

Effects of Dioxins on Human Health : A Review

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The toxicity of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) has been known since 1950s. TCDD is a by-product of herbicide 2,4-dichloroacetophenol (2,4-D) and 2,4,5-trichloroacetophenol (2,4,5-T), but it was first found in flyash of municipal incinerator in 1979 in Japan. In 1998, the survey of municipal incinerators revealed that 105 out of 1,641 produced above the allowed emission level of 80 ng TEQ/m³. Total annual release of dioxins is estimated to be about 5,000 g TEQ in 1997 in Japan. Japanese government started a comprehensive survey for dioxin levels in milk and blood of residents around incinerators, and their health effects.

Human effects by dioxin exposures in Western countries were mostly acute and at high level in accidentally and/or occupationally. Health effects of low-dose and long lasting exposure has not been well understood.

Certain amount of polychlorinated dibenzo-p-dioxins (PCDD), dibenzofurans (PCDF) and polychlorinated biphenyls (PCB) is accumulated in our body. Mother's milk is also contaminated by PCDD/PCDF. Health effects of the polychlorinated chemicals are summarized, and the necessity of regulations and recommendations for making a guideline is discussed in this review. *J Epidemiol*, 1999 ; 9 : 1-13

Polychloro-dibenzodioxins, Dibenzofurans, Health effect, Human, Epidemiology

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/PCDF) occur as unwanted by-products of thermal processes, so the major part of their environmental distribution is caused by human activity¹⁾. Burning waste materials that may contain chlorine, such as plastics, pesticide-treated wastes, wood treated with pentachlorophenol (PCP), other polychlorinated chemicals can produce PCDD/PCDF¹⁾. Waste incineration is the most important sources of emission now, although herbicide industry, chlorine and paper industry, melting processes, car traffic, etc. yield PCDD/PCDF. Sakurai et al²⁾ studied on the origin of dioxin pollution from the isomer pattern in Kasumigaura, Ibaragi, Japan, and estimated that it was attributable to air (39%), CNP (22%), and PCP (22%). PCDDs have also been detected in cigarette smoke. Side stream smoke contained more PCDD/PCDF (1.3-9.2 pg-TEQ/pack)(Shiosaki et al, personal communication). The Environmental Agency estimated annual release of dioxins in Japan being about 5,000 g³⁾(Table 1). Japan becomes one of the most heavily polluted

Table 1. Estimated amount of dioxins in Japan.

Origin	Amount of emission (g TEQ/year)
Municipal incinerator	4,300
Industrial incinerator	547-707
Metal refining	250
Oil supplement (lubricant)	20
Cigarette smoke	15
Collected waste oil boiler	3
Burning of waste wood	0.2
Exhaust gas of cars	0.07
Bleaching process of pulp	0.78
Herbicide industry (PCNB)	0.06
Total	5,140-5,300

Environmental Agency, 1997

Received November 14, 1998 ; accepted December 24, 1998.

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country by dioxins in the world.

The US Environmental Protection Agency (EPA) has regulated dioxin (2,3,7,8-TCDD) as a carcinogen based on the positive animal data and the compatible epidemiological findings of occupational exposure³. 2,3,7,8-TCDD is classified to group 1 carcinogen at the IARC review work in February 1997⁴. PCDD and PCDF have many isomers. Of the 75 PCDD isomers and 135 PCDF isomers, only 7 and 10, respectively, are likely to have toxic properties similar to 2,3,7,8-TCDD. Thirteen polychlorinated biphenyl (PCB) are also have toxicity among 206 different PCB congeners. The toxicity are summed based upon their toxicity equivalency factor (TEF)(Figure 1.). Determination of TEF still contained some problem, but, so far, it is practical for evaluating risk for public health.

Biological effects other than cancer caused by a background level exposure is unclear. Lowered immune function and possible activity as endocrine disrupter are plausible human toxicity from animal experiments⁵. It works at very low concentration at level of ng/kg (pg/g) body weight. The determination of human burden (cumulative dose) in such a low concentration had been impossible until the high resolution mass-spectrometry have been developed for quantitative measurement.






























WHO and several countries develop regulations and recommendations to protect public health. Regulations should be

enforced by the law. This review deals with recent situation of dioxin research in Japan and world, mainly focused on human health effects.

PRODUCTION OF DIOXINS AND RELATED SUBSTANCE

Dioxins are produced from chlorinated materials and aromatic carbohydrates by burning trash in an incinerator. When there is catalysts, like iron, the producibility becomes high. When two oxygens combine two benzenes (or chlorinated benzene), it becomes para-dibenzodioxins, and when one oxygen combines two benzenes, it is called dibenzofuran. Both chemicals could have up to 8 chlorines, so the number of isomers is many; 75 for PCDDs, and 135 for PCDFs. All these compounds are called congeners. Some PCBs (polychlorinated biphenyls) have similar character with dioxins, because of their flat structure. These four isomers among 206 isomers are called coplanar PCBs. Toxicity of these congeners is evaluated based on the 2,3,7,8-TCDD, and expressed as toxic equivalency factor (TEF). Presence of four chlorine atoms at positions 2,3,7, and 8 on the dioxin molecules seems to cause toxicity (Figure 1.). The toxicity are summed up based upon their toxicity equivalents concentration (TEQ), and expressed as pg TEQ/g lipid (ppt).

A general observation for human background contamination

PCDDs (dioxins)		PCDFs (furans)		PCBs with no chlorine at orthopositions ('coplanar'PCBs)		PCBs with one chlorine atom at ortho position	
	TEF		TEF		TEF		TEF
2,3,7,8-TCDD (TCDD)	 1	2,3,7,8-TCDF	 0.1	3,3',4,4'-TCB	 0.0001	2,3,3',4,4'-PeCB	 0.0001
1,2,3,7,8-PeCDD	 1	1,2,3,7,8-PeCDF	 0.05	3,4,4',5-TCB	 0.0001	2,3,4,4',5-PeCB	 0.0005
1,2,3,4,7,8-HxCDD	 0.1	2,3,4,7,8-PeCDF	 0.5	3,3',4,4',5-PeCB	 0.1	2,3',4,4',5-PeCB	 0.0001
1,2,3,7,8,9-HxCDD	 0.1	1,2,3,4,7,8-HxCDF	 0.1	3,3',4,4',5,5'-HxCB	 0.01	2',3,4,4',5-PeCB	 0.0001
1,2,3,6,7,8-HxCDD	 0.1	1,2,3,7,8,9-HxCDF	 0.1			2,3,3',4,4',5-HxCB	 0.0005
1,2,3,4,6,7,8-HpCDD	 0.01	1,2,3,6,7,8-HxCDF	 0.1			2,3,3',4,4',5'-HxCB	 0.0005
OCDD	 0.0001	2,3,4,6,7,8-HxCDF	 0.1			2,3',4,4',5,5'-HxCB	 0.00001
		1,2,3,4,6,7,8-HpCDF	 0.01			2,3,3',4,4',5,5'-HpCB	 0.0001
		1,2,3,4,7,8,9-HpCDF	 0.01				
		OCDF	 0.0001				

T = tetra (4 chlorine atoms)
 Pe = penta (5 chlorine atoms)
 Hx = hexa (6 chlorine atoms)
 Hp = hepta (7 chlorine atoms)
 O = octa (8 chlorine atoms)

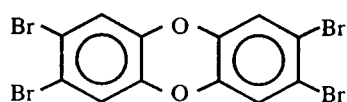
Figure 1. Dioxin congeners with TEF.

PCDDs, PCDFs, and PCBs which have toxicity equivalency factor (TEF) for human beings and mammals.

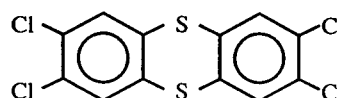
Modified from Bernes C, Persistent Organic Pollutant, Swedish Environmental Protection Agency, 1998.

Table 2. The TEQ concept by European Center of Environmental Health of WHO and International Program on Chemical Safety.

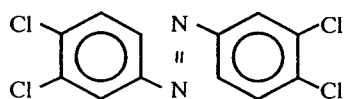
1.	A compound must show a structure relationship to the PCDDs and PCDFs.
2.	A compound must bind to the Ah receptor.
3.	A compound must elicit Ah receptor-mediated biochemical and toxic responses.
4.	A compound must be persistent and accumulate in the food chain.



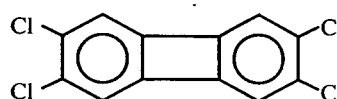
2,3,7,8-Tetrabromodibenzo-*p*-dioxin



2,3,7,8-Tetrachlorodibenzothiantrene



3,3',4,4'-Tetrachloroazobenzene



2,3,7,8-Tetrachlorobiphenylene

Figure 2. Dioxins and related compounds.

Various compounds which are considered to show similar effects of PCDDs/PCDFs.

is that OCDD is the most abundant isomer, followed by the 2,3,7,8-substituted hepta- and hexa-chloro-congeners. 2,3,7,8-TCDD is normally less abundant than PeCDD.

The toxicity is considered to be additive. The determination of TEF is based upon the activation of Ah receptor, induction of CYP1A1, in animal experiment, for chemicals with dioxin-like structure (Table 2). So the other effects that are not via Ah receptor are not evaluated by TEQ expression.

The oxygen bond between two benzenes could be substituted to sulfur or azide (Figure 2.). Similarly, chlorine could be replaced by bromium. Toxicity of bromodioxins is considered to be similar to that of chlorinated dioxins⁵⁶. Recent increase of polybromobiphenyl (PBB) calls attention by environmentalists, whether or not the chemicals have human health effects. PBB is now widely used for inflammable clothes. More sophisticated experimental design is necessary to screen toxicological effects⁷.

Until 1970, PCDD/PCDF had been made as a contaminant in the manufacturing process of certain chlorinated organic

chemicals, such as 2,4-D and 2,4,5-T. 2,4,5-T has been widely sprayed for weed control on lands, and along roadways throughout the world. 2,4,5-T was a component of Agent Orange, which was heavily used by the US military in the Vietnam War⁸⁸. Currently emission from incinerator sources becomes problem. Management practices and applied technologies influenced the emission of dioxins¹⁰⁻¹². Recent report from Osaka showed extremely high concentration of PCDD/PCDF around incinerator due to smoke washing system (Watanabe et al. in preparation).

UPTAKE BY HUMANS AND ACCUMULATION

Three routes are considered for human exposure; eating food, breathing polluted air, and skin contact with contaminated soil and materials¹³⁻¹⁶.

Plants take up only very small amounts of PCDDs by their roots. However, spinach and several vegetables contained dioxins at certain level¹⁷. Most of the PCDDs found on the

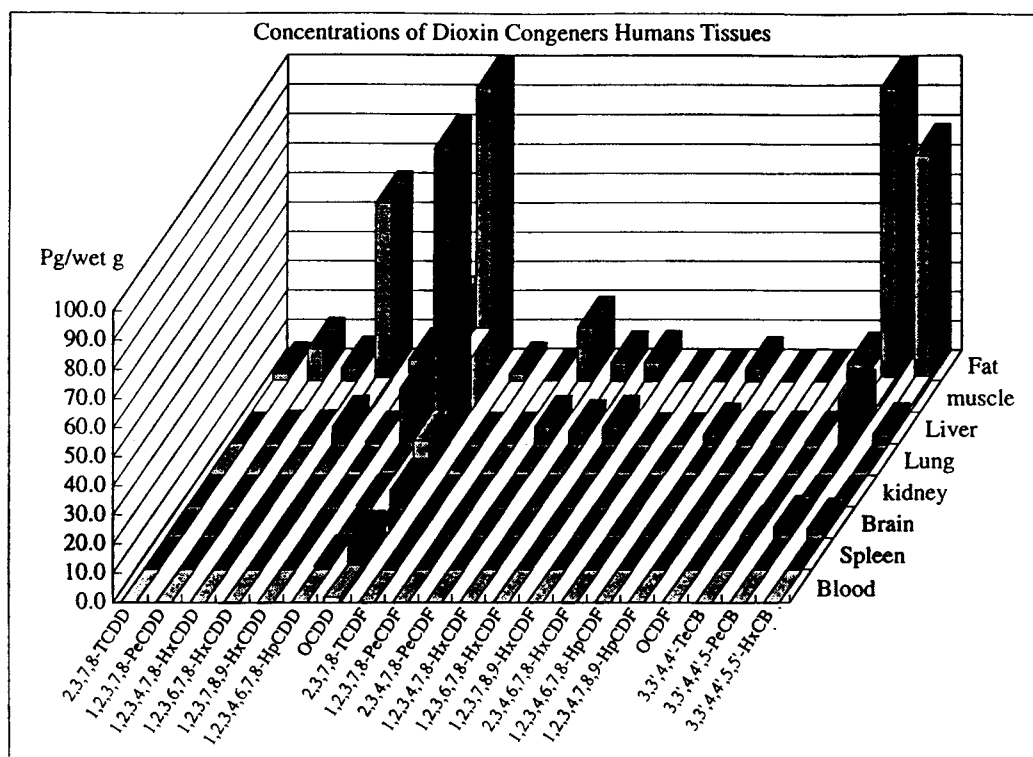


Figure 3. Tissue distribution of dioxin congeners.

OCDD, coplaner PCBs and hexachlorodioxins are commonly found in the fat, liver, and muscle.

parts of plants above the ground probably comes from air and dust of soil erosion or surface runoff. Several plants can accumulate organic substances in their lipophilic cuticle. Transfer from air to plants and the accumulation in plants is considered in investigations concerning the surveillance of emissions. Pine needles and kales were used as convenient bioindicator of PCDD/F pollution^{18,19}.

Plants and animals can condense through respiration or food chains. We are exposed to 0.52-4.52 pg TEQ/kg/day in urban area, 0.5-3.5 pg TEQ/kg/day in towns, and 0.29-3.29 pg TEQ/kg/day in background area³(Table 3). The actual intake of PCDDs from food for any one person will depend on the amount and type of food consumed and the level of contamination¹⁵. Japanese takes a lot of fishes, so the frequent fish eaters consume more PCDD/PCDF/PCB. Coast-side fishes, especially from areas contaminated with chemicals, contained more dioxins¹⁷. Dioxin congeners are known to be accumulated mostly in fat and liver of fishes and animals.

In Japan, 90% of the daily intake of PCDDs, PCDFs, and other dioxin-like compounds is estimated to come from food, primarily fish and meat in general population. Japanese levels

of PCDD/PCDF in blood from the general population ranged from 0.13 to 29 pg TEQ/g lipid (8.21 pg TEQ/g lipid in average) in Saitama prefecture. The concentrations in humans are higher in industrialized countries, being about 15 pg TEQ/g lipid and normally below 10 pg TEQ/g lipid. These values are several orders of magnitude lower than those observed in accidentally or occupationally exposed individuals⁶.

Table 3. Estimated intake of dioxins by average Japanese.

Origin	Urban area	Towns	Background area
Food	0.26-3.26	0.26-3.26	0.26-3.26
Air	0.18	0.15	0.02
Water	0.001	0.001	0.001
Soil	0.084	0.084	0.008
Total	3.59	3.56	3.35
Range	0.52-4.52	0.49-3.5	0.29-3.29

If fish consumption is large, dioxins from food becomes 3.32 pg-TEQ/kg/day in each area.

Breast milk contains relatively high levels of PCDDs. In Saitama prefecture, it was 2.8-76 pg TEQ/g lipid in milk, in average, 15 ± 8 pg TEQ/g lipid. Breast feeding infants are exposed to higher levels of dioxins on a body-weight basis than are adults (Tada, personal communication). This causes anxiety whether or not they have the future problem.

BODY DISTRIBUTION

Most of the PCDDs pass from the gastrointestinal tract to the blood stream after eating food containing PCDDs, and then distributed to the liver and other adipose tissue. PCDDs can also enter our body through the lungs by breathing polluted air and pass into the blood stream. Body burden or whole body wet weight dioxin concentration can be calculated from the blood PCDD/PCDF levels. It is assumed that the concentration of 2,3,7,8-TCDD in serum lipid is in equilibrium with total body lipid 2,3,7,8-TCDD concentrations⁷⁰.

Half-life of 2,3,7,8-TCDD in the body is variable and may take 7 to 12 years¹⁴⁸(Table 4). More chlorinated TCDD seemed to have shorter half-life. Half-life-adjusted serum levels and body burdens were calculated using a half-life of 8.7 years and lipid dose of 22% of body weight²⁰.

Congener-specific distribution in dioxin levels between various tissues is present²¹. Iida et al²⁰ measured PCDD/PCDF congeners in various tissues in autopsy cases, in which liver and fat tissues showed the highest concentrations (Fig. 3). Muscle and lung also contained certain level of PCDD/PCB. PCDDs are eliminated from the body primarily from the sebaceous gland, in the stool, and only a small amount left the body in the urine^{23,20}. Moderate amount of PCDDs leaves the body in the breast milk of nursing mother²⁹.

In breast fed baby, the estimated dietary intakes of PCDDs and PCDFs often exceeded the TDL²⁰. The breast feeding period is short compared with the long biological half-lives of 2,3,7,8-TCDD, so it will be diluted by rapidly increasing body weight²⁷. Taken over a lifetime, the eventual body burden would not be greatly increased by this short period. Breast feeding gives considerable benefits on the baby in terms of immunological protection, nutrition and mother-infant relation-

ship. The WHO Working Group which considered this issue in 1998 recommended that breast feeding should be encouraged and promoted in the basis of convincing evidence on its benefits to the overall health and development of the infant, despite the presence of dioxins in human milk²⁷. Effects on development, endocrine and immune system, however, need further study on breast fed infants.

HEALTH EFFECTS

Various health effects associated with exposure to PCDD (primarily 2,3,7,8-TCDD) have been reported²⁸⁻³¹. Most effects are considered to occur through PCDDs binding to Ah receptor, and following gene activation (Figure 4). Other pathways seem to be present¹.

Chloracne was a classical manifestation caused by chronic exposure. Others are systemic, immunological, neurological, developmental, reproductive, genotoxic, and carcinogenic effects. The LD50 of dioxins is 1,000 ng/kg body weight in rat. LD50 is different by species, but 60 μ g TCDD could kill 60 kg man, if the LD50 of rat is similar to human. Human death, however, has not been reported so far by examining human acutely exposed to high concentrations of TCDD/TCDF.

Only populations come from accidental, occupational, and residential exposure have been evaluated^{1,2,8,9,31-35}. Instead of clinical symptom like chloracne as a surrogate marker, recent studies have used blood lipid PCDD levels as a measure of internal dose in order to quantify exposure in individuals.

CANCER

Exposure to 2,3,7,8-TCDD have occurred occupationally in factory workers handling 2,4-D and 2,4,5-T. The first industrial exposure was 2,4,5-T producing factory in West Virginia in 1949. Three other explosive releases were reported from the BASF AG facility in Germany in 1953, from Philips-Duphar facility (Netherlands) in 1963, and at 2,4,5-T manufacturing facility in England in 1973³¹⁻³³. An industrial accident at the ICMESA plant in Seveso, near Milan, Italy caused population-sized exposure³⁴⁻³⁹. The reactor overheated during hydrolysis of 1,2,4,5-tetrachlorobenzene with alkali in ethylene glycol and the safety valve ruptured releasing a cloud containing trichlorophenatate and TCDD. It was estimated that more than 1300 g TCDD was released and that more than 17,000 people were exposed. Other large sized exposures were those in Missouri, where about 29 kg of TCDD contaminated waste oil was sprayed on horse areas, parking lots and residential roads for dust control in 1971 and 1972²⁰, and those in the Vietnam War where 76 million L of herbicides were dispersed²⁹. These cases were precisely reviewed at IARC review committee and the carcinogenicity of TCDD was defined². The overall relative risk was 1.4, the sites of cancer were various, and long

Table 4. Biological half-life (year) of dioxins in human body.

Chemicals	Child blood	Blood	Adult	
			Fat	Whole body
PCB congeners	0.28	7.1		
2,3,7,8-TCDD		7.1-11.3	9.7	5.8
2,3,4,7,8-PCDF		7.2	4.7	
1,2,3,4,7,8-HCDF		4.4	2.9	
1,2,3,6,7,8-HCDF		4.3	3.5	
1,2,3,4,6,7,8-HCDF		4.1	6.5	

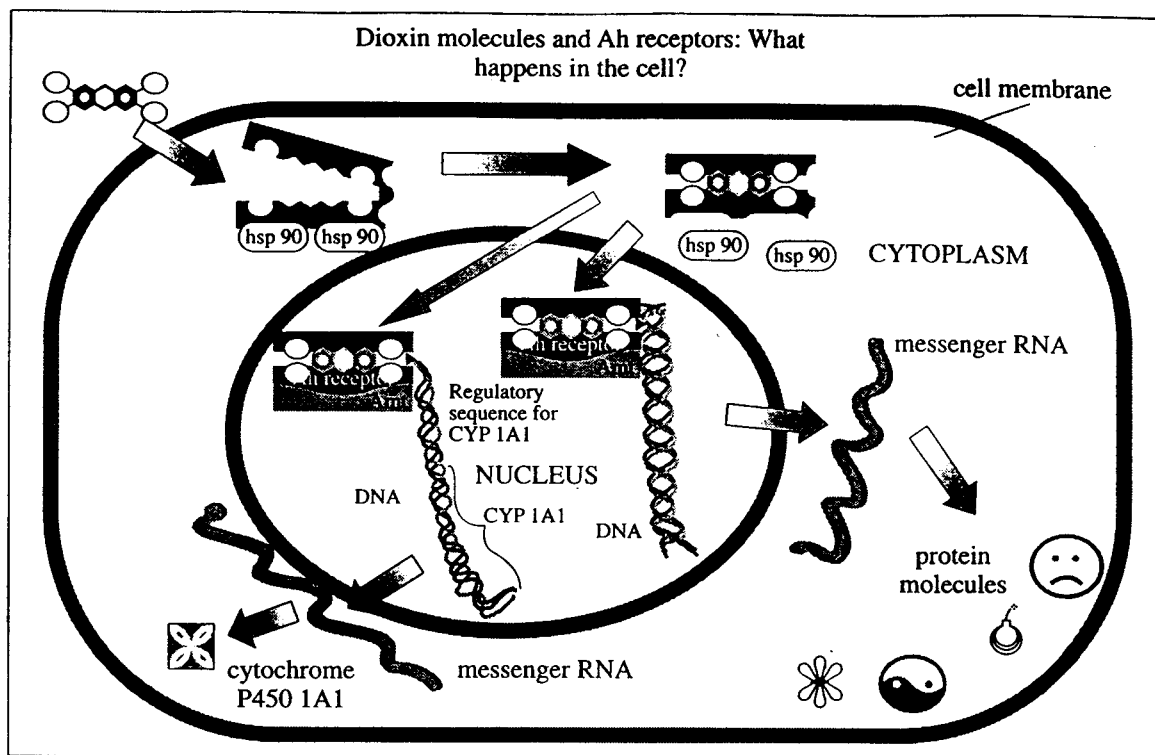


Figure 4. Dioxin molecules and Ah receptors.

Dioxins bind Ah receptor in cytoplasm, and the complex moves into the nucleus, where it binds to Arnt to bind gene activator sites. CYP (p450) 1A1 is a representative product, but many other products would be yielded.

Table 5. Evaluation of carcinogenicity of dioxins (IARC summary).

	All cancer			Lung			Lymphoma			Soft tissue sarcoma			Digestive tract			
	n	RR	95%CI	n	RR	95%CI	n	RR	95%CI	n	RR	95%CI	n	RR	95%CI	
IARC Int Cohort	394	1.2	1.1-1.3	127	1.2	1.0-1.4	14	1.6	0.9-2.7	3	2.3	0.5-6.6	190	1.0	0.9-1.2	
USA	114	1.5	1.2-1.8	40	1.4	1.0-1.9	2	0.9	0.1-3.4	3	9.2	1.9-27.0	28	1.4	0.9-2.0	
Germany	105	1.3	1.0-1.5	33	1.4	1.0-2.0	6	4.6	1.7-10.0	0	0.0	-	27	0.9	0.6-1.4	
Nether land	51	1.5	1.1-1.9	14	1.0	0.5-1.7	3	3.8	0.8-11.0	0	0.0	-		NR		
BASF Cohost	18	1.9	1.1-3.0	7	2.4	1.0-5.0		NR			NR		6	1.8	0.7-4.0	
Total	288	1.4	1.2-1.6	94	1.4	1.1-1.7	11	2.6	1.3-4.7	3	4.7		61	1.2	0.9-1.5	
P value		< 0.001			< 0.01			< 0.01						0.23		

IARC (Kogevinas, et al) Limited to persons who spent more than 20 years after the exposure. NR, not reported.

USA (Fingerhut, et al) Males who exposed more than one year and passed more than 20 years.

Germany (Becher, et al) Totalled males in Boehringer-Ingelheim and Bayer-Uerdingen cohorts.

Dutch cohort (Hooiveld et al) Both males and females in the factory A.

BASF accident (Ott et al)

latency periods suggest its promoter activity (Table 5).

Spindler³⁹ evaluated the carcinogenic risk of soot on basis of the unit risk concept for relative carcinogenic risk of dioxins compared to polyaromatic hydrocarbons (PAH). PAHs, like benzo(a)pyrene, dibenzo(a)anthracene, etc., are much more important by factors of 25 to 500 in comparison to dioxins.

CARDIOVASCULAR EFFECTS

A statistically significant increase in deaths from circulatory disease was observed in the 15-year following the Seveso accident³⁰. In Seveso, the relative risk (RR) of ischemic heart disease was 1.5 (95%CI; 0.8-2.8), chronic heart disease 3.0 (95%CI; 1.2-7.3), and of cerebrovascular disease was 1.5 (95%CI; 0.6-3.7) in males. It is noteworthy that RR of chronic rheumatic heart disease deaths was 15.8 (95%CI; 5.0-50.8) in females.

Vene et al³⁰ found by the international study comprising 36 cohorts from 12 countries followed from 1939 to 1992 that an increased risk for ischemic heart disease (RR 1.67; 95%CI 1.23-2.26) was present among PCDD/higher chlorinated dioxin-exposed workers. The RR of cerebrovascular disease was 1.54 (95%CI; 0.83-2.88) and diabetes mellitus was 2.25 (95%CI; 0.53-9.5).

Follow-up study of the Ranch Hand cohort showed the increased mean diastolic blood pressure in those with current serum lipid TCDD levels from 15 to 33.3 pg/g lipid⁹. The RR of mortality from heart and circulatory diseases increased to 1.96 (95%CI; 1.15-3.34) and 2.48 (95%CI; 1.32-4.66), respectively, in BASF workers with serum TCDD lipid levels of >348 pg/g.

IMMUNE FUNCTION

Many animal studies have shown adverse effects of PCDD/PCDF and PCBs on the immune system. The most consistent finding is thymic atrophy. In utero and lactational exposure is a more sensitive period for the immunotoxic effects than adult exposure³⁰. In vitro studies of human venous blood and lymphocyte fractions incubated with low doses of TCDDs, demonstrated a decrease in B-cells and CD4+ (helper) T-cells and in relative increase in CD8+(suppressor) T cells⁴⁰. In human studies, there is no consistent exposure-related changes in lymphocyte subset and stimulation. Nagayama et al⁴¹ reported that the increased T4/T8 ratio according to the plasma dioxin level.

The changes in the T-lymphocyte population could persist into later child- or adulthood and could result in immune suppression, allergy or autoimmunity. Zober et al³¹ found a significant increase in the incidence of infectious and parasitic diseases in TCDD exposed workers in the 35 year period after the BASF accident. A two fold increase in the incidence of

upper respiratory tract infection and higher incidence of appendicitis were reported. Respiratory involvement was also present in Yusho patients⁴². Although increased WBC counts was reported in Missouri study²⁰, a follow-up study of the same population found no difference in WBC, RBC and platelet count between exposure and non-exposure.

ENDOCRINE DISRUPTER

Several organochlorines have been identified as endocrine disrupting agents^{43,44}. o,p'-DDT and p,p'-DDE act directly through binding to steroid hormone receptors^{45,47}. Other dioxin-like compounds operate through a variety of mechanisms, one of them being the alteration of hormone metabolism through induction of cytochrome CYP dependent enzymes⁴⁷⁻⁴⁹.

The effects of TCDD exposure on gonadal function has not been well studied. A health study of US veterans showed a significant association between testicular size and serum TCDD levels, although no alterations in sperm count or the percentage of abnormal sperm was observed⁹. In workers at two 2,4,5-T factories, serum TCDD levels were positively correlated with follicle stimulating hormone (>140 pg-TEQ/g lipid) and luteinizing hormone levels (>1,860 pg-TEQ/g lipid) and inversely correlated with total testosterone levels (>140 pg TEQ/g lipid)⁵⁰.

Pregnancy outcomes seems to show no significant alterations in the incidence of spontaneous abortions among Vietnam War ranchers. An increased incidence of spontaneous abortion was reported in women living close to a herbicide manufacturing factory in Sweden⁵¹. The residents were exposed to phenoxy acids, chlorophenols, TCDD and PCDF which were released into the soil and groundwater. The sex ratio is another interesting point, because female/male ratio in Seveso between 1977 and 1984 was 48/26, but it became 64/60 between 1985 and 1994⁵². Thus, the results of the human reproductive toxicity studies are inconclusive.

There are some other hormonal effects. A 35-year follow-up study of TCDD exposed workers at BASF accident found a significant increase in the incidence of thyroid disease, as compared to an age-matched referent group²¹. A strong positive association was found between glucose intolerance or increased risk of diabetes mellitus and TCDD serum levels⁹. Interference of TCDD with secretion or synthesis of bioactive ACTH is considered to affect adrenal steroidogenesis by animal experiment⁵³.

DEVELOPMENT

Small amounts of PCDD/Fs pass the placental barrier during pregnancy. The embryo and fetus are exposed during a critical period of organ growth and development⁷⁰. After birth the

breast-fed infant is exposed to relatively high levels of these contaminants.

The overall risk of having a child with birth defects was not significantly increased in the Vietnam veterans (OR of 0.97, 95% CI=0.83-1.14), but some birth defects, such as spina bifida, cleft lips, and congenital tumors (dermoid cysts, teratomas, hepatoblastomas, central nervous system tumors, and Wilms tumors), were present at higher proportion among veterans father. Malformations of nervous system, cardiovascular system, genital system and urinary system may be caused by father's exposure to TCDD. Koopman-Esseboom et al.⁵⁰ reported relationship between maternal and cord blood plasma level of dioxins in 418 mother-infant pairs and neurological and psychomotor development. They recognized a small delay in psychomotor development, and alterations in the thyroid hormone status.

Ilse et al.⁵⁰ observed signs of enhanced neurological maturation in children at 2 years and 7 months in relation to the perinatal load of dioxins (19 low exposure group; 8.7-28.0 pg TEQ/g milkfat, 19 high exposure group; 29.2-62.7 pg TEQ/g milkfat.) Although all psychomotor, neurological and laboratory findings were within the normal range, the signs of higher reflexes and enhanced neuromotor maturation should be regarded as a warning sign. These findings may be due to thyroxine agonistic action of dioxins^{43,44}. Nagayama et al.⁴¹ reported lowered plasma T4 levels in breast fed babies.

Newborns from Yu-Cheng mothers who were exposed in utero to both PCBs and PCDFs were smaller at birth than newborns from unexposed mothers. Correlation analysis revealed a statistically significant inverse relationship between aryl hydrocarbon hydroxylase (AHH) activity and weight of the newborn⁵⁰.

OTHER EFFECTS

Chloracne has been a well known skin lesion and often used as a surrogate marker of dioxin exposure⁵⁷⁻⁶⁰. Chloracne is characterized by follicular hyperkeratosis (comedones) occurring with or without cysts and pustules^{61,62}. Unlike adolescent acne, chloracne may involve almost every follicle in an involved area, such as face, neck, upper arms, back, chest, abdomen, outer thighs, and genitalia. In mild cases, the lesion may clear several months after exposure ceases, but they may persist 30 years in severe cases^{60,63,64}. Porphyria cutanea tarda, hypertrichosis, hirsutism, and hyperpigmentation were also reported in TCDD-exposed workers. In case Kanemi Yusho, many patients are still suffering from various skin lesions 30 years later⁶⁰.

In Seveso, erythema and edema of exposed areas, vesiculobullous and necrotic lesions and papulonodular lesions were found in 447 people 20-40 days after the accident, and 34 of these people later developed chloracne⁶⁵. Chloracne level in

Seveso was 1,770-10,400 pg TEQ/g lipid, in Missouri 577-2,310 pg TEQ/g lipid, and in Operational Ranch Hand veterans 618-4,477 pg TEQ/g lipid. In Seveso children, 12,100-56,000 pg TEQ/g lipid blood level was associated with type 4 chloracne, and 823-7,420 pg TEQ/g lipid with type 3 chloracne. Chloracne is a good marker of dioxin exposure, but its absence does not preclude such exposure. Schuster et al.⁶⁶ estimated that mean body burden of workers with chloracne was 3,200 ng versus 72 ng for general population.

Hepatic toxicity was observed in acute and subacute exposure of TCDD. Hypercholesterolemia, hyperlipemia, and hyperphospholipidemia are observed in about half patients with chloracne. At 10 years post exposure, most of the biochemical changes were not detected except for high cholesterol level⁶⁷. A slight increase in GGT and ALT occurred in the highest exposure group in Seveso residents, but returned to baseline levels within 3 years of the initial exposure⁶⁸. A positive trend for GGT and alkaline phosphatase was observed in Missouri residents living in a TCDD-contaminated area.

Uroporphyrin or urinary porphyrins did not indicate any definitive changes in the residents exposed for up to 11 years. In general, the effect is mild and transient. Kidney lesions have not been reported in any of the several studies on occupational exposure or in the cohort from Seveso.

Neurological effects also appear by dioxin exposure. Subjective signs, such as lassitude, weakness of the lower limbs, muscular pains, sleepiness or sleeplessness, increased perspiration, loss of appetite, headaches, and mental and sexual disorders were reported in several workers with severe chloracne who had been exposed to TCDD^{69,70}. Neurological symptoms persisted in these workers for up to 10 years. In German accident, peripheral neuropathy, sensory impairment, tendency to orthostatic collapse, and reading difficulties were reported⁷¹. Depression, hypochondria, hysteria, and schizophrenia were found more often in Vietnam veterans exposed to herbicides than in the control groups of veterans²⁹. The blood concentration of dioxins was 16.1-80.4 pg TEQ/g lipid with a mean of 31 pg TEQ/g lipid in Peper's study in Germany⁷¹. Gastrointestinal effects, such as peptic ulcer, was once reported, but a study of Vietnam veterans failed to find such effects. Occupational cohort study by Calvert et al.⁷² did not find any gastrointestinal disease difference between cases (220 pg TEQ/g lipid) and controls (7 pg TEQ/g lipid). In Seveso residents, increased risk of gastric cancer was reported once³⁵.

LEGISLATION

WHO and several countries develop regulations and recommendations to protect public health. Regulations can be enforced by law (Table 6). Environmental Agency of Japan set the level of dioxin emission to be less than 80 ng TEQ/m³, in which situation human exposure would be less than 10 pg

TEQ/kg/day. WHO, Japan and several other countries have used a TDI approach, based on the two-year Kociba's experiment⁷⁹, which focused on the liver tumors in female Sprague-Dawley rats. It resulted in an upper bound estimate of an excess of one in a million cancer risk from exposure to 6 fg/kg/day. They suggested that 1 ng/kg/day was a NOAEL for carcinogenesis. Applying a 10-fold safety factor for inter-species extrapolation and a 10-fold "correction" for rat/human

pharmacokinetic differences, resulted in a TDI of 10 ng/kg/day. The WHO also suggested that a TDI would be appropriate based on their estimation of a NOAEL of 1 ng/kg/day based on the Murray⁵⁰ multigeneration reproduction study. WHO's TDI is proposed to change to 1-4 pg TEQ/kg/day in 1998 considering risk of lowered spermatogenesis or risk of endometriosis.

The linearized multi-stage (LMS) model was used as a

Table 6. Legislation of dioxins in Germany and Italy.

	Germany pg TEQ/dry g	Italy pg TEQ/dry g	$\mu\text{g}/\text{m}^2$
Room			<0.01
Farm land	<5	<6	<0.75
Agriculture forbidden		6-40	0.75-5
Agriculture possible until no PCDD is found in products	5-40		
Agriculture forbidden except for less absorption by plant	40<		
Play ground repair	100<		5-50*
Movement of residents		400<	50<movement
Residential area repair	1,000<		
Industrial area repair	10,000<		

* Residents may stay in polluted area, but pregnant women and children should stay outside during daytime.

Table 7. ATSDR's decision framework for sites contaminated with dioxin and dioxin-like compounds in U.S.A.

SCREENING LEVEL	EVALUATION LEVELS	ACTION LEVEL
≤ 50 ppt (0.05 ppb) TEQs	>0.05 ppb but < 1 ppb TEQs	≥ 1 ppb TEQs
<ul style="list-style-type: none"> The EMEG for TCDD is 50ppt This is based on an MRL of 1 pg/kg/day for TCDD (ATSDR 1989). For screening purposes 50 ppt TCDD is assumed to be equivalent to 50 ppt TEQs 	Evaluation of sitespecific factors, such as <ul style="list-style-type: none"> Bioavailability Ingestion rates Pathway analysis Soil cover Climate Other contaminants Community concerns Demographics Background Exposures 	Potential public health actions considered, such as <ul style="list-style-type: none"> Surveillance Research Health studies Community education Physician education Exposure investigations

The toxicity equivalent (TEQ) of TCDD is calculated by multiplying the exposure level of a particular dioxin-like compound by its toxicity equivalency factor (TEF).

Table 8. Research Program in Japan.

Environmental Agency 5-year project for dioxin control strategy:
PCDDs/PCDFs levels from Incinerator for waste and Steel maker
Other emission site
Monitoring of air, soil and water
Ministry of Health and Welfare: Mother's milk survey.
Dioxin levels in foods
Background exposure level of residents near the incinerator
Ministry of Labor. Health check up of laborers at the incinerator
Ministry of Agriculture and Fishery: Survey of PCDDs/PCDFs levels in rice and other vegetables.
Saitama Prefecture: Exposure level of residents near the municipal incinerator by health check up
Osaka Prefecture: Same as above
Ibaragi Prefecture: Same as above

EPA's default position. Application of this model also to the bioassay results from Kociba⁷⁹. Approaches for extrapolation beyond the range of observation are being explored. The EPA has not regulated dioxin based on its non-cancer effects, although believing that the use of the LMS model for carcinogenesis would be protective for non-cancer effects as well. Given that current exposure to total TCDD equivalents is approximately 1-3 pg/kg/day, EPA deemed it inappropriate to establish RfD which would likely be less than the current average daily intake.

FDA recommends against consuming fish and shellfish with TCDD levels greater than 50 pg TEQ/g. Such levels have resulted in the closing of several commercial fishing areas. EPA advises that children should not have more than 1 ng/L water in one day, or more than 10 pg/L per day for long-term exposure. For adult, it should be less than 40 pg/L in drinking water.

In Japan, the concentration of dioxins in the air around exhausted smoke from the chimney is restricted under 0.8 pg/m³. Soil levels for legislation in Germany and Italy is shown in Table 6. Strict legislation should be effective to decrease background level of dioxins in the body^{74,75}. Such legislation has not yet determined in Japan. First removal of contaminated soil (more than 8,000 pg/g dry weight) was recently performed around the municipal incinerator in Osaka. Agency for Toxic Substances and Disease Registry in U.S.A. proposed a policy guideline^{76,79}. They proposed decision framework for sites contaminated with dioxin and dioxin like compounds (Table 7). Comprehensive survey for the environmental pollution and health effects around several polluted incinerators is carried out under the collaboration between the Ministry of Labor, Ministry of Health and Welfare, Environmental Agency and local government. Current programs are shown in Table 8.

CONCLUSION

Data from Europe suggest that dioxin levels in human tissues may have decreased rapidly over the past 4-6 years. Environmental sources, regulations, and enforcement may vary between the United States and Germany, so a clear decrease was shown in levels from the 1980's and 1990's only in Germany⁷⁰. Annual release of dioxins in Germany now is only 40 g. It is more than 100 times less than that of Japan. Urgent survey of the municipal incinerators revealed nearly 1% incinerators emit dioxins above allowable emission level. Health check-up of residents around such incinerators including control is carried out in 6 different prefectures, so the result should be shown in the near future. Holistic environmental control system in these areas and follow-up system for the residents are necessary in Japan.

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