TCDD IS A POTENT DEVELOPMENTAL TOXIN, BUT DOES NOT AFFECT SPERM NUMBER IN OFFSPRING OF CRL:WI(HAN) RATS FOLLOWING CHRONIC DIETARY EXPOSURE

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Abstract
This study examined whether fetal exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) causes defects in the male reproductive system of the rat through sub-chronic exposure, using rats fed TCDD via the diet. Rats were exposed to a diet containing TCDD, to attain an average dose of control, 2.4, 8 and 46 ng TCDD kg^{-1} day^{-1} for thirteen weeks, whereupon the rats were mated, and allowed to litter; rats were switched to control diet after parturition. Male offspring were allowed to develop until kills on PND70 (25 per group), or PND120 (all remaining animals). Offspring from the high dose group showed an increase in total litter loss, and the number of animals alive on PND4 in the high dose group was ~26% less than control. The high and medium dose offspring showed decreased weights at various ages. Balano-preputial separation was significantly delayed in all three dose groups, compared to control. There were no significant effects of maternal treatment seen in various other tests carried out including sperm and spermatid parameters at PND70 and 120, with the exception that there was an increase in the proportion of abnormal sperm in the high dose group at PND70.

Introduction
2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent toxin, and the prototypical member of a series of related chlorinated dioxins, furans, and PCBs. It is present at low levels in the environment at levels that give rise to concern. One of the most potent recorded effects of TCDD is on the developing male reproductive system, where exposure of the pregnant dam on Gestational Day (GD) 15 induces a spectrum of effects, including decreased epidymal sperm numbers, after a single dose of as little as 64 ng kg^{-1}.[1] The UK Committee on Toxicity Food, Consumer Products and the Environment (COT) considered that the developmental effects of TCDD on F1 male epidiymal sperm levels were consistent,[1-3] and proposed a Tolerable Daily intake for dioxins of 2 pg kg day using this endpoint.

We set out to replicate this endpoint, whilst taking contemporaneous measurements of TCDD in target tissues. Our previous experiment with an acute dose of TCDD on GD15 showed that there was no effect of developmental exposure on F1 epidymal sperm levels. In order to exclude the possibility that there is a period during development of sensitivity to TCDD, we now present an experiment with chronic exposure of the dam to TCDD.

Methods
5-6 week old CRL:WI(Han) female rats were given diet supplemented with 0, 28, 93 and 530 ng TCDD kg . Rats were dosed for 12 weeks, then during mating and pregnancy, and dosing terminated at parturition. Females were

killed for tissue samples at weeks 10 and 12 of dosing, and at GD16 and 21. ~30 dams per group littered, and F1 males were killed at PND70 or PND120 for tissue and seminology investigations. F1 males were also subject to a functional observational battery, and 20 males per group were mated. TCDD analysis was by High Resolution Mass Spectrometry, using a method accredited to the ISO17025 standard.

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 were exposed to 2.4, 8 and 46 ng TCDD kg day . There was no evidence of adverse effects in the dams, and pre-coital time, mating, fertility and fecundity indices were similar in all groups. However, there was a significant effect and fewer pups surviving between days 1 and 4. There was a dose related reduction in mean pup body weight on day 1 post partum, and this remained so throughout lactation. High dose group males were lighter than control throughout the study, and the medium dose group showed a trend towards lighter weight at PND70-120 (Figure 1). Balanopreputial separation (BPS) was delayed in all three dose groups (Figure 2), by 1.8, 1.9 and 4.4 days for low, medium and high dose respectively; however, there was no association between body weight at PND42 and day of BPS. The F1 males were subjected to a functional observational battery, and with the exception of lower activity in the high dose group, these results were not different from control. 20 males per group were mated in week 16; there was no significant difference in uterine/ implantation data, and no change in the % of male offspring between groups. In the seminology at PND70, the high dose group showed an increase in abnormal sperm from 3.7 (control) to 5.8%, and testicular spermatids were decreased by ~15%, but there were no other significant differences. At PND120, there were no significant differences in the number of epididymal sperm, the % of abnormal sperm, or testicular spermatids (Figure 3). A comparison of reported epididymal sperm values with maternal dose of TCDD is presented (Figure 4).

This experiment had a 95% power for detecting a 30% difference in control epididymal sperm which meant that at $P<0.05$, based on the data from the control group, this was sufficient to detect the ~50% decrease in sperm numbers reported by Malby et al,[1] and there is 70% power to detect the ~20% decrease in sperm reported by Faqui[2] and Gray.[3] The weight of ventral prostate was directly measured , and this showed no significant decrease from control; given the variability of the control animals, our experiment had a ~90% power for detecting a 10% decrease from control values in ventral prostate weight, which could be sufficient to detect the ~40% decrease in prostate weight described.[1]

Conclusions

- Maternal exposure to 1 μg kg^{-1}, or 46 ng kg^{-1} day^{-1} of TCDD yields 15-25% lethality in the offspring. At this dose, it is difficult to separate the direct effects of TCDD from indirect effects caused by pup lethality.
- Maternal exposure to TCDD does not reduce epididymal sperm levels in F1 CRL:WI(Han) rats.
- A survey of the literature demonstrates that the initial reports of developmental exposure to TCDD causing a decrease in epididymal sperm have not been reproduced since 2000, at doses below 300 ng kg^{-1}.
- Delay in BPS is a potent adverse effect of TCDD that is sensitive to whether TCDD is administered on an acute or sub-chronic basis.
- It is unclear whether the delay in BPS results from *in utero* or lactational exposure of the F_1 to TCDD.

References

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Figure 1A

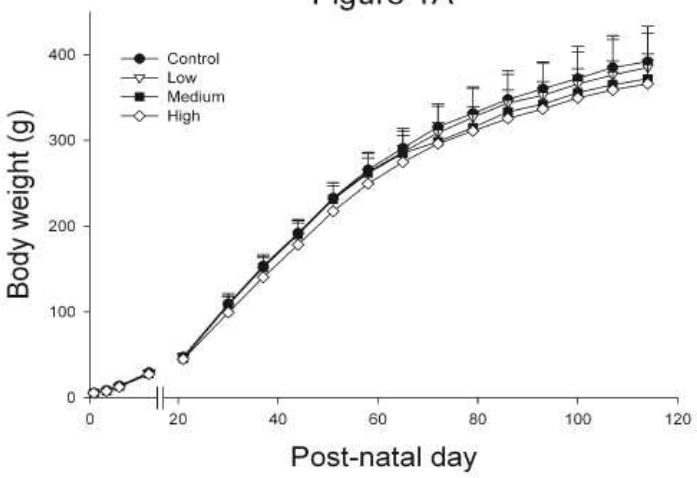

Figure 1B

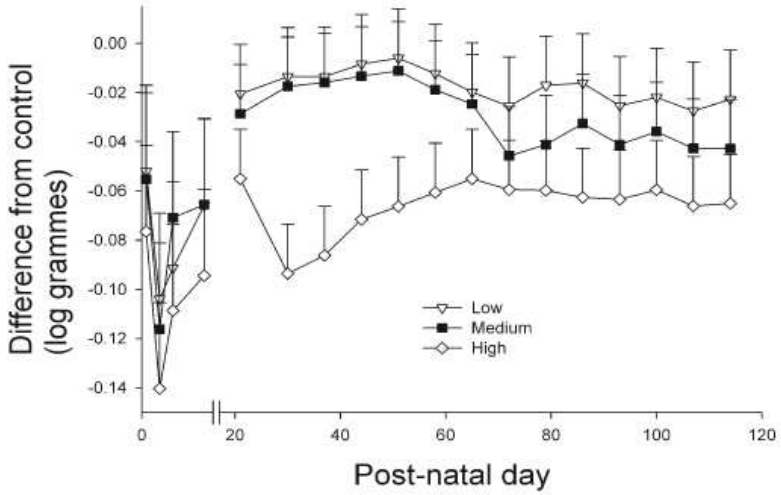

FIG. 2

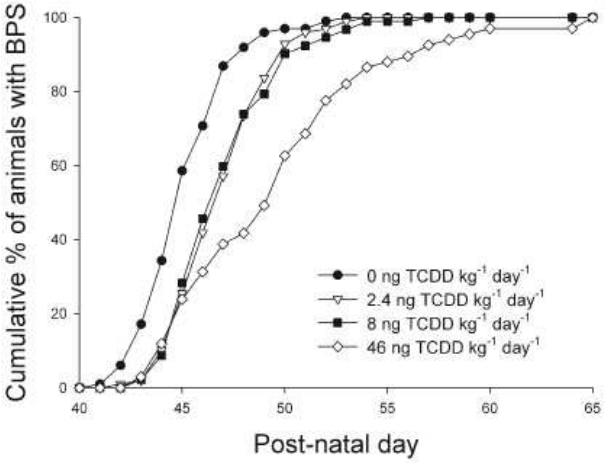

FIGURE 3

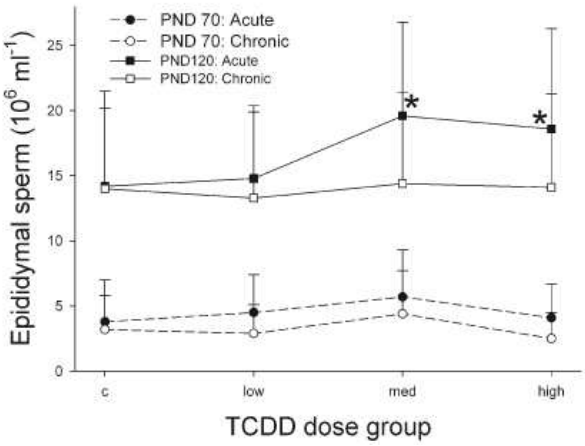

Fig 3A

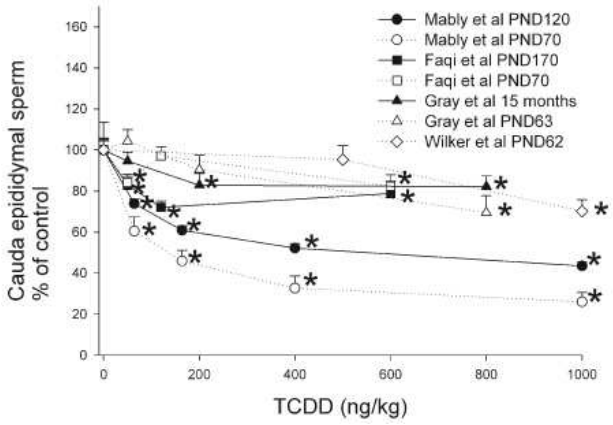

Fig. 3B