RELATIONSHIPS BETWEEN TISSUE TCDD LEVELS, MRNAS AND TOXICITY IN THE DEVELOPING WISTAR(HAN) RAT

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Introduction

The most sensitive marker of TCDD toxicity comes from animal studies that demonstrate adverse effects on the reproductive function of adult offspring born to dams receiving doses of TCDD as low as 50 ng/kg body weight **[1] [2-5]**. However, examination of the published studies (Figure 1) finds a lack of consistency in low dose effects; subsequently, an array of studies were unable to repeat the effect of low doses of TCDD on offspring sperm levels **[6-12] [13]**.

We undertook two animal studies, where dams were dosed either with a single acute dose **[6]**, or sub-chronic exposure **[7]**, to TCDD, and offspring were allowed to litter and were examined for various reproductive endpoints. These animal experiments were performed to GLP, and used large group sizes (~20-25 dams) for enhanced reliability. These experiments showed that TCDD had minimal adverse effects on sperm parameters, but that TCDD was a potent toxin, killing ~15-25% of the offspring after maternal doses on the order of 1 microgramme per kilogramme. Additionally, TCDD had stronger adverse effects after sub-chronic dosing of the dams, as compared with a single acute dose, on the delay in puberty and on weight gain in the offspring. Analysis of tissue TCDD and RNA levels was undertaken on samples obtained from these studies **[14]**, and the results are presented herein.

Methods

Acute study. WI(Han) rats of 16-18 weeks old were mated, and dosed with 0-1000 ng TCDD/kg on gestation day (GD) 15; tissue samples were taken on GD16 and 21. Sub-chronic study. WI(Han) rats of 5-6 weeks of age were placed on a diet containing TCDD, mated at week 17, and transferred to control diet at parturition. The dams were allowed to litter, and male offspring killed on post-natal day (PND) 70 or 120 for evaluation.

TCDD analysis was performed by high resolution GC-MS, using a method accredited to the ISO17025 standard **[15]**. RNA analysis was performed using real-time PCR methodology, with Taqman probes. The PCR co-amplified β-actin, AhR and CYP1A1, or β-actin and CYP1A2, in the same reaction. PCR efficiencies were verified.

Results

TCDD concentration was verified in the diet, and found to be stable; there was batch-to-batch variation in TCDD concentration of up to 30%. Taking into account food consumption, the

treated groups in the sub-chronic trial were exposed to 2.4, 8 and 46 ng TCDD kg day. Measurement of TCDD, and TEQ, in the control animals showed that these were detectable, and that they were at least forty-fold below the corresponding tissue concentrations in the lowest TCDD treatment groups. Thus there is no contamination with TCDD and congeners in the control groups which could perturb the interpretation of the study.

The experimental design was that the dose of TCDD in the sub-chronic study should yield approximately equivalent TCDD concentrations in tissue, as seen in the GD16 samples from the acute dose study. Body burdens of TCDD in the acute study were ~55, 110 and 118% of the corresponding sample from the low, medium and high dose groups from the chronic study. This shows that the dose of TCDD is roughly comparable between the low, medium and high dose groups of the acute and sub-chronic studies, hence enabling a comparison of the toxic effects send in these two studies.

However, there was considerable evidence for markedly different pharmacokinetics between the two studies. The liver: adipose ratio of TCDD concentrations was much higher in the acute GD16 studies, and disposition to adipose tissue was marked by GD21. The sub-chronic studies showed lower hepatic concentrations of TCDD. The disposition of TCDD to extrahepatic tissues in the chronic study was dose-dependent, with a greater proportion of TCDD dose reaching extrahepatic tissues in the lowest dose group. TCDD half-life was dose-dependent, and increased by ~50% in the low dose groups of the sub-chronic study. Thus TCDD body burden and disposition is critically dependent upon dose and dosing regime.

TCDD concentration in the fetus on GD16 or 21 showed a poor relationship with endpoints of toxicity. So while fetal concentrations of TCDD in the three dose groups were roughly comparable between the acute and sub-chronic studies, the sub-chronic study had significant delay of puberty (BPS) in all three dose groups where the acute study only showed an effect at the highest dose group. This confirms that TCDD has more potent effects after sub-chronic administration, and queries the relevance of fetal TCDD concentration to the toxic response.

Measurement of the induction of CYP1A1 RNA provides a sensitive measure of activation of the AhR. While there was minor (less than two-fold) variation in the control RNAs β-actin and AhR, the CYP1A1 RNA was induced by ~1000 fold in maternal liver in a dose-dependent fashion, and hepatic RNA induction levels were comparable between acute and sub-chronic studies. However, although fetal CYP1A1 RNA was highly induced in the medium and high dose groups, there was no statistically significant induction of CYP1A1 RNA in the low dose group. This suggests that activation of the AhR in the low dose fetus is minimal, and that TCDD is not mediating its potent effects on delay in puberty through activation of the AhR in the fetus. These data suggest that sub-chronic dosing of TCDD causes delay in puberty through a mechanism which does not involve activation of the fetal AhR between GD16 and GD21, and we propose that lactational transfer of maternal TCDD to the pup may be responsible for toxicity.

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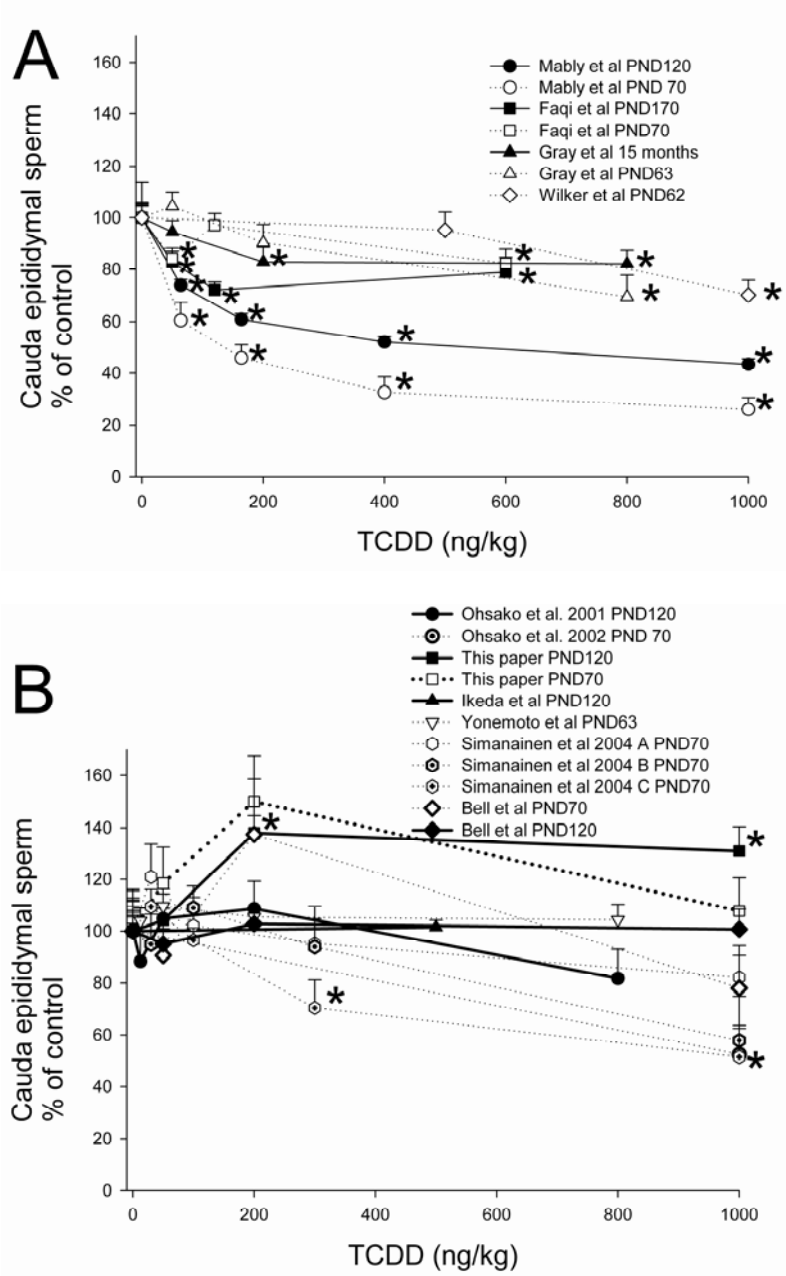

Figure 1. Epididymal sperm values from studies (A) pre-2000 or (B) post-2000 were normalised to the control value of 100%, and are plotted as mean and standard error of the mean. * indicates a $P<0.05$. Filled symbols and continuous lines are for analyses with animals older than PND 100, and open symbols and dotted lines are for younger animals. The relevant studies are listed in the key. Maternal dose of TCDD is shown on the x-axis, and doses greater than 1000 ng/kg are not shown. Faqi et al (1988) used a sub-chronic dosing protocol, and this is converted to the equivalent acute dose on the basis of liver TCDD concentrations (DRB, unpublished data).