2,3,7,8-TETRABROMODIBENZOFURAN (TBDF): 4 WEEK SUBCHRONIC TOXICITY STUDY IN THE RAT**

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ABSTRACT

TBDF was administered to Sprague Dawley rats by gavage at 0, 1, 10, 50, 150 or 500 ug/kg five days per week for four consecutive weeks. A high mortality rate was found at the 150 and 500 ug/kg dose levels. Treatment-related lesions were noted in the liver and thymus in the 10 and 50 ug/kg dose levels. No treatment-related lesions were found in the 1 ug/kg group.

INTRODUCTION

2,3,7,8-Tetrachlorodibenzodioxin (TCDD) and 2,3,7,8-Tetrachlorodibenzofuran (TCDF) produce toxic effects which are similar in nature but different in potency (1). TCDD and TCDF have both produced progressive weight loss, thymic atrophy, hepatomegaly, immunosuppression and lesions in the skin, bladder, adrenals and kidneys in laboratory animals. However, the chlorinated furan, as a rule, is less toxic than the chlorinated dioxin. Wide species differences in sensitivity to the toxic effects of either compound also exist (2). For instance, the LD50 of TCDD varies 5000-fold between the least (hamster) and most (guinea pig) sensitive animal species.

2,3,7,8-Tetrabromodibenzodioxin (TBDD) and 2,3,7,8-Tetrabromodibenzofuran (TBDF) have been less well studied than their chlorinated counterparts. Initial work indicates the brominated compounds produce a response in the rat which is qualitatively similar to the chlorinated dioxins and furans. In acute studies designed to determine LD50 values, the liver, thymus and spleen were identified as target organs in the Sprague Dawley rat following treatment with either TBDD or TBDF (4,5). The present study was designed to provide

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preliminary information on the repeated-dose toxicity of TBDF in the Sprague Dawley rat.

MATERIALS AND METHODS

Five groups of five male and female Sprague Dawley rats each were administered TBDF in a corn oil/acetone vehicle by gavage at 0, 1, 10, 50, 150 or 500 ug/kg five days per week for four consecutive weeks. Body weights were measured weekly. Animals were housed individually in stainless steel wire mesh cages and provided food and water ad libitum. A 12/12 hour light cycle with a temperature range of 60-75 degrees Fahrenheit was maintained in the animal room. Animals were subjected to a gross necropsy at terminal sacrifice and the liver, spleen, kidneys, adrenals, thymus, and testes weighed. The liver, kidney, spleen, adrenal, heart, thymus and gross lesions were examined under the light microscope (hematoxylin and eosin staining) in the 0, 1, 10 and 50 ug/kg dose groups. Statistical analysis was performed under the General Linear Models Procedure by the Ryan-Einot-Gabriel-Welsch Multiple Range test.

RESULTS

All animals in the 500 ug/kg groups died or were sacrificed in a moribund condition between study days 17 and 24. In the 150 ug/kg group, 3 of 5 males and 4 of 5 females died or were sacrificed in a moribund condition between study days 18 and 24. No animals were lost prior to scheduled termination in the 1, 10 and 50 ug/kg dose groups. Organ and body weight data are presented only on those groups with 2 or more animals surviving through day 28.

Group mean body weight, expressed relative to Day 0 body weight, was depressed in a time- and dose-dependent manner in both sexes (Table 1). Mean thymus weight, expressed relative to Day 28 body weight, was significantly less than the control mean for males in the 150 ug/kg and females in the 10 and 50 ug/kg groups (Table 1). The relative mean kidney and testes weight were increased in males, but not females (kidney), of the 50 and 150 ug/kg groups. This effect was probably related to the decrease in body weight in these groups, rather than due to a specific effect on these organs. No significant differences in relative mean liver, adrenal or spleen weights were detected (data not shown).

Treatment-related alterations were noted in the liver and thymus from the 50, and to a lesser extent, the 10 ug/kg dose groups. Liver changes consisted of panlobular hypertrophy of the hepatocytes with associated panlobular

hepatocyte vacuolation and focal necrosis. Hepatocyte hypertrophy was widespread and consisted of enlargement of hepatocytes and their nuclei with occasional multinucleated cells and small numbers of mitotic figures. Some of the enlarged hepatocytes in male, but not female, rats were vacuolated. Multiple scattered focal areas of hepatocyte necrosis were present in most of the affected livers. Thymic atrophy consisting of overall depletion of the lymphoid elements was present in all 50 ug/kg male and female rats from which thymus was available and in most animals from the 10 ug dose group. Alterations noted in the remaining organs evaluated in the 10 and 50 ug/kg dose groups were considered incidental. No treatment-related alterations were observed in the 1 ug/kg group.

TABLE I. Group Mean Body and Organ Weight Data.

Dose (ug/kg)	Body Weight (Normalized to Day 0)				Organ to Body Weight Ratio (Org Wt/Bdy Wt, Day 28) X 100		
	D7	D14	D21	D28	Thymus	Kidney	Testes
Males							
0	1.18	1.35	1.53	1.48	0.001	0.008	0.011
1	1.13	1.28	1.46	1.43	0.001	0.008	0.012
10	1.11	1.26	1.40	1.29	0.0007	0.009	0.013
50	1.11	1.12*	1.07*	0.86*	0.0004	0.010*	0.017*
150	1.03*	0.91*	0.97*ˆ	0.84*ˆ	0.0001*ˆ	0.010*ˆ	0.018*ˆ
500	1.00*	0.87*	0.89*#	-	-	-	-
Females							
0	1.12	1.14	1.34+	1.25+	0.002+	0.009+	-
1	1.06	1.13	1.26	1.21	0.002	0.008	-
10	1.07	1.13	1.22	1.06	0.0009*	0.008	-
50	1.09	1.04	1.15	0.98*	0.0008*	0.009	-
150	0.96*	0.97	0.97*#	-	-	-	-
500	0.85*	0.80*	-	-	-	-	-

*p ≤ 0.05
n=5/sex/group except as noted; +:n=4, #:n=3, ˆ:n=2.

DISCUSSION

In acute studies designed to determine LD50 values, the liver, thymus and spleen were identified as target organs in the Sprague Dawley rat following treatment with either TBDD or TBDF (4,5). A single TBDD dose of 150 ug/kg induced histologic lesions in the liver, thymus and spleen; 50 ug/kg was without effect except for a mild thymic lymphoid atrophy in several female, but not male, rats (4). A single dose of TBDF at 500 ug/kg did not induce compound-related histologic effects (5). A TBDF dose of 1000 ug/kg induced sporadic liver changes in male, but not female, rats. Effects on the thymus

were not observed until a dose level of 2500 ug/kg was reached. The LD50 values of TBDD and TBDF in the Sprague Dawley rat were determined to be > 500 and > 5000 ug/kg, respectively.

In comparison, TCDD when administered in a single dose of 100 ug/kg produced 43% mortality and severe thymic atrophy and liver damage (6). Liver damage was not observed at 25 ug/kg, but slight to moderate thymic atrophy was produced. An LD50 of between 25-60 ug/kg was determined in the Sprague Dawley rat (3). Comparable studies on TCDF were not found in the literature.

The no effect level for TBDF in the present study was 1 ug/kg based on body and organ weight data and histopathology. The liver and thymus changes observed are similar to those induced on a subchronic basis by TCDD. However, TCDD at 1 ug/kg for 31 days induced slight to moderate liver damage and thymic and renal degeneration in the rat (6). At 10 ug/kg for 31 days, TCDD produced 94% mortality and severe hepatic damage. Significant microscopic changes were not observed in rats given TCDD at a dose level of 0.1 ug/kg for up to 31 days. In comparison, no treatment-related lesions were detected in rats receiving TBDF at a dose level of 1 ug/kg over a 4 week period.

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