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

Kazuo Asaoka[1], Hiroko Iida[1], Kunio Watanabe[2], Hiroshi Goda[3], Toshio Ihara[4], Ryoichi Nagata[4], Mineo Yasuda[5], Shunichiro Kubata[6]

[1]Department of Molecular and Cellular Biochemistry, Primate Research institute, Kyoto University
[2]Field Research Center, Primate Research institute, Kyoto University
[3]Towa Kagaku Co., Ltd.
[4]Safety Research Facility, Shin Nippon Biomedical Laboratories, Ltd.
[5]Department of Clinical Engineering, Faculty of Health Sciences, Hiroshima International University
[6]Department of Life Science, Graduate School of Arts and Sciences, The University of Tokyo

## *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**

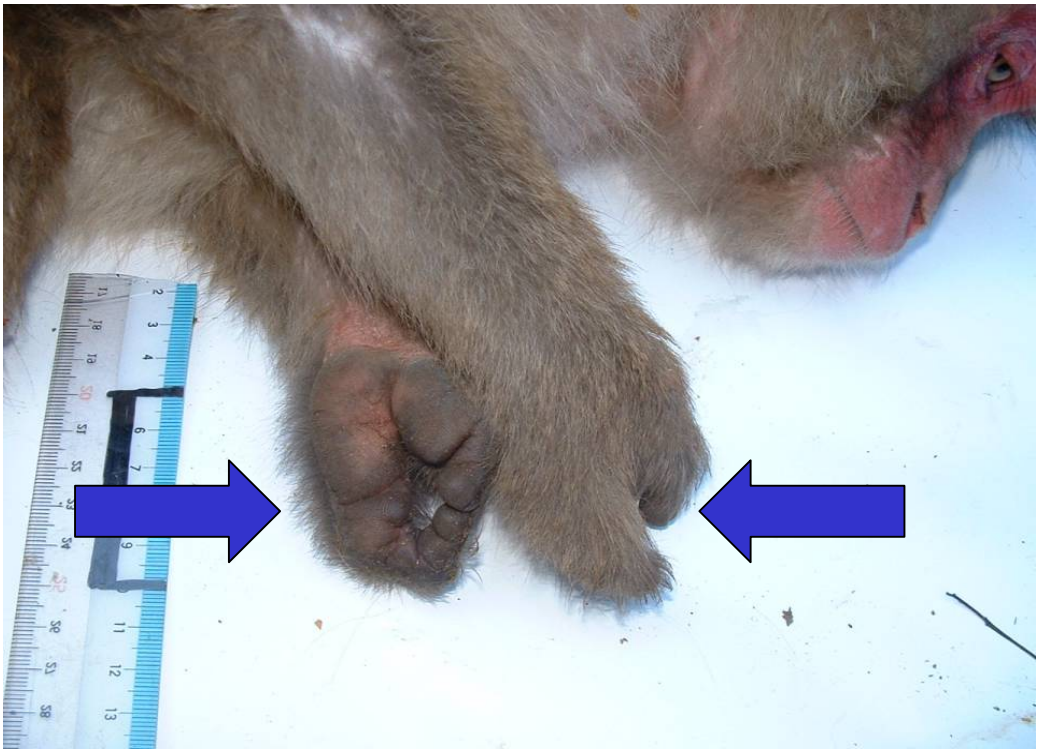
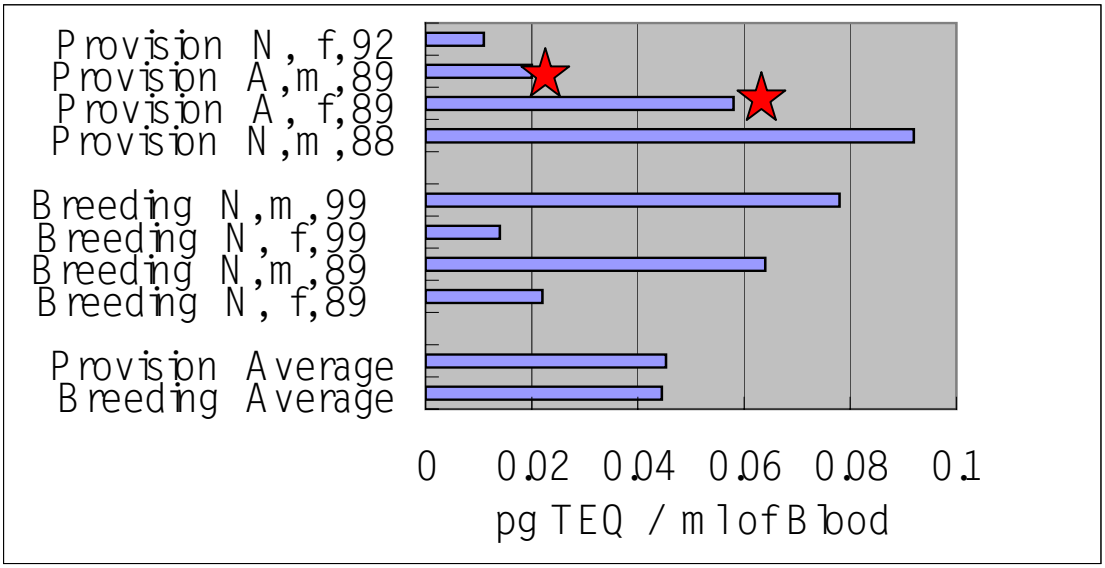


**Figure 2: The comparison of TEQ in the blood of monkeys with between malformation limbs and normal limbs**
**★A, abnormal; N, normal; f, female; m, male; number, birth year**

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