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**Title:**

Contamination of Dioxins in Free Ranging and Breeding Monkeys in Japan and Relationship Analysis between Limb Malformations and Administration with 2, 3,7,8- Tetrachlorodibenzo-p-dioxin (TCDD) on Macaque Monkeys

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Key words: dioxins, TCDD, monkey, limb malformation, contamination, blood

**(Abstract)**

In this study, we investigate to be fine that relations are presence or not between dioxins and the limb malformations to be born in wild in macaque monkeys. The contamination of dioxins in the monkey blood between the malformed and the normal was measured using mass-spectrometer. The dioxin isomers except 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, and 1,2,3,7,8,9-HxCDF were detected in the bloods of the two groups of monkeys. TEQs from the monkeys with the abnormal were lower than TEQs from the monkeys with the normal. The TEQs of the monkeys with abnormal limbs, ID 2 and ID 3ID in wild monkey are much lower than that of the monkeys ID 1 with normal limb in the same group of wild monkey. And also pregnant monkeys were exposed with 2,3,7,8-Tetrachlorodibenzo-p-dioxin from the day 20 of gestation to the day 90 after birth and the limb was examined on neonates born from the exposed pregnant. These 42 neonates exposed with TCDD had observed with normal limbs. Finally present investigations resulted to indicate that dioxins including TCDD had no or more weak effect than that of thalidomide on the limb malformations at the range of 30-300 ng TEQ /kg of body weight to macaque monkeys.

## 1. Introduction

Among the environmental pollutants, dioxin is highly toxic even at low levels on immune (Pohjanvirta and Tsuomisto, 1994), reproductive (Birnbaum, 1994) and developmental systems (Birnbaum, 1995) in human, wildlife, and experimental animals.

Comparing the many reports on the effects of dioxins in rodents (Abraham et al., 1989, Nagao et al., 1996, Michalek et al., 1996, Gray et al., 1997), not much is studied on the effects in non-human primates. In the point of view among species differences, the studies using primates are needed to estimate dioxin affects on human health. For experimental animals, macaque monkey has advantages for human models because of the most resemblance in physiological properties.

In the wild life of the macaque monkeys, the limb malformations of monkeys in Japan have been reported since 1957 (Itani and Mizuhara, 1957, Yoshihiro et al., 1979) and that is still to be seen in monkeys in Japan. Their chromosomal abnormalities are excluded from the cause of the limb malformations (Minezawa et al., 1990). Therefore the environmental pollutant chemicals are most suspected the influence on the malformations. The Dioxin contaminations in monkey in Japan have reported by the survey of the Ministry of Environment in Japan (the Ministry of Environment in Japan, 1999), but it has not been available information about the contamination of dioxins in wild living monkeys with limb malformations.

The development of limb buds is established as stage 12 for human embryo and comes apparent at day 28 of gestation in the embryo of the macaque monkeys (Hendrickx and Peterson, 1997). Limb malformations are exerted by the administration of chemicals such as thalidomide to pregnant monkeys before the day 28 of gestation (Hendrickx and Peterson, 1997). The macaque monkeys are an animal model to be inducted the limb defects in human thalidomide syndrome that have not been produced in rodent embryo because of species differences between rodent and primates including humans.

Considering the advantage of monkeys for human models, we have been studying the effects of administration of TCDD on the tissue functions and the organ development in the pregnant monkeys (Kubota et al, 2000, Kubota et al, 2001, Asaoka et al, 2003, Asaoka et al, 2004, Yasuda et al, 2005).

In this study, we investigate to be fine that relations are presence or not between dioxins and the limb malformations to be born in wild in macaque monkeys. The contamination of dioxins in the monkey blood between the malformed and the normal was measured using mass-spectrometer. And also pregnant monkeys were exposed with 2,3,7,8-Tetrachlorodibenzo-p-dioxin from the day 20 of gestation to the day 90 after birth and the limb was examined on neonates born from the exposed pregnant. Finally present investigations resulted to indicate that dioxins including TCDD had no or more weak effect than that of thalidomide on the limb malformations at the range of 30-300 ng TEQ /kg of body weight to macaque monkeys.

## 2. Methods and Materials

### *2.1. Chemicals.*

2, 3,7,8-TCDD dissolved in toluene and DMSO (1:2, V/V) was purchased from Daiichi Pure Chemicals Co., Ltd. Tokyo, Japan.

### *2.2. Measurement of dioxin isomers in blood and TEQ calculation*

The isomers of dioxins in 10 ml of blood samples from the monkeys was measured in Towa Kagaku Co., Ltd., Hiroshima, Japan, using a high resolution mass spectrometer by the methods of the provisional manual for analyzing the dioxin in blood by the Ministry of Health, Labour and Welfare (22 Dec, 2000). TEQ of the monkey blood was calculated by the amount of dioxin isomers using WHO-TEF (WHO, 1998). Isomers that were detected at levels below the lower limit of determination were assigned as not detected (ND), and concentrations below the lower limit of determination were converted to TEQ values equivalent to zero values.

*2.3. Blood samples*

Blood samples were collected from two groups of Japanese monkeys living in different districts on February-March 2003. One is provisional group of free ranging wild monkeys including limb malformations at Arashiyama, Kyoto Japan and the other is breeding monkeys at Primate Research Institute, Kyoto University, Aichi, Japan. The birth at Primate Research Institute has not been found with limb malformation since the establishment of breeding facilities on 1980. Both monkeys were captured and treated according to the animal control program and the guide for the care and use of laboratory primates. Four blood samples were obtained from each group. Two abnormal monkeys with limb malformations were from the group at Arashiyama. The blood samples were collected after anaesthetization with Ketaral and kept in freezing until used. The ages of the monkeys tested were conformed by the records of birth.

*2.4. Pregnant monkeys and TCDD administration*

Rhesus monkeys purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijing, China). The monkeys (6-9 years old and 4.5-6.5 kg in body weight) were kept in Shin Nippon Biomedical Laboratories, Ltd, Kagoshima, Japan. The breeding conditions were described previously (Ihara et al., 1999). The monkeys were mated, and the pregnancies were administered 2, 3,7,8-TCDD (30-300 ng / kg of body weight) via subcutaneous on day 20 of their gestation. Every 30 days interval, 5% of the initial dose of TCDD was given to the pregnancies until day 90 after birth for maintaining the body burden. Controls were given the solvent only.

**3. Results and Discussion**

*3.1. Blood analysis*

The monkeys captured at the wild group have two monkeys with limb malformations in total four monkeys. The abnormality was types of split hands and lack of arms shown in figure 1. This type of limb abnormality categorized in absence deformities, which are the most frequently observed in the limb malformations in Japan (Yoshihiro et al., 1979). The breeding monkeys at Inuyama had normal llimbs as same shown in figure 1.

The contamination of dioxin in the blood from wild and breeding monkeys in Japan was measured using mass-spectrometer as summarized in Table I. The dioxin isomers except 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, and 1,2,3,7,8,9-HxCDF were detected in the bloods of the two groups of monkeys. Amount of each isomer in the monkey blood was no difference among the limb properties, different districts, and sexes. By sum of dioxin isomers measured, the amounts of coplanar polychlorobiphenyl,

polychrolodibenzo-p-dioxine, and polychlorinated dibenzofran were detected in the range of 830-31,000 pg/ g lipid, 22-82 pg/ g lipid, and 4.1-67 pg/ g lipid, respectively, in the bloods of monkeys at both places.

Although lack of blood samples and abnormal monkeys, the survey reports almost same range of the dioxins and the presence of 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, and 1,2,3,7,8,9-HxCDF in wild monkeys by the Ministry of Environment in Japan (The Ministry of Environment in Japan, 1999). The amount of dioxins in the blood of monkeys with abnormal limbs was within the same ranges of the amount of dioxins in that of normal monkeys by the present data and the reported by the Ministry.

TEQ was calculated by the amount of the isomers using WHO-TEF as summarized in Table II. The TEQ levels in the monkey blood were also no difference among the limb properties, different districts, and sexes. The Total TEQ calculated by the amount of all isomers in monkey blood with limb malformations was also in the same ranges of that with normal limbs, 5.5-17 pg TEQ / g lipid, and 3.6-34 pg TEQ/ g lipid, respectively.

The TEQs of each individual was pointed against their birth year as shown figure 2. The TEQ from the monkey with the abnormal was pointed in the range of TEQ from the normal breeding monkeys. TEQs from the monkeys with the abnormal were lower than TEQs from the monkeys with the normal. The TEQs of the monkeys with abnormal limbs, ID 2 and ID 3ID in wild monkey are much lower than that of the monkeys ID 1 with normal limb in the same group of wild monkey. This shows the no relations between limb abnormality and the effects of dioxins incorporated to the monkeys.

*3.2. Exposed analysis*

Twenty neonates were born from 20 pregnancies administered with 30 ng / kg of body weight of TCDD, the 22 neonates were born from 29 pregnancies administered with 300 ng / kg of body weight of TCDD, and 21 neonates were born from 23 pregnancies of control. These 42 neonates exposed with TCDD had observed with normal limbs and the control neonates were also born with normal limbs. The 9 abortions could not be detected.

The TCDD administration was started to monkey pregnancies from the day 20 of gestation that is pre-periods before the day 28 of gestation that begins the limbs formation in monkey embryo (Hendrickx and Peterson, 1997). Present study was enough at the stage of limb formation to examine the possibility of the effect of TCDD on limb malformations because the defects in thalidomide syndrome are inducted by chemical administration at day 24-30 of gestation in macaque monkeys of rhesus and Japanese monkeys (Wilson and Gavan, 1967, Tamimura, 1972).

Finally present investigations resulted to indicate that dioxins including TCDD had no or more weak effect than that of thalidomide on the limb malformations at the range of 30-300 ng TEQ /kg of body weight to macaque monkeys. The limb malformations in wild may be occurring with other environmental material(s) or their complex and/or with TCDD in the environments surrounding the monkeys. The abnormal birth in wild monkeys are serious for humans because the physiological resemblance between humans and monkeys. Further studies are necessary to clarify the relationships between environmental pollutants and the limb malformation to be born in wildlife.

*Acknowledgment*

We are deeply thanks to the heads of Mr. Nobuo Asaba and Mr. Shinsuke Asaba, Arashiyama Monkey Park Iwatayama, Kyoto, Japan to keep the birth record of monkeys and help handling monkeys. This study was supported by Health Labor Sciences Research Grants for Research on Food and Chemical Safety from the Ministry of Health Labor and Welfare of Japan, MEXT Grant-in-Aid for Scientific Research (No. 13027240), and a Grant for the Biodiversity Research of 21st Century COE (A14) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Table I. The physical conditions of monkeys examined and dioxins detected in the blood of monkeys living in free ranging and breeding places in Japan, 2003. The amounts were expressed as pg/g of lipid of the blood.

| | Place | Arashiyama | | | | Inuyama | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | living | free ranging | | | | breeding | | | | | | |
| Monkey | ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | AVERAGE | STDEV | TEF |
| | Sex | male | female | male | female | female | male | male | female | | | |
| | Birth year | 1988 | 1989 | 1989 | 1992 | 1989 | 1989 | 1999 | 1999 | | | |
| | Limb | normal | abnormal | abnorma | normal | normal | normal | normal | normal | | | |
| | Body weight (Kg) | 8.3 | 7.5 | 10 | 5.5 | 8 | 10.2 | 4.5 | 5.4 | | | |
| polychrolodibenzo-p-dioxin | 2,3,7,8-TeCDD | 3.6 | ND | ND | ND | ND | ND | 1.6 | ND | 2.6 | 1.4 | ×1 |
| | 1,2,3,7,8-PeCDD | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ×1 |
| | 1,2,3,4,7,8-HxCDD | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ×0.1 |
| | 1,2,3,6,7,8-HxCDD | 2.5 | ND | ND | ND | ND | ND | ND | ND | 2.5 | ND | ×0.1 |
| | 1,2,3,7,8,9-HxCDD | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ×0.1 |
| | 1,2,3,4,6,7,8-HpCDD | ND | 3 | 1.3 | 5.2 | 11 | 3.5 | 7.5 | ND | 5.3 | 3.5 | ×0.01 |
| | OCDD | 26 | 79 | 21 | 24 | 49 | 31 | 43 | 58 | 41.4 | 20 | ×0.0001 |
| | Total PCDDs | 32 | 82 | 22 | 29 | 61 | 35 | 52 | 58 | 46.4 | 20.3 | |
| polychlorinated dibenzofran | 2,3,7,8-TeCDF | 5.7 | 11 | ND | ND | ND | ND | ND | ND | 8.4 | 3.7 | ×0.1 |
| | 1,2,3,7,8-PeCDF | ND | 9.3 | ND | ND | ND | ND | 3.6 | 2.1 | 5 | 3.8 | ×0.05 |
| | 2,3,4,7,8-PeCDF | 27 | 16 | ND | ND | 11 | 9.7 | 4.7 | 3.4 | 12 | 8.7 | ×0.5 |
| | 1,2,3,4,7,8-HxCDF | 11 | 8.9 | ND | 8.2 | 8 | 6 | 7.1 | ND | 8.2 | 1.7 | ×0.1 |
| | 1,2,3,6,7,8-HxCDF | 10 | 8.8 | ND | 6.4 | 9.2 | 5.1 | 6 | ND | 7.6 | 2 | ×0.1 |
| | 2,3,4,6,7,8-HxCDF | 12 | 9.6 | 4.1 | ND | ND | 7.6 | ND | ND | 8.3 | 3.3 | ×0.1 |
| | 1,2,3,7,8,9-HxCDF | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND | ×0.1 |
| | 1,2,3,4,6,7,8-HpCDF | ND | ND | ND | 3.7 | 12 | ND | 3.5 | ND | 6.4 | 4.9 | ×0.01 |
| | 1,2,3,4,7,8,9-HpCDF | ND | ND | ND | ND | 7.1 | ND | 5 | ND | 6.1 | 1.5 | ×0.01 |
| | OCDF | ND | ND | ND | ND | 10 | ND | 3.2 | ND | 6.6 | 4.8 | ×0.0001 |
| | Total PCDFs | 67 | 63 | 4.1 | 18 | 57 | 28 | 33 | 5.6 | 34.5 | 25.2 | |
| coplanar polychlorobiphenyl | 3,3',4,4'-TeCB #77 | 100 | 67 | 98 | 39 | 38 | 2100 | 2600 | 67 | 638.6 | 1064.9 | ×0.0001 |
| | 3,4,4',5-TeCB #81 | 17 | 9.3 | 11 | 4.9 | ND | 280 | 270 | ND | 98.7 | 136.7 | ×0.0001 |
| | 3,3',4,4',5-PeCB #12 | 110 | 42 | 43 | 19 | 7 | 110 | 130 | 21 | 60.3 | 48.6 | ×0.1 |
| | 3,3',4,4',5,5'-HxCB #1 | 30 | 8.4 | 17 | 3.4 | ND | 15 | 37 | 6.5 | 16.8 | 12.5 | ×0.01 |
| | 2,3,3',4,4'-PeCB #10 | 930 | 420 | 940 | 150 | 200 | 8100 | 6600 | 680 | 2252.5 | 3185.5 | ×0.0001 |
| | 2,3,4,4',5-PeCB #11 | 110 | 40 | 110 | 20 | 23 | 850 | 730 | 75 | 244.8 | 339.9 | ×0.0005 |
| | 2,3',4,4',5-PeCB #11 | 2500 | 860 | 2900 | 400 | 580 | 17000 | 16000 | 2400 | 5330 | 6963 | ×0.0001 |
| | 2',3,4,4',5-PeCB #12 | 71 | 32 | 67 | 10 | 14 | 600 | 500 | 50 | 168 | 238.3 | ×0.0001 |
| | 2,3,3',4,4',5-HxCB #1 | 480 | 140 | 340 | 85 | 46 | 870 | 1200 | 190 | 418.9 | 414.8 | ×0.0005 |
| | 2,3,3',4,4',5'-HxCB #1 | 150 | 46 | 100 | 24 | 16 | 240 | 260 | 61 | 112.1 | 95.5 | ×0.0005 |
| | 2,3',4,4',5,5'-HxCB #1 | 260 | 68 | 180 | 45 | 29 | 480 | 670 | 140 | 234 | 229.4 | ×0.00001 |
| | 2,3,3',4,4',5,5'-HpCB #1 | 140 | 30 | 82 | 25 | 8.7 | 110 | 230 | ND | 89.4 | 78.5 | ×0.0001 |
| | Total Coplanar PCBs | 4900 | 1800 | 4900 | 830 | 970 | 31000 | 29000 | 3700 | 9637.5 | 12680.3 | |

TEF used the WHO-TEF (WHO,1998). Isomers that were detected at levels below the lower limit of determination were assigned ND and concentrations below the lower limit of determination were converted to TEQ values equivalent to zero values.

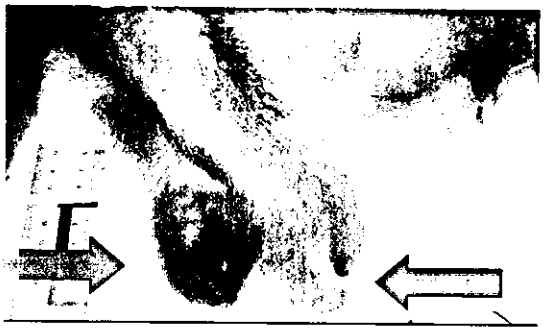
Table II. TEQs of dioxins detected in the blood of monkeys living in free ranging and breeding places in Japan, 2003.

The amounts were expresssed as pg-TEQ/g of lipid of the blood..

| Place | Arashiyama | | | | Inuyama | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| living | free ranging | | | | breeding | | | | | |
| ID | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | AVERAGE | STDEV |
| TEQ of total PCDDs | 3.9 | 0.038 | 0.015 | 0.055 | 0.12 | 0.039 | 1.7 | 0.0058 | 0.7 | 1.4 |
| TEQ of total PCDFs | 18 | 12 | 0.41 | 1.5 | 7.4 | 6.7 | 4 | 1.8 | 6.5 | 6 |
| TEQ of total Coplanar PCBs | 12 | 4.6 | 5.1 | 2.1 | 0.83 | 15 | 17 | 2.6 | 7.4 | 6.3 |
| TEQ of total PCDDs+ PCDFs+Coplanar PCBs | 34 | 17 | 5.5 | 3.6 | 8.3 | 22 | 23 | 4.4 | 14.7 | 11.1 |

TEF used the WHO-TEF (WHO,1998). Isomers that were detected at levels below the lower limit of determination were assigned ND and concentrations below the lower limit of determination were converted to TEQ values equivalent to zero values.

a) ID-1 Arashiyama, normal limbs



b) ID-2 Arashiyama, abnormal limbs, sprite fingers

c) ID-3 Arashiyama, abnormal limbs, arms' less

d) ID-4 Arashiyama, normal limbs

igure 1: Free ranging monkeys analyzed in this study including with limb malformations to be born at rashiyama in Japan. Arrows are pointed the parts of abnormal limbs. More details of the individuals were hown in Table I.

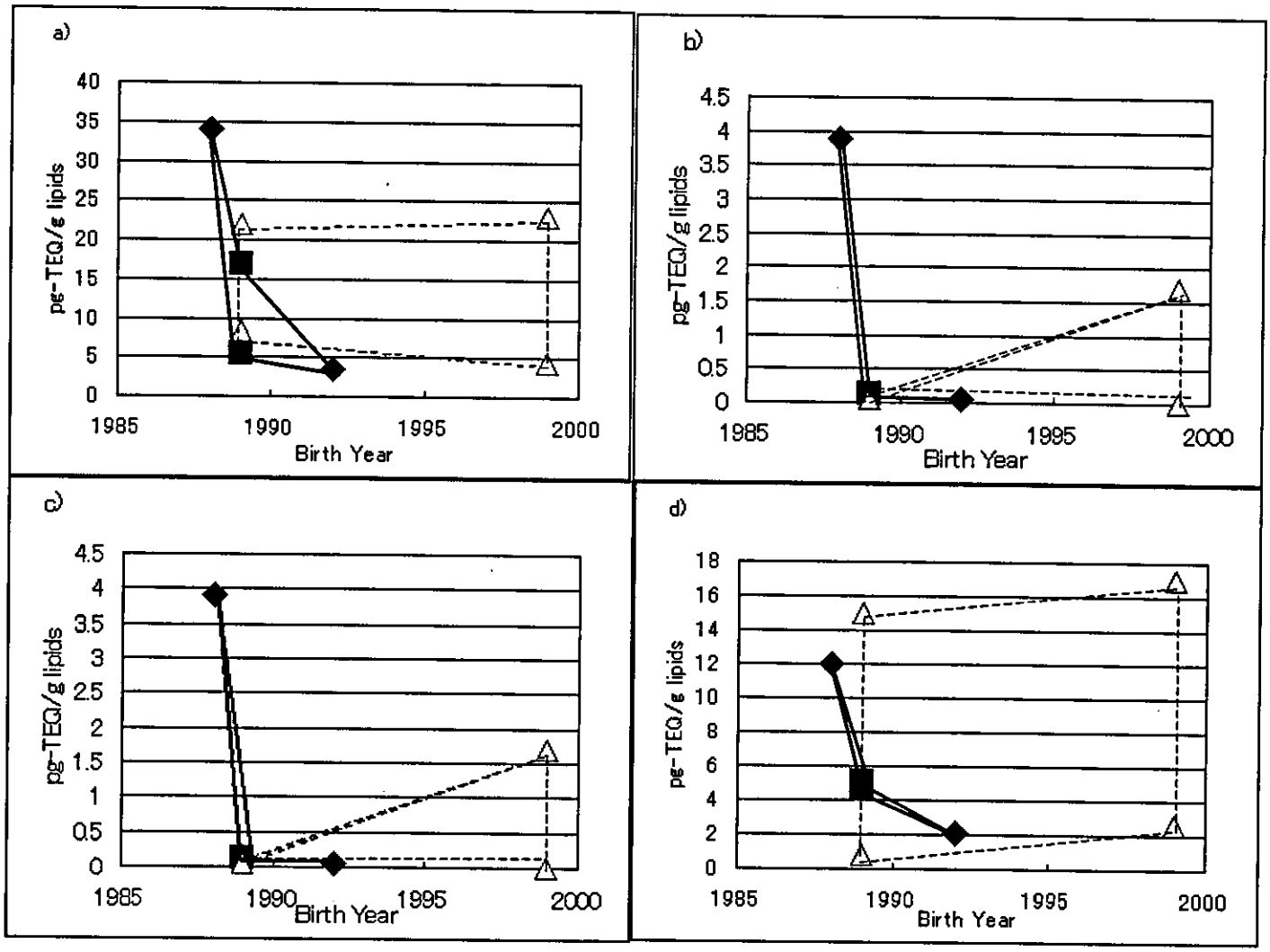



Figure 2. The comparison of TEQs of dioxins contaminated in blood between free ranging and breeding monkeys including with limb malformations. TEQs of dioxins contaminated in the monkey bloods are expressed as pg-TEQ/g lipids versus the year at the birth of each monkey. a) TEQ of total PCDDs+ PCDFs+Coplanar PCBs, (b) TEQ of total PCDDs, (c) TEQ of total PCDDs, (d) TEQ of total Coplanar PCBs. Square: wild monkeys with abnormal limbs on 1989; diamond: wild monkeys with normal limbs, open triangle: breeding monkeys with normal limbs: solid line: TEQ's area detected in the wild monkeys; and dashed line: TEQ's area detected in the breeding monkeys. For more details, see the table I.

# NO EFFECTS OF DIOXIN SINGLY ON LIMB MALFORMATIONS IN MACAQUE MONKEYS THROUGH EPIDEMIOLOGICAL AND TREATED STUDIES

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## *Introduction*

Human populations exposed with highly dioxin were suspected to be caused immunological dysfunctions, carcinogenesis, and developmental and reproductive dysfunctions. Because of species resemblances, the dioxin effects have been investigating using monkeys as a model for assessment of dioxin exposure on human health [1-6]. Since 1957 the limb malformations of monkeys in Japan have been reported [7-8]. The higher frequency of them was found in provisional groups of monkeys who were given the same kind of food for human. The chromosomal abnormalities are excluded from the factor for the congenital limb malformations [9] that are still producing in Japan. In this study, the relations between dioxin and the limb malformations of macaque monkeys were estimated by the epidemiological and administered researches. The dioxin levels in monkeys were measured at two districts that one has the provisional groups including monkeys with limb malformations and the other has breeding groups never seeing the malformations for a long time. TEQ was calculated by the levels of dioxin isomers in the monkeys and the values show no difference between the two places and between the individuals with and without the limb malformations. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) was administered via subcutaneous to pregnant rhesus monkeys from the day 20 of gestation to the day 90 after birth. The exposed babies, including the offspring and died in neonatal, had observed normal limbs in the range of 30-300 ng TCDD /kg of body weight.

## *Methods and Materials*

**Chemicals.** 2, 3,7,8-TCDD dissolved in toluene and DMSO (1:2, V/V) was purchased from Daiichi Pure Chemicals Co., Ltd. Tokyo, Japan.

**Animals.** For epidemiological studies, blood samples were collected from Japanese monkeys living in two different districts, provisional groups of semi-wild monkeys and breeding monkeys. Some in the former are born with limb malformations and in the later no records of birth with malformation. The monkeys captured and treated according to the animal control program and the guide for the care and use of laboratory primates. Four samples including two abnormal monkeys were obtained from the provisional group of monkeys at Arashiyama, Kyoto Japan. The other four samples were obtained from the breeding facility at Primate Research Institute, Kyoto University, Inuyama, Aichi, Japan. The ages of all the monkeys tested were conformed by records of birth. The samples were collected after anaesthetization with Ketaral and kept in freezing until used. For exposure studies, rhesus monkeys purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijing, China). The monkeys (6-9 years old and 4.5-6.5 kg in body weight) were kept in Shin Nippon Biomedical Laboratories, Ltd, Kagoshima, Japan. The breeding conditions were described previously [10]. The rhesus monkeys were mated, and the pregnancies were administered 2, 3,7,8-TCDD (30-300 ng/kg of body weight) via subcutaneous on day 20 of their gestation. Every 30 days interval, 5% of the initial dose of TCDD was given to the pregnancies until day 90 after birth for maintaining the body burden. Controls were given the vehicle.

**Measurement of dioxin isomers in blood.** The isomers of dioxins in 10 ml of blood samples from the monkeys was measured using a high resolution mass spectrometer by the methods of the provisional manual for analyzing the dioxin in blood by the Ministry of Health, Labour and Welfare (22 Dec, 2000).

## Results and Discussion

Epidemiological analysis – In total four monkeys at the provisional groups, two monkeys had limb malformations that were split hands as shown in figure 1. This type categorized in absence deformities is the most frequently observed in the limb malformations that occurred under 5% of the groups at Arashiyama in 1972-1979 [8]. The other monkeys including breeding monkeys had normal llimbs. Among the measured dioxin isomers, polychlorinated dibenzofran and coplanar polychlorobiphenyl were detected in major, and polychrolodibenzo-p-dioxine was detected in minor in the bloods of monkeys living at both semi-wild and breeding. TEQ was calculated by the amount of the dioxin isomers among the monkeys. Figure 2 show the values in the bloods of the two malformation monkeys were pointed in the range of the values of the other normal monkeys. The difference of TEQ between the two districts also was not detected in the values of their averages.

Exposure analysis – TCDD is the most toxic in dioxin isomers. In utero and lactational exposure, TCDD affects morphological abnormalities that are reported on teeth development especially at incisor and molar in rodents[11-12] and monkeys[6]. But in the case of TCDD administration in the range of 30-300 ng /kg of body weight, the babies exposed with TCDD had observed normal limbs including the offspring and died in neonatal. The TCDD administration was started on day 20 of gestation that is enough, if TCDD is teratogic on the limbs, to effect on the limb malformation in

macaque monkeys because famous thalidomide as a limbs teratogen acts sensitively on the embryo of rhesus monkey in the period of 24-30 days after gestation [13]. The limb malformations with thalidomide also occur among macaque species including Japanese monkeys during almost same periods [14].

Above the results show dioxin isomers including TCDD may have no effects on the limb malformation when TCDD acts singly in the same period of thalidomide as a teratogen.
The limb malformations may be occurring with other material(s) or their complex in the living environments surrounding the monkeys. Those abnormalities are serious for humans because the physiological resemblance based on the molecular similarity between humans and monkeys. Further studies are necessary to clarify the materials and limb malformation relationships.

**Figure 1: Provisional semi-wild monkey with congenital finger less in Japan**

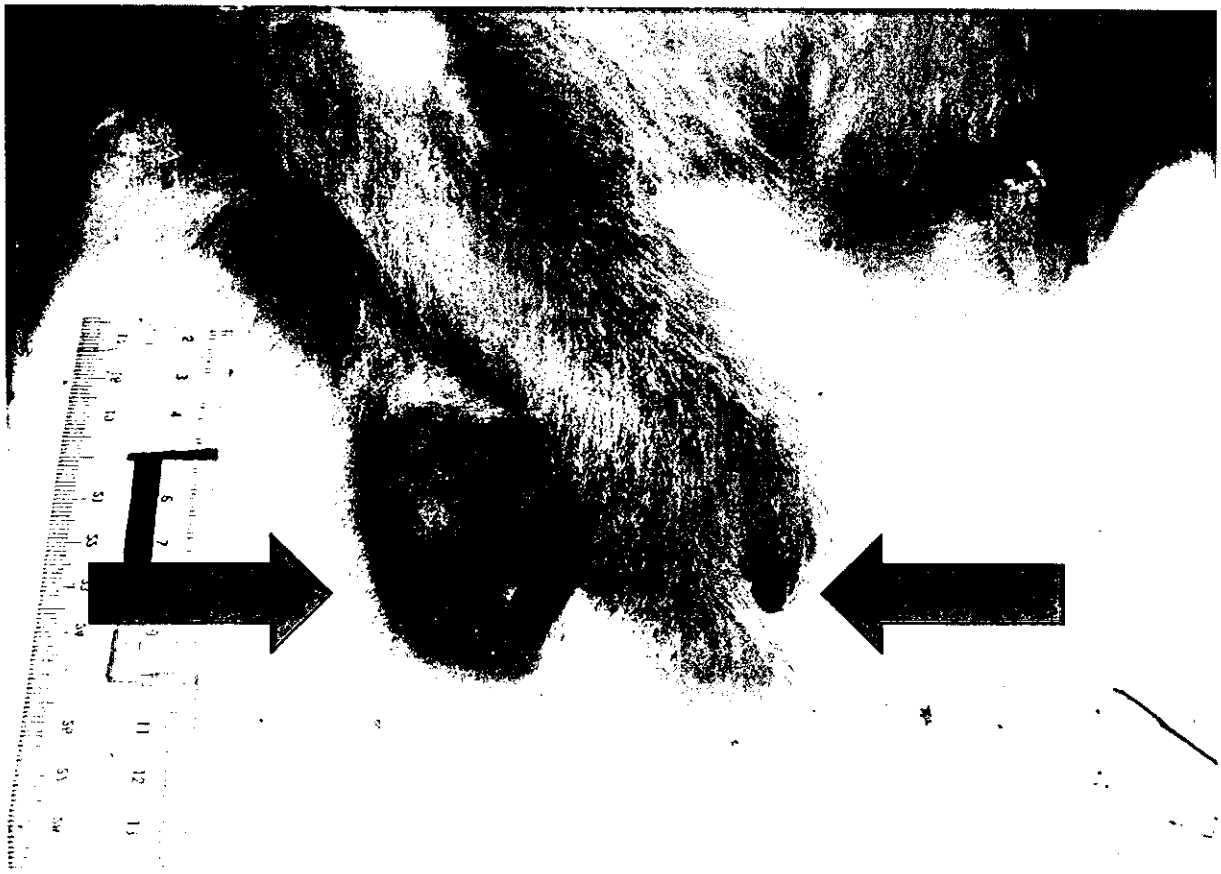
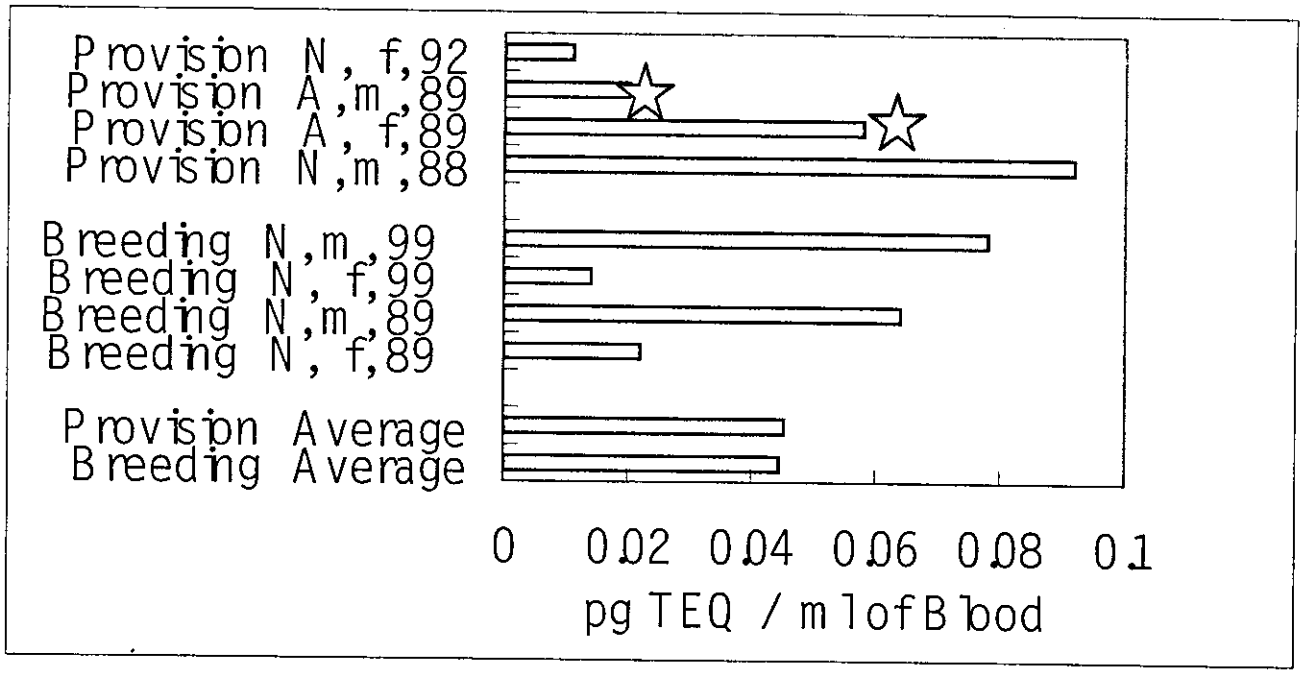


**Figure 2: The comparison of TEQ in the blood of monkeys with between malformation limbs and normal limbs**
**☆A, abnormal; N, normal; f, female; m, male; number, birth year**




***Acknowledgment***
This study was supported by Health Labour Sciences Research Grants for Research on Food and Chemical Safety from the Ministry of Health Labour and Welfare of Japan, and MEXT Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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2004年10月13日-16日 第77回日本生化学会大会（パシフィコ横浜）

# Phylogenic Characteristics of Genes Expressed in Macaque Monkeys and The Application for Ecotoxicogenomics of Dioxin Disorder

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[Introduction] In experimental animals, macaque monkey has advantages for human models because of the most resemblance physiologically. Information on gene sequences is limited in the monkeys especially in an early developmental stage that is important in ecotoxicogenomics. The analyses based on mRNA have to be started. [Methods] Full-length cDNA was converted from mRNA expressed in the monkey embryo and transfected to *E. coli*. Their clones were selected randomly from about 3,000 plaques and were sequenced. Pregnant monkeys were administered with TCDD and the amount transported in the tissues was detected. The changing of genes expression was analyzed using RT-PCR and DNA microarray. [Result & Conclusion] The resemblance of genes expressed in the monkey embryo versus human genes was estimated about 94 % and 96 % based on the structure and the gene frequencies, respectively. Those are closer to human genes because both those of rats and mice versus that of humans are reported about 80%. The TCDD was detected in many tissues of the fetal and changed the gene expression. It shows TCDD transports from the mother to the fetal via placenta. In the analyses using DNA microarray, some were changed the gene levels up or down depending on the amount TCDD exposed. Those genes may be candidate TCDD affection on humans.

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## P3-I-62

### 妊娠および授乳期ダイオキシン暴露による牝親アカゲザルの肝障害

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【目的】ダイオキシン類は生体に対して様々な毒性を示すことが知られているが, 2.3.7.8-テトラクロロジベンゾ-*p*-ダイオキシン (TCDD) は最も毒性が強い. 今回, 妊娠期及び授乳期のアカゲザルにおけるTCDD投与後の肝障害について検討した. 【方法】妊娠20日目の牝アカゲザルに0ng, 30ng, 300ng/kgのTCDDを皮下投与し後, 分娩後90日まで30日毎に初回投与の5%を追加投与した. 3年後に屠殺後病理学的及び生化学的検討を行った. 【成績】TCDD非投与群に肝病変は見られず, 投与群で肝の巣状脂肪化, 出血, 梗塞, 類洞内の微小血栓形成, 胆管上皮の過形成が認められた. 投与群では, 免疫染色で平滑筋アクチン陽性細胞の増加, Western blottingでAh受容体及びVE-カドヘリンの発現減少, CYP 1A1の発現増加が認められた. 電顕では, 類洞内皮細胞の腫大, 細胞膜の不明瞭化, 細胞質の不規則な形の拡大など類洞内皮細胞の変性所見が見られ, 類洞内腔の狭窄が認められた. 【結論】TCDD投与群で肝内に認められた所見は類洞内皮障害と局所循環障害の存在を示唆していた. その原因がダイオキシン投与による可能性が考えられた.

## 2-F-18

### 妊娠および授乳期ダイオキシン暴露による仔アカゲザルの新規腎障害

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【目的】ダイオキシン類暴露による次世代への影響が問題になっている. 今回, 最も毒性が強い2.3.7.8-テトラクロロジベンゾ-*p*-ダイオキシン (TCDD) を妊娠期及び授乳期の牝アカゲザルに投与後, 仔アカゲザル (F1) にみられる臓器障害を検討した. 【方法】妊娠20日目の牝アカゲザルに0ng, 30ng, 300ng/kgのTCDDを背部皮下投与し, 分娩後90日まで30日毎に初回投与の5%を追加投与した. 約3年後にF1を屠殺後病理学的検討を行った. 【成績】TCDD非投与群及び30ng/kg投与群のF1（各々6頭, 3頭）で腎病変は見られず, 300ng/kg投与群からの9頭中5頭でのみ特異な腎線維化が認められた. いわゆる間質性腎炎とは異なり, 炎症細胞浸潤は軽度で, 尿細管周囲・糸球体周囲・血管周囲の間質に強い線維化が認められ, 同時に腎盂・腎杯周囲の強い線維化も伴い, 腎乳頭萎縮や腎機能不全も認められた. 免疫染色ではビメンチン陽性細胞とI型コラーゲンが増加し, シリウス赤染色後の画像解析で腎間質に線維化領域の拡大が有意に認められた. 【結論】TCDD投与後次世代 (F1) における特異な腎間質線維化の報告は初めてである. 300ng/kg投与群のF1のみに認められた点はTDI（耐容1日摂取量）の設定に有用な情報を提供すると判断される.

2005

# 解剖学雑誌

Vol. 80 No. 1

## 目　　次



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