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Risk Assessment of Dioxin in Japan

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ABSTRACT *: In 1990, Tolerable Daily Intake (TDI) of 10 pg TCDD/kg/day for dioxins based on carcinogenicity and reproductive toxicity was determined by WHO/EURO, that resulted in the establishment of TDIs in other countries. In Japan, Ministry of Health and Welfare and Environment Agency, respectively established the TDI of 10 pg TCDD/kg/day and Health Risk Assessment Index of 5 pg TCDD/kg/day in 1996. Accumulation of new scientific data, especially by molecular toxicology since 1990, resulted in the reevaluation of TDI by WHO-ECEH and IPCS in May, 1998. At this meeting, it was stressed that ① toxic effects of dioxin is mediated through Ah-receptor in both animals and humans, ② use of ëbody burdení concept is better than the use of traditional NOAEL/UF approach, ③ inclusion of coplanar PCBs in the TDI by the use of new WHO-TEF. LOAELs (0.16~200 ng TCDD/kg/day) obtained from reproductive toxicity and immunotoxicity in rats, and neurobehavioral toxicity and induction of endometriosis in rhesus monkeys are calculated to be the body burden of 10~50 ng TCDD/kg that is 14~37 pg TEQ/kg/day as human daily intake. Finally TDI of 1~4 pg TEQ/kg/day was established by applying the UF of 10. In Japan, reproductive toxicity and immunotoxicity in rats were used to obtain LOAELs (100~200 ng TCDD/kg/day). Finally TDI of 4 pg TEQ/kg/day was established in June 1999 by applying the UF of 10 to human daily intake of 43.6 pg TEQ/kg/day which corresponds to the body burden of 86 ng TCDD/kg.*

Key Words *: Dioxin, Risk assessment, Toxicity*

I. INTRODUCTION

The TDI of dioxins is an important index and have been established by the WHO and several countries, based on scientific knowledge, to help design sound measures to prevent the effects of dioxins on human health.

In Japan, the EA and the MHW have respectively established a TDI and a Health Risk Assessment Index for dioxins in 1996, as indices to evaluate the effects of pollution on human health and for policies relating to dioxins.

A variety of studies on the health effects of dioxins have been conducted internationally since the first WHO consultation in 1990. For this reason, WHO-ECEH and IPCS held the second expert consultation in Geneva, Switzerland in May 1998 to review the data and to reevaluate the TDI.

Thereafter similar movements have been taken in Japan. Namely, the EA and the MHW have established expert committees (the Dioxin Risk Assessment Subcommittee, Environmental Health Committee, Central Environment Council, and the Special Dioxin Health Effects Evaluation Committee, Food Sanitation Investigation Council, the Living Environment Council) in June 1998. The committee experts analyzed the TDI of dioxins by assessing the discussions of the 1998 WHO Consultation and by contributing new information and established the TDI in June, 1999 (EA and MHW, 1999).

In this paper, "Dioxins" means polychlorinated

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List of Abbreviations: Ah Receptor, Arylhydrocarbon Receptor; Co-PCB, Coplanar polychlorinated biphenyl; EA, Environment Agency, Japan; ECEH, European Centre for Environment and Health; EPA, United States Environmental Protection Agency; IPCS, International Program for Chemical Safety; MHW, Ministry of Health and Welfare; Japan; LOAEL, Lowest Observed Adverse Effect Level; LOEL, Lowest Observed Effect Level; NOAEL, No Observed Adverse Effect Level; ng, nano gram (billionth of a gram = 10^{-9} g); pg, pico gram (trillionth of a gram = 10^{-12} g); PCDD, Polychlorinated dibenzo-p-dioxin; TCDD, 2,3,7,8-Tetrachlorodibenzo-p-dioxin; PeCDD, Pentachlorodibenzo-p-dioxin; HxCDD, Hexachlorodibenzo-p-dioxin; HpCDD, Heptachlorodibenzo-p-dioxin; OCDD, Octachlorodibenzo-p-dioxin; PCDF, Polychlorinated dibenzofuran; TCDF, Tetrachlorodibenzofuran; PeCDF, Pentachlorodibenzofuran; HxCDF, Hexachlorodibenzofuran; HpCDF, Heptachlorodibenzofuran; OCDF, Octachlorodibenzofuran; 2,4,5-T, 2,4,5-Trichlorophenoxyacetic acid; TDI, Tolerable Daily Intake; I-TEF, International Toxic Equivalency Factor; TEQ, Toxic Equivalent; UF, Uncertainty Factor; VSD, Virtually Safe Dose

dibenzo-p-dioxin (PCDDs) and polychlorinated dibenzofurans (PCDFs) and "Dioxin" includes co-planar polychlorinated biphenyls (co-planar PCBs) in addition to dioxins.

II. OUTLINE OF THE HISTORY OF TDI

1. WHO Consultation in 1990

A WHO meeting held at Bilthoven, the Netherlands, proposed a TDI of 10 pg/kg for TCDD, based on the information available at the time. NOEL was considered to be 1 ng/kg/day, based on the results of several studies using various species which demonstrated the hepato-, reproductive-, and immunotoxicity of TCDD. Then by applying novel toxicokinetic analysis (at that time), NOEL of 1 ng/kg/day was calculated to be 540 ng/kg in the liver of rat, which equals to the human daily intake of 100 pg/kg/day. This value was divided by the UF which was set at 10 by the lack of human data on reproduction and the TDI of 10 pg/kg/day was determined.

It should be noted that the WHO adopted the so-called 'threshold model' to set the TDI. It is because they concluded that TCDD is carcinogenic in animals, but not in humans, and it is a nongenotoxic promoter (WHO, 1991).

2. TDIs in Other Countries

This method used by WHO to set the TDI was principally adopted by relevant administrative agencies of other countries (with the exception of the US), although there are some changes regarding the selection of data and the value of UFs. As shown in Table 1, most countries used NOEL or NOAEL of 1 ng/kg/day derived from rat carcinogenicity study and/or rat reproductive toxicity study, and applied UF of 100.

In the Netherlands, LOAEL of 100 pg/kg/day was derived from long term rhesus monkey study, in which disturbances in neurodevelopmental behavior and endometriosis were observed. They applied UF of 100 and TDI of 1 pg/kg/day was proposed.

The USEPA took the approach of 'non-threshold

Table 1. TDI levels based on 'Threshold models'

		References	NOAEL (pg TCDD/kg bw/d)	UF or SF	TDI (pg TCDD/kg/d)
Germany	1985	Kociba *et al.* Murray *et al.*	1,000 (NOEL)	100~1000	10 1 (final goal)
Nordics	1988	Kociba *et al.* Murray *et al.*	1,000 (NOEL)	200	5 (0~35/W) (TWI)
WHO/EURO	1990	Kociba *et al.* Murray *et al.*	1,000 (NOEL)	10*	10
Canada	1990	Murray *et al.*	1,000	100	10
New Zealand	1991	Adopted WHO TDI	1,000	100	10
Holland	1991	Adopted WHO TDI	1,000	100	10
UK COT	1991	Adopted WHO TDI	1,000	100	10
Switzerland	1993	Adopted WHO TDI	1,000	100	10
Australia	1994	Adopted WHO TDI	1,000	100	10(proposed)
Holland	1996	Rier *et al.*	100 (LOAEL)	100	1 (proposed)
Japan MHW	1996	Kociba et al. Murray et al.	1,000	100	10 (temporary)
Japan Environment Agency	1997	Kociba *et al.* Rier *et al.*	1,000	100	5 (Health Risk Assessment Index)

*UF 10 was applied to the human daily intake value of 100 pg/kg/day which was calculated by toxicokinetic analysis.

Table 2. Risk specific doses on dioxins in the USA

	Reference	Extrapolation model	Acceptable risk	q1* (mgTCDD/kg/bw/d)-1	Risk Specific Doses (pgTCDD/kg bw/d)
US EPA 1994	Kociba *et al.*	LMS	1×10^{-6}	1.56×10^{5}	0.006
US FDA	Kociba *et al.*	LMS	1×10^{-6}	1.75×10^{4}	0.057

*Tumor incidence at a dose of 1 mg/kg. Coulston (1994).

model' by using the VSD, which differs from the concept used by WHO and resulted in the much lower levels demonstrated as Risk Specific Doses as shown in Table 2 (USEPA,1994).

3. Establishment of TDI and Health Risk Assessment Index in Japan in 1996

The "Dioxin Risk Assessment Study Group" of the MHW proposed in 1996 a TDI of 10 pg/kg/day for TCDD. This figure was reached based on the WHO estimation formula and consideration of data on 3-generation reproduction test using rats in addition to the 2-year rat study noted above. Evaluation of in utero deaths, litter size, and inhibition of postnatal body weight gain etc led to the conclusion of NOAEL of 1 ng/kg/day, to which UF of 100 was applied to produce the TDI. However, considering the new scientific data on dioxin toxicity in the near future, TDI was set as provisional (MHW,1996).

The EA's "Dioxin Risk Evaluation Committee", while also adopting the WHO estimation formula as the basis for discussion, took into consideration possibility of induction of endometriosis in rhesus monkeys. Therefore they applied UF of 200 (10 for species difference, 10 for individual difference, 2 for addition factor) to NOAEL of 1 ng/kg/day from rat study which resulted in the establishment of 5 pg/kg/day as the Health Risk Assessment Index for dioxins. This index is a value that serves as a guideline for environmental protection measures to protect human health in terms of exposure to dioxins, but not for the tolerable limit for maintaining human health (EA, 1997).

4. Re-evaluation by WHO Consultation in 1998

WHO-ECEH and IPCS held the second expert consultation in May 1998 to review the TDI based on new scientific data accumulated and to discuss the health risks for infants, cancer and non-cancer endpoints in humans and animals, mechanistic aspects, toxicokinetics, modeling, exposure, the applicability of the TEQ concept, and risk assessment approaches for dioxin in various countries (WHO, 1999. Assessment of the health risk of dioxins, 2000).

It should be noted that, for applying the results of toxicity studies to humans, the ëbody burdení approach was introduced for this reevaluation as a metric of choice from pharmacokinetic point of view, in contrast to using the traditional NOAEL/UF approach. The minimum toxic dose for humans was considered to be the lowest body burden values at which adverse effects were observed in toxicity studies. In addition, the concept that the toxic effects of dioxin in both animals and humans is mediated through Ah-receptor became the background for the reevaluation. Also it was agreed to use WHO-TEF 1997 which means that the new TDI includes PCDD, PCDF and coplanar PCBs.

Actual data used as the basis for body burden approach were, decrease in sperm counts, increase in female genital malformations and immune suppression in rats, and induction of endometriosis and effects on neurobehavior in rhesus monkeys. LOAELs from these studies ranged from 0.16 to 200 ngTCDD/kg and the body burden values were 10~50 ngTCDD/kg which are equal to the human daily intake of 14~37 pg TEQ/kg . After applying a composite UF of 10 to these values, the TDI was considered to be 1~4 pg TEQ/kg (rounded figures).

The Executive Summary of the WHO final report concludes that this value for TDI is considered to be the provisional tolerable value, in view of the fact that the current exposure conditions in industrialized countries are 2~6 pg TEQ/kg/day. Although subtle effects may occur at these exposure levels, no confirmed manifestations of toxic effects have yet been reported. In addition, the influence of other chemical substances cannot be ruled out in regard to the effects that have been observed by dioxin exposure. Finally, while the consultation considered the upper range of 4 pg TEQ/kg/day to be the "maximal tolerable intake on a provisional basis", it stressed that the ultimate goal should be to reduce human intake levels to less than 1 pg TEQ/kg/day.

5. Toxic Equivalency Factors (TEFs) and Toxic Equivalents (TEQs)

① Toxic Equivalency Factors (TEFs)

The term "dioxins" refers to the general name for 210 different PCDD and PCDF congeners. In addition, some PCBs possess a planar-type molecular

structure and toxicity similar to that of dioxins, and are referred to as "co-planar PCBs" or dioxin-like Compounds.

The toxic manifestations of the above substances appear to share a common mechanism of action mediated by the Ah receptor. The method used to express the degree of the toxicity of the individual congeners is based on utilization of toxic equivalency factors (TEFs), with the toxicity of TCDD set equal to "1".

TEFs have been established by WHO committee and others by comparing test results on long-term toxicity, short-term toxicity, and in vivo and in vitro biochemical reactions for different congeners. The TEF figures have been revised from their previous values, and they are expected to improve as new scientific data are acquired in the future.

② Toxic Equivalents (TEQs)

Since dioxin is usually present in the environment in the form of a mixture of congeners, the degree of toxicity when ingested can be expressed as the toxic equivalent (TEQ) by multiplying the amount of each congener by its TEF, and adding up the products. Dioxin toxicity is evaluated internationally on the basis of the TEQs expressed as numerical values.

③ Calculation of TEQs according to New TEFs

Because TEFs have been corroborated to be generally correct by numerous studies, it is now considered valid to calculate TEQs based on the new TEFs re-evaluated by the WHO in 1997, and to use them in evaluations of exposure to dioxin. As shown in Table 3, dioxin which has been given TEF values include 7 PCDDs, 10 PCDFs, and 12 co-planar PCBs.

Table 3. Toxic Equivalency Factors (TEFs) of Dioxin Based on the Re-evaluation in 1997 by the WHO Meeting

	Congener	TEF value
PCDD (Polychlorinated dibenzo-p-dioxin)	2,3,7,8-TCDD	1
	1,2,3,7,8-PeCDD	1
	1,2,3,4,7,8,-HxCDD	0.1
	1,2,3,6,7,8-HxCDD	0.1
	1,2,3,7,8,9-HxCDD	0.1
	1,2,3,4,6,7,8-HpCDD	0.01
	OCDD	0.0001
PCDF (Polychlorinated dibenzofuran)	2,3,7,8-TCDF	0.1
	1,2,3,7,8-PeCDF	0.05
	2,3,4,7,8-PeCDF	0.5
	1,2,3,4,7,8-HxCDF	0.1
	1,2,3,6,7,8-HxCDF	0.1
	1,2,3,7,8,9-HxCDF	0.1
	2,3,4,6,7,8-HxCDF	0.1
	1,2,3,4,6,7,8-HpCDF	0.01
	1,2,3,4,7,8,9-HpCDF	0.01
	OCDF	0.0001
Co-planar PCB	3,4,4',5-TCB	0.0001
	3,3',4,4',-TCB	0.0001
	3,3',4,4',5-PeCB	0.1
	3,3',4,4',5,5'-HxCB	0.01
	2,3,3',4,4'-PeCB	0.0001
	2,3,4,4',5-PeCB	0.0005
	2,3',4,4',5-PeCB	0.0001
	2',3,4,4',5-PeCB	0.0001
	2,3,3',4,4',5-HxCB	0.0005
	2,3,3',4,4',5'-HxCB	0.0005
	2,3',4,4',5,5'-HxCB	0.00001
	2,3,3',4,4',5,5'-HpCB	0.0001

TEF value: Dioxins and dioxin-like compounds consist of many congeners and the levels of toxicity vary among congeners. Thus the degree of the toxicity of the individual congeners is expressed relative to the toxicity of 2,3,7,8-TCDD, which is assigned a TEF of 1.

III. ESTABLISHMENT OF TDI IN JAPAN IN 1999

1. Basic Approach

The expert committees first of all considered it appropriate to base TDI estimates on concepts 1) to 2) below, in view of the pharmacokinetic and toxic mechanisms of dioxin. These concepts are the same as the policy adopted by the WHO expert consultation (EA and MHW, 1999).

① Genotoxicity

Since almost all of the genotoxicity tests conducted both in vitro and in vivo have demonstrated the negative results, TCDD can be judged to have no direct genotoxicity. Therefore thresholds exist in toxicity studies and either NOAEL or LOAEL can be used as the basis to derive the TDI.

② Body burden

Continuous intake of a highly bioaccumulative chemicals over a long period, the amount that accumulates initially increases because more is absorbed than is metabolized and excreted. However, as the amount that bioaccumulates continues to increase, metabolism and excretion also increase, and eventually the amount present in the body (i.e. body burden) reaches a state of equilibrium at a certain level that corresponds to the amount of intake. Toxic manifestations caused by chemicals generally depend on the amount present in the body. An important factor to assess toxicity of a highly bioaccumulative chemicals is the amount of continuous intake that will lead to the level at which the body burden will manifest toxic-

ity. Moreover, because there are large species differences in the elimination half-life of dioxin from the body, the dosage itself is not appropriate to extrapolate the results of toxicity tests to humans. Rather, it is more appropriate to calculate the body burden from the dosage at which effects develop in the tests, to obtain the amount that if taken continuously would reach that body burden in humans.

Accordingly, it is concluded body burden is the measure to be selected for dioxin known to bioaccumulate with large species differences.

③ Evaluation of toxicity data

TDI is to be estimated based on the lowest body burden derived from the toxicity tests in which adverse reactions are observed. For the evaluation of data it is necessary to consider the toxicological significance of the endpoint, its dose-dependency, and the reliability and reproducibility of the tests.

④ Uncertainty factors

The significance of uncertainty factors is especially important when evaluating the toxicity of chemicals such as dioxin, whose adverse effects are highly diverse and for which large species and strain differences have been observed.

2. Survey of Body Burden in Various Toxicity Tests

Most of the toxicity tests on dioxins have been conducted by using the most toxic congener, TCDD. Body burdens calculated from data since 1990 on very low doses that caused toxic effects are shown in Table 4. This Table also incorporates new literature published after the WHO Consultation, but both are basically compatible since the Table includes all of the toxicity tests used for assessment in the WHO Consultation.

Because very little appropriate data to derive the NOAELs were available from the various tests surveyed, the LOAELs were used to calculate the TDI estimates. In addition, calculations to estimate body burden were based upon either actual experimental data that were considered reliable or findings reported in the literature.

It should be noted that the experimental results for estimating body burden obtained by Gray *et al.* were presented in the Executive Summary Document by the WHO Consultation, but the estimation method was not clearly described. Accordingly, several committee members visited investigators at the US EPA who had submitted the figures, and confirmed that the values were derived from actually determined data. In addition, it was confirmed that some of the body burden values presented at the WHO Consultation were based on conditions that differed from the dosage conditions when the toxic reactions were investigated. Accordingly, this paper utilizes newly calculated values instead of the noted body burden values.

3. Body Burden Levels that Served as the Basis for Estimating TDI

The results of the various toxicity tests described above, especially those in which the effect was observed at low body burden levels, were carefully assessed in regard to their validity as data for the basis of TDI estimates after considering toxicological significance, dose-dependency, and the reliability and reproducibility of the tests.

① Enzyme induction in rat and mouse

Induction of drug-metabolizing enzymes (CYP1A1) was observed in rats at a body burden level of 0.86 ng/kg, and a similar effect was observed in mouse liver at 20 ng/kg. However, it is more valid to regard these findings as an adaptive reaction of the body than a toxic reaction to TCDD (Table 4, Nos. 1 and 5).

② Changes in lymphocyte composition in marmoset

Alterations in lymphocyte composition were observed in marmosets at body burden levels of 9 ng/kg and 10 ng/kg (Table 4, Nos. 2 and 4). However, since the effect on T-lymphocyte subset composition ratios observed at higher doses were opposite of that seen at low doses, it is concluded to be inappropriate to use this data.

③ Chloracne in rabbit and human

Chloracne was observed in rabbits at a dose (topical application to the skin) of 4.0 ng/kg. However, this experiment showed the effect of local exposure and it does not appear appropriate to use this data as a basis for calculating body burden (Table 4, No. 6). Moreover, since human findings have been obtained in regard to chloracne, the human data take priority in calculating the TDI. The minimal body burden

Table 4. Summary of Experimental Results on Low Level Effects of 2,3,7,8-TCDD

No.	Species	Biological Effects	Exposure (LOEL or LOAEL)*		Body Burden ng/kg	Exposure Level for Humans** pg/kg/day	References	***
1	rat	Induction of P450 enzymes	1	Single injection (sc)	0.86	0.44	Van den Heuvel *et al.* (1994)	1
2	marmoset	Altered lymphocyte subsets	0.3	1 injection/week (sc), 24 weeks, thereafter 1.5 ng/kg/week(sc), 12 weeks	9	4.56	Neubert *et al.* (1992)	1
3	mouse	Enhanced viral susceptibility	10	Single injection (sc), infection 7days after TCDD injection.	9	4.56	Burleson *et al.* (1996)	1
4	marmoset	Altered lymphocyte subsets	10	Single injection (sc)	10	5.06	Neubert *et al.* (1990)	1
5	mouse	Induction of P450 enzymes	1.5	5 injections/week (po), 13 weeks	20	10.13	DeVito *et al.* (1994)	1
6	rabbit	Chloracne	4.0	Spread on skin 5times/week, 4 weeks	22	11.14	Schwetz *et al.* (1973)	1
7	rat	Decreased spermatid count in testes	25	Single injection (maternal sc), thereafter 5 ng/kg/week (maternal sc) till weaned. Start mating 2 weeks after the first injection.	27	13.67	Faqi *et al.* (1998)	1
8	monkey	Object learning	0.151	20.2 months in mothers diet	29	14.69	Schwetz & Bowman (1989)	1
9	monkey	Endometriosis	0.15	4years in the diet	40	20.26	Rier *et al.* (1993)	1
10	rat	short anogenital distance	50.0	Single injection (maternal po), dissolved in corn oil.	43	21.77	Ohsako *et al.* (1999)	1
11	rat	Decreased spermatid count in testes	64	Single injection (maternal po), dissolved in corn oil.	55	27.85	Mably *et al.* (1992)	1
12	rat	Immunotoxicity	100	Single injection (maternal po), dissolved in corn oil.	86	43.55	Gehrs *et al.* (1997)	1
13	rat	Malformation of reproductive organ	200	Single injection (maternal po), dissolved in corn oil.	86	43.55	Gray *et al.* (1997)	2
14	rat	Decreased sperm count in epididymis	200	Single injection (maternal po), dissolved in corn oil.	86	43.55	Gray *et al.* (1997)	2
15	mouse	Immunotoxicity	100	Single injection (ip), dissolved in corn oil.	100	50.64	Narashimhan *et al.* (1994)	1
16	monkey	Increased offspring death rate (Decreased offspring viability)	0.76	4 years in the diet	202	102.3	Bowman *et al.* (1989)	1
17	rat	Decreased birth weight	400	Single maternal ig intubation, dissolved in corn oil.	344	174.2	Mably *et al.* (1992)	1
18	monkey	Chloracne	1000	9 ig intubations in 4 animals, or single ig intubation in 12 animals.	500	253.2	McNulty *et al.* (1985)	1
19	rat	Kidney abnormalities	500	Single injection (sc)	500	253.2	Courtney *et al.* (1971)	1
20	rat	Increased offspring death rate (Decreased offspring viability)	1000	Single injection (po)	860	435.5	Gray *et al.* (1997)	1
21	rat	Delayed developmental milestones	1000	Single maternal ig intubation, dissolved in corn oil.	860	435.5	Bjerke & Peterson *et al.* (1994)	1
22	mouse	Cancer	71.4	2 ig intubations/week, 104 weeks	979	495.7	NTP No. 209 (1982)	1
23	rat	Cancer	100	in the diet, 2 years	1710	865.8	Kociba *et al.* (1978)	1
24	hamster	Decreased birth weight	2000	Single maternal ig intubation (unidentified)	1720	870.8	Schueplein *et al.* (1991)	1
25	mouse	Hydronephrosis	3000	ig intubation dissolved in corn oil, 30 weeks	2580	1306	Couture *et al.* (1990)	1
26	rat	Down regulation of EGFR	125	ig intubation dissolved in corn oil, 30 weeks	3669	1858	Sewall (1993)	1
27	rat	Cancer promotion	125	ig intubation dissolved in corn oil, 30 weeks	3669	1858	Maronpot *et al.* (1993)	1

*: po; per oral administration, sc; subcutaneous administration, ip; intraperitoneal administration ig;intragastric. **: Human daily intake on normal condition was calculated on the assumption that a half-life for elimination of 7.5 years and absorption of 50%. Human daily intake = (body burden *ln2)/(T1/2*absorption rate). ***: 1: Calculated from the exposure condition of original report (assumes a gastrointestinal absorption of 50% in the dioxins containing diet and 86% peroral treatment with corn oil.). 2: Body burden was calculated based on results at gestational day 16 and 21 (Hurst *et al.*, personal commu unication).

level at which chloracne has been observed in humans is reported to be 95 ng/kg.

④ Immunotoxicity in rat and mouse

Toxicity to the immune system, for which delayed hypersensitivity was used as an index, was observed in the offspring of rats at a body burden of 86 ng/kg (Table 4, No. 12). Also immunotoxicity in the adult mice, for which inhibition of antibody formation was used as the index, was observed at 100 ng/kg (Table 4, No. 15). Since these findings also showed dose-dependency, they are considered to be the effects of TCDD.

By contrast, the experiment which showed that viral infections increased at 10 ng/kg was considered to be inappropriate as a basis for TDI estimate, since this effect occurred without dose-dependency (Table 4, No. 3).

Since the immune system is an extremely complex network that is composed of various cell populations and soluble factors, detailed studies using multiple indices will be definitely needed to analyze the effects of dioxins on this system.

⑤ Effects on the male rat reproductive system

Effects on spermatogenesis which were observed at low body burdens include decreases in numbers of spermatids in the testes and of sperms in the cauda epididymis at body burdens of 27 ng/kg and above, 55 ng/kg and above, and 86 ng/kg and above (Table 4, Nos. 7, 11, 14).

These changes could be regarded as toxic effects. However, consistency with other tests is not apparent with regard to the effects on the male reproductive system, in terms of associations between body burden levels and manifestation of the effects. More specifically, no effects have been observed in the sperm count in semen at these body burden levels but the effects have been reported at 425 ng/kg, and a statistically significant difference in fertility of the offspring from the control group have not even been observed at 860 ng/kg. Moreover, experiments conducted under the same conditions as those by Mably *et al.* did not demonstrate any effect on the spermatid count in the testes or sperm numbers in the cauda epididymis even at a body burden of 688 ng/kg, although anogenital distance was observed to be shorter at the 43 ng/kg level (Table 4, No. 10).

As described above, the relationship between the manifestation of effects on the male reproductive system and body burden levels differ among the endpoints, the test parameters, or the laboratories that conducted the tests. Accordingly, the minimal body burden that caused the effects should be determined on the basis of comprehensive assessment of multiple related experiments, not on the basis of a particular single experiment.

⑥ Endometriosis and reduced learning ability of offspring in rhesus monkeys

Technical flaws have been pointed out relating to animal care conditions and other factors in the experiments which demonstrated an increased incidence of endometriosis in rhesus monkeys at a body burden of 40 ng/kg (Table 4, No. 9). Accordingly, the reliability of the test is considered inadequate for estimation of TDI.

Moreover, although a decrease in scores on learning ability test was observed in the offspring of the rhesus monkeys at a body burden of 29~38 ng/kg at the same research institution, it appeared to be a mild effect from which the animals could recover by training (Table 4, No. 8). It should be noted that in this case evaluation was made based on behavioral tests alone, and neurochemical, anatomical, or histopathological examinations were not performed.

⑦ Female genital anomalies in rat

Genital anomalies that were observed in the female offspring of rats (Table 4, No. 13) are considered to be significant toxic endpoints. The test was judged to be valid in terms of dose-dependency and the reliability of the test.

In this test, rats were given TCDD on gestational day 15. Measurements of body burden yielded 97 ng/kg on day 16 and 76 ng/kg on day 21. Since the embryological critical period is thought to be between gestational day 16 and day 21, the value midway between these measurements, 86 ng/kg, is calculated and used as the body burden in the critical period.

4. Human Body Burden

There have been no reports of systematic studies on the relationship between body burden and species differences with regard to toxic manifestations of dioxin. However, when results of existing toxicity tests and epidemiological surveys are integrated, it is con-

sidered that major differences do not emerge between humans and animals for body burden values that cause toxic effects. A similar conclusion was made at the 1998 WHO Consultation. In view of the above, it is reasonable to assume that the minimal body burden level that produces a certain toxic effect in animal toxicity tests is the minimal body burden level that exerts a toxic effect in humans as well.

5. Estimation of Human Daily Intake

The following formula is used in this paper to estimate the daily intake necessary for humans to reach a certain body burden as a result of life-long exposure. This is the same as adopted at the WHO Consultation:

$$\text{Human daily intake} = \frac{\text{body burden} \times \ln 2^*}{\text{7.5-yr half-life} \times 50\% \text{ absorption rate}}$$

*ln2 = 0.693

6. Determination of UF

In order to compensate for uncertainties when calculating the human TDI based on the LOAEL for humans inferred from toxicity test data, it is necessary to apply UF. This paper uses an UF of 10, after taking the following points into consideration.

① The LOAELs are used instead of the NOAELs as the value for the basis of TDI calculations.

② The body burden value is used when calculating the minimal toxic level in humans. Therefore species differences factor that arises from pharmacokinetics need not be considered, as discussed above.

③ There is no clear evidence showing that humans are more sensitive to dioxins than experimental animals. In fact, there are data from studies on affinity for the Ah receptor that suggest that humans are less sensitive.

④ Data related to individual differences in toxic manifestations in humans are insufficient.

⑤ Data are inadequate on the half-life of each of the dioxin congeners.

7. Derivation of TDI

① Report of the WHO Consultation

Based on the results of various toxicity tests, the WHO Consultation set 1~4 pg TEQ/kg/day as the range of TDI values. The daily intake in industrialized countries is 2~6 pg TEQ/kg/day, and subtle effects may be manifested in people of these countries. However, the subtle effects that have been reported do not appear to be overtly adverse effects and other chemical substances may be involved in the effects. Accordingly the WHO Consultation considered the current exposure levels to be tolerable, setting 4 pg TEQ/kg/day as the maximal tolerable intake, while stating that the ultimate goal should be to reduce the human intake levels to less than 1 pg TEQ/kg/day.

The mean daily dioxin intake of the Japanese population is currently approximately 2.6 pg TEQ/kg/day, and the decreasing concentration of dioxins in breast milk indicates that the exposure level is also decreasing. Therefore the existing exposure conditions in Japan are within the tolerable range.

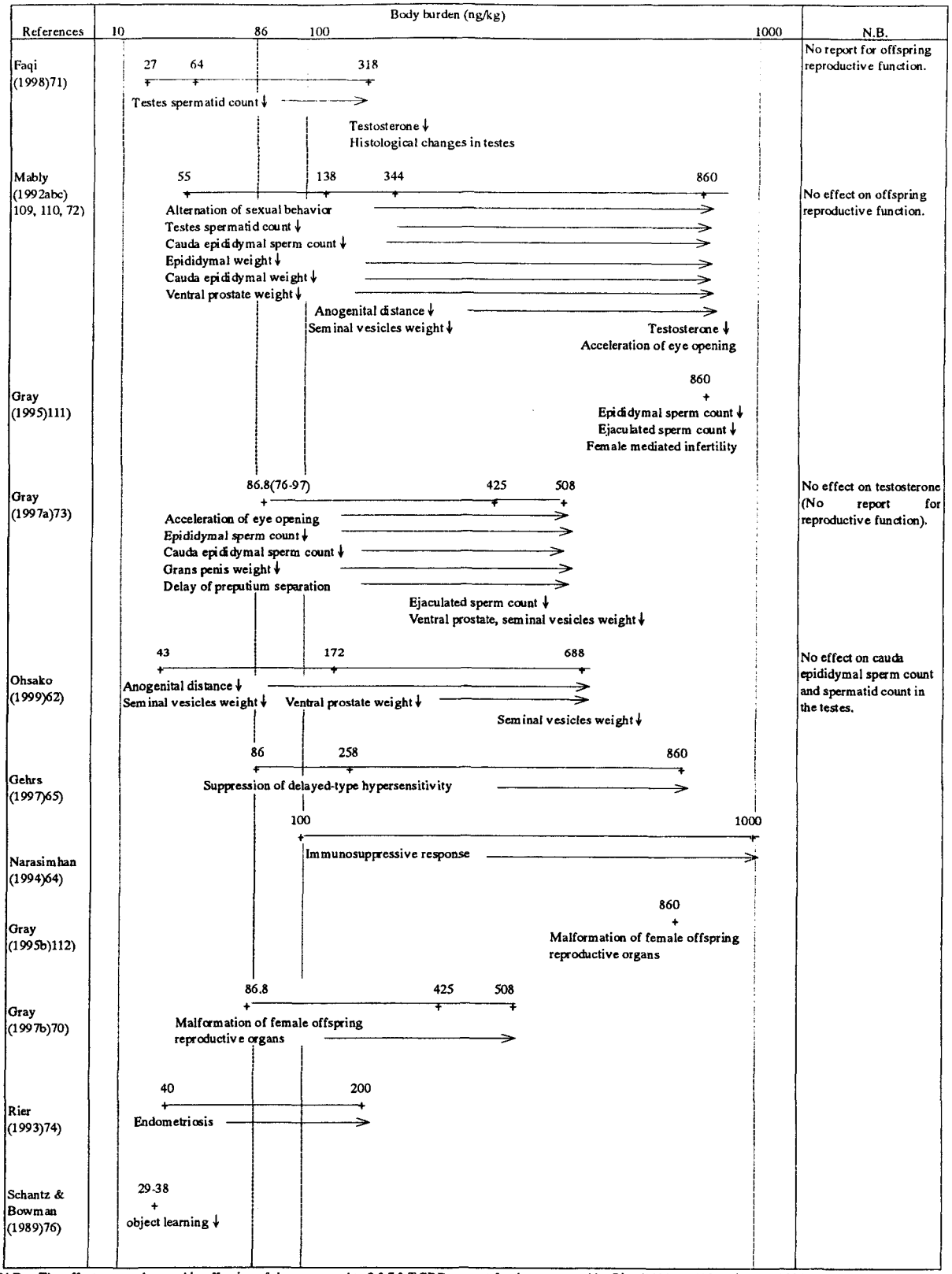
② Selection of body burden value as the basis for calculating TDI in Japan

The relation between body burden and manifestation of effects in each experiment is shown in Fig. 1. A level of approximately 86 ng/kg is the lowest body burden value just below or above that at which clearly toxic effects are manifested, including female genital anomalies. In some experiments effects have been observed at lower body burden values, but when dose-dependency, reliability, reproducibility, and the toxicological significance of the data are comprehensively taken into consideration, the numerical values have relatively low reliability, and they are considered inadequate to use as indices for human health effects.

For these reasons, it is generally appropriate to use 86 ng/kg as the basis to calculate the TDI. This view is based on the perspective that body burden as a basis for estimating TDI should be decided after carrying out a comprehensive evaluation of test results, rather than specific numerical values from specific tests.

③ Conclusion on TDI in Japan

While some aspects of the human health effects of dioxin remain unresolved, it is reasonable to set the provisional TDI for dioxin (including co-planar PCBs) at 4 pg TEQ/kg/day. This derived by applying an UF of 10 to the human daily intake of 43.6 pg TEQ/kg/day, which corresponds to a body burden of 86 ng/kg of TCDD (Fig. 2).



N.B. The effects were observed in offspring of dams exposed to 2,3,7,8-TCDD, except for those reported by Rier (1993) and Narasimhan (1994). The dose dependent effects observed in each experiment are presented in this table.

Fig. 1. Relationships between Body Burden and Effects Observed at Low Levels.

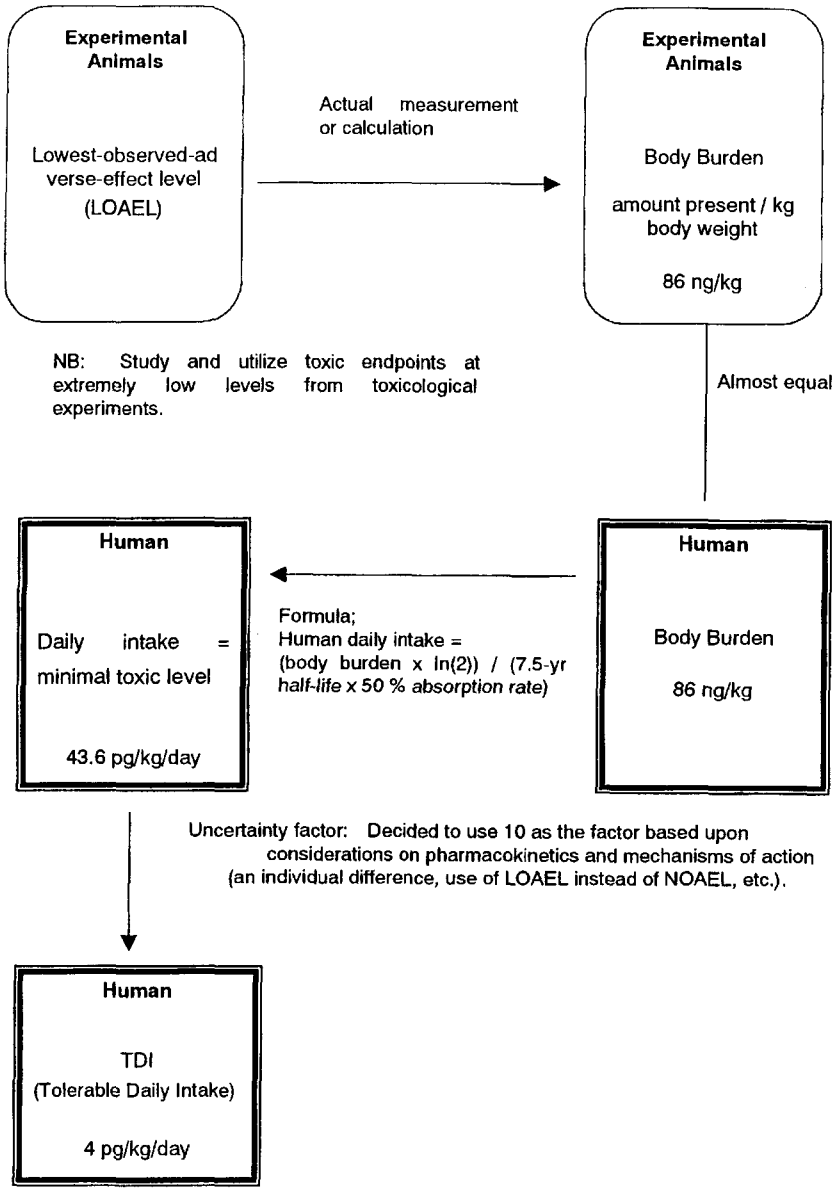


Fig. 2. Establishment of Dioxin TDI by Using Body Burden.

Since subtle effects have been observed at body burden levels below 86 ng/kg in some studies, further research is necessary including those on the toxicological significance of these effects.

IV. DISCUSSION

1. Differences from Earlier Methods of Estimating TDI

In both the 1990 WHO report and 1996 MHW report, the human TDI was calculated by directly applying UF to the NOEL or NOAEL. However, in the WHO report of 1998 and the Japanese evaluation, body burden values are used instead as the basis for calculating the TDI.

When the TDI was calculated from the NOEL or NOAEL in the past, UF was applied empirically, using a standard value of 100. However, this has changed in recent years due to more appropriate risk assessment methods for humans, with UF that recognizes species and individual differences, taking into account findings related to the pharmacokinetics and mechanisms of action of test substances. This paper

also sets the UF at 10, the reasons for which has been stated above.

When making risk assessment on dioxin in the past, generally the results of long-term tests were used as the basis. However, it is now reasonable to assume that most of the toxicity of dioxin is mediated by binding to the Ah receptor. It has become possible to apply the results of single and short-term toxicity tests to long-term low level exposure in humans, by using body burden approach. As a result, highly sensitive endpoints observed in short term reproductive toxicity tests have been used in this paper.

2. Points to consider on TDI

① TDI is an index of life-long exposure

It should be stressed that TDI is a value calculated as an index of effects on health when daily intake continues throughout life. This means that there will be no damage to health even if intake temporarily slightly exceeds the TDI during the course of a lifetime, as long as the average intake over the long period is within the TDI.

② TDI is derived from the most sensitive endpoints

It is important to remember that effects observed in the critical period, considered to be the most sensitive period, were used to calculate the TDI in this paper. Thus, it can be regarded as being on the safe side for evaluation of the human population as a whole, and effect such as carcinogenicity, for example, would occur as a result of higher exposure.

④ TDI uses an uncertainty factor.

The fact that UF has been applied to the TDI means that allowances have been made for differences in sensitivity between humans and animals as well as for individual differences.

REFERENCES

Assessment of the health risk of dioxins: reevaluation of the tolerable daily intake (TDI) (2000): eds. F.X.R. Van Leeuwen and M.M. Younes. Food Additives & Contaminants. Vol. 17, No. 4.

Environment Agency (Japan) (1997): Dioxin Risk Assessment Committee Report (in Japanese).

Environment Agency and Ministry of Health and Welfare (1999): Report on Tolerable Daily Intake (TDI) of Dioxins and Related Compounds (Japan).

Ministry of Health and Welfare (Japan) (1996): Interim Report of Studies on Dioxin Risk Assessment.

US. EPA (1994): Health assessment document for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds.

WHO (1991): Summary Report of "Consultation on Tolerable Daily Intake from Food of PCDDs and PCDFs".

WHO (1999): Executive Summary Report of "Assessment of the health risks of dioxins: re-evaluation of the Tolerable Daily Intake (TDI).