EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN ON REPRODUCTION IN RHESUS MONKEYS

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Introduction

In June, 1999, the Environmental Health Committee of the Central Environmental Council (Environmental Agency), the Living Environment Council, and Food Sanitation Investigation Council (Ministry of Health and Welfare) of Japan adopted 4 pg/kg/day as the tolerable daily intake (TDI) of dioxin and dioxin-related compounds[1]. This value was based on the body burden in experimental animals (86 ng/kg) at which some slight adverse effects were observed. However, most of the experimental studies reviewed for the TDI determination were those with rodents. One of the highly sensitive indicators of adverse effects was sperm production in male offspring of rats from dams treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) prior to mating and throughout mating, pregnancy, and lactation[2]. For proper extrapolation to humans, data from studies in non-human primates are urgently needed. This is a preliminary report of a reproductive study in Rhesus monkeys treated with TCDD being undertaken in Japan.

Materials and Methods

Animals and Chemicals. Sexually mature Rhesus monkeys purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijing), 5-7 years old and 4-6 kg in body weight, were used. The monkeys were kept in Shin Nippon Biomedical Laboratories, Ltd (Kagoshima). The breeding conditions, mating, and diagnosis of pregnancy have been described previously[3]. TCDD was dissolved in a mixture of toluene and DMSO (1:2, v/v) at a concentration of 30 or 300 ng/mL by Kanto Chemical (Tokyo), and subcutaneously administered to pregnant monkeys on day 20 of gestation at an initial dose level of 30 or 300 ng/kg body weight. The lower dose of 30 ng/kg was selected because Faqi et al. reported reduced sperm production in male rat offspring from dams with an initial loading dose of 25 ng/kg and weekly maintenance dose of 5 ng/kg. Control animals received the vehicle. For maintenance of a certain body burden, 5% of the initial dose was given to dams every 30 days during pregnancy and lactation until 90 days after delivery. Pregnancy was monitored on days 25, 40, 60, 80, 100, 120, and 140 by an ultrasound device. In a preliminary study for measurement of TCDD in serum, 3 non-pregnant adult female monkeys were injected TCDD subcutaneously at 30 or 300 ng/kg. Approximately 20 mL of blood was drawn from the femoral vein on 1, 4, 7, 10, 13, 20, 27, 34, 41, 48, and 49 days after injection.

Analyses of TCDD. Approximately 10 g of serum samples were used for analysis. After spiking of 100 pg of $^{13}C_{12}$ labeled PCDDs/PCDFs including TCDD, lipid was extracted according to a method by Patterson et al[4]. The extracted lipid was determined gravimetrically and subjected to a column chromatographic clean-up procedure. This consisted of a multi-layer silica column, an aluminum oxide column, and if necessary HPLC (porous graphitized carbon)[5] was applied. HRGC-HRMS was performed on a Micromass Autospec Ultima with a Hewlett Packard HP6980 fitted with a DB-17 (30 m, 0.32 mm, 0.25 μm) capillary column for PCDDs/PCDFs. The MS was operated in selected ion monitoring mode (SIM) at a resolution >10,000. Two ions were monitored for each congener group.

Results and Discussion

General Conditions of Animals. No animals died in any group. Reddening of the skin and crust formation were noted at the injection site. Food intake and body weight were not appreciably affected by the TCDD treatment.

Measurement of TCDD in serum. Our preliminary experiment showed that successive measurements of TCDD was possible in individual monkeys even with the lower dose level of 30 ng/kg. Changes in serum TCDD concentration after single injection are shown in Table 1.

Table 1. Plasma TCDD Concentration in Female Rhesus Monkeys (pg/g wet weight)

Dose (ng/kg)	Day 5	Day 21	Day 49
0	<0.04	<0.04	<0.04
30	0.92	0.82	0.52
300	9.20	20.00	11.00

These data indicate that the serum concentration of TCDD began to decrease between day 21 and day 49 (day of administration = day 0). Administration of monthly supplemental doses seems appropriate to keep the TCDD body burden at a certain level. The adequacy of a 5% supplement will be examined soon.

Effects on Pregnancy. At the time of submission of this abstract, approximately 20 monkeys in each dose group became pregnant. The pregnancy outcome is summarized in Table 2.

Table 2. Pregnancy Outcome in Rhesus Monkeys Treated with TCDD

Dose (ng/kg)	No of Pregnant Monkeys	Abnormal Outcome
0	20	Fetal death: 1 (Day 40 of gestation) Stillbirth: 1 (Day 146 of gestation, breech birth)
30	20	Stillbirth: 1 (Day 156 of gestation, breech birth)
300	20	Abortion: 2 (Days 40 and 128 of gestation)

No external abnormalities were noted in stillborn or aborted conceptuses. The frequencies of abnormal pregnancy outcomes in the TCDD treated groups were comparable to that in the

concurrent control group and historical background data. McNulty[6] reported that three out of four pregnancies resulted in abortion when pregnant Rhesus monkeys were treated with a total of 1 μg/kg TCDD divided into 9 doses. The higher dose used in this experiment is lower than that used by McNulty, and the present dose levels seem to exert no adverse effects on the maintenance of pregnancy.

Further Studies. Examination of the newborns are in progress. The newborns are exposed to TCDD contained in maternal milk. We will raise these offspring prenatally and postnatally exposed to low dose levels of TCDD, and examine reproductive indices in these F1 monkeys. Data obtained from these further studies will provide us with information useful for determination of a more reliable TDI.

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