# KINETIC PROFILE OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN IN PREGNANT AND LACTATING RHESUS MONKEYS AND THEIR INFANTS

Shunichiro Kubota[1], Toshio Ihara[2], Satoru Oneda[2], Minoru Inouye[2], Masaki Sato[3], Takumi Takasuga[4], Mineo Yasuda[5], Toshio Fukusato[6], Hisae Hori[7], Motoyoshi Nomizu[8], Takashi Kobayashi[9], and Ryoichi Nagata[2]

[1]Department of Physiological Chemistry and Metabolism, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[2] Shin Nippon Biomedical Laboratories, Ltd., 2438 Miyanoura, Yoshida-cho, Kagoshima 891-1394, Japan
[3] Shin Nippon Biomedical Laboratories, Ltd., Pharmacokinetics and Bioanalysis Center, 216-1 Minamiakasaka, Kainan, Wakayama 642-0017, Japan
[4] Shimadzu-Techno-Research Inc., 1 Nishinokyo Shimoai-cho, Nakagyo-ku, Kyoto 604-8436, Japan
[5] Department of Health Sciences, Hiroshima International University, 555-36 Gakuendai, Kurose-cho, Kamo-gun, Hiroshima 724-0695, Japan
[6]Department of Pathology, Faculty of Medicine, Gunma University, Maebashi 371-0034, Japan.
[7]Department of Molecular Pathogenesis, Medical Res. Inst., Tokyo Medical and Dental University, Kanda Surugadai, Chiyoda-Ku, Tokyo 101-0062, Japan
[8]Division of Bioscience, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan.
[9]Department of Dermatology, Faculty of Medicine, Chiba University, Chiba 260-8670, Japan.

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) belongs to a class of chemicals known as polyhalogenated aromatic hydrocarbons including polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs). TCDD is the most toxic member of dioxins which are environmentally and biologically stable. Exposure to these dioxin-like compounds results in a wide variety of effects, including a wasting syndrome, immunological dysfunctions, chloracne, tetragenecity, and carcinogenesis, as well as developmental and reproductive dysfunctions [1-4]. Faqi et al. reported that exposure to low doses of TCDD throughout pregnancy and lactation elicited delay in vaginal opening and reduced uterine weight in F1 females. In the male offspring rats of the same dams exposed to the equal doses of TCDD, the number of sperm per cauda epididymis and daily sperm production were reduced in all TCDD groups at puberty and at adulthood[5]. Assessment of TCDD exposure on human health including reproductive functions requires more information concerning the distribution of TCDD in target organs, and toxicity of TCDD in non-human primates exposed to TCDD. Although considerable amount of information is available from studies in rodents[6-10], not much is known of the pharmocokinetics of TCDD in non-human primates[11, 12].

Considering the pronounced species differences observed in some studies on TCDD, pharmacokinetic studies using primates are required for the assessment of TCDD exposure on human health, especially on developmental and reproductive dysfunctions. The final goal of the present study is to determine whether developmental and reproductive functions are affected by a relatively low dose of TCDD. In the present study TCDD was subcutaneously administered to

pregnant rhesus monkeys on day 20 of pregnancy. Plasma TCDD levels on days 80 and 140 of pregnancy in dams were compared in abnormal delivery and normal delivery groups. $^{3}$H-TCDD was also subcutaneously administered to lactating rhesus monkeys on day 30 of delivery. Distribution of TCDD in the tissues of dams and infants were investigated by the measurement of TCDD concentrations in different tissues of dams and infants.

**Materials and Methods**

***Chemicals.*** 2,3,7,8-TCDD and $^{3}$H-2,3,7,8-TCDD (3.84 GBq/mg) dissolved in toluene and DMSO (1:2, v/v) were purchased from Kanto Chemicals and Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan), respectively.

***Animals.*** Rhesus monkeys were purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijing, China). All procedures involving animal care were in accord with institutional guidelines in compliance with national laws. The monkeys (5-7 years old and 5.3-6.7 kg in body weight) were kept in Shin Nippon Biomedical Laboratories, Ltd (Kagoshima, Japan). The breeding conditions, mating and diagnosis of pregnancy were described previously[13]. 2,3,7,8-TCDD (0, 30 and 300 ng /kg body weight) was subcutaneously administered to three groups of twenty pregnant monkeys on day 20 of pregnancy. Every 30 days, five percent of the initial doses of TCDD was subcutaneously administered as a maintenance dose. Abnormal delivery was observed in three groups; 2, 5 and 5 cases out of twenty animals, respectively. The levels of plasma TCDD on days 80 and 140 of pregnancy were compared with those in the normal delivery and abnormal delivery groups.

$^{3}$H-2,3,7,8-TCDD (30 or 300 ng/kg body weight) was subcutaneously administered to two groups of two pregnant monkeys on day 30 of delivery. After 7 days the radioactivity in the blood and tissues was counted.

***Measurement of TCDD in blood and tissues.*** TCDD in plasma was analyzed using HRGC-HRMS. The concentration of TCDD in the blood, plasma, mammary gland, placenta, liver, and fetal ovary or testis was measured. Tissues obtained from mother and fetus were homogenized and solubilized. After the addition of 10 ml of scintillation cocktail, samples were analyzed by scintillation counting. The concentrations of TCDD were expressed as pg eq/g or ml, and tissue/plasma ratio (T/P ratio).

**Results and Discussion**

Plasma TCDD levels on 80 and 140 day of pregnancy in dams were compared in normal delivery group and abnormal delivery group. The results are shown in Tables 1 and 2, respectively. The concentrations of TCDD were expressed as pg / g and pg/g fat. In normal delivery group, the concentrations of TCDD on 80 and 140 days after administration of 30 (n=5) and 300 (n=5) ng/kg weight TCDD were (321±130 and 188±73) and (1680±460 and 952±215) pg/ g fat, respectively, significantly higher compared to untreated control (undetectable level) (n=5). In abnormal delivery group the concentrations of TCDD on 80 and 140 days after administration of 30 (n=3) and 300 (n=2) ng/kg weight were (203±85 and 97±11) and (2,650 and 4,700) pg/ g fat, respectively. The number of abnormal delivery in control, 30 ng/kg group and 300 ng/kg one, was 2, 5 and 5 cases, respectively. As TCDD was reported to cause abortion when given early in pregnancy[12], it is possible that TCDD caused fetotoxicity and induced abnormal delivery in TCDD-treatment groups. However, further study is needed to confirm and clarify whether TCDD induced abnormal delivery.

$^{3}$H-2,3,7,8-TCDD (30 and 300 ng/kg) was subcutaneously administered to lactating rhesus monkeys on day 30 of delivery. Seven days after administration radioactivities of TCDD were measured in tissues of dams and infants transferred via milk. Table 3 summarizes the concentrations of TCDD in the blood, plasma, liver, mammary gland, fat ovary and testis of mothers or infants. In group 1 (1G)(n=2) administered with 30 ng/kg TCDD, the higher concentrations were observed in fat (mean level; 77.7 pg eq/g), liver (45.3 pg eq/g) and mammary gland (21.9 pg eq/g) of mothers. In infants the higher concentrations were observed in fat (55.1 pg eq/g), liver (24.0 pg eq/g), ovary (10.8 pg eq/g) and testis (7.7 pg eq/g). The concentration of blood and plasma of both mothers and infants were lower compared to other tissues. In group 2 (2G)(n=2) administered with 300 ng/kg TCDD, the highest concentrations were observed in fat of both mothers (383.1 pg eq/g) and infants (271.6 pg eq/g). The higher concentrations were observed in the liver (204.9 pg eq/g) and mammary gland (100.3 pg eq/g) of mothers, and in the liver (135.0 pg eq/g) and ovary (116.8 pg eq/g) of infants. The concentrations of TCDD in milk (73.3 pg eq/g) and plasma (3.8 pg eq/g) or blood (13.3 pg eq/g) of infants are relatively lower than that in mammary gland, indicating that TCDD was effectively transferred from mammary gland to infant liver, fat and ovary via blood of infants. As TCDD was effectively transferred to testis and ovary of the infants, TCDD may lead to reproductive dysfunction.

This study is ongoing. We will examine the effects of TCDD on pathological and functional changes in various target organs including the reproductive ones.

**Acknowledgment**

This study was supported by Health Science Research Grants for Research on Environmental Health from the Ministry of Health and Welfare of Japan.

**References**

1. Sweeney MH and Mocarelli P. (2000) Food Addit Contam 17:303-316.
2. Signorini S, Gerthoux PM, Dassi C, Cazzaniga M, Brambilla P, Vincoli N, and Mocarelli P. (2000) Andrologia 32:263-270.
3. Birnbaum LS. (1994) Environ Health Perspect 102: 157-167.
4. Colborn T, vom Saal FS and Soto AM. (1993) Environ Health Perspect 101:378-384.
5. Faqi AS, Dalsenter PR, Merker H-J, Chahoud I. (1998) Toxicol Appl Pharmacol 150: 383-392.
6. Abraham K, Krowke R, and Neubert D. (1988) Arch Toxicol 62: 359-368.
Gray LE, Kelce WR, Monosson E, Ostby JS, and Birnbaum LS. (1995) Toxicol and Appl Pharmacol 131: 108-118.
7. Gray LE, Wolf C, Mann P, and Ostby JS. (1997) Toxicol and Appl Pharmacol 146: 237-244.
8. Abbott BD, Birnbaum LS, and Diliberto JJ. (1996) Toxicol and Appl Pharmacol 141: 256-263.
10. Nagao T, Yamashita K, Golor G, Bittmann H, Korner W, Hagenmaier H, and Neubert D. (1996) Life Sci 58:325-336.
11. Hagenmaier H, Wiesmuller T, Golor G, Krowke R, Helge H, and Neubert D. (1990) Arch Toxicol 64: 601-615.
12. McNulty WP. (1985) Environ Health Perspect. 60:77-88.
13. Ihara T, Oneda S, Yamamoto T, Boudrel L, Lau D, Miller D and Nagata R. (1999) Cong Anom. 39: 223-242.

Table 1. Plasma concentration of 2, 3, 7, 8-TCDD on days 80 and 140 of pregnancy (normal delivery)

| group (ng/kg) | Animal no. | Plasma levels pg/g (wet weight) | | Plasma levels pg/g fat | |
|---|---|---|---|---|---|
| | | 80 day | 140 day | 80 day | 140 day |
| 0 | 1 | N.D. | N.D. | N.D. | N.D. |
| | 2 | N.D. | N.D. | N.D. | N.D. |
| | 3 | N.D. | N.D. | N.D. | N.D. |
| | 4 | N.D. | N.D. | N.D. | N.D. |
| | 11 | N.D. | N.D. | N.D. | N.D. |
| | Mean | - | - | - | - |
| | S.D. | - | - | - | - |
| 30 | 16 | 0.72 | 0.63 | 380 | 230 |
| | 18 | 0.63 | 0.65 | 420 | 280 |
| | 19 | 0.52 | 0.50 | 350 | 110 |
| | 21 | 0.15 | 0.19 | 94 | 120 |
| | 22 | 0.47 | 0.43 | 360 | 200 |
| | Mean | 0.50 | 0.48 | 321 | 188 |
| | S.D. | 0.22 | 0.19 | 130 | 73 |
| 300 | 31 | 1.9 | 1.8 | 1300 | 1100 |
| | 32 | 4.5 | 2.8 | 1900 | 1000 |
| | 33 | 3.3 | 1.9 | 2200 | 730 |
| | 35 | 2.6 | 2.2 | 1100 | 1200 |
| | 38 | 2.9 | 1.6 | 1900 | 730 |
| | Mean | 3.0 | 2.1 | 1680 | 952 |
| | S.D. | 1.0 | 0.5 | 460 | 215 |

Table 2. Plasma concentration of 2, 3, 7, 8-TCDD on days 80 and 140 of pregnancy (abnormal delivery) after subcutaneous administration

| group ng/kg | Animal No. | Plasma levels pg/g (wet weight) | | Plasma levels pg/g fat | |
|---|---|---|---|---|---|
| | | 80 day | 140 day | 80 day | 140 day |
| 30 | 17 | 0.41 | 0.30 | 290 | 91 |
| | 20 | 0.29 | 0.25 | 120 | 110 |
| | 29 | 0.34 | 0.30 | 200 | 91 |
| | Mean | 0.35 | 0.28 | 203 | 97 |
| | S.D. | 0.06 | 0.03 | 85 | 11 |
| 300 | 34 | 6.3 | - | 2100 | - |
| | 37 | 8.6 | 7.0 | 3200 | 4700 |
| | Mean | 7.5 | 7.0 | 2650 | 4700 |

Table 3 TCDD concentrations in body fluids and tissues on 7 days after subcutaneous administration of 3H-TCDD to lactating Rhesus monkeys on day 30 of delivery

| Tissue | Radioactivity concentration (pg TCDDeq/g or mL) | | | | | | | | | | | | 2G/1G |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 ng/kg (1G) | | | | | | 300 ng/kg (2G) | | | | | | |
| | No.3 | (T/P ratio) | No.4 | (T/P ratio | Mean | (T/P ratio) | No.7 | (T/P ratio | No.8 | (T/P ratio | Mean | (T/P ratio) | |
| Mother | | | | | | | | | | | | | |
| Plasma | 0.891 | (1.0) | 0.199 | (1.0) | 0.545 | (1.0) | 6.008 | (1.0) | 3.349 | (1.0) | 4.679 | (1.0) | 8.59 |
| Blood | 7.646 | (8.6) | 2.109 | (10.6) | 4.878 | (9.0) | 9.077 | (1.5) | 9.073 | (2.7) | 9.075 | (1.9) | 1.86 |
| Liver | 73.108 | (82.1) | 17.518 | (88.0) | 45.313 | (83.1) | 211.574 | (35.2) | 198.322 | (59.2) | 204.948 | (43.8) | 4.52 |
| Mammary gland | 29.317 | (32.9) | 14.465 | (72.7) | 21.891 | (40.2) | 105.667 | (17.6) | 94.865 | (28.3) | 100.266 | (21.4) | 4.58 |
| Fat | 115.743 | (129.9) | 39.595 | (199.0) | 77.669 | (142.5) | 449.305 | (74.8) | 316.856 | (94.6) | 383.081 | (81.9) | 4.93 |
| Milk | 12.015 | (13.5) | 5.677 | (28.5) | 8.846 | (16.2) | 96.667 | (16.1) | 49.849 | (14.9) | 73.258 | (15.7) | 8.28 |
| Infant | (Female) | | (Male) | | | | (Female) | | (Male) | | | | |
| Plasma | 0.227 | (1.0) | 0.134 | (1.0) | 0.181 | (1.0) | 3.750 | (1.0) | 3.934 | (1.0) | 3.842 | (1.0) | 21.23 |
| Blood | 2.580 | (11.4) | 3.565 | (26.6) | 3.073 | (17.0) | 11.527 | (3.1) | 14.982 | (3.8) | 13.255 | (3.5) | 4.31 |
| Liver | 27.609 | (121.6) | 20.398 | (152.2) | 24.004 | (132.6) | 154.614 | (41.2) | 115.347 | (29.3) | 134.981 | (35.1) | 5.62 |
| Fat | 88.766 | (391.0) | 21.474 | (160.3) | 55.120 | (304.5) | 285.630 | (76.2) | 257.585 | (65.5) | 271.608 | (70.7) | 4.93 |
| Ovary | 10.849 | (47.8) | | | 10.849 | (59.9) | 116.784 | (31.1) | | | 116.784 | (30.4) | 10.76 |
| Testes | | | 7.733 | (57.7) | 7.733 | (42.7) | | | 39.008 | (9.9) | 39.008 | (10.2) | 5.04 |

-: No sample or not calculated T/P ratio: Tissue/plasma concentration ratio