DOSE- AND TIME-DEPENDENT EFFECTS OF 2,3,7,8-TETRABROMODIBENZO-*P*-DIOXIN ON RAT LIVER

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ABSTRACT — Dose- and time-dependent effects of 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD) on the liver were examined by single administration of TBDD by gavage to male and female rats. Fifteen Wistar rats of each sex per group received 0, 10, 30, 100 or 300 µg TBDD/kg body weight. Rats surviving to scheduled necropsy on Day 2, 7 or 36 after the TBDD administration were examined for hepatic histopathology, activities of hepatic microsomal enzymes and serum levels of lipids, total cholesterol and transaminases and hepatic concentrations of TBDD. Tigroid basophilic cytoplasm and hepatocellular hypertrophy were observed at 10 µg/kg on Day 2 or 7 through 36, whereas degenerative and aggressive lesions such as necrosis, fibrosis, multinucleated hepatocytes and disarrangement of hepatocytes occurred later at higher dose levels. Persistently increased activities of hepatic aryl hydrocarbon hydroxylase (AHH), ethoxycoumarin *O*-deethylase (ECOD) and ethoxyresorufin *O*-deethylase (EROD), increased serum levels of total cholesterol and phospholipid and increased relative liver weight were observed in all groups dosed 10 µg/kg and above, suggesting that hepatic microsomal monooxygenases and basophilic cytoplasm of hepatocytes were early and sensitive indicators among those TBDD-induced effects. A dose-dependent increase in liver concentrations of TBDD on Day 2 was followed by logarithmic decreases in TBDD concentrations against the days elapsed after the TBDD administration. An elimination half-life ($t_{1/2}$) of TBDD from the liver was estimated to range from 12 to 16 days. It was suggested that females were more susceptible to TBDD than males, and that acute hepatotoxicity of TBDD was as potent as that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

KEY WORDS: 2,3,7,8-Tetrabromodibenzo-*p*-dioxin, Rat, Liver, Hepatotoxicity, Multinucleated hepatocytes

INTRODUCTION

Polybromodibenzo-*p*-dioxins (PBDDs) and polybromodibenzofurans (PBDFs) are unintentionally produced during the thermal decomposition reactions of plastics, carpets, textiles and other materials containing brominated flame retardants in incinerators and fires (Buser, 1986; Zelinski *et al.*, 1993). It has also been reported that PBDDs and PBDFs were formed during extrusion and molding processes of polybrominated flame-retardant polymers, and that workplace air was contaminated with tetra-BDDs and tetra-BDFs during these manufacturing processes (Brenner and Knies,

1990 and 1993). There has been a paucity of medical case reports and epidemiological studies on PBDD/F-exposed humans except for a case report of chloracne in a chemist accidentally exposed to 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD) in his laboratory (Schechter and Ryan, 1992). There are a few experimental toxicology data of TBDD available for health risk assessments. Ivens *et al.* (1992 and 1993) reported overall evaluation of hematological, hepatic and systemic toxicities and target organ doses of TBDD after single and repeated administrations of TBDD by gavage to rats. Single oral and intraperitoneal administrations of TBDD were reported to induce teratogenic

effects in mice (Birnbaum *et al.*, 1991) and to induce thymic atrophy, body weight loss and induction of hepatic microsomal enzymes in immature male rats (Mason *et al.*, 1987). Yamamoto *et al.* (2006) demonstrated that a single administration of TBDD by gavage to rats induced systemic and myelotoxic effects associated with a wasting syndrome.

The toxicity of TBDD has been reported to be less potent than that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Ivens *et al.*, 1992 and 1993; Birnbaum *et al.*, 1991; Yamamoto *et al.*, 2006). TCDD is known to target the liver in humans and experimental animals (IARC, 1997; Gupta *et al.*, 1973; Jones and Butler, 1974; Kociba *et al.*, 1976; Plüss *et al.*, 1988; Pohjanvirta *et al.*, 1989). Since Ivens *et al.* (1992) evaluated various acute toxicities of TBDD including hepatotoxic effects in rats, this study focused on further examination of dose- and time-dependencies of various hepatotoxic responses to TBDD and relationships of those responses to hepatic levels of TBDD after the single TBDD administration to male and female rats. The hepatotoxic responses to TBDD thus obtained were compared with those to TCDD reported to date.

MATERIALS AND METHODS

Animals and administration of TBDD

Crj:Wistar rats of both sexes were obtained at the age of 4 weeks from Charles River Japan, Inc. (Tsukuba, Japan). Animal maintenance and dosing procedures were performed in a high-hazard area that was designed to prevent exposure of personnel to, and environmental contamination with, animal waste. After quarantine and acclimation for 2 weeks, the animals were housed individually in stainless steel wire hanging cages. The temperature, relative humidity and air change rate in the high-hazard area were maintained constant at $23\pm 2^{\circ}\text{C}$, $55\pm 15\%$ and 12 air changes/hr, respectively. Lighting was controlled automatically to give a 12-hr light/dark cycle. All rats were given a sterilized commercial pellet diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and sterilized drinking water *ad libitum*. The animals were cared for in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). The present study was approved by the ethics committee of the Japan Bioassay Research Center (JBRC).

TBDD (purity >98%) was purchased from Cambridge Isotope Laboratories, Inc. (MA, USA). The

TBDD 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 $\mu\text{g/kg}$ body weight. The control groups of both sexes received corn oil containing 5% toluene. The total volume administered to rats was 5 ml/kg body weight. The route of administration and dose levels of TBDD used in this study were similar to those used by Ivens *et al.* (1992). The preparation of the TBDD solution and dosing procedures as well as pretreatment of the liver for analysis of TBDD were performed under yellow fluorescent light, because TBDD is subject to photolytic decomposition (Buser, 1988).

Clinical observations and analysis, and pathological examinations

The animals were observed every day for clinical signs and mortality. Animals surviving to the scheduled necropsy on Days 2, 7 or 36 or found dead during the 36-day observation period underwent complete necropsy. Blood samples were taken under ether anesthesia from the abdominal aorta of all surviving animals. Blood biochemical parameters were measured with an Automatic Analyzer (Hitachi 7070, Hitachi, Ltd., Tokyo, Japan). The liver was examined for macroscopic lesions, removed and weighed at necropsy. The wet liver weight as a percent of the final body weight was used as one of the hepatotoxic parameters, because the relative liver weight was reported to be best expressed for the hepatotoxicity (Popp and Cattley, 1991). The liver tissue of the rats surviving to the scheduled necropsy was fixed in 10% neutral buffered formalin and embedded in paraffin. Tissue sections of 5 μm thick were prepared and stained with hematoxylin and eosin (H & E) for microscopic examination. Postmortem examination was attempted for hepatic histopathology of the TBDD-dosed animals that died during the 36-day observation period.

Analysis of aryl hydrocarbon hydroxylase (AHH), ethoxycoumarin *O*-deethylase (ECOD) and ethoxresorufin *O*-deethylase (EROD) in the liver

At the scheduled necropsy on Days 2, 7 and 36, the liver was removed from two rats of both sexes administered TBDD at a dose of 0, 30, 100 or 300 $\mu\text{g/kg}$, perfused by ice-cold KCl solution of 1.15% from the portal vein, frozen by liquid nitrogen and stored at -80°C until analysis. Analysis of hepatic ECOD, EROD and AHH activities was commissioned to Mit-

subishi Chemical Safety Institute, Ltd (Tokyo, Japan).

Determination of TBDD in the liver

At the scheduled necropsy, the liver was quickly removed from two rats of both sexes administered TBDD at a dose of 30, 100 or 300 µg/kg on Day 2 and at a dose of 100 or 300 µg/kg on Days 7 and 36, and stored at -20°C. The pretreatments, procedures and instruments for the analysis of TBDD were the same as those described in our previous paper (Yamamoto *et al.*, 2006).

Statistical analysis

Relative liver weights and blood biochemical parameters were analyzed by Dunnett's multiple comparison test (Dunnett, 1964). A two-sided analysis with *p* values of 0.05 and 0.01 was performed to determine statistical significance.

RESULTS

Histopathology

Hepatic histopathology of the TBDD-dosed rats that survived to the scheduled necropsy revealed that different types of hepatic lesions were induced, depending on the dose levels and the days elapsed after the single TBDD administration, whereas none of these hepatic lesions occurred in the control animals

(Table 1). The earliest lesion was characterized by tigroid basophilic cytoplasm in all the TBDD-dosed groups on Day 2 and its persistent occurrence until Day 36 (Photo 1.-A). Hepatocellular hypertrophy (Photo 1.-A) was induced persistently on Days 7 and 36 in all the TBDD-dosed groups except for the 10 µg/kg-dosed male group on Day 7. Cytoplasmic inclusion was observed in the hepatocytes of both the males and females dosed 300 µg/kg on Day 7, and one male dosed 100 µg/kg and two females dosed 300 µg/kg on Day 36. Single-cell necrosis and centrilobular fibrosis were observed on Day 7 only in the female groups dosed 100 and 300 µg/kg. However, on Day 36, fibrosis was extensively observed in one 300 µg/kg-dosed male and in all the female groups dosed 10 µg/kg and above. Disarrangement of hepatocytes (Photo 1.-B) occurring on Day 36 in one male dosed 300 µg/kg and in the female groups dosed 100 and 300 µg/kg was characterized by irregular arrangement of hepatic cords and multilayered hepatocytes. Multinucleated hepatocytes (Photo 1.-B) were manifested on Day 36 only in the female groups dosed 100 and 300 µg/kg. The three 300 µg/kg-dosed females that died on Days 21, 23 and 27 exhibited both the prominent disarrangement of hepatocytes accompanied by a tubule-like structure in the parenchymatous tissue of the liver (Photo 1.-C) and the peliosis-like lesion characterized by markedly dilated and irregularly sinusoidal spaces

Table 1. Number of surviving rats of both sexes bearing the hepatic lesions, sacrificed on Day 2, 7 or 36 after a single administration of TBDD by gavage at 4 different doses or vehicle as a control.

Findings	Males (µg/kg)					Females (µg/kg)				
	0	10	30	100	300	0	10	30	100	300
Day 2 (Number of animals examined)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Cytoplasmic basophilia: tigroid	0	5	5	5	5	0	5	5	5	2
Day 7 (Number of animals examined)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
Cytoplasmic basophilia: tigroid	0	5	5	5	5	0	5	5	5	5
Hepatocellular hypertrophy	0	0	5	5	5	0	5	5	5	5
Cytoplasmic inclusion	0	0	0	0	3	0	0	0	0	2
Necrosis: single cell	0	0	0	0	0	0	0	0	4	4
Fibrosis: centrilobular	0	0	0	0	0	0	0	0	4	4
Day 36 (Number of animals examined)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(2)
Cytoplasmic basophilia: tigroid	0	5	5	5	5	0	5	5	5	2
Hepatocellular hypertrophy	0	5	5	5	4	0	5	5	5	2
Cytoplasmic inclusion	0	0	0	1	0	0	0	0	0	2
Multinucleated hepatocytes	0	0	0	0	0	0	0	0	4	2
Disarrangement	0	0	0	0	1	0	0	0	1	2
Fibrosis: centrilobular	0	0	0	0	1	0	1	4	5	2

a) Each of the groups examined was comprised of 5 animals except for two 300 µg/kg-dosed female rats surviving until Day 36.

filled with erythrocytes (Photo 1.-D). Although their livers were seriously damaged, the primary cause of their deaths was attributed not to liver failure but to hemorrhage in the brain and spinal cord (Yamamoto *et al.*, 2006).

Liver weights

Relative liver weight was significantly increased on Day 2 in both the male and female groups dosed 30 $\mu\text{g/kg}$ and above, and on Day 7 in all the TBDD-dosed male and female groups (Table 2.). On Day 36, an increase in the relative liver weight still persisted in the male groups dosed 30 $\mu\text{g/kg}$ and above and in the female groups dosed 100 and 300 $\mu\text{g/kg}$. On the other hand, a statistically significant decrease in body weight was observed only on Day 36 in the 300 $\mu\text{g/kg}$ -dosed male group and on Days 7 and 36 in the 300 $\mu\text{g/kg}$ -dosed female group (data not shown here but presented in detail in our previous paper (Yamamoto *et al.*, 2006)). Therefore, a dose-related increase in the relative liver weights of the TBDD-dosed males and

females was attributed not to their decreased body weights but to the increased liver weight.

Hepatic enzymes and biochemistry

The hepatic activities of AHH, ECOD and EROD were markedly increased on Day 2 through 36 in all the TBDD-dosed groups, compared with the respective controls (Fig. 1.). The marked increase was not dose-related but persisted without apparent attenuation of the enzyme activities along the time course elapsed after the single TBDD administration. All the TBDD-dosed female groups exhibited significantly increased serum levels of total cholesterol and phospholipid on Days 7 and 36 (Table 2). In the males, the serum levels of total cholesterol were increased on Day 2 at 30 and 100 $\mu\text{g/kg}$ and on Days 7 and 36 at 30 $\mu\text{g/kg}$ and above, while the serum levels of phospholipid were increased on Day 36 at 30 $\mu\text{g/kg}$ and above. Significantly increased release of liver-associated aspartate transferase (AST) into serum was observed on Day 7 in the female groups dosed 30 $\mu\text{g/kg}$ and above and on

Photo 1. Light microscopic pictures of hepatic lesions in a 300 $\mu\text{g/kg}$ -dosed female rat sacrificed on Day 7 (A), noting tigroid basophilic cytoplasm and hepatocellular hypertrophy, in a 100 $\mu\text{g/kg}$ -dosed female rat sacrificed on Day 36 (B), noting both disarrangement and multinucleating of hepatocytes, in a 300 $\mu\text{g/kg}$ -dosed female rat on Day 27, indicating a tubule-like structure (C) and a peliosis-like lesion (D), in addition to the prominent disarrangement of hepatocytes. H & E stain. The bars indicate 100 μm for Photo A and D and 50 μm for Photo B and C.

Acute hepatic effects of 2,3,7,8-tetrabromodibenzo-*p*-dioxin in rats.

Day 36 in the female groups dosed 100 and 300 µg/kg.

Hepatic levels of TBDD

The mean concentrations of TBDD in the liver collected on Day 2 from the 30, 100 and 300 µg/kg-dosed rats of both sexes were increased proportionally with an increase in dose level (Table 3.), indicating a dose-dependent increase in the hepatic levels of TBDD. In addition, time course changes in the liver concentrations of TBDD were found to follow a linear relationship between the logarithmic concentrations of TBDD in the liver and the days elapsed after the single administration, suggesting that the first-order rate held for the elimination of TBDD from the liver. It was estimated on the basis of both the least square regression line and the first-order elimination rate that an elimination half-life ($t_{1/2}$) of TBDD from the liver was 13 and

15 days for the male groups dosed 100 and 300 µg/kg and 16 and 12 days for the female groups dosed 100 and 300 µg/kg, respectively. No difference in TBDD elimination from the liver was found either between the males and the females or between the 100 and 300 µg/kg-dosed groups of each sex. Liver-to-adipose tissue ratios of TBDD in both males and females were greater on Day 2 than on Days 7 and 36, suggesting the preferential accumulation of TBDD in the liver on Day 2 compared with Days 7 and 36.

DISCUSSION

According to Ivens *et al.* (1992, 1993), acute hepatotoxicity induced by single administration of TBDD by gavage to male and female rats at dose levels of 10 to 300 µg/kg was histopathologically character-

Table 2. Relative liver weights and serum levels of total cholesterol, phospholipid and AST in surviving rats of both sexes sacrificed on Day 2, 7 or 36 after a single administration of TBDD by gavage at 4 different doses or vehicle as a control.

Items	Male (Days after administration)			Female (Days after administration)		
	2	7	36	2	7	36
Relative liver weights (%)						
0	5.05 ± 0.18	4.63 ± 0.51	3.83 ± 0.27	5.05 ± 0.27	4.59 ± 0.22	4.07 ± 0.22
10	6.16 ± 0.48	5.66 ± 0.29**	4.32 ± 0.31	5.73 ± 0.56	5.66 ± 0.35**	4.45 ± 0.45
30	6.54 ± 0.50**	6.43 ± 0.51**	4.67 ± 0.28*	6.55 ± 0.46**	6.63 ± 0.48**	4.52 ± 0.24
100	6.81 ± 0.62**	7.20 ± 0.52**	5.79 ± 0.69**	6.96 ± 0.55**	7.07 ± 0.80**	5.20 ± 0.24**
300	7.49 ± 1.16**	7.06 ± 0.19**	6.12 ± 0.26**	6.48 ± 0.74**	6.97 ± 0.41**	6.39 ± 0.10 ^{a)}
Total cholesterol (mg/dL)						
0	78 ± 10	82 ± 5	62 ± 9	68 ± 6	77 ± 5	69 ± 11
10	87 ± 6	109 ± 26	96 ± 12	82 ± 3	129 ± 20**	93 ± 7*
30	98 ± 9*	127 ± 16**	117 ± 15**	81 ± 12	115 ± 7**	110 ± 14**
100	107 ± 14**	126 ± 5**	139 ± 38**	79 ± 8	128 ± 18**	117 ± 21**
300	96 ± 12	118 ± 21*	131 ± 26**	82 ± 9	163 ± 18**	120 ± 70 ^{a)}
Phospholipid (mg/dL)						
0	171 ± 19	175 ± 7	135 ± 15	143 ± 11	157 ± 10	163 ± 25
10	184 ± 24	199 ± 36	186 ± 8	170 ± 10	231 ± 30**	201 ± 14*
30	200 ± 17	207 ± 22	213 ± 23**	169 ± 23	211 ± 17*	218 ± 7**
100	206 ± 11	212 ± 13	231 ± 53**	164 ± 15	224 ± 30**	212 ± 29**
300	201 ± 26	217 ± 35	235 ± 47**	160 ± 10	292 ± 36**	226 ± 110 ^{a)}
AST : Aspartate aminotransferase (IU/L)						
0	56 ± 2	63 ± 4	60 ± 7	54 ± 5	53 ± 5	48 ± 8
10	58 ± 5	59 ± 5	62 ± 7	62 ± 7	57 ± 5	60 ± 18
30	58 ± 8	57 ± 5	73 ± 17	62 ± 6	61 ± 8 *	62 ± 10
100	63 ± 4	76 ± 26	81 ± 21	64 ± 5	78 ± 15 **	97 ± 16**
300	62 ± 4	81 ± 33	181 ± 195	61 ± 4	311 ± 281 **	186 ± 81 ^{a)}

Values are expressed as mean ± S.D. of 5 rats in each group without 300 µg/kg-dosed group at Day 36 in female rats.

* and **: Significant difference at $p < 0.05$ and $p < 0.01$ by Dunnett test, respectively.

^{a)} : Number of 300 µg/kg-dosed female rats surviving until Day 36 was only 2, and statistical analysis was not applied.

ized by cytoplasmic vacuolation, hepatocellular hypertrophy accompanied by nuclear swelling and basophilic cytoplasm, single-cell necrosis, dissociation of liver-cell plates and dislocation of hepatocytes, while peliosis hepatis, focal necrosis and increased connective tissue were additionally observed by repeated

Fig. 1. Hepatic activities of AHH, ECOD and EROD in male and female rats on Days 2, 7 and 36 after a single administration of TBDD by gavage at a dose of 10, 30, 100 or 300 µg/kg and vehicle as a control. Each bar indicates the mean of two rats.

administration of TBDD for 13 weeks at dose levels of 0.01 to 10 µg/kg/day. The present study confirmed Ivens *et al.*'s histopathological findings and might extend their findings to novel ones that a broad spectrum of these TBDD-induced hepatic lesions is induced in dose- and time-dependent manners. The dose- and time-dependent occurrence of the hepatic lesions was characterized as follows. The lowest dose level of 10 µg/kg induced tigroid basophilic cytoplasm of hepatocytes on Day 2 through 36 and hepatocellular hypertrophy on Days 7 and 36. These two hepatocellular signs occurring at low dose levels during the early period are thought to be classified as an adaptive response of hepatocytes to TBDD. The relatively high dose levels induced single-cell necrosis and fibrosis during the middle and late period, and might reflect degenerative and aggressive changes in the liver. Both disarrangement and multinucleation of hepatocytes are thought to represent the most pronounced changes in the liver that occurred on Day 36 in the surviving female rats dosed 100 and 300 µg/kg. Occurrence of these severe hepatic lesions as well as the deaths only in the TBDD-dosed female rats can be taken to indicate that females are more susceptible to TBDD than males. The dead females had the peliosis-like lesion characterized by markedly dilated and irregularly sinusoidal spaces filled with erythrocytes, in addition to the prominent disarrangement of hepatocytes. Ivens *et al.* (1992) noted the prepeliotic foci characterized by a dissociation of liver-cell plates and dislocation of single hepatocytes in surviving rats treated with 300 µg TBDD/kg. Our microscopic observation of those severely affected lesions induced by TBDD is essentially similar to the reported findings showing both the disorganized hepatic cord and the tubule-like structure in the parenchymatous tissues of liver in rats dosed 10 µg TCDD/kg/day for 31 days (Gupta *et al.*, 1973), prepeliotic ectasis of the liver sinusoids in rats fed 20 µg TCDD/kg-containing diet for 13 weeks (Plüss *et al.*, 1988) and peliosis-like distension of hepatic sinusoids in rats dosed intragastrically with 300 µg TCDD/kg (Niittynen *et al.*, 2003). Especially, since the dose level used in Niittynen *et al.*'s study was the same as that used in the present study, our and their findings suggested that the acute hepatotoxicity of TBDD is as potent as that of TCDD.

Notably, the multinucleated hepatocytes occurred on Day 36 only in the female rats dosed 100 and 300 µg TBDD/kg, although Ivens *et al.* (1992, 1993) did not state the TBDD-induced hepatocellular multinucleation. Our present finding is consistent with the pre-

viously reported results showing that single and repeated oral administrations of TCDD to rats induced multinucleated hepatocytes (Jones and Butler, 1974; Kociba *et al.*, 1976; Pohjanvirta *et al.*, 1989; NTP, 2006). Two bioassay studies of 2-year oral administration of TCDD also demonstrated the occurrence of multinucleated hepatocytes, in addition to significant increases in the incidence of hepatocellular carcinomas only in female Sprague-Dawley rats (Kociba *et al.*, 1978) and the incidence of cholangiocarcinomas and hepatocellular adenomas only in female Harlan Sprague-Dawley rats (NTP, 2006). Shi and King (2005) reported that binucleated polyploid cells resulting from a spontaneous failure of cytokinesis were tightly coupled to chromosome nondisjunction, suggesting that the nondisjunction would provide a potential explanation for the prevalence of aneuploid cells and centrosome amplification in many cancers. Fujiwara *et al.* (2005) reported that tumorigenesis was promoted by polyploid cells resulting from binucleated *p53*^{-/-} cells by using a cytokinesis inhibitor. Cytokinesis failure of a normal mononuclear cell is known to cause the multinucleated cell having polyploidy with more than 4n genome, resulting in intracellular conditions that cause genomic instability. On the basis of these two reports on polyploid cells as a causative factor of tumorigenesis, the induction of multinucleated hepatocytes might be tightly coupled to the TCDD-induced hepatotumorigenesis. Therefore, the TBDD-induced hepatocellular multinucleation in the female rat might be predicted as a causative factor for possible development of neoplasm.

It is noteworthy in the present study that the single administration of TBDD at dose levels of 10 to 300 µg/kg induced persistent elevation of hepatic AHH, ECOD and EROD activities, which appeared not to be

dose-related. Our finding can be contrasted with two studies demonstrating that hepatic EROD activity was increased dose-dependently in the rats orally administered TBDD at dose levels ranging from 0.1 to 100 nmol/kg (Kedderis *et al.*, 1991b) and that a dose-dependent increase in EROD activity occurred in the rats subcutaneously injected 0.1 to 3 µg TBDD/kg (Nagao *et al.*, 1996). Since the dose levels of TBDD examined in these two studies were lower than those used in the present study, the persistently elevated hepatic AHH, ECOD and EROD activities found in the present study can be interpreted as these hepatic enzyme activities being saturated in response to the excessively high hepatic levels of TBDD. It can be inferred, therefore, that the hepatic AHH, ECOD and EROD activities and the tigroid basophilic cytoplasm of hepatocytes are the most sensitive endpoints among TBDD-induced hepatic lesions. Furthermore, the tigroid basophilic cytoplasm may morphologically reflect the induction of hepatic microsomal enzyme activities of AHH, ECOD and EROD. Indeed, association between increased amounts of smooth endoplasmic reticulum (SER) and rough endoplasmic reticulum (RER) in the hepatocytes of rats following a single oral dose of TCDD by gavage and induction of hepatic microsomal enzymes by TCDD was suggested (Fowler *et al.*, 1973; Lucier *et al.*, 1973). Prominent proliferation of SER in the hepatocytes of TCDD-dosed rats (Jones and Butler, 1974) and hamsters (Olson *et al.*, 1980) and marked elevation of microsomal enzyme activity of EROD by administration of TCDD to rats (Abraham *et al.*, 1988; Yamashita *et al.*, 1992; Nagao *et al.*, 1996) have been reported.

It has been reported that toxicity of TBDD is less potent than that of TCDD (Ivens *et al.*, 1992 and 1993; Birnbaum *et al.*, 1991; Yamamoto *et al.*, 2006). How-

Table 3. Liver concentrations and liver-to-adipose tissue ratios of TBDD on Days 2, 7 and 36 after a single administration of TBDD by gavage at a dose of 30, 100 or 300 µg/kg.

Sex	Dose of TBDD (µg/kg)	Concentrations of TBDD (ng/g) (liver-to-adipose tissue ratios)		
		Day 2	Day 7	Day 36
Male	30	68 (1.45)		
	100	250 (2.00)	215 (1.08)	42 (1.08)
	300	585 (1.33)	465 (0.93)	120 (1.00)
Female	30	46 (1.35)		
	100	200 (1.43)	160 (0.76)	45 (0.73)
	300	710 (1.38)	450 (0.83)	87 (0.73)

Values are represented as means of two rats in each group.

The adipose tissue levels of TBDD were obtained from the TBDD study by Yamamoto *et al.* (2006).

ever, comparison between the dose- and time-dependent hepatocellular responses to 10 to 300 µg TBDD/kg found in the present study and the dose levels of TCDD and hepatotoxic responses to TCDD reported in the following four studies suggested that acute hepatotoxicity of TBDD is as potent as that of TCDD. First, hepatocellular hypertrophy was induced by a single oral administration of 200 µg TCDD/kg to male and female rats (Jones and Butler, 1974). Second, marked distortion of the hepatocellular architecture with necrosis, and swelling and multinucleated hepatocytes were induced in rats dosed 10 µg TCDD/kg/day for 10 to 13 days (Gupta *et al.*, 1973). Third, a single intraperitoneal injection of TCDD to rats at dose levels of 5 to 500 µg/kg significantly increased both relative liver weights and serum levels of cholesterol as well as multinucleated and swelling hepatocytes (Pohjanvirta *et al.*, 1989). Fourth, a dose-dependent increase in hepatic EROD activity was induced by a single subcutaneous administration of TCDD to rats at dose levels of 3 to 3000 ng/kg (Abraham *et al.*, 1988). Indeed, this suggestion is compatible with the findings by two TBDD studies showing that hepatic EROD activity was induced by TBDD at such low dose levels as 0.1 to 100 nmol/kg (0.05 to 50 µg/kg) (Kedderis *et al.*, 1991b) and 0.1 to 3 µg/kg (Nagao *et al.*, 1996).

The elimination half-life ($t_{1/2}$) of TBDD from the liver was found to range from 12 to 16 days in the present study. Our $t_{1/2}$ values are slightly longer than the graphically estimated $t_{1/2}$ value of 9 days (Ivens *et al.*, 1992), but are in agreement with the reported elimination half-life of 17 days from the liver of the rats intravenously injected TBDD (Kedderis *et al.*, 1991a) and that of 13.3 days from the liver of the rats subcutaneously injected TBDD (Nagao *et al.*, 1996). Notably, the elimination half-lives of TBDD from the liver found in the present study were in good agreement with the reported half-life of 13.6 days from the liver of the rats subcutaneously injected TCDD (Abraham *et al.*, 1988; Nagao *et al.*, 1996). Also, consistent with the results of earlier studies (Kedderis *et al.*, 1991a; Nagao *et al.*, 1996), the present finding of a higher liver-to-adipose tissue ratio of TBDD on Day 2 than on Days 7 and 36 revealed that TBDD was preferentially accumulated in the liver during the early phase of TBDD administration. The early preferential accumulation of TBDD in the liver might be due to higher affinity and subsequent saturation of TBDD to the microsomal binding proteins specific to the liver through the Ah receptor mechanism (Leung *et al.*, 1988; Poland *et al.*, 1989).

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