

tibia and femur), hepatic retinoid levels (HPLC of total retinyl ester, all-trans-retinol, and atRA levels), and CYP1A1/2, 1A2, and 2B1/2 activities (E/P/MROD assay) in adult male CRBPI-KO mice before and after a single dose of TCDD. Wildtype (WT) mice of the same strain background were used as controls. We saw no differences in bodyweight or bone length between untreated CRBPI-KO and WT mice. However, bone mineral density (BMD) and strength were lower in CRBPI-KO mice. After TCDD exposure, CRBPI-KO bones were unaffected, whereas WT bones displayed reduced BMD and strength. Retinyl esters in CRBPI-KO livers were significantly lower than WT at all times and doses. After TCDD exposure CRBPI-KO mice lost nearly all their retinyl ester stores, whereas WT mice were almost unaffected. TCDD-treated CRBPI-KO livers displayed reduced atRA levels, whereas atRA levels in WT were unchanged. Baseline CYP1A activities were lower in CRBPI-KO than in WT but after TCDD exposure, CYP1A was twice as much induced in the CRBPI-KO than in WT. This study clearly shows that a binding protein specific for the vitamin A system plays a role not only for proper retinoid storage as earlier shown but also for bone status and for the homeostasis of the hormone RA. The effects of TCDD on bone could however not be associated with an altered RA homeostasis. Nonetheless, the absence of CRBP I was indeed protective against the bone-disrupting effects of TCDD and the role of this protein in bone homeostasis must therefore be further explored.

### 1762 REGULATION OF CYCLIN D1 GENE EXPRESSION IN THE MOUSE UTERUS BY ESTROGEN AND 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN.

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Induction of uterine cyclin D1 mRNA levels was investigated in 25 day-old B6C3F1 mice treated with 200 ng of 17 $\beta$ -estradiol (E2) for 1, 3, 6, and 12 h followed by *in situ* hybridization of uterine sections. Increased cyclin D1 mRNA staining was observed in the luminal epithelium 6 and 12 h after treatment, whereas background cyclin D1 mRNA staining in stromal cells was observed in solvent (corn oil)-treated animals and was not increased after 6 and 12 h treatment with E2. Comparable hormone-responsiveness was observed in wild-type estrogen receptor  $\alpha$  (ER $\alpha$ ) knockout ( $\alpha$ ERKO)/+ and aryl hydrocarbon receptor knockout (AhRKO) mice, whereas responsiveness was not observed in homozygous  $\alpha$ ERKO/- mice, confirming the role of ER $\alpha$  in E2-induced cyclin D1 expression in the uterus. Moreover, since previous studies indicate that induction of uterine cyclin D1 mRNA is not affected by cycloheximide, our results suggest that induction of this gene in luminal epithelial cells is a direct effect of E2 and not related to induced stromal factors. Inhibition of E2-responsiveness in the uterus by the AhR agonist 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD) is well established. TCDD alone does not affect stromal or epithelial expression of cyclin D1; however, in B6C3F1 mice cotreated with E2+TCDD, there was a decrease in E2-induced uterine cyclin D1 mRNA expression in the luminal epithelial cells. This inhibitory TCDD-ER $\alpha$  crosstalk on cyclin D1 mRNA was not observed in AhRKO mice confirming the role of the AhR in mediating this response which was similar to inhibitory AhR-ER crosstalk observed in breast cancer cells and rodent mammary tumors. (Supported by NIH ES09106 and ES04176)

### 1763 HEPATIC RETINOID LEVELS IN A TCDD-SENSITIVE (LONG-EVANS) AND TCDD-RESISTANT (HAN/WISTAR) RAT STRAIN FOLLOWING LONG-TERM LOW-DOSE TCDD EXPOSURE.

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2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent environmental contaminant that alters retinoid homeostasis by mechanisms believed to be mediated by the aryl hydrocarbon receptor (AhR). In this study we investigated whether the noted structural differences between the AhRs of Long-Evans (L-E) and Han/Wistar (H/W) rat strains influenced polar and apolar retinoid homeostasis following long-term TCDD treatment. Female L-E and H/W rats were given TCDD by s.c. injection once per week at calculated daily doses of 0, 1, 10, 100, or 1000 (H/W only) ng/kg bw/day for 20 weeks. Total hepatic retinoid levels were dose-dependently decreased in both strains. However, effects were seen at 1 ng/kg bw/day in the L-E strain whereas significant effects were observed at 10 ng/kg bw/day in the H/W strain. BMD05 levels, defined as a 5% change from mean control values, were approximately 140 and 1400 pg/kg bw/day for L-E and H/W rats respectively.

Preliminary analyses of hepatic polar retinoid levels showed no change in retinoic acid levels of either strain. On the other hand, a recently discovered retinoic acid metabolite, 9-cis-4-oxo-13, 14-dihydro-retinoic acid was all but eliminated in the liver of both strains at the low-dose. Thus the data suggest that long-term low-dose TCDD exposure markedly increases hepatic retinoid turnover without significant effects on hepatic retinoic acid levels. Moreover, the 10-fold difference between the L-E and H/W strain provide further support for a role of the AhR in TCDD altered retinoid homeostasis.

### 1764 USE OF CYP1A2 (-/-) KNOCKOUT AND CYP1A2 (+/+) C57BL/6N PARENTAL STRAINS OF MICE TO COMPARE METABOLISM OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD).

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The most toxic dioxin congener, TCDD, induces hepatic cytochrome CYP1A2 to which it subsequently binds, resulting in whole body half lives of 5-11 years in humans and 30 days in rats. Metabolism of TCDD is very limited in both species. Whether TCDD is a poor substrate for metabolizing enzymes or is unavailable for metabolism due to its strong affinity to CYP1A2 has not been firmly established. Thus, we tested the hypothesis that sequestration of TCDD by CYP1A2 makes TCDD unavailable for metabolism that would readily occur in the absence of CYP1A2. Male C57BL/6N mice which possess or lack the CYP1A2 gene were given a single oral dose of 156 ug[14C]TCDD/kg. After 4 days, the mice (housed in metabolism cages with separate collection of urine and feces every 24h) were killed and tissues collected. Tissue deposition and overall metabolism in urine and feces were quantitated. Similar to previously reported studies, liver:fat ratio of the two groups was vastly different, i.e. 4.09 (C57BL/6N) vs. 0.57 (knockout, KO). Slightly higher levels of 14C-derived TCDD were excreted in urine and feces of the parental strain at each time point when compared to KO mice. The overall level of metabolism of TCDD was determined as sum of 14C in 0-96h urine, non-extractable feces, and metabolites in extractable feces. The parental strain of mice had greater overall metabolism than the KO mice, i.e. 11.1% vs. 6.5% of the dose, respectively. The lower overall metabolism in the KO than the parental strain of mice is probably due to low hepatic retention and rapid redistribution of TCDD into lipophilic tissues for storage, which made the TCDD unavailable to hepatic metabolizing enzymes. In conclusion, the data presented in this study contradicts the original hypothesis and confirms that TCDD has an inherently slow metabolism in mammals, perhaps *via* the inducible CYP1A1, 1A2, and 1B1 isozymes and/or non-P450 dependent mechanisms. (This abstract does not reflect USEPA and USDA policies.)

### 1765 A COMPARISON OF THE METABOLISM OF METHOXYRESORUFIN, ACETANILIDE AND CAFFIENE IN RAT AND HUMAN CYP1A2 SUPERSOMES AND THEIR INHIBITION BY 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN (TCDD).

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CYP1A2 is highly expressed in both rats and humans. TCDD and related chemicals induce and bind to these proteins. The binding of TCDD and related chemicals to CYP1A2 leads to their sequestration in hepatic tissue. The present study compares the metabolism of prototype CYP1A2 substrates in rat and human CYP1A2 + P450reductase SUPERSOMES (GenTest Corporation, Woburn, MA) and the ability of TCDD to inhibit these reactions. For the O-demethylation of methoxyresorufin, the  $K_M$  was similar between human and rat CYP1A2, 0.10 and 0.09 nM respectively. Rat supersomes had a slightly higher  $V_{max}$  than human supersomes, 3.5 and 2.4 pmol/min/mg CYP1A2. TCDD inhibited methoxyresorufin metabolism with  $K_i$  values of 0.3 and 0.06  $\mu$ M in the human and rat supersomes respectively. Caffeine was metabolized by both human and rat CYP1A2 supersomes. The estimated  $V_{max}$  was higher in the human compared to the rat supersomes, 5.0 and 2.2 nmol/min/mg CYP1A2, respectively. The  $K_m$  was also higher in the human (6.5 mM) compared to the rat (0.9 mM) supersomes. The metabolism of acetanilide to 4-hydroxy acetanilide was similar in the human and rat supersomes. The  $V_{max}$  was 2.8 and 5.9 nmol/min/mg protein, in human and rat supersomes, respectively. The  $K_M$  was 19.1 mM in human supersomes and 74.5 mM in rat supersomes. Initial studies indicate that TCDD inhibits both caffeine and acetanilide metabolism in rat and human supersomes. These data demonstrate that the *in vitro* metabolism of prototype substrates is similar between the rat and human CYP1A2 supersome preparations and that TCDD inhibits the metabolism of these substrates by both rat and human CYP1A2. Because of the potential for inhibition of CYP1A2 activity by TCDD, studies examining CYP1A2 induction in TCDD exposed populations using these substrates should be viewed cautiously. (This abstract does not reflect EPA policy)