Relationships between Tissue Levels of 2,3,7,8-Tetrachlorodibenzo-\(p\)-dioxin (TCDD), mRNAs, and Toxicity in the Developing Male Wistar(Han) Rat

David R. Bell,*1 Sally Clode,† Ming Qi Fan,* Alwyn Fernandes,§ Paul M. D. Foster,‡ Tao Jiang,* George Loizou,§ Alan MacNicoll,§ Brian G. Miller,|| Martin Rose,*† Lang Tran,|| and Shaun White*†

*School of Biology, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom; †Covance Laboratories, Ltd., Harrogate, North Yorkshire HG3 1PY, United Kingdom; ‡NIEHS, Research Triangle Park, North Carolina 27709; §Health & Safety Laboratory, Harpur Hill, Buxton, Derbyshire SK17 9JN, United Kingdom; ‖Central Science Laboratory, Environment, Food and Health, Sand Hutton, York YO41 1LZ, United Kingdom; and ||Institute of Occupational Medicine, Research Park North, Riccarton, Edinburgh EH14 4AP, United Kingdom

Received May 23, 2007; accepted July 5, 2007

We compared the effects of a single acute dose, or chronic fetal exposure, to 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin (TCDD) on the male reproductive system of the Wistar(Han) rat. Tissue samples were taken from dams on gestation day (GD)16 and GD21, and from offspring on postnatal days (PND)70 and 120. Steady-state concentration of TCDD was demonstrated in the chronic study; body burdens were comparable in both studies. Fetal TCDD concentrations were comparable after acute and chronic exposure, and demonstrate more potent toxicity after chronic versus acute dosing. In maternal liver, cytochrome P450 (CYP)1A1 and CYP1A2 RNA were induced. In fetus, there was induction of both CYP1A1 and CYP1A2 RNA at medium and high doses, but inadequate evidence for induction at low dose in either study. The low level induction of CYP1A1 RNA at low dose in fetus argues against AhR activation in fetus as a mechanism of toxicity of TCDD in causing delay in balanopreputial separation (BPS), and the greater induction of CYP1A1 RNA in PND70 offspring liver from chronically-dosed dams suggests that lactational transfer of TCDD is crucial to this toxicity. These data characterize the maternal and fetal disposition of TCDD, induction of CYP1A1 RNA as a measure of AhR activation, and suggest that lactational transfer of TCDD determines the difference in delay in BPS between the two studies.

Key Words: dioxin; sperm; developmental; toxicity; balanopreputial separation; puberty.

2,3,7,8-Tetrachlorodibenzo-\(p\)-dioxin (TCDD) is a ubiquitous toxin, and a prototypical representative of a series of chemicals which effect toxicity through a common mechanism, binding to the Ah receptor (Poland and Knutson, 1982). Much investigation has focused on the toxicity of TCDD, on the basis that other chemical congeners will show the same toxicity as TCDD, but with altered potency determined by their relative agonism of the Ah receptor, and pharmacokinetics (Haws et al., 2006; Van den Berg et al., 2006). We have investigated the effects of TCDD on the developing fetus after acute (Bell et al., in press-a) or chronic (Bell et al., in press-b) exposure, and have shown that maternal exposure to TCDD causes a delay in BPS in male offspring, which is more pronounced after chronic (vs. acute) exposure to TCDD. However, understanding of such data on the basis of administered dose is limited, and elucidation of the tissue concentration and the biological effects caused by that concentration of TCDD is required for understanding and extrapolation from these data.

In many studies, TCDD is given as a single acute dose, whereas exposure of the human population to TCDD arises from chronic exposure through the diet and from lactational transfer (COT, 2001; Fries, 1995). The pharmacokinetics of TCDD are complex with multiple uptake and elimination phases (Weber et al., 1993), and thus the disposition of TCDD at 24 h after a single dose on gestation day (GD)15 is likely to vary from steady state. There is induction of metabolism of TCDD during chronic exposure (Fries and Marrow, 1975), and it takes ~13 weeks to attain steady-state levels of tissue TCDD in the rat (Rose et al., 1976). This is important to understanding reported effects of TCDD that have a narrow temporal window of susceptibility (Ohshako et al., 2002), between GD15 and GD18. Indeed, there are clear differences in disposition at GD16 between acute and chronic dosing regimes that give similar concentration in liver tissue (Hurst et al., 2000a,b). Thus, it is possible that the dosing regimen (i.e., acute vs. chronic dosing protocol) may be a determinant of sensitivity for the toxic effects of TCDD.

We have directly compared the developmental toxicity of TCDD after chronic administration (Bell et al., in press-b) with acute administration of a single dose on GD15 (Bell et al., in press-a): surprisingly, chronic administration yielded delay in balanopreputial separation (BPS) at all three dose groups, whereas only the highest dose group yielded a delay in BPS in
the acute dose study; this finding was unexpected, since the chronic dosing study was designed to give similar body burdens of TCDD to those seen in the acute dose study. This experimental system is complicated by the postparturition delivery of TCDD via milk (Korte et al., 1990; Moore et al., 1976; Nau et al., 1986). The majority of TCDD in offspring of dosed dams arises from lactational transfer of TCDD (Hurst et al., 2000a; Li et al., 1995), and there is evidence that chronic dosing protocols can result in higher TCDD delivery to the offspring (Korte et al., 1992). Indeed, it has been shown that lactational transfer of TCDD is responsible for the thyroid toxicity seen in Holtzmann rats (Nishimura et al., 2003, 2005, 2006). There are multiple explanations for the greater potency of TCDD for inducing delay in BPS by chronic (as opposed to acute) dosing, and these include (1) that there is a higher concentration of TCDD in the fetus after chronic versus acute dosing, (2) that there is a window of sensitivity of the fetus to TCDD’s effect before GD15, and (3) that there is greater lactational transfer of TCDD to the pups after lactational dosing, and that the lactational transfer of TCDD results in the delay in BPS. It is therefore important to measure the concentration of TCDD in relevant organs, and to determine the biological effects of TCDD using a suitable marker gene, such as CYP1A1 (Vandenheuvel et al., 1994).

In a direct comparison of the effects of acute and chronic administration of TCDD (Bell et al., in press-a, in press-b), we have shown that a single acute dose of TCDD to the pregnant CRL:WI(Han) rat on GD15 causes a delay in BPS in offspring after a dose of 1000 ng TCDD/kg, whereas chronic administration of 2.4 ng TCDD/kg/day yielded a significant delay in puberty in offspring. In order to relate the administered dose of TCDD with the retained tissue levels of TCDD, and consequently the biological effects seen in these studies, we have undertaken analysis of rat tissues for concentration of TCDD. Reverse transcription–PCR (RT-PCR) analysis was undertaken as a sensitive means of measuring biological effects of TCDD exposure, i.e., activation of the Ah receptor, by looking at induction of sensitive marker genes. The CYP1A1 gene has been shown to be highly responsive to TCDD (Vandenheuvel et al., 1994), and the CYP1A2 gene was examined because of its inducibility by TCDD and its role in hepatic sequestration of TCDD (Poland et al., 1989a,b).

MATERIALS AND METHODS

Materials

TCDD was obtained from Cambridge Isotope Laboratories, MA, and purity (99%) was verified by high-resolution mass spectrometry (MS). All other chemicals were also of the highest quality available and were checked for contamination by dioxins and PCBs as appropriate.

Animal Study

The animal studies were performed at Covance (Harrogate, UK), and were Good Laboratory Practice compliant; the full data set is published in Bell et al. (in press-a, in press-b). CRL-WI(Han) rats were provided food (SQC rat and mouse breeder diet No. 3, expanded; Special Diets Services, Ltd., Witham, Essex, UK) and water ad libitum, and were housed singly (the parental generation postpairing), or in groups of five for the parental generation prepairing and the F1 generation. Briefly, the dosing schedules are shown schematically in Figure 1, and were as follows.

Acute. Animals of 16–18 weeks of age (204–294 g) were time mated, with the day after mating designated as day 0 of gestation (GD0), delivered to Covance by GD9, and assigned to treatment groups on GD12 using a randomization procedure based on body weight. Seventy-five animals were treated with control vehicle (corn oil) by oral gavage, and 55 animals with...
50, 200, and 1000 ng TCDD/kg body weight on GD15; the concentration of TCDD in the dosing vehicle was verified by gas chromatography–MS (GC–MS) (104.6–106.1% of target concentration). Twenty-five vehicle-treated rats and 15 TCDD-treated rats were killed on GD16 and GD21 for tissue sampling prior to TCDD analysis and messenger RNA analysis; the remaining females were allowed to litter and rear their offspring until weaning (postnatal day [PND] 21). For the control animals, tissues were pooled from five animals, and five samples of pooled tissue were analyzed. In the TCDD-treated groups, blood samples were pooled from five animals, and three pools analyzed; adipose and liver tissue from five individual animals were analyzed, except for the high dose group fat on GD16, where six samples were analyzed. Fetuses from five individual animals were pooled and analyzed. Where possible, blood, fat, fetus, and liver were analyzed from the same animal; animals were selected randomly for sampling.

Chronic. Animals of 5–6 weeks of age (100–146 g) were assigned to treatment groups using a randomization procedure based on body weight. Animals (75, 65, 65, and 65, respectively) were provided with diet containing 0, 28, 93, and 530 ng TCDD/kg diet (the TCDD was dissolved in acetone, mixed with the feed, and then acetone evaporated by air drying) ad libitum. After 12 weeks of treatment of the parental females, one female was housed with one untreated male for up to 15 days, and mating confirmed by a vaginal plug. The concentration of TCDD in the diet was verified by GC–MS. Five and 10 animals per group were killed in weeks 10 and 12 after starting on the diet, and on GD16 and 21, 15 animals from the control group and 10 animals from the treated group were killed; tissue samples from these culls were used for TCDD and RNA analysis. TCDD treatment of the dams (and offspring) was discontinued after parturition. The remaining females were allowed to litter and rear their offspring until weaning (PND21) and killed on PND21. Litters were reduced to a maximum size of eight on PND4, and to five males (where possible) on PND21. For the control and treated animals, adipose, fetus, and liver tissues were obtained from five individual animals, and individual tissues were analyzed; the exceptions were analysis of six samples in medium dose group, GD21 adipose, and GD16 liver, and GD21 high-dose group, for fetus and fat. Blood samples were pooled from five animals, and three pools analyzed for all dose groups. Fetuses from five individual animals were pooled and analyzed.

Thereafter, males were then maintained untreated, until killed (25 per group) at PND70, and all remaining animals at PND120. Although kill days are referred to as PND70 and 120, the number of animals involved required that the kills were conducted during postnatal weeks 10 and 17.

TCDD Analysis

Tissue TCDD levels were determined on GD16 and 21, since there was a prior expectation that GD16 would reflect a period of sensitivity of the fetus to TCDD (Gray et al., 1995; Ohsako et al., 2002), and GD21 would reflect on accumulation of TCDD in the fetus through pregnancy. The maternal liver and adipose tissue were sampled, as TCDD is known to accumulate in these two organs, and fetal tissue was taken as the presumed site of action of the TCDD. Samples were stored frozen until analyzed. Adipose tissue and liver samples were analyzed individually and fetus samples from individual females were combined, but the volumes of blood samples were too low for individual analysis, and were pooled. The tissue samples were homogenized (pulping for fetus or liver, chopping for adipose), and an aliquot was taken for analysis. Sample aliquots were fortified with 13C-labeled dioxins, and exhaustively extracted using mixed solvents. The extracts were initially purified by acid hydrolysis, fractionated on activated carbon, and further purified using adsorption chromatography, on alumina. The eluent was concentrated under nitrogen and sensitivity standardized for measurement using additional 13C-labeled dioxins. TCDD was measured using high-resolution GC with high-resolution mass spectrometric detection at a resolution of ~10,000 (defined at 10% of peak height). Instrument performance was monitored during the measurement interval by the use of a calibrant (perfluorokerosene) lock mass and ions corresponding to native and 13C-labeled dioxins were recorded. Data were processed using Masslynx and Microsoft Excel software to provide tissue concentration data. The analytical data met published acceptance criteria (Ambidge et al., 1990) for dioxins. The method used is accredited to the ISO17025 standard and has been validated and published after peer review (Fernandes et al., 2004). Each batch of samples analyzed incorporated a full reagent blank, and analytical results were validated by the analysis of an in-batch reference material (RM) (Maier et al., 1995), for which results were compared with certified or assigned data. The contribution from the batch blanks was found to be negligible and quality standards were always met or exceeded.

Essentially, the same procedure was used for total polychlorinated dibenzo-p-dioxin and furan World Health Organization TCDD toxic equivalent (WHO-TEQ) (Van den Berg et al., 1998) measurements where these were performed. The 13C-labeled mix described above provides internal standardization for all 17 laterally substituted PolyChlorinated DibenzoDioxins (PCDDs) and PolyChlorinated DibenzoFurans (PCDFs) that contribute to the WHO-TEQ (Van den Berg et al., 1998) and ions corresponding to these compounds as well as native PCDDs and PCDFs were recorded and measured using the same instrument parameters as used for TCDD measurement. WHO-TEQ values were calculated by multiplying the measured concentration of each of the 17 compounds by the appropriate WHO TEF (Van den Berg et al., 1998) and summed to give S WHO-TEQ. The methodology, including quality criteria, is described in Fernandes et al. (2004).

The tissue TCDD concentration from one sample (acute study GD16 fat from animal 200, high dose group) had a TCDD concentration at ~1% of the value of other adipose tissues from the group, and was held to be anomalous. A further determination was performed on adipose tissue from animal 199, and these data were used instead. Four samples were rechecked; for liver and fetuses, duplicate determinations were within 5% of the original value. For adipose tissue, where the sample is chopped, duplicates were 115 and 130% of the first determinations.

Calculation of tissue TCDD burden. The average tissue weights (for liver, fetus) from the appropriate kill point were determined by weighing, and adipose tissue was estimated from the data of Bailey et al. (1980). The average weights were used to determine the mean tissue burden, and the standard deviation (SD) is shown as the corresponding SD from the TCDD determination. The whole-body burden is calculated from summation of the adipose, liver, and fetus TCDD burden, and an indicative estimate of variation, which is the mean multiplied by the coefficient of variation of the TCDD analyses in the acute (36%) and chronic (27%) studies, respectively, is shown. The apparent half-life of TCDD in the chronic study was calculated on the basis that animals were at steady-state body burden of TCDD; under this assumption, TCDD excretion equals the rate of TCDD consumption, which is known. Apparent half-life is then deduced by solving $C_t = C_0e^{-kt}$. The proportion of TCDD dose disposing to tissues was calculated as follows. The average TCDD concentration in a given tissue at high dose was set as 100%, and the average concentration at low and medium doses was expressed as a percentage of the high dose concentration. The medium and low doses were expressed as a percentage of the maximum dose (i.e., 5 and 20% for the acute study, 5.2 and 17.3% for the chronic study). The tissue concentration (as a percentage of maximum concentration) was divided by the dose (as a percentage of maximum dose) to give a ratio, which represents the relative proportion of TCDD dose disposing to a given tissue with dose.

RNA and RT-PCR analysis. Total RNA was extracted from liver or fetus using the Absolutely RNA Minipur Kit (Stratagene 1101 CB Amsterdam Zuidoost, The Netherlands). A total of 20–25 mg of frozen tissue was transferred to 0.4 ml of lysis buffer and homogenized at room temperature using a minihomogenizer (Kontes Pellet Pestle Fisher Scientific UK Ltd, Loughborough, UK). The resulting homogenates were stored at ~80°C, prior to isolation and treatment with DNase I according to manufacturer’s instruction. The purified RNA was stored at ~-80°C. The quality of the RNA was assessed by electrophoresis on 1% denaturing agarose gel based on the integrity of the 28S and 18S bands after ethidium bromide staining. Total RNA was determined by measurement of a fluorescent RNA–binding probe, RiboGreen.
time PCR reactions were one cycle at 95°C for 20 s, and 58°C for 90 s. All PCR reactions were performed in 12.5 µl of buffer solution containing the following: 6.25 µl of 2 × master mix; 100–300 nM of starter probes; 100–300 nM of each 5' fluorescent tag at the 5' end and fluorescence quencher Black-Hole at the 3' end. The specificity of CYP1A1 primers was confirmed by sequencing its RT-PCR amplicon. Real-time PCR (RT-PCR) was performed using Mx3005P instrument (Stratagene). Initial characterization showed little or no primer-dimer or genomic amplification was detected and all amplicons were at the size expected. Multiplex real-time PCR was performed using Brilliant Multiplex QPCR Master Mix (Stratagene) according to manufacturer’s protocol with slight modification. Briefly, multiplex PCRs were set up in a total volume of 12.5 µl of buffer solution containing the following: 6.25 µl of 2 × master mix; 100–300 nM of starter probes; 100–300 nM of each 5’ and 3’ of starter primer pairs for CYP1A1, AhR, and β-actin (combination 1) or for CYP1A2 and β-actin (combination 2); and 5 ng of cDNA. The conditions for the multiplex real-time PCR reactions were one cycle at 95°C for 10 min, followed by 40 cycles at 95°C for 20 s, and 58°C for 90 s. All PCR reactions were performed in duplicate. Negative controls were processed in the same manner, except that the no-RT product was used or the template was omitted. In order to determine the real-time PCR efficiencies of both targets and reference genes, a standard curve was generated using fivefold serial dilution of the cDNA produced from a TCDD-treated rat liver. The PCR efficiencies varied from 90 to 115% (data not shown). In each experiment, an independent control sample was run side by side with the samples, and the Ct was used as a calibrator to determine the relative quantification of a gene in each sample assayed, and samples were normalized to input cDNA quantity. Amplification efficiency was used to determine copy number (Pfaffl, 2001):

\[
\text{Change of copy number} = E^{\Delta C_{t} (\text{control-sample})}
\]

where \( E \) is the predetermined PCR efficiency for each transcript; \( \Delta C_{t} \) (control-sample) is the \( C_{t} \) of the external control transcript minus sample. All data were presented as percent of the control group.

**Statistical Analysis**

TCDD concentrations in tissues were summarized as mean ± SD. Differences in TCDD concentrations between dose groups were analyzed by analysis of variance (ANOVA) on the log scale. The RNA levels were compared by ANOVA of the logs of the values, followed by \( t \)-test to compare means with a common control; \( p < 0.05 \) was considered significant. Because of the small numbers of samples, no allowance was made for litter differences.

**RESULTS**

All dioxin analysis was performed with negative control (reagent blanks) and positive control (certified RMs) samples; all of which were within acceptable limits. Limits of detection varied between analyses and analytes, but were approximately 0.03 ng TCDD/kg of analyte, depending on available sample

---

**TABLE 1**

Sequence of Oligonucleotides Used in RT-PCR

<table>
<thead>
<tr>
<th>Gene name primer</th>
<th>Sequence</th>
<th>GenBank accession number</th>
<th>Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1 Primer (f)</td>
<td>CCACGAGACCATAAGAGATACAAG</td>
<td>X00469</td>
<td>FAM-BH1</td>
</tr>
<tr>
<td>Primer (r)</td>
<td>CCGGAATCTGTTGGATCACC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probe</td>
<td>ATAGTTCCCTGCTGATGTAAGCTGACAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP1A2 Primer (f)</td>
<td>ACCATCCACCCACAGTACA</td>
<td>K02422</td>
<td>CY5-BH3</td>
</tr>
<tr>
<td>Primer (r)</td>
<td>GITTGACCTGCCATGTTTAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probe</td>
<td>ACATTCACAGGAGGCCTGAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AhR Primer (f)</td>
<td>GCAGCTTATCTGCTGCTACA</td>
<td>A082124</td>
<td>HEX-BH1</td>
</tr>
<tr>
<td>Primer (r)</td>
<td>CATGCGCTTCTTTCACGCTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probe</td>
<td>TACATGTTTACGCGCCGCTGAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Actin Primer (f)</td>
<td>CTGACAGGATGAGAAGGAG</td>
<td>V01217</td>
<td>ROX-BH2</td>
</tr>
<tr>
<td>Primer (r)</td>
<td>GATAGAGCCACAATCCACA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probe</td>
<td>CAAAGATCATTTGCCCTGAGCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH Primer (f)</td>
<td>GTATCCGACGCCTGTTAC</td>
<td>Ab017801</td>
<td>ROX-BH2</td>
</tr>
<tr>
<td>Primer (r)</td>
<td>ACTGGAACATGTAGACCATGTTTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probe</td>
<td>TTGCAATCAACGACCCCTTCA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note. The sequence of primers used for qPCR is shown from 5' to 3'. Forward (f) and reverse (r) primers, and probe sequence, are indicated. The genbank accession number from which these sequences are derived is indicated, and the gene name is given. The labels for the probe are abbreviated: BHx, Black-Hole quencher 1-3; FAM, iscarboxy fluorescein; CY5,Cy5 dye; Hex, hexachlorofluorescein; ROX, 5(6)-carboxy-X-rhodamine.*
weight and the levels of TCDD observed in reagent blanks. Measurement uncertainty was determined at an analyte concentration of 1 ng/kg to be approximately 20%. The experimental schedule for the acute and chronic dosing protocols is outlined in Figure 1.

**Acute Study**

TCDD was administered as a single dose on GD15, and the concentration of TCDD at GD16 and 21 in maternal and fetal tissues is shown in Table 3. The coefficient of variation in these samples was 36% of mean values (Table 3); the variation seen in these biological samples was consistently higher than that seen with reference samples (data not shown). However, upon repeated assay of liver or fetus samples, the reproducibility was within 10% (A.F., S.W., M.R., unpublished data), suggesting that the observed variability is due to interanimal differences in pharmacokinetics of TCDD. The TCDD analytical method was sufficiently sensitive to accurately quantify TCDD levels in control tissues (Table 3). The concentration of TCDD in control tissues was above the limit of quantitation, and was at least 40 times below the levels in the corresponding tissue from the lowest dose group, 50 ng TCDD/kg. ANOVA demonstrated that there was a statistically significant difference in concentrations in liver: adipose tissue was approximately equal to blood, and blood was approximately equal to maternal tissues (data not shown).

**GD16**

Adipose tissue and liver showed higher concentrations of TCDD than fetus or blood, and although the ratio of TCDD concentrations in maternal tissues roughly paralleled that in control animals, the relative concentration of TCDD in liver increased with increasing dose (Table 3). Estimation of the total body burden of TCDD as the sum of adipose, liver, and fetus (Table 5) shows that ~38.3, 43.8, and 42.6% of the administered TCDD has been taken up into these organs at low, medium, and high dose, respectively.

**GD21**

At GD21, total body burden of TCDD was 39.5, 33.2, and 37.5% of the administered dose, respectively (Table 5). Compared with GD16 data, liver concentrations had reduced, and adipose tissue concentrations had doubled (Table 3). Although fetal concentrations of TCDD had remained at ~90% of the

### Table 2

<table>
<thead>
<tr>
<th>Tissue WHO-TEQ concentration (ng/kg)</th>
<th>GD16</th>
<th>GD21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose</td>
<td>1.93 ± 0.199</td>
<td>1.7 ± 0.319</td>
</tr>
<tr>
<td>Fetus</td>
<td>0.28 ± 0.079</td>
<td>0.08 ± 0.0</td>
</tr>
<tr>
<td>Liver</td>
<td>0.44 ± 0.106</td>
<td>0.43 ± 0.081</td>
</tr>
</tbody>
</table>

**Note.** Animals of 5–6 weeks of age were provided with diet that had been treated with acetone control vehicle (see Supplementary Materials) ad libitum, *i.e.*, this is the control group. After 12 weeks of treatment, animals were mated and mating confirmed by a vaginal plug. Fifteen animals per group were killed on GD16 and GD21 (as indicated in the table), and tissues were analyzed from individual animals (*n* = 5). Results are presented as mean ± SD.

### Table 3

<table>
<thead>
<tr>
<th>Tissue</th>
<th>TCDD Concentration in Maternal Tissue and Fetus after a Single Dose on GD15</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tissue TCDD concentration (ng/kg) (ng per tissue)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>GD16</td>
<td>Adipose</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>Fetus</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>GD21</td>
<td>Adipose</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
</tr>
<tr>
<td></td>
<td>Fetus</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
</tr>
</tbody>
</table>

**Note.** Animals of 16–18 weeks of age were time mated, with the day after mating designated as day 0 of gestation (GD0). Seventy-five animals were treated with control vehicle (corn oil) by oral gavage, and 55 animals with 50, 200, and 1000 ng TCDD/kg body weight on GD15; the concentration of TCDD in the dosing vehicle was verified by GC–MS (104.6–106.1% of target concentration). Twenty-five vehicle-treated rats and 15 TCDD-treated rats were killed on GD16 and GD21 for tissue sampling prior to TCDD analysis. For the control animals, tissues were pooled from five animals, and five samples of pooled tissue were analyzed. In the TCDD-treated groups, blood samples were pooled from five animals, and three pools analyzed; adipose and liver tissue from five individual animals were analyzed. The adipose, blood, and liver are maternal tissues. Fetuses from five individual animals were pooled and analyzed. The exceptions, where six animals per group are analyzed, are set out in “Materials and Methods” section. Liver and fetus weight were directly determined, and adipose tissue calculated relative to body weight (as described in “Materials and Methods” section) in the calculation of amount of TCDD per tissue. Results are presented as mean ± SD. The tissue concentrations are shown in ng/kg, and the ng per tissue are shown in brackets.
levels on GD16, the increase in fetal mass resulted in fetal TCDD burden increasing by ~eightfold between GD16 and 21.

**Chronic Study**

The coefficient of variation for TCDD determinations on tissue samples in this study was 27%. TCDD concentrations in control animal tissues were comparable with those seen in the acute study (Table 4), and were substantially less than those in the lowest dose group; however, some blood and fetus samples showed marginally higher levels or limits of detection due to the prevailing measurement conditions such as the weight of available sample and the concurrent solvent blanks. In order to

**TABLE 4**

TCDD Concentration in Maternal Tissue and Fetus after Chronic Dosing

<table>
<thead>
<tr>
<th>Tissue TCDD concentration (ng/kg) (ng per tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose of TCDD (ng/kg/day)</td>
</tr>
<tr>
<td>---------------------------</td>
</tr>
<tr>
<td><strong>Week 10</strong></td>
</tr>
<tr>
<td>Adipose NP</td>
</tr>
<tr>
<td>Liver NP</td>
</tr>
<tr>
<td><strong>Week 12</strong></td>
</tr>
<tr>
<td>Adipose NP</td>
</tr>
<tr>
<td>Liver NP</td>
</tr>
</tbody>
</table>

**Note.** Animals of 5–6 weeks of age (100–146 g) were provided with diet containing 0, 28, 93, and 530 ng TCDD/kg diet (the TCDD was dissolved in acetone) *ad libitum*. After 12 weeks of treatment of the P females, one female was housed with one untreated male for up to 15 days, and mating confirmed by a vaginal plug. Five and 10 animals per group (treated groups only) were killed in weeks 10 and 12 after starting on the diet, and on GD16 and 21. Animals from the control group and 10 animals from the treated group were killed; tissue samples from these culls were used for TCDD analysis. For the control and treated animals, adipose, fetus, and liver tissues were obtained from five individual animals, and individual tissues were analyzed. Blood samples were pooled from five animals, and three pools analyzed for all dose groups. Fetuses from five individual animals were pooled and analyzed. The exceptions, where six animals per group are analyzed, are set out in "Materials and Methods" section. Liver and fetus weight were directly determined, and adipose tissue calculated relative to body weight (as described in "Materials and Methods" section) in the calculation of amount of TCDD per tissue. Results are presented as mean ± SD. The tissue concentrations are shown in ng/kg, and the ng per tissue are shown in brackets. NP = no analysis performed.
be certain that control tissue levels of dioxin and dioxin-like compounds were below the level of the TCDD-treated groups, the levels of TEQ (Van den Berg et al., 1998) were determined (Table 2). These data demonstrate that the level of TEQ in the control rat liver and adipose tissue is orders of magnitude below the levels of TCDD seen in the lowest dose group. The chronic study dose levels were designed to give approximately the same hepatic level of TCDD as seen in the acute study; the hepatic levels of TCDD in the chronic study were bracketed by the levels of hepatic TCDD at GD16 and 21 in the acute study (Table 3). The chronic dosing study was designed to achieve steady-state burdens of TCDD with a 12-week feeding period, before mating of the animals. Comparison of tissue concentrations of TCDD in animals in weeks 10, 12 and GD16 and 21, shows that both adipose tissue and liver TCDD concentrations were at equilibrium (Table 4); however, body weight increases over this time period, and since the body fat was calculated on a metric where fat increases with body weight (Bailey et al., 1980), this resulted in a higher total body burden of TCDD, expressed both as an amount, and in ng/kg (Table 5). ANOVA demonstrated that there was a statistically significant difference between dose groups in the level of TCDD for all tissues (data not shown), with the exception of hepatic TCDD as a result of the decrease in liver mass accounted for ~5% of total body burden of TCDD. The fetal TCDD concentrations had approximately doubled by comparison to GD16, and with increased fetal mass, the total burden of TCDD in the fetal compartment had risen by ~20-fold compared to GD16 (Table 4).

**Fetal Disposition of TCDD and Relationship with BPS**

The ratio of fetal TCDD concentration to maternal body weight was plotted against maternal body weight for both acute and chronic studies (Fig. 2A). At GD16, this ratio was two- to threefold lower after chronic administration of TCDD, compared with acute administration. At GD21, the ratio was close to 1.0, indicating that the ratio was constant with treatment, although the treated samples from the acute study, but high dose (46 ng/kg/day) required for induction of CYP1A2 in the chronic study, the CYP1A2 values for GD16 and GD21 were much closer, and the TCDD concentrations were similar. The threshold doses for induction of CYP1A2 were distinct in the two studies, with low dose (50 ng/kg) giving a ~threefold increase in the acute study, but high dose (46 ng/kg/day) required for induction in the chronic dosing study, i.e., a liver concentration of TCDD that is ~30-fold higher to achieve similar induction of CYP1A2. CYP1A1 was a much more sensitive indicator of treatment with TCDD, with statistically significant induction, and the lowest exposure groups giving >10-fold induction of CYP1A1 (Fig. 3D), at ~100–300 ng TCDD/kg liver. In the acute study, the CYP1A1 levels at GD16 were consistently lower than GD21 values, in spite of the hepatic TCDD concentrations being three- to fivefold higher at GD16 than at GD21. There was a similar trend to lower CYP1A1 RNA at GD16 than GD21 in the chronic study, but hepatic TCDD concentrations were approximately equal.

**RT-PCR Analysis**

RT-PCR was established with primers and an internal fluorescent probe, the Taqman methodology, for enhanced specificity (Table 1); the primer probe sets were validated to be resistant to contamination by genomic DNA (data not shown). Assays were run in the presence of a no template control, which routinely produced C, values of > 40, demonstrating an absence of contaminating cDNA or cross-reacting genomic DNA.

Figure 3 shows the analysis of β-actin, AhR, CYP1A2, and CYP1A1 RNA from maternal liver at GD16 and 21 from the two studies: CYP1A1 and CYP1A2 are assayed (Fig. 3B). Figure 3C shows that there was statistically significant induction of CYP1A2 RNA and that, in the acute study, the GD16 values for induction of CYP1A2 were lower than the values for GD21, in spite of the higher TCDD concentrations at GD16; whereas in the chronic study, the CYP1A2 values for GD16 and GD21 were much closer, and the TCDD concentrations were similar. The threshold doses for induction of CYP1A2 were distinct in the two studies, with low dose (50 ng/kg) giving a ~threefold increase in the acute study, but high dose (46 ng/kg/day) required for induction in the chronic dosing study, i.e., a liver concentration of TCDD that is ~30-fold higher to achieve similar induction of CYP1A2. CYP1A1 was a much more sensitive indicator of treatment with TCDD, with statistically significant induction, and the lowest exposure groups giving >10-fold induction of CYP1A1 (Fig. 3D), at ~100–300 ng TCDD/kg liver. In the acute study, the CYP1A1 levels at GD16 were consistently lower than GD21 values, in spite of the hepatic TCDD concentrations being three- to fivefold higher at GD16 than at GD21. There was a similar trend to lower CYP1A1 RNA at GD16 than GD21 in the chronic study, but hepatic TCDD concentrations were approximately equal.
Given that TCDD was administered on GD15, it is likely that the GD16 values in the acute study did not represent an organ at equilibrium in the TCDD induction response, but the comparison between GD16 and 21 values in the chronic study should have been at equilibrium. However, it is clear from considering liver weights that the liver was enlarged at GD16 (Bell et al., in press-a, in press-b; Weitman et al., 1986), but that the liver weight has decreased by ~15% on GD21; thus it is likely that the physiological functionality of the liver may be different at these two times.

Fetal RNA analysis used samples of RNA from total fetus at GD16, but fetal liver from GD21; note that TCDD analysis was on fetus at both time points. Figures 4A and 4B show that β-actin RNA and AhR RNA stayed relatively constant with no statistically significant effect of TCDD dosing, under both dosing regimes. However, while the GD16 total fetus samples showed no effect of TCDD on CYP1A2, the GD21 fetal liver samples showed a strong induction effect, with > 20-fold induction of CYP1A2 at medium dose, rising to > 1000-fold induction at high dose; thus CYP1A2 was more inducible in fetal liver (compared to maternal liver), and there was little difference between chronic and acute dosing schedules. The CYP1A1 RNA also showed statistically significant and high inducibility, with total fetus showing > 100-fold induction on GD16 in acute and chronic studies (tissue concentrations of 45 and 27 ng TCDD/kg, respectively). At GD21 in fetal liver, high levels of induction of CYP1A1 were detectable at the medium dose level, and the high-dose induction was > 1000-fold. However, induction of CYP1A1 RNA in fetal liver was not statistically significant at low-dose level after either chronic or acute dosing.

**FIG. 2.** Role of fetal TCDD concentration. (A) Dose dependency of fetal disposition in acute and chronic dosing. The fetal TCDD concentration was divided by the total maternal concentration, and plotted against maternal body concentration. Data points from the acute study are circles on a solid line, whereas the chronic study is represented by squares on a dashed line. GD16 points are in black, and GD21 data points are in white. Results are presented as mean ± SD; note that the SD is shown both for the ratios, and for TCDD concentration. (B) Correlation between fetal TCDD concentration and delay in BPS. Fetal TCDD concentration at GD16 (black symbols) or GD21 (white symbols) was plotted against days of developmental delay compared to control, after dosing with TCDD by acute (circles) or chronic (squares) dosing. Values are shown as mean and SD; note that the SD is shown for both parameters. (C) Delay in BPS (in days) is shown as the percentage decrease in the incidence rate of BPS (Bell et al., in press-a, in press-b), and plotted against the relative reduction in body weight on PND4. The relative reduction in body weight on PND 4 is derived by subtracting the natural log of the weight of the appropriate treatment group from the corresponding control group; an increasing value shows a greater weight reduction in a treated group. Acute study data points are circles, and chronic study data points are squares. Linear regression was undertaken with SigmaPlot, and the line of best fit is shown.
acute administration of TCDD (but note that \( n \) is small). There was clear evidence for induction of CYP1A1 RNA in fetus at the medium dose in both acute and chronic studies, showing that TCDD had activated the AhR signaling pathway in the fetus at these higher dose levels.

Figure 4E compares the induction of CYP1A1 RNA in maternal liver and fetal tissue, as a function of tissue TCDD concentration; this shows that fetal tissue had reached comparable levels of fold-induction as maternal liver, at lower concentrations of TCDD in the tissue.

RT-PCR analysis of RNAs in the PND70 rats showed that \( \beta \)-actin RNA (Fig. 5A), and AhR RNA (Fig. 5B), were not statistically significantly perturbed by maternal treatment. By contrast, CYP1A1 RNA levels were statistically significantly induced in medium and high-dose groups from the acute study, but at all three treatment groups in the chronic study; moreover, the fold-induction of CYP1A1 RNA was higher in the chronic study, compared to the acute study, for all three dose groups (Fig. 5C).

### DISCUSSION

Analysis of TCDD concentrations using high-resolution MS, with internal isotope-labeled standards, brings high specificity, sensitivity, and quality control methods to the determination of TCDD concentrations. This method enables detection of TCDD in samples from control animals, and thereby allows comparison of the TCDD burden in control and in treated animals, thereby showing that there were at least 40-fold higher concentrations of TCDD in the adipose or liver of the lowest dose group of the treated animals than in the controls (Tables 3, 4). Moreover, we undertook a direct determination of the WHO-TEQ burden, and Table 2 also shows that the levels of WHO-TEQ in the control animals from the chronic study are considerably lower than the TCDD levels in the lowest dose group. This clear separation of TCDD concentration in control, versus treated, tissues, is reflected in the marked induction of hepatic CYP1A1 RNA in the low-dose groups, compared to control (Fig. 3), at GD16 or 21, in both studies. This shows that a biological effect of TCDD, which is dependent upon activation of the AhR by ligand, is orders of magnitude above comparable levels in control animals in the low-dose groups.

The chronic dosing study (Bell et al., in press-b) was designed by extrapolation from (Hurst et al., 2000a,b) to achieve comparable concentrations of hepatic TCDD to those obtained on GD16 in the acute dose study (Table 3) (Bell et al., in press-a). Table 4 shows that the concentrations of hepatic
TCDD obtained are approximately 50% of those obtained in the acute study, and cover a ~10-fold range in total body burden of TCDD (Table 5). Thus, the dosing regimen yielded an appropriate range of tissue concentrations of TCDD for comparability to the acute study. An important criterion for the chronic dosing regimen was that the tissue concentrations of TCDD should be at equilibrium, and the dosing period of 12 weeks prior to mating was based on Rose et al. (1976).

Table 4 shows that the concentration of TCDD in the liver and adipose tissue does not change appreciably from week 10 of dosing through GD21 (i.e., ~5 weeks later), thus demonstrating that the tissue concentrations are at steady state. However, there are differences in total body burden of TCDD over this period, whether expressed as an amount, or as a concentration (Table 5). This is due to the increase in body mass and in relative organ mass of the animals over this time, and due to pregnancy; a limitation of our study is that the estimates for adipose tissue mass (Bailey et al., 1980) were based on data from Sprague–Dawley rats, and may not accurately represent the adipose depot in pregnant CRL:WI(Han) rats.

**FIG. 4.** RT-PCR analysis of fetal samples. Total fetus RNA (GD16), or fetal liver RNA (GD21), was analyzed by RT-PCR for β-actin (A), AhR (B), CYP1A2 (C), and CYP1A1 (D). This was performed as described in “Materials and Methods” section. (A), (B), (C), and (D) are presented as % of control. (A) and (B) are plotted against dose group, whereas (C) and (D) are plotted against the group mean TCDD concentration, and have error bars both for RNA level, and TCDD concentration. Acute dose samples are shown as circles, and chronic study samples are triangles; GD16 samples are shown by black symbols, and GD21 samples as white symbols. (E) Comparison of CYP1A1 induction and tissue TCDD concentration. CYP1A1 RNA is plotted as percent of control on a log scale, and TCDD concentration was determined as described in “Materials and Methods” section. Maternal values are with black symbols, whereas fetal samples are white; chronic GD21 samples are circles, and acute GD16 samples are squares. Samples are mean ± SD.
There are complications arising from the physiological consequences of pregnancy; for example, the food intake of pregnant rats increases by ~20%, resulting in a greater dose of TCDD when expressed on a ng/kg basis (Bell et al., in press-a, in press-b). A further issue is that the liver mass decreased by ~14% between GD16 and 21 (Bell et al., in press-a, in press-b; Weitman et al., 1986); the hepatic TCDD concentration remains unchanged during this period (Table 4), so this must result in ~5% of the total body burden of TCDD redistributing during this period. There is some evidence for altered hepatic functionality between GD16 and GD21, insofar as GD21 CYP1A1 levels are consistently higher than GD16 levels (Fig. 3D), even though TCDD concentrations are approximately equal; it is not clear what the basis of this effect is.

Disposition of TCDD into the fetus shows dose dependency, with a greater proportion of the dose reaching the fetus at lower doses of TCDD. It has been shown that both AhR and CYP1A2 null mice show enhanced disposition of TCDD to the fetus (Dragin et al., 2006; Thomae et al., 2004), showing that this phenomenon is dependent on both AhR and also the CYP1A2 gene. Figure 2A shows that the dosing regimen is a variable; after acute dosing with TCDD, the ratio of TCDD concentration in fetus: dam remains constant between GD16 and 21. However, this is a different pattern from the chronic dosing regimen, where GD16 ratios are approximately half the level at GD21. Acute and chronic dosing regimens thus result in dose- and time-dependent differences in the proportion of TCDD dose reaching the fetus. The absolute concentration of TCDD in the fetus doubled between GD16 and 21 in the chronic study (Table 4), whilst staying constant in the acute study (Table 3); this is particularly notable since fetal mass increases by ~10-fold during this period, and suggests that there is disposition of TCDD from lipid reserves into the fetus during this period of fetal growth.

The absolute fetal concentrations of TCDD in treated animals are > 30-fold below the corresponding level in maternal liver, but are 20-fold greater than the concentration of TCDD in control fetus. CYP1A1 and CYP1A2 RNA are not significantly induced at low dose in either study, but this is not due to fetal tissue being refractory to induction of CYP1A2 RNA; a plot of CYP1A1 RNA versus tissue TCDD concentration shows that equivalent fold-induction of CYP1A1 RNA is obtained at approximately 30-fold lower TCDD concentrations in fetus/fetal liver, as compared to maternal liver (Fig. 4E). The higher apparent potency of TCDD in fetus/fetal liver, as compared to adult liver, may be due to a lower
proportion of free TCDD in adult liver, as a consequence of greater biochemical sequestration of TCDD by CYP1A2 in adult liver (Poland et al., 1989a,b); the absolute levels of CYP1A2 RNA are \( \sim 10^4 \) lower in fetal liver than in maternal liver (Fig. 4, supplementary data). Vandenheuvel et al. (1994) have shown that a hepatic concentration of 2.5–21 ng TCDD/kg represents the limit of detection for induction of CYP1A1 RNA, consistent with our data, and Kawakami et al. (2006) have found a similar threshold for tissue TCDD concentration to induce CYP1A1 RNA in fetal liver on GD20. Both CYP1A1 and CYP1A2 are highly inducible (\( \sim 10^3 \)-fold) in fetal liver, whereas CYP1A2 shows much lower induction (10-fold) in maternal liver; this could be due to the lower basal levels of CYP1A2 in fetal, as compared to maternal, liver. While fetal TCDD concentrations are sufficient to activate AhR-mediated gene transcription of CYP1A1 and CYP1A2 in total fetus (GD16) or fetal liver (GD21) at medium and high doses, the evidence for AhR-mediated gene activation (CYP1A1/2) at low dose does not attain statistical significance; moreover, given that the acute study only showed toxicity (lethality, delay in BPS) at high dose, whereas the chronic study led to delay in puberty in all three dose groups, the pattern of AhR-mediated gene activation in fetus or fetal liver at GD16 and GD21 fails to correlate with these endpoints. The induction of CYP1A1 is regarded as one of the most sensitive endpoints of AhR activation (Vandenheuvel et al., 1994), and so the failure to detect significant and marked induction of CYP1A1 RNA in fetus at low dose levels argues against a toxic effect of TCDD mediated by AhR either between GD16 and GD21, or before GD16; however, it is not possible to exclude the possibilities either that there is a tissue within the fetus which accumulates TCDD to higher levels, or that there is an AhR responsive gene which is more sensitive to TCDD levels than CYP1A1, and which mediates toxicity.

Acute administration of TCDD on GD15 has much less effect on delay of BPS than chronic administration of TCDD (Bell et al., in press-a, in press-b) at doses that yield comparable concentrations of TCDD either in the fetus (Tables 3, 4), or in total maternal body burden (Table 5), and this finding demonstrates that TCDD is a more potent developmental toxin after chronic, as opposed to acute, administration. A plot of fetal TCDD against delay in puberty seen in the acute or chronic studies fails to correlate simply at either GD16 or 21, e.g., Figure 2B. This finding shows that the greater potency of TCDD after chronic administration is not due to enhanced disposition of TCDD to the fetus between GD15 and 21, and suggests that if the offspring have a window of enhanced susceptibility to TCDD, it is either prior to GD15 or after GD21. The fact that fetal CYP1A1 RNA is barely perturbed at low dose in fetus in both studies (Fig. 4) strongly suggests that the AhR has not been sufficiently activated to mediate a physiologically relevant response at GD16–21. However, a plot of relative decrease in body weight on PND4 versus delay in BPS (Fig. 2C) shows a correlation (\( r^2 \sim 0.9 \)) between these two variables. It is possible that the decrease in body weight, and subsequent delay in BPS, is due to lactational transfer of comparatively large amounts of TCDD. The disposition of TCDD from the diet into breast tissue (and milk) may not be accurately reflected in the abdominal adipose depot concentrations of TCDD, since dietary fats (and presumably, dietary TCDD) can be directly taken up by the mammary gland (Neville and Picciano, 1997); thus when comparing the dose of TCDD administered by lactation between the chronic and acute studies, this may not be simply reflected in the liver and abdominal adipose depot concentrations. This is important, since lactational transfer of TCDD accounts for the majority of pup TCDD after an acute dose of TCDD on GD18 (Li et al., 1995) or GD15 (Nishimura et al., 2005), and a chronic dosing regimen can achieve high concentrations of TCDD in the offspring (Hurst et al., 2000a; Korte et al., 1992). Further, TCDD causes hypothyroidism and hydropnephrosis in F1 rats via lactational transfer of TCDD (Nishimura et al., 2003, 2005, 2006). Thus it is not clear whether acute and chronic TCDD dosing regimens result in a different lactational delivery of TCDD to the offspring.

To examine the possibility that lactational transfer of TCDD to the pups was different under chronic and acute dosing regimens, we examined the levels of CYP1A1 in liver RNA of rats at PND70 (Fig. 5). This experimental design is inherently limited, since the pups were principally dosed through lactation, which finished on PND21; the time from PND21 to PND70 is threefold the apparent whole-body half-life of TCDD in the dams, without considering the dilution of TCDD caused by the \( \sim 90\% \) growth in body mass between PND21 and 70. Hence, TCDD concentrations would be expected to be considerably lower than at puberty, and the comparison between groups would be complicated by any dose-dependent decrease in half-life. There was no reliable correlation between day of puberty and CYP1A1 RNA abundance in either the acute or chronic study (data not shown). However, the induction of CYP1A1 RNA was significantly elevated above control values in all three dose groups in the chronic dosing study, whereas only the highest dose group showed an elevation above control in the acute dosing study (Fig. 5C). Since the induction of CYP1A1 is both AhR- and TCDD dependent, this suggests that the pups in the chronic dosing study received a higher dose of TCDD lactationally than the pups in the acute dosing study.

Although we have restricted our analysis in this paper principally to explaining the biology of delay in BPS, the information in Tables 3 and 4 also provides insight into maternal pharmacokinetics of TCDD, which is determinative of the fetal and pup exposure. The Supplementary Data contain analysis of the pharmacokinetics, and show that TCDD disposition is dependent on dose, and whether dosing is acute or chronic; that the proportion of the TCDD dose reaching extrarepithelial tissues increases with decreasing dose; and that the apparent half-life of TCDD is dose dependent.

In summary, we have characterized the concentration of TCDD and marker genes in tissue samples from acute and chronic dosing studies. Our data confirm that TCDD was
adequately dosed, that the chronic study animals were at steady state, and that the tissue TCDD concentrations in the chronic study were comparable to those in the acute dosing study. Whereas maternal CYP1A1 RNA is highly induced at all dose levels, the concentration of TCDD in fetus is insufficient to induce fetal CYP1A1 at the low-dose group in either study, suggesting that activation of the AhR in fetus is insufficient to account for the subsequent delay in puberty. In agreement with a role for lactational transfer of TCDD in delay in puberty, hepatic CYP1A1 RNA in PND70 males from the acute study were at higher levels than the corresponding group from the chronic study, showing that there was greater transfer of TCDD to pups during lactation in the chronic study. These results illustrate the complexity of pharmacokinetic and biological responses to TCDD, and are key to understanding, and interspecies extrapolation, of the toxicity of TCDD.

SUPPLEMENTARY DATA

Supplementary data are available online at https://toxsci.oxfordjournals.org/. FUNDING

U.K. Food Standards Agency contract (T01034).

ACKNOWLEDGMENTS

The authors wish to thank the Food Standards Agency, its expert reviewers (Professors G. Gibson, A.G. Renwick, and Dr A.G. Smith), and Peter R. Sinclair for helpful comments and guidance. We also wish to thank the reviewers and editors for advice.

REFERENCES


