

Lactational rather than *in utero* exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin caused hypothyroxinemia at weaning of Holtzman rats

J. Yonemoto,^{1,3} N. Nishimura,^{2,3} C. Yokoi,^{1,3} Y. Takeuchi,¹ and C. Tohyama^{1,2,3}

¹Endocrine Disruptors and Dioxin Research Project, and ²Environmental Health Sciences Division, National Institute for Environmental Studies (NIES), Tsukuba 305-8506, Japan; and ³CREST, Japan Science and Technology, Kawaguchi 332-0012, Japan

[Introduction] We previously reported that a low dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) given orally on gestational day 15 (GD15) caused a reduction in the serum T4 level in pups on PND21 but not on PND49 in Holtzman rats. T4 suppressing effect of TCDD has shown to be caused by the enhanced biliary excretion of T4-glucuronide, which is conjugated by UDP-glucuronosyltransferase (UGT-1), a TCDD inducible enzyme. We hypothesize that the effect of TCDD on T4 is reversible and that lactational exposure has more impact on T4 than *in utero* exposure since most of the TCDD in pups is transferred via lactation. In the present study, the relative impact of *in utero* versus lactational exposure to TCDD on serum T4 level was compared using cross-fostering protocol to test the hypothesis.

[Materials and Methods] Pregnant Holtzman rats were given an oral dose of 1000ng TCDD/kg bw on GD15 or an equivalent volume of corn oil as vehicle-control. Pregnant rats were allowed to deliver, and half of the treated litters and that of the control litters were cross-fostered on PND1, which resulted in the following groups: C/C (control), T/C (perinatal exposure), T/C (prenatal exposure only), and C/T (postnatal exposure only). Pups were sacrificed on PND21 and PND49. Body weight and organ weight were measured, and sera and tissues were collected. Serum levels of T4 and T3 were determined by RIA, and TSH was determined by EIA. Expression of mRNA of dioxin responsive genes, CYP1A1, UGT1A6 and UGT1A7 in the liver was determined semi-quantitatively by RT-PCR. The liver was stained immunohistochemically for CYP1A1 and UGT1A6.

[Results and Discussion] In male pups, the body weight in the T/C and T/T group was significantly smaller than that in the C/C group on PND21, but no difference was observed among groups on PND49. Serum total T4 level of male pups was significantly decreased in the C/T and T/T group but not in the T/C group compared to the C/C group on PND21, whereas, on PND49, no reduction in serum total T4 level in treatment groups but elevation in C/T group was observed. The result on the time-course of serum total T4 level of the pups perinatally exposed to TCDD was consistent with the result of our earlier study. The serum total T3 Level and TSH level of male pups in the treatment groups were not different from those of the C/C group both on PND21 and PND49. In female pups, the body weight was reduced only in the T/T group compared to C/C group on PND21, while no difference was observed among groups on PND49. Effects of the cross-fostering regimen on the serum total T4 level in female pups were almost the same as in male pups on PND21. There was no difference in total T4 level among groups on PND49 in female pups.

CYP1A1 mRNA expression was significantly elevated in the T/C, C/T and T/T groups in the livers of pups on PND21. The elevation was very prominent in the C/T and T/T groups. The mRNA expression of UGT1A6 and UGT1A7 was significantly elevated in the C/T and T/T groups but not in the T/C group compared to the C/C group on PND21. An induction of UGT1A6 and CYP1A1 in the liver of the C/T and T/T groups but only a trace in the T/C group was visualized immunohistochemically, supporting the molecular biological data on PND21.

In conclusion, the T4-suppressing effects of TCDD on PND21 were mainly due to lactational exposure and this effects were suggested to be caused by the enhanced biliary excretion of T4-glucuronide through induction of UGT-1.

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