A robust examination of the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the developing male reproductive system

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Introduction

The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT), a UK independent expert committee, has established a tolerable daily intake of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds[1]. Human epidemiological data was insufficient for risk assessment, and the most sensitive measure of animal toxicity (developmental effects on sperm number in male offspring) was identified. Extrapolation to human is on the basis of body burden of TCDD.[2-5] The COT identified uncertainties relating to the body burden of TCDD required to cause toxicity.

The aims of the study described in this paper were:

- To repeat the TCDD single dose experiments causing developmental male toxicology
- To measure the TCDD burden in the target organs in a contemporaneous experiment
- To prepare for a chronic study with dietary administration of TCDD

Methods

Pregnant CrlWistar (Glx/BRL/Han)BR rats were dosed on gestational day (GD) 15 with TCDD by oral gavage at 0, 50, 200 or 1000 ng TCDD kg^{-1}. Rats were killed at GD16 and 21 for analysis of TCDD by HRGC-HRMS to ISO17025 standards in adipose, blood, foetus and liver in n=5 animals/ dose group. About 20 dams per group were allowed to litter, and male offspring retained. 25 F_1 males per group were killed on post-natal day (PND) 70, and the remainder (ca. 60/group) at PND 120. Animals were subjected to a functional observational battery, and kills were conducted at PND 70 and 120. This report deals principally with the PND120 data. Animals were subjected to necropsy and seminology. The *in vivo* experiments were performed to GLP.

Results

Analysis of TCDD by HRGC-HRMS passed quality control measures for sensitivity and specificity. TCDD was quantified in tissues from control animals at ~1% of the values for the 50 ng TCDD kg^{-1} treatment group. There was evidence for dose-dependent distribution of TCDD to tissue; for example, Figure 1 shows that ~40% more TCDD goes to liver at the higher two dose levels. Figure 2 shows that the TCDD redistributes between organs between GD16 and GD21.

Four out of 20 of the dams exposed to 1000 ng TCDD kg^{-1} had total litter loss; there were significantly fewer pups from day 1 onwards (Figure 3); the body weight of the F1 males was lower than control (Figure 4); balano-preputial separation (an indicator of onset of puberty) was delayed [45.8 vs 48.6 days] (Figure 5) and brain and testis weights were reduced (by 2.7 and 5.7%, respectively; not shown). This dose of TCDD causes frank toxicity.

Figure 6 shows that the sperm count in PND 120 males is marginally higher in the 200 and 1000 ng kg^{-1} dose groups. Although this effect is statistically significant, these measures are within the normal range, and the biological significance of this finding is unclear. At 1000 ng TCDD kg^{-1}, there was no effect on sperm motility, sperm average path velocity, number of testicular spermatids, prostate weight, seminal vesicle weight or epididymis weight, but there was a significant increase in inflammatory cell foci in the epididymis.

The 200 ng TCDD kg^{-1} group had a transient lowering of pup body weight, and balano-preputial separation was delayed by ~1 day. There were no other significant and dose-related findings in the 200 or 50 ng TCDD kg^{-1} groups.

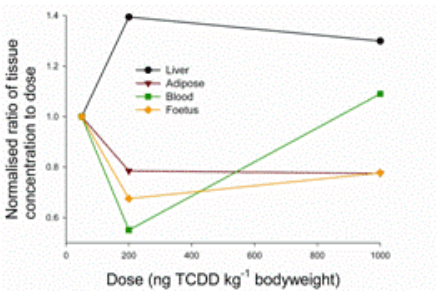

Figure 1. GD16 tissue concentration of TCDD normalised to dose, and to the low dose group value, plotted against dose.

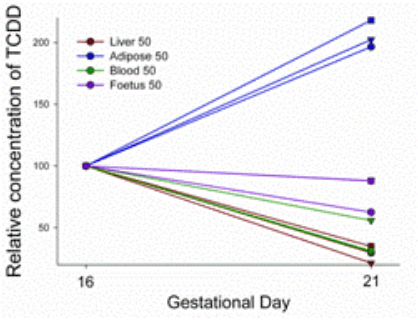

Figure 2. Tissue concentration of TCDD normalised to GD16 against time.

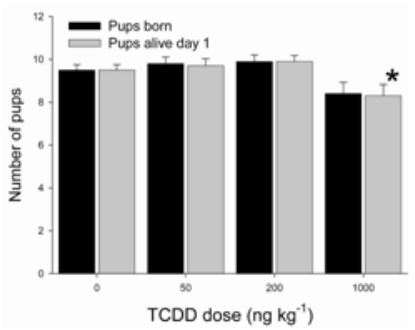

Figure 3. Number of pups born, and alive on day 1, per litter, plotted against dose. [Mean +SEM. *= P<0.05]

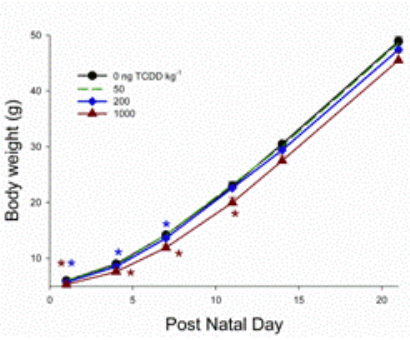

Figure 4. Body weight of F1 animals from PND1 to 21. [Mean +SEM, * =P<0.05]

Discussion

These results show that TCDD is a potent toxin, causing pup loss, body weight gain deficiencies and some more subtle changes at doses as low as 1 µg kg^{-1}. There were no clear-cut adverse effects on sperm production, although puberty was delayed at this dose. There was a transient reduction in pup body weight and delay in balano-preputial separation at 200 ng kg^{-1} and no significant effects at 50 ng TCDD kg^{-1}.

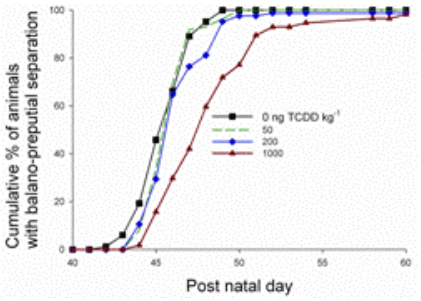

Figure 5. Percentage of animals with balano-preputial separation.

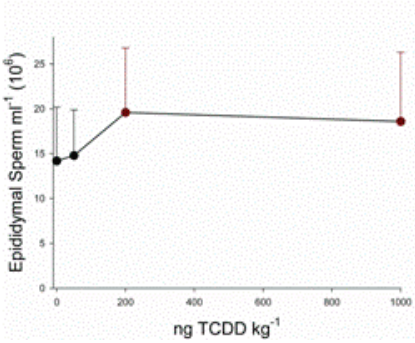

Figure 6. Epididymal sperm count against dose group at PND 120. [SEM. Red= P<0.05]

The developmental reproductive toxicity of TCDD at doses as low as ~64 ng kg^{-1} is the basis for the WHO, EU and UK risk assessments of TCDD. An extension to the study to examine chronic dietary exposure to TCDD will provide further data for risk assessment.

References

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