[Environmental Health and Preventive Medicine 11, 136–144, May 2006]

Original Article

Systemic and Myelotoxic Effects of Single Administration of 2,3,7,8-Tetrabromodibenzo-*p*-Dioxin in Rats

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Abstract

Objective: Systemic and myelotoxic effects of 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD) were examined by the single administration of TBDD by gavage to rats.

Methods: Fifteen Wistar rats of both sexes per group received 0, 10, 30, 100 or 300 µg TBDD/kg body weight. Rats surviving to the scheduled necropsy on Days 2, 7 and 36 after TBDD administration were examined for growth rate, organ weight, hematology, histopathology and adipose tissue levels of TBDD.

Results: Three 300 µg/kg-dosed females died on Days 21, 23 and 27, and exhibited a marked decrease in body weight, severe thymic atrophy, decreased bone marrow hematopoiesis and hemorrhage in the subarachnoid space of brain and spinal cord. TBDD-dosed surviving rats exhibited growth retardation, decreased bone marrow hematopoiesis, decreases in red blood cell counts, hemoglobin concentrations, and hematocrit values, an increase in reticulocytes and decreases in platelet counts, white blood cell counts and eosinophils. These signs suggested TBDD myelotoxicity. Splenic extramedullary hematopoiesis was increased in both sexes given TBDD, whereas atrophy of the splenic white pulp occurred only in TBDD-dosed females. Marked decreases in body weights and the size and weight of the thymus, severe thymic atrophy and death in TBDD-dosed females suggested a wasting syndrome. The adipose tissue level of TBDD culminated on Day 7 and decreased to 20–30% of the Day 7 level on Day 36.

Conclusions: The TBDD-induced effects were characterized by a wasting syndrome and myelotoxicity that appeared at the dose levels of 30 µg/kg and higher and caused death in 300 µg/kg-dosed females.

Key words: 2,3,7,8-tetrabromodibenzo-*p*-dioxin, rat, hematotoxicity, myelotoxicity, wasting syndrome

Introduction

Polybromodibenzo-*p*-dioxins (PBDDs) and polybromodibenzofurans (PBDFs) are not intentionally produced, but are formed during the thermal decomposition processes of plastics, carpets, textiles and other materials containing brominated flame retardants in incinerators and fires (1–4) and during the extrusion and molding processes of polybrominated biphenyl and diphenylether flame retardants in workplaces (1, 5, 6). Waste residues from various products of brominated flame retardants incinerated in a laboratory-scale incinerator were reported to contain total PBDDs/PBDFs ranging between 3000 and 130,000 ng/g (4). The atmospheric concentrations of total PBDDs/PBDFs in Kyoto were reported to range between 1.1 and 11 pg/m^3 in the gaseous state and between 0.38 and 1.2 pg/m^3 in particulates in 2000 and 2001 (7). Workplace air concentrations of tetra-BDDs and tetra-BDFs measured during the extruder production and injection molding of polybutyleneterephthalate polymer blended with the glass fiber, Sb_2O_3 and decabromodiphenylether (DBDPE) were reported to be 2 and 34 ng/m^3, respectively (5). The same manufacturing process using tetrabromobisphenol was reported to contaminate workplace air with total PBDDs/PBDFs ranging from 30 to

Received Nov. 7, 2005/Accepted Mar. 15, 2006
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260 pg/m^3 (6). The concentration of 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD) in human adipose tissue collected from the general Japanese population was reported to have a median value of 1.7 pg/g with a range of 0.8–4.2 pg/g in 1970 and a median value of 0.5 pg/g with a range of 0.1–2.0 pg/g in 2000 (8). However, little is known about the toxicities of PBDDs and PBDFs. There are a few experimental toxicology data of TBDD available for the characterization and assessment of human health risk, although many studies on experimental toxicology and epidemiology of a chlorinated analogue, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and its related compounds have been reported so far (9). Single oral and intraperitoneal administrations of TBDD were reported to induce teratogenic effects in mice (10) and thymic atrophy, body weight loss and induction of hepatic microsomal enzymes in immature male rats (11), respectively. Furthermore, by studying overall evaluation of hematological, hepatic and systemic toxicities and target organ doses after single administration of TBDD by gavage to male and female rats, Ivens et al. (12) reported that most effects and toxic symptoms of TBDD seemed to be comparable to those seen after TCDD administration. However, myelotoxic effects of TBDD have not been reported so far, although bone marrow and its hematopoietic stem cells have been shown to be sensitive targets for treatment in rats and mice with TCDD (13–19). These myelotoxic effects seem to be associated with a wasting syndrome, reported as the most characteristic sign of acute toxicity that appeared in experimental animals treated with lethal and sublethal levels of TCDD (20–22).

This study was designed to examine myelotoxic effects of TBDD and the wasting syndrome developed after single administration of TBDD by gavage to Wistar rats of both sexes. The adipose tissue levels of TBDD were determined on Days 2, 7 and 36 after TBDD administration to clarify a relation between the TBDD effects and internal dose. Dose-response relationships for those effects were characterized with reference to the acute toxicity of TBDD and TCDD reported thus far.

Materials and Methods

Chemicals

TBDD (purity >98%) and all-^{13}C-labeled TBDD were purchased from Cambridge Isotope Laboratories, Inc. (MA, USA). Corn oil of biochemistry grade and toluene of reagent grade (purity >99.5%) used for the oral administration of TBDD, and dichloromethane, hexane, silica gel, 10% $AgNO_3$ silica gel and 44% H_2SO_4 silica gel used for the analysis of TBDD were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Animals

This study was approved by the ethics committee of the Japan Bioassay Research Center (JBRC). Crj:Wistar rats were obtained at the age of 4 weeks from Charles River Japan, Inc. (Tsukuba, Japan). The animals were quarantined and acclimated for 2 weeks, before the start of the experiment. The animals were housed individually in stainless-steel wire hanging cages (150 [W]×216 [D]×176 [H] mm). All dosing and animal maintenance were performed in a high-hazard area designed to prevent exposure of personnel to, and environmental contamination with, animal wastes. The high-hazard area was maintained at a constant temperature of 23±2°C and a relative humidity of 55±15% with 12 air changes/hr. Fluorescent lighting was controlled automatically to give a 12-hr light and 12-hr dark cycle. All rats were given a sterilized commercial pellet diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and sterilized water *ad libitum*.

Experimental design

The same administering methods and dose levels of TBDD as those used by Ivens et al. (12) were employed in this study. The test substance was predissolved in degassed toluene and added to corn oil. The toluene concentration in corn oil was 5% for all treated groups. TBDD was orally administered by gavage to groups of 15 rats of both sexes each at a dose of 10, 30, 100 or 300 μg/kg body weight. The control groups of both sexes received corn oil containing 5% toluene. The total volume administrated to rats was 5 ml/kg body weight. Any deleterious effects persisting on Day 2 and longer, after the oral administration of 250 mg toluene/kg by gavage as a vehicle were absent when compared with findings in the literature (23), showing that a marginal increase in sleeping time, induced by an intraperitoneal injection of 200 mg toluene/kg in rats, was observed only on Day 1 after the injection, together with a maximum blood level of 24.7 μg/ml and an elimination half life of 3 hours in blood. Five males and 5 females per dose group were necropsied on Days 2, 7 and 36 after TBDD administration. The preparation and dosing procedures were performed under yellow fluorescent light to protect TBDD from decay by ultraviolet light (24).

Clinical observations and analysis, and pathological examinations

The animals were observed every day for clinical signs and mortality. Body weights were measured on Days 2, 4, 7, 10, 14, 21, 28 and 36 after TBDD administration. Animals surviving up to the scheduled necropsy on Days 2, 7 and 36 or found dead during the 36-day observation period underwent complete necropsy. Blood samples were taken under etherization from the abdominal aorta of all surviving animals, and hematological parameters were measured using an Automatic Blood Cell Analyzer (ADVIA120, Bayer HealthCare, NY, USA). Organs were examined for gross lesions, removed and weighed at necropsy. Wet organ weight measured at the time of necropsy was defined as absolute organ weight, and the wet organ weight as percentage of final body weight was defined as relative organ weight. The relative organ weight was reported to be best expressed for the organ toxicity as in the case of toxicity affecting the liver (25), when the body weight was decreased by toxicant administration. In this study, however, both absolute and relative organ weights were used for the evaluation of organ toxicity. Bone marrow, thymus, spleen, brain, spinal cord, lung and other organs affected were fixed in 10% neutral buffered formalin and embedded in paraffin. The femoral bone was fixed, decalcified and embedded in paraffin. Tissue sections 5 μm thick were prepared, and

stained with hematoxylin and eosin (H & E) for microscopic examination.

Determination of TBDD in adipose tissue

At the scheduled necropsy, adipose tissue was quickly collected from the perirenal region for two rats of both sexes administered with TBDD at a dose of 30, 100 or 300 μg/kg and stored at –20°C. The samples were handled in the dark, because TBDD is subject to photolytic decomposition (24). Extraction was carried out by refluxing the adipose tissue with dichloromethane in a Soxhlet-apparatus for 12 hours. An aliquot of the extract was mixed with all-^{13}C-labeled TBDD, and concentrated under vacuum and chromatographed on a 5-layer column consisting of silica gel, 10% $AgNO_3$ silica gel, silica gel, 44% H_2SO_4 silica gel and silica gel. The column was eluted with 5% dichloromethane/hexane. TBDD was determined using a mass spectrometer (Autospec Ultradetector, Waters, MA, USA) equipped with a gas chromatograph (HP6890, Agilent Technol., CA, USA) with a DB17HT fused silica capillary column (Agilent Technol.).

Statistical analysis

Body weights, organ weights, and hematological parameters were analyzed using the previously described algorithms (26, 27). Bartlett's test was used to test whether the variance was homogeneous or not. When variance was homogeneous, one-way ANOVA was performed. When variance was not homogeneous, the Kruskal-Wallis rank sum test was performed by arranging all data from the control and exposed groups in descending order. Statistical differences in the means and the rank means among the groups were analyzed by Dunnett's multiple comparison test and the same multiple comparison test by rank, respectively. A two-sided analysis with p values of 0.05 and 0.01 was performed, to determine statistical significance.

Results

Survival, clinical observation and body weights

Three females from the 300 μg/kg group died on Days 21, 23 and 27 after TBDD administration. These animals exhibited decreased locomotor activity and emaciation, before death. Their terminal body weights had fallen below their initial body weights measured just before the TBDD administration (Fig. 1). All other TBDD-dosed animals survived to the scheduled necropsy on Days 2, 7 and 36.

Growth rates of TBDD-dosed rats of both sexes were retarded in a dose-related manner (Fig. 1). A decrease in terminal body weight occurred in the 300 μg/kg-dosed females on Day 7 and in the females dosed at 30 μg/kg and higher on Day 36. On the other hand, only the 300 μg/kg-dosed males exhibited decreased body weights on Days 7 and 36.

Hematology

Decreases in red blood cell counts (RBC), hemoglobin concentrations (Hb) and hematocrit values (Hct) were noted in both males and females dosed at 30 μg/kg or higher on Day 36 (Table 1). The decreases in these erythrocyte parameters were accompanied by an increase in reticulocytes on Day 36, although a transient decrease in reticulocytes was noted in the males dosed at 30 μg/kg and higher and in the females dosed at 100 μg/kg on Day 7.

Platelet counts were decreased dose-dependently in both males and females dosed at 100 and 300 μg/kg on Days 7 and 36. White blood cell counts (WBC) were decreased dose-dependently in both males and females dosed at 30 μg/kg or higher on Day 36. Percent eosinophils were decreased dose-dependently throughout Days 2, 7 and 36. Although no significant change in percent lymphocytes, neutrophils or other types of leukocytes occurred in any of TBDD-dosed rats (data not shown), the significantly decreased WBC in the TBDD-dosed

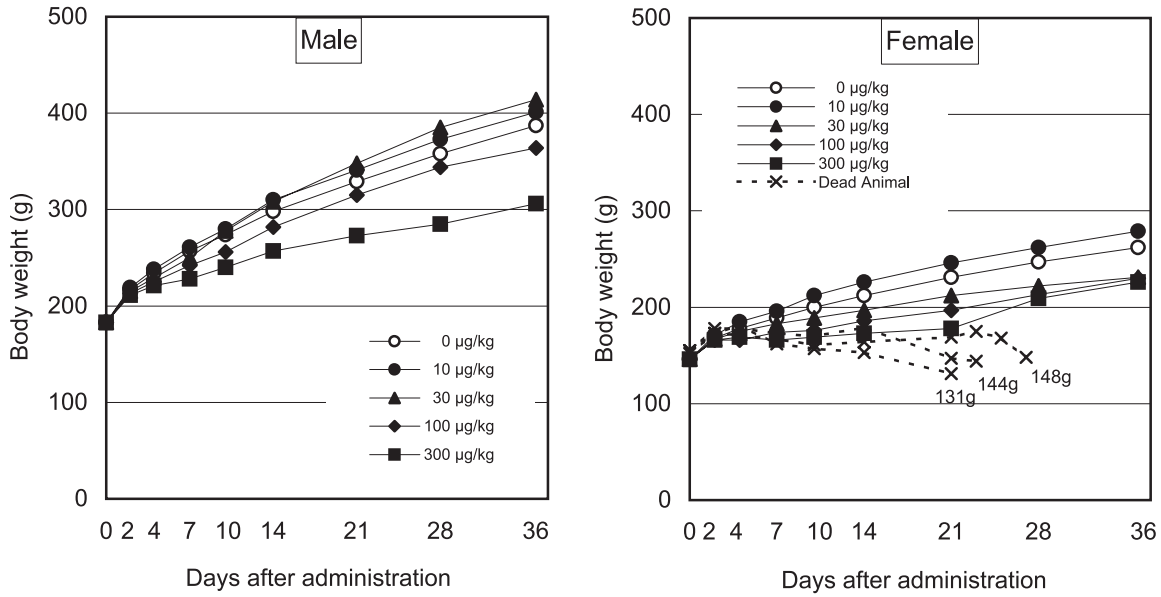

Fig. 1 Time course changes in mean body weights of male and female rats after single administration of TBDD by gavage at 0 (vehicle), 10, 30, 100 or 300 μg/kg. The crosses linked with a dotted line indicate the body weights of the 3 dead females dosed at 300 μg/kg, and their final body weights at the time of death are shown at the end of the dotted lines.

Table 1 Hematology of male and female rats on Days 2, 7 and 36 after single administration of TBDD by gavage at 0 (vehicle), 10, 30, 100 or 300 μg/kg

Item	Dose (μg/kg)	Male (Days after administration)			Female (Days after administration)		
		2	7	36	2	7	36
Red blood cell ($\times10^6$/μl)	0	6.05±0.23	6.63±0.25	8.19±0.49	6.45±0.38	6.74±0.36	8.00±0.51
	10	6.16±0.14	6.50±0.46	8.12±0.46	6.42±0.35	6.95±0.26	7.66±0.30
	30	6.45±0.45	6.31±0.40	7.66±0.24	6.54±0.49	6.82±0.42	7.46±0.29
	100	6.53±0.53	6.49±0.26	7.03±0.80	6.59±0.41	7.06±0.49	6.95±0.67**
	300	6.35±0.63	6.94±0.65	5.84±1.25**	6.70±0.30	7.72±0.57**	5.97±0.31[a]
Hemoglobin (g/dl)	0	12.9±0.4	13.7±0.2	14.9±0.7	12.9±0.8	13.6±0.5	14.6±0.7
	10	13.1±0.3	13.5±0.7	15.0±0.4	13.4±0.4	13.9±0.5	14.0±0.5
	30	13.6±0.5	12.9±0.6	13.8±0.7*	13.4±0.4	13.5±0.9	13.3±0.3*
	100	13.5±0.7	13.0±0.4	12.5±1.0**	13.5±0.3	13.7±1.0	12.3±1.3**
	300	13.3±0.7	14.1±1.4	10.3±2.3**	13.2±0.6	15.2±1.2	10.5±0.8[a]
Hematocrit (%)	0	37.1±1.2	40.4±0.7	42.6±1.9	37.0±1.7	39.0±2.1	41.2±1.9
	10	38.1±1.0	40.2±2.2	43.7±1.9	38.4±1.1	40.3±1.3	39.9±1.5
	30	39.5±2.7	37.5±1.9	39.4±1.3	38.3±3.2	39.2±2.2	37.5±0.6**
	100	39.8±2.7	38.3±1.7	36.7±2.9*	38.6±2.1	39.7±2.4	35.3±3.8**
	300	38.9±2.5	41.1±4.1	30.8±6.0**	38.5±1.3	43.8±3.7	30.9±1.8[a]
Platelet ($\times10^3$/μl)	0	1326±131	1298±123	1140±211	1440±114	1357±85	1148±102
	10	1284±157	1410±62	1106±178	1349±146	1311±86	1112±158
	30	1293±153	1260±93	1123±176	1324±210	1144±131*	998±149
	100	1180±69	907±251**	907±330	1182±169	749±156**	821±152**
	300	1297±115	828±166**	510±339**	1262±154	762±149**	354±192[a]
Reticulocyte (%)	0	3.5±0.5	5.7±0.8	2.5±0.2	2.4±0.4	4.2±1.1	2.5±0.6
	10	3.4±0.8	6.0±0.5	2.9±0.4	2.4±0.3	3.9±1.2	2.8±0.5
	30	3.2±0.3	4.0±0.8**	2.9±0.3	2.6±0.2	2.9±0.6	2.8±0.3
	100	3.0±0.4	2.9±0.5**	5.0±1.4**	2.7±0.6	2.4±0.2*	4.5±1.6
	300	2.9±0.4	2.3±0.9**	8.1±5.5**	3.8±0.6**	2.8±1.1	5.3±0.7[a]
White Blood Cell ($\times10^3$/μl)	0	6.62±0.98	8.06±2.59	11.73±3.53	3.83±0.92	4.52±1.03	7.87±0.65
	10	7.36±2.51	6.94±0.96	8.62±1.26	5.00±1.17	6.57±1.36	5.65±1.78
	30	7.12±1.49	5.98±1.90	7.27±3.33	6.10±1.93	5.82±1.34	4.64±1.82*
	100	6.65±2.46	7.98±2.41	8.27±3.66	6.25±1.11	9.52±3.38*	5.18±1.53*
	300	7.06±2.39	9.60±3.21	3.96±1.60**	4.99±0.99	9.63±4.38*	4.14±0.10[a]
Eosinophil (%)	0	1.2±0.3	1.0±0.3	1.3±0.2	2.5±1.0	1.5±0.4	2.3±1.0
	10	1.2±0.4	0.9±0.3	1.4±0.5	1.2±0.5*	1.2±0.5	1.6±0.7
	30	1.0±0.4	0.4±0.2**	0.6±0.3*	1.8±0.4	0.9±0.4	1.4±0.2
	100	0.8±0.2	0.2±0.1**	0.6±0.6*	1.2±0.5**	0.5±0.3**	0.6±0.1**
	300	0.5±0.3*	0.3±0.1**	0.5±0.2*	1.2±0.2**	0.3±0.1**	0.4±0.0[a]

Values are expresed as mean±SD of 5 rats in each group.
Significantly different at $p<0.05$ (*) and $p<0.01$ (**) by Dunnett's test.
[a] Statistical analysis was not applied, because the number of animals examined was 2.

rats on Day 36 may indicate that both lymphocytes and neutrophils were also decreased by TBDD administration.

Gross findings, organ weights, and histopathology

Dead animals

Macroscopically visible lesions from the 3 dead females were hemorrhage in the cranial cavity or vertebral foramen, the subcutaneous tissue of the neck and the nasal cavity, congestion and edema in the lung and a markedly reduced size of the thymus. The histopathological finding was characterized by hemorrhage in the subarachnoid space (Fig. 2) and ventricle of the brain, the subarachnoid space of the spinal cord, the subcutaneous tissue of the neck and the lamina propria and cavity of the nose. The primary cause of death was attributed to the hemorrhage in the brain and spinal cord. Notably, those 3 dead females exhibited the decreased bone marrow hematopoiesis characterized by decreased number of hematopoietic cells including erythroblasts, granulocytes and megakaryocytes, as well as marked thymic atrophy characterized by the disappearance of lymphocytes in the entire region of the cortex. Decreased number of lymphocytes in the slightly atrophied white pulp of the spleen was also observed in the 3 dead females, in addition to congestion and edema in the lungs.

Surviving animals

A 300 μg/kg-dosed male necropsied on Day 36 exhibited hemorrhage in the subcutaneous tissue and muscle of the right limb as a macroscopically gross lesions.

On Day 2, absolute and relative thymus weights were

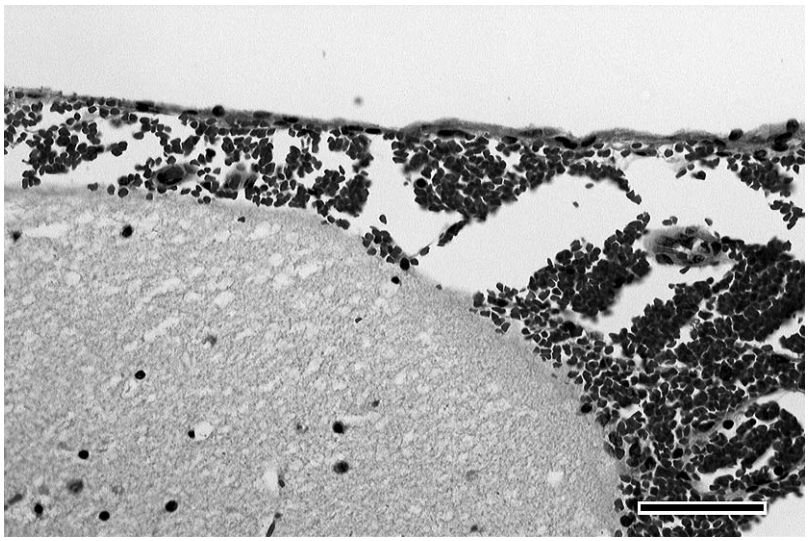

Fig. 2 Hemorrhage in subarachnoid space of brain in 300 μg/kg-dosed female rat that died on Day 27. H & E stain. Bar indicates 100 μm.

Table 2 Absolute and relative organ weights of male and female rats on Days 2, 7 and 36 after single administration of TBDD by gavage at 0 (vehicle), 10, 30, 100 or 300 μg/kg

Item	Dose (μg/kg)	Male (Days after administration)			Female (Days after administration)		
		2	7	36	2	7	36
Body weight (g)	0	220±9	259±9	387±20	167±11	189±17	262±9
Schedule necropsied animals	10	219±13	264±9	401±18	166±7	190±11	279±16
	30	220±7	242±13	414±26	171±8	189±9	231±11*
	100	212±9	242±12	364±42	166±7	175±16	230±21*
	300	209±11	224±16**	306±54**	164±7	163±9*	226±25[a]
Thymus	0	615±78	652±130	666±128	431±100	501±57	526±86
Absolute weight (mg)	10	591±151	599±71	639±139	459±118	485±99	504±152
	30	428±47*	441±87**	602±60	388±75	355±98*	341±37*
	100	577±86	290±83**	360±136**	425±53	240±48**	177±94**
	300	423±111*	263±65**	187±79**	446±107	207±59**	138±42[a]
Thymus	0	0.279±0.024	0.252±0.047	0.172±0.028	0.256±0.049	0.266±0.020	0.201±0.037
Relative weight (%)	10	0.268±0.052	0.227±0.026	0.159±0.033	0.275±0.062	0.254±0.038	0.180±0.051
	30	0.195±0.022*	0.181±0.031*	0.146±0.019	0.226±0.035	0.187±0.050**	0.148±0.024
	100	0.273±0.043	0.120±0.033**	0.098±0.037**	0.256±0.029	0.136±0.018**	0.078±0.041**
	300	0.201±0.047*	0.116±0.024**	0.060±0.020**	0.271±0.063	0.126±0.031**	0.061±0.012[a]
Spleen	0	677±70	950±165	856±115	562±76	638±140	700±85
Absolute weight (mg)	10	758±62	948±105	900±118	487±59	609±43	729±115
	30	770±103	818±102	1043±179	593±49	601±49	565±43
	100	671±61	827±200	1039±128	502±90	587±114	685±78
	300	583±198	825±181	1198±513	546±92	551±51	688±33[a]
Spleen	0	0.308±0.020	0.366±0.057	0.222±0.031	0.335±0.034	0.336±0.049	0.268±0.037
Relative weight (%)	10	0.346±0.011*	0.360±0.051	0.224±0.024	0.292±0.029	0.321±0.005	0.262±0.036
	30	0.351±0.044	0.337±0.030	0.251±0.038	0.347±0.043	0.317±0.020	0.245±0.017
	100	0.317±0.021	0.345±0.092	0.286±0.027*	0.302±0.049	0.334±0.044	0.298±0.025
	300	0.277±0.091	0.367±0.072	0.401±0.181**	0.332±0.055	0.339±0.028	0.306±0.019[a]

Values are expressed as mean±SD of 5 rats in each group.
Significantly different at $p<0.05$ (*) and $p<0.01$ (**) by Dunnett's test.
[a] Statistical analysis was not applied, because the number of animals examined was 2.

decreased only in the males dosed at 30 and 300 μg/kg TBDD, but not in any of the TBDD-dosed females (Table 2). Absolute and relative thymus weights were decreased in both males and females dosed at 30 μg/kg and higher on Day 7, and in both males and females dosed at 100 and 300 μg/kg on Day 36. Absolute and relative spleen weights were increased in the males dosed at 100 and 300 μg/kg on Day 36 but not in any of TBDD-dosed females.

The lesions of the bone marrow, thymus and spleen, and their severities, in the TBDD-dosed rats of both sexes surviving

Table 3 Number of animals bearing the histopathological lesions on Days 7 and 36 after single administration of TBDD by gavage at 0 (vehicle), 10, 30, 100 or 300 μg/kg

Finding	Male (μg/kg)					Female (μg/kg)				
	0	10	30	100	300	0	10	30	100	300
Day 7 (Number of animals examined)	5	5	5	5	5	5	5	5	5	5
Bone marrow										
Decreased hematopoiesis (1+)	0	0	0	0	2	0	0	0	0	2
Thymus										
Atrophy (1+)	0	0	0	2	3	0	0	1	4	1
(2+)	0	0	0	1	2	0	0	0	1	4
Day 36 (Number of animals examined)	5	5	5	5	5	5	5	5	5	2
Bone marrow										
Decreased hematopoiesis (1+)	0	0	0	2	1	0	0	0	2	0
(2+)	0	0	0	1	2	0	0	0	3	0
(3+)	0	0	0	0	2	0	0	0	0	2
Thymus										
Atrophy (1+)	0	0	0	3	0	0	0	0	3	0
(2+)	0	0	0	0	2	0	0	0	0	0
(3+)	0	0	0	0	3	0	0	0	2	2
Spleen										
Atrophy: white pulp (1+)	0	0	0	0	0	0	0	0	0	1
(2+)	0	0	0	0	0	0	0	0	0	1
Increased extramedullary hematopoiesis (1+)	0	0	0	1	4	0	0	0	0	1

The values indicate the number of animals bearing the lesions with 3 different grades of severity, i.e., 1+: slight, 2+: moderate, 3+: marked.
Three 300 μg/kg-dosed females died on Days 21, 23 and 27.

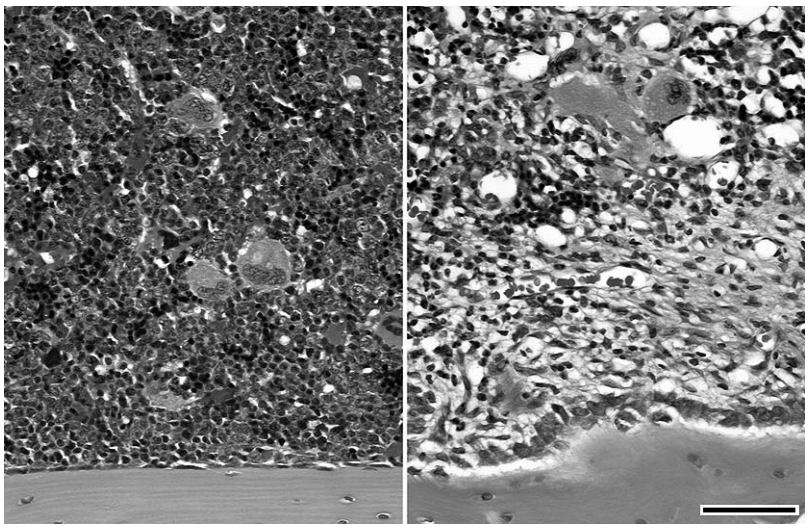

Fig. 3 Decreased hematopoiesis in bone marrow of 300 μg/kg-dosed male rat necropsied on Day 36 (right), compared with bone marrow of control male rat necropsied on Day 36 (left). H & E stain. Bar indicates 100 μm.

to the scheduled necropsy on Days 7 and 36 are summarized in Table 3. On Day 2, no histopathological lesions were found at any dose level in those three organs of TBDD-dosed rats. On Day 7, decreased hematopoiesis with slight grade of severity was seen in the bone marrow of both males and females dosed at 300 μg/kg. On Day 36, almost all surviving animals dosed at 100 and 300 μg/kg exhibited the decreased bone marrow hematopoiesis that progressed to a more severe grade (Fig. 3). On Day 36, increased extramedullary hematopoiesis in the spleen was noted in the males dosed at 100 and 300 μg/kg and in a female dosed at 300 μg/kg, whereas two 300 μg/kg-dosed females exhibited atrophy of the splenic white pulp. On Day 7, thymic atrophy with slight and moderate grades of severity was observed in both males and females dosed at 100 and 300 μg/kg. This was characterized by a decrease in the number of lymphocytes in the thymic cortex. On Day 36, more severe thymic atrophy was noted in both males and females dosed at 100 and 300 μg/kg. The macroscopically visible hemorrhage in the subcutaneous tissue and muscle of the right limb was confirmed by the histopathological examination. However, no hemorrhage in the subarachnoid space of the brain and spinal cord was detected histopathologically in any of the surviving TBDD-dosed rats necropsied on Day 7 or 36.

Table 4 Adipose tissue levels of TBDD on Days 2, 7 and 36 after single administration of TBDD by gavage at 30, 100 or 300 μg/kg

Adipose tissue level of TBDD (ng/g)	Dose (μg/kg)	Days after administration		
		2	7	36
Male	30	47	—	—
	100	125	200	39
	300	440	500	120
Female	30	34	—	—
	100	140	210	62
	300	515	545	120

Values are expressed as mean of two rats in each group.

Adipose tissue levels of TBDD

The mean levels of TBDD in the adipose tissue collected on Day 2 were increased proportionally with an increase in dose levels (Table 4). The TBDD levels culminated on Day 7, and decreased to 20–30% of the Day 7 levels for the 100 and 300 μg/kg-dosed rats of both sexes on Day 36, when the TBDD levels on Day 7 were taken as 100%. There were no gender differences in the adipose tissue levels of TBDD.

Discussion

In this study, we confirmed that the TBDD-induced systemic effects previously reported by Ivens et al. (12) who demonstrated growth retardation, decreased locomotor activity, emaciation, deaths of females accompanied by marked weight loss, and reduced size and weight of the thymus seen after a single administration of TBDD by gavage to rats of both sexes. The lethal wasting syndrome, which was characterized by the marked weight loss and emaciation resulting in death, was found to appear only in the 300 μg/kg-dosed females in this study, whereas the lethal wasting syndrome occurred in the 100 and 300 μg/kg-dosed females in Ivens et al.'s study (12). It was found from the present macroscopic observations of the cranial cavity and vertebral foramen and the histopathological examinations of the brain (Fig. 2) and spinal cord that the primary cause of death in the 300 μg/kg-dosed female rats was attributed to the hemorrhage in the brain and spinal cord. Single administration of TCDD by gavage to rats was reported to produce the lethal wasting syndrome at the dose levels of 50 and 100 μg/kg (20), 25 and 50 μg/kg (21), and 37 and 75 μg/kg (22). Therefore, comparison between the results of the two TBDD studies, that is, the results reported by Ivens et al. (12) and found in this study, and the results of the three TCDD studies (20–22) revealed that the lethal wasting syndrome of TBDD was induced at higher dose levels than that of TCDD, indicating less potent toxicity of TBDD than TCDD. Higher sensitivity of females to TBDD than males observed in this study and in Ivens et al.'s study (12) was similar to the finding reported by Harris et al. (20) who showed that female rats appeared to be more sensitive to acute toxicity of TCDD administered by gavage than males.

The organs of TBDD-dosed rats in which hemorrhage was observed in this study were different partly from those reported by Ivens et al. (12) who showed that TBDD induced hemorrhage in the gastrointestinal tract, the genital region and the subcutaneous tissue of the front paws of rats. They did not observe hemorrhage in the brain or spinal cord, whereas we found the hemorrhage in the subarachnoid space (Fig. 2) and ventricle of the brain and the subarachnoid space of the spinal cord in the 300 μg/kg-dosed females in this study. It is noteworthy in this study that platelet counts were decreased dose- and time-dependently at a dose level of 30 μg/kg or higher. Circulating platelets are known to be a major constituent of the hemostatic system (28). It can be inferred, therefore, that the TBDD-induced hemorrhage was causally related to the decreased platelet counts, through an impaired clotting mechanism. Furthermore, the TBDD-induced thrombocytopenia and hemorrhage are essentially consistent with the reported findings demonstrating that single or repeated administrations of TCDD by gavage to rats induced a decrease in platelet counts (13), and the decreased platelets, diminished clot retraction and prolonged prothrombin consumption time (14), and subcutaneous hemorrhage in the tail and paws (15).

The present histopathological observations revealed that the single administration of TBDD to rats induced a decreased bone marrow hematopoiesis (Fig. 3), marked thymic atrophy and atrophy of splenic white pulp. The decreased bone marrow hematopoiesis was characterized by a decreased number of hematopoietic cells including erythroblasts, granulocytes and megakaryocytes, while a decrease in the number of lymphocytes was observed in both the thymus and spleen of TBDD-dosed animals. However, Ivens et al. (12) reported that the histopathological findings of bone marrow (data not shown) did not indicate any changes in the number of cells. Both the decreased bone marrow hematopoiesis and the decreases in the number of erythrocytes, leukocytes and platelets found in this study may reflect possible TBDD-induced alterations in bone marrow hematopoietic stem cells and their proliferation, differentiation and maturation, since erythrocytes, leukocytes and platelets are known to originate from the hematopoietic stem cells in the bone marrow (29). Consistent with this inference, it has been demonstrated in mice that TCDD adversely affected the multilineage hematopoiesis in the hematopoietic stem cells of the bone marrow, mediated through aryl hydrocarbon receptors (AhR) in the target tissue (16–19). It can be inferred, therefore, that the adverse effects of TBDD on bone marrow hematopoiesis exist behind possible impairment of the clotting mechanism induced by the TBDD-induced thrombocytopenia and the resulting hemorrhage in the brain and spinal cord that primarily caused death in the 300 μg/kg-dosed female rats.

In this context, the possible impairment of the cellular immune system in the TBDD-dosed rats is also suggested by the present histopathological findings of the thymus. Since the thymus is known to perform a function in the cellular immune system in rodents (29), the thymic atrophy which was characterized by a disappearance or decreased number of lymphocytes in the thymic cortex might be a morphologic correlate reflecting the possible impairments of differentiation and maturation of thymocytes in the cellular immune system of TBDD-dosed rats. This inference is comparable with the reported findings that TCDD administration causes thymic atrophy, by directly affecting the cortical epithelium of the rat thymus (30) and

through the induction of apoptosis in mouse thymocytes (31), and by directly targeting the hematopoietic compartment of the mouse thymus originating from the bone marrow hematopoietic stem cells through the AhR, resulting in thymic atrophy (32).

Although a statistically significant change in only one hematological parameter (decreased eosinophils) was observed in the 10 μg/kg-dosed females on Day 2, toxicologically significant and interrelated changes were thought to occur at a dose of 30 μg/kg. The reason for this is that this dose level caused significant decreases in erythrocyte, leukocyte and platelet counts, thymus weights and body weights of the female group on Day 36, as well as slight thymic atrophy in a female on Day 7. Therefore, the nonlethal wasting syndrome and the myelotoxic effects seen after the single administration of TBDD by gavage to rats would be manifested at the dose levels of 30 and 100 μg/kg, which corresponded to the adipose tissue levels of 47 and 125 ng/g in males and 34 and 140 ng/g in females on Day 2.

The present results of the adipose tissue levels of TBDD on Days 2, 7 and 36 are in sharp contrast to both the adipose tissue levels of TBDD and their temporal patterns in the 100 μg/kg-dosed rats reported by Ivens et al. (12) who showed that the adipose tissue level of TBDD was 20 ppb (ng/g) on Day 2 and below the detection limit on Day 28. The persistence of TBDD in the adipose tissue until Day 36 after the single oral administration is comparable with the findings of the adipose tissue levels of TBDD after single intravenous and subcutaneous administrations of TBDD reported by Kedderis et al. (33) and Nagao et al. (34), respectively, although the route of administration and the dose levels of TBDD used in the present study were different from those used in these two studies.

Acknowledgments

This study was financially supported by the Health and Labour Sciences Research Grant, Research on Occupational Safety and Health (Grant-in-Aid No. H14-Labour-26).

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