COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

FSA FUNDED STUDY INVESTIGATING THE DEVELOPMENTAL EFFECTS OF DIOXIN (TCDD) IN RATS

Non Technical Summary

1. Dioxins and dioxin-like polychlorinated biphenyls (PCBs) are persistent organic pollutants that are known to cause a wide range of toxic effects in animals, some of which have been seen at very low doses. These effects may have significant consequences for human health.

2. In 2001, the COT assessed the risks posed by dioxins and dioxin-like PCBs. They identified a number of studies in which treatment of pregnant rats with dioxin resulted in toxicity to the developing reproductive system of male offspring. Changes in sperm quality occurred at lower doses than the other effects of dioxin; therefore, the COT used these data to set a tolerable daily intake (TDI) for dioxins, which would protect humans from all the toxic effects of these chemicals. However, the Committee also noted that there were several limitations in the data, which led to uncertainties in their risk assessment.

3. The Food Standards Agency (FSA) has funded a developmental toxicity study which aimed to address some of the limitations identified by the Committee. This study examined the effect of dioxins on developing rats, whilst measuring the level of dioxin in the mother and in the fetus, termed ‘body-burden’.

4. The Committee considered this study was valuable in clarifying some of the uncertainties in their 2001 risk assessment. In the new study, the most sensitive effect of dioxin was a delay in puberty, rather than altered sperm quality. However, this was observed at levels of dioxin exposure that were similar to those used as the basis for the 2001 risk assessment. Therefore, the Committee considers that this study provides additional evidence that the current tolerable daily intake (TDI) of 2 pg/kg bw/day is protective for the developing male fetus.

Background

5. The term “dioxins” is commonly used to refer to a group of 75 polychlorinated dibenzo-p-dioxin (PCDD) and 135 polychlorinated dibenzofuran (PCDF) congeners, of which fewer than 20 are considered to be biologically active. Dioxins are produced in a number of thermal reactions, including incineration of municipal waste, domestic fires and bonfires, forest fires and in internal combustion automobile engines. They are also generated as trace contaminants during the synthesis of many organochlorine compounds and during some industrial processes

6. PCBs are environmentally stable, lipophilic chemicals that were widely manufactured for a range of industrial applications between the 1930s and 1970s. Use of PCBs for industrial purposes has been discontinued but these substances may still be released to the environment during disposal of materials and obsolete equipment. There are 209 theoretically possible PCB congeners, of which 12 non-ortho or mono-ortho compounds exhibit similar biological activity to PCDDs and PCDFs, and are therefore referred to as “dioxin-like PCBs”.

7. These compounds are persistent in the environment and tend to accumulate in biological systems, particularly in fatty tissues. One of the most potent and extensively studied PCDD congeners, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), exhibits a broad range of toxic effects in laboratory animals, some at very low doses. Since the toxicity of the various dioxins and dioxin-like congeners is generally accepted to be mediated by the aryl hydrocarbon receptor (AhR), and experiments using mixtures of congeners are consistent with an additive model; a system of toxic equivalency factors (TEFs) has been devised by the World Health Organisation (WHO) to enable total TCDD toxic equivalents (TEQ) to be calculated. This was initially developed in the 1980s and has subsequently been subjected to periodic review, to ensure the system incorporates newly acquired data and knowledge [1]. Estimates of dietary exposure are expressed in terms of WHO TEQ. The TEQ is defined as the sum of the products of the concentration of each congener, multiplied by the TEF.

2001 COT Evaluation

8. In 2001, the COT undertook an extensive review of dioxins and dioxin-like PCBs. The Committee on Carcinogenicity (COC) advised that TCDD should be regarded as probably carcinogenic to humans, based on the available data and, although there were uncertainties regarding the mechanism of action, it was likely that a threshold approach to risk assessment was appropriate [2].

9. The COT concluded that the available human data did not provide a sufficiently rigorous basis for establishment of a tolerable intake because:

- the epidemiological studies did not reflect the most sensitive population identified by animal studies;
- there were considerable uncertainties in the exposure assessments and inadequate allowance for confounding factors; and
- the patterns of exposure did not reflect the main route of exposure experienced in the general UK population, which is mainly from diet.

It was, therefore, considered necessary to base the evaluation on the data from studies conducted in experimental animals [3].

10. The Committee concluded that the most sensitive indicators of TCDD toxicity were the effects on the developing reproductive system of male rat fetuses exposed *in utero*. These endpoints had also been used to derive tolerable intakes by Joint Food and Agriculture Organisation (FAO) / WHO Expert Committee on Food Additives (JECFA), 70 pg WHO TEQ/kg bw per month [4]; and EU Scientific Committee on Food (SCF), 14 pg WHO TEQ/kg bw per week [5].

11. The key studies used different strains of rats and tended to give contradictory findings. A change in anogenital distance (AGD) was found after single oral doses given on day 15 of gestation (GD15) of 50 ng/kg bw [6], 200 ng/kg bw [7] and 1000 ng/kg bw [8]. However, the Committee considered that the data on AGD were not robust because of lack of correction for body weight or other means of normalisation, and should be regarded as an intermediate marker with no functional significance in itself. Decreases in sperm numbers, production, reserve or morphology were found shortly after puberty (postnatal day (PND) 49-70) and in adulthood (PND120 onwards), after single oral doses of 50 ng/kg bw and above on GD15 [7,8,9] and following weekly subcutaneous dosing to give a body burden of 25 ng/kg bw [10]; although these changes were not seen in one acute oral study, dosing 800 ng/kg bw on GD15 [6]. Changes in the weight of the urogenital complex, including the ventral prostate were reported after an oral dose of 200ng/kg bw on GD15 [6] but not at 300ng/kg bw subcutaneously [10].

12. Despite these inconsistencies, the Committee considered that the effects on sperm production and morphology represented the most sensitive effects. These were indicative of the functional adverse reproductive effects in the rat that were produced by long-term administration in a multigeneration study, at doses resulting in a 10-fold higher body burden than those in the studies of sperm production [11]. The Committee also noted that the sperm reserve in the human male is much less than that in the rat, and therefore these changes were considered relevant. The study of Faqi [10] provided the lowest LOAEL, but no NOAEL. Limitations in this study were noted but it was considered that the results could not be discounted; therefore, this was used as the basis for deriving the tolerable

intake. The Committee considered that a tolerable intake based on these effects would also protect against any risk of carcinogenicity from dioxins and dioxin-like PCBs since a significant increase in incidence of tumours was only found at doses that were higher than the LOAEL in the Faqi study.

13. Several studies [6,7,8,9] reported adverse effects in male rat offspring following a single oral dose of TCDD given on GD15, and one [10] following repeated weekly subcutaneous injections. In all cases the effects were observed postnatally , although the pattern of both *in utero* and postnatal exposure would be different between single and repeat dose studies. The JECFA and SCF evaluations [4,5] used the data from toxicokinetic studies [12,13] to model the fetal body burdens on GD16, on the assumption that the postnatal effects were the result of exposure of fetal tissue at GD16.

14. The COT used a similar approach, albeit with simpler toxicokinetic modelling[3]. Derivation of a tolerable intake for humans involved: calculation of the fetal body burden of rats under the experimental conditions; correction of the corresponding maternal body burden in rats to represent chronic daily intake via the diet; the use of uncertainty factors to give an equivalent tolerable human maternal body burden; and finally, derivation of a daily intake by humans that would result in the tolerable human maternal body burden.

The FSA funded Dioxins Risk Assessment project (T01034)

15. In evaluating the available toxicity data, the Committee identified gaps in knowledge related to the risk assessment of dioxins during pregnancy. In light of this, the FSA commissioned a developmental toxicity study, conducted in accordance with Good Laboratory Practice (GLP), using Computer Assisted Sperm Analysis (CASA) for robust collection of seminology data, and using large group sizes to increase the statistical power and reliability of the analyses. This project aimed to relate dose of TCDD to maternal burden, fetal burden and biological endpoints, within the same study. In view of the complexity of TCDD toxicokinetics, it was considered essential to have both an acute dose study, so as to be directly comparable with previous studies which dosed on GD15; and a sub-chronic repeat dose dietary study where TCDD administration is more representative of human exposure.

16. The Committee was presented with prepublication drafts of the papers discussing the acute[14] and sub-chronic[15] administration studies, together with the toxicokinetic data[16]

Acute Study

17. In the acute dosing study[14], groups of 75 control (vehicle alone) or 55 (50, 200 or 1000 ng of TCDD/kg bw) pregnant female Wistar(Han) rats were dosed by oral gavage on GD15. Tissues were harvested from 25 (control) and 15 (treated groups) animals killed on GD16 and 21. Tissue levels of

dioxin were determined at GD16 and GD21 using a sensitive and specific gas chromatography-mass spectrometry (GC-MS) analytical method. These tissue levels were used to determine maternal and fetal body burdens[a]. About 25 animals per group were allowed to litter.

18. During the study, 4 dams experienced total litter loss in the 1000 ng/kg dose group, compared to 1 in the control group and none in the lower dose groups. There was no statistically significant effect of maternal treatment on the sex ratio of the F1 offspring. The offspring of the 1000 ng/kg dose group showed reduced body weight throughout the study, reduced pup body weights were also seen in the 200 ng/kg dose group in the first week *post partum*.

19. There were no adverse effects of maternal treatment when the pups were subjected to a functional observational battery and no significant findings when reproductive capability was assessed. There was a significant delay in balano-preputial separation (BPS), a marker of puberty, in the offspring of the 1000 ng/kg dose group. Although body weight on PND21 had a borderline significant effect on delay on BPS; adjusting for reduced bodyweight as a covariate did not materially affect the differences between the treatment groups. Therefore, there was no evidence that the delay in BPS was related to reduced body weight.

20. Seminology was assessed on PND70 and 120. A small but statistically significant increase in sperm count was observed at PND120 in the 200 and 1000 ng/kg dose group; however, this was not seen at PND70, was within the range of historical control data, and was not reflected in testicular sperm counts. The proportion of abnormal sperm was elevated at PND70, particularly in the high dose group, although this was not seen at PND 120.

Sub-Chronic Study

21. In the sub-chronic dosing study, groups of 75 control (diet alone) or 65 (diet with 28, 93 or 530 ng TCDD/kg diet) female Wistar(Han) rats were provided with respective diets *ad libitum*. These dietary levels equated to 2.4, 8 and 46 ng/kg bw/day. Doses were selected to give hepatic TCDD concentrations approximately equivalent to the acute study, as determined by extrapolation from Hurst's toxicokinetic data [12,13]. Dosing continued for 12 weeks pre-mating (to reach steady-state), throughout mating and pregnancy, and stopped after parturition. Tissue TCDD concentrations were compared on weeks 10 and 12 in the conditioning period and on GD16 and 21, which showed that the animals had reached equilibrium. Hepatic TCDD concentrations on GD16 were approximately 50% of the acute concentrations and covered a 10-fold range in total body burdens; thus making the acute and sub-chronic studies comparable[a].

[a] The tissue levels, and body burdens are not quoted in this statement, so as to not prejudice subsequent publication of these results. The Committee was provided with this information in confidence during their deliberations.

22. During the study, 8 dams experienced total litter loss in the high dose group, compared to 4 in each of the lower dose groups and 3 in the control group. The level of pup death was statistically significant in the high dose group.

23. A delay in BPS was statistically significant in all three dose groups following sub-chronic maternal administration, with the greatest delay apparent in the high dose group. The delay in BPS in treated groups remained significantly different from controls when both body weight and litter were considered as covariates.

24. As with the acute study there was no statistically significant effect on F1 sex ratio; and when reproductive capacity was assessed in male F1 offspring, no statistically significant effect was seen on mating parameters or on the sex ratio in F2 offspring. No statistically significant effects were observed when F1 offspring were subjected to a functional observational battery and learning tests, with the exception of a deficit in motor activity in the high dose group.

25. Seminology was assessed on PND70 and 120. Maternal exposure had no statistically significant effects upon these parameters, with the exception that the proportion of abnormal sperm was elevated at PND70 in the high dose group, although this was not seen at PND120.

Committee Discussion

26. The Committee discussed the relevance of the delay in BPS being greater during the sub-chronic study than in the acute study. It was questioned whether this might be evidence that increased lactational exposure in the sub-chronic study was contributing to the greater delay; however, it was considered possible that exposure prior to GD15 may have increased the delay. Members considered it would have been useful to have measured AGD, since this is now a routine technique in studies of this type. Similarly measurement of hormone levels, particularly testosterone, might have yielded insights into the delay in BPS; however, the Committee acknowledged that examination of hormonal endpoints had not been essential for the initial study objectives.

27. The delay in BPS was not clearly adverse since there were no changes in fecundity in F1 generation. However, a significant delay in puberty in animals treated at doses below lethality was a matter of concern. Members considered it possible that a functional deficit resulting from BPS may manifest itself as a subtle generational effect, or may not be apparent in the endpoints examined in this study.

28. The issue of variability amongst rat strains was discussed, since it is plausible that a strain that is less susceptible to the acute toxicity of dioxins might reveal an effect on androgen synthesis or spermatogenesis.

However, it was noted that the original studies by Mably *et al.* which demonstrated the potent effect on sperm count in Holtzman rats[9] could not be repeated by Ohsako *et al.* in the same strain[6]. Furthermore, the study by Faqi and colleagues observed a reduction in sperm counts in Wistar rats[10], a closely related strain to the Wistar Han rats used in the FSA funded study[14,15]. Therefore, strain differences in dioxin susceptibility to these effects were considered unlikely, although it was noted that these are out-bred strains and the potential for strain drift cannot be ignored.

Implications for the 2001 TDI

29. Previously, a LOAEL maternal body-burden of 33 ng/kg bw had been calculated for the Faqi study[10] using toxicokinetics data from Hurst *et al.*[13]. Uncertainty factors accounting for human variation in toxicokinetics (3.2) and the use of a LOAEL (3) were applied to yield a human maternal body burden. This was converted to a human maternal dietary intake using a bioavailability of 50% and a half-life of 7.5 years for TCDD. This resulted in a dietary intake of 1.7 pg/kg bw/day, which was rounded to a TDI of 2 pg/kg bw/day due to the various uncertainties in the risk assessment.

30. In the FSA funded sub-chronic study, delay to BPS was observed in the lowest dose group, hence this study also provides a LOAEL. The maternal steady-state body burden for this dose group was determined analytically[a] to be very similar to that calculated for the Faqi study[10], which was used in the 2001 risk assessment. Thus, the LOAEL body burden from the FSA funded study provides additional evidence that the current TDI of 2 pg/kg bw/day is protective for the developing male fetus.

31. Data considered during the 2001 risk assessment, indicated that GD16-21 represents a critical window of exposure in the rat. However, whilst delay in BPS was seen following acute dosing on GD15 in the FSA funded study; sub-chronic administration, at doses which gave rise to similar maternal and fetal body burdens, resulted in a greater delay in BPS. The difference in magnitude of the effect highlights uncertainty regarding the critical window of exposure in the rat.

32. The more pronounced delay in BPS in the sub-chronic study may be due to fetal exposure to the maternal body burden *in utero* prior to GD15, possible increased postnatal exposure prior to puberty, or a combination of the two. The 2001 risk assessment assumed that the effects on the reproductive system of the male offspring resulted from *in utero* exposure to the maternal TCDD body burden. However, if the critical window of exposure is post natal, the differences in toxicokinetics and relative onset of puberty between rats and humans are likely to affect the relative susceptibility of the two species. In the absence of robust data relating to the critical window of exposure, it is appropriate to assume that the effects occurred *in utero*, since basic modelling of rat and human TCDD toxicokinetics indicated that this would result in a more conservative risk assessment.

Committee Conclusions

33. Having reviewed the FSA funded study[14,15,16], we are content with the study design and are satisfied that the statistical power of the study, seminology and analytical data are robust.

34. We consider that the new study provides additional evidence that the current TDI of 2 pg/kg bw/day is protective for effects on the developing male fetus. Therefore, review of the TDI is not a priority on the basis of this study.

COT Statement 2007/02
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