (1.4 fold, $p < 0.001$), dry weight (1.2 fold, $p < 0.01$), and heart myosin content (1.8 fold, $p < 0.07$). In addition, on day 10 TCDD-exposed hearts exhibited a high incidence (77%) of enlarged right and left ventricles, thickened ventricular septa, and increased ventricular trabeculation; and a moderate incidence (31%) of ventricular septal defects. TGF β 3 stimulates the formation of valves and septa and collagen I expression in valvular and septal mesenchyme. The decrease of TGF β 3 and collagen I on day 5 suggests that TCDD is altering valve and septa development which could lead to reduced cardiac output. Furthermore, TCDD-induced changes observed on day 10 (hypertrophy, increased myosin content, septal defects) are all indicative of progressive cardiac insufficiency. (Supported by NRSA ES-05673 to MKW).

1109 POSSIBLE BLADDER CANCER RISKS IN UNDERGROUND COAL MINERS BY PAH.

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Case-control studies in coal mining areas in several countries indicated an elevated bladder cancer risk in underground coal miners. To further elucidate the causes of carcinogenicity, 179 bladder cancer in-patients in an area of former hard coal mining were investigated for possible shifts in the distribution of polymorphic enzymes involved in the metabolism of aromatic amines (N-acetyltransferase 2, NAT2) and of polycyclic aromatic hydrocarbons (glutathione S-transferase M1, GSTM1).

The patients were phenotyped by using the caffeine test method. Ninety-two patients were additionally genotyped, using lymphocyte DNA, for NAT2, 89 patients for GSTM1. All patients were interviewed for occupations ever performed for more than 6 months.

In the subgroup of 32 phenotyped underground coal miners, an ordinary distribution of acetylator phenotypes (18 slow acetylators) was observed. By contrast, GSTM1 was lacking in 16 out of 19 underground coal miners. A similar distribution of NAT2 and GSTM1 was observed in other patient subgroups, which were highly exposed to polycyclic aromatic hydrocarbons. There was an ordinary distribution of acetylator phenotypes (24 slow acetylators out of 41) and of GSTM1 genotypes (7 GSTM1 negative out of 13) in bladder cancer patients having worked as businessmen and administratives (not exposed to bladder toxicants).

This study is the first pointing to an involvement of PAH on elevated bladder cancer risks in underground coal miners.