Kidney: Nephropathy is a common occurrence in aging F344/N rats and was observed in nearly all males and the majority of females in this study. In comparison to the control group, the severity of nephropathy was significantly increased in 2,500 ppm females both at 15 months and 2 years (Table 10). The number of females with a moderate severity of nephropathy was much higher in the 2,500 ppm group than in the control group, whereas the reverse was true for minimal nephropathy. The severity of nephropathy was similar among all groups of male rats.

TABLE 10
Incidences and Severity of Nephropathy in Female Rats in the 2-Year Feed Study of 4,4′-Thiobis(6-*t*-Butyl-*m*-Cresol)

Dose (ppm)	0	500	1,000	2,500
15-Month Interim Evaluation				
Kidney[a]	10	10	10	10
Nephropathy[b]	9	10	10	10
Absent (Grade 0)	1	0	0	0
Minimal (Grade 1)	6	8	9	0
Mild (Grade 2)	3	2	1	8
Moderate (Grade 3)	0	0	0	2
Marked (Grade 4)	0	0	0	0
Group average severity grade	1.2	1.2	1.1	2.2**
2-Year Study				
Kidney	50	50	50	50
Nephropathy	44	41	46	48
Absent (Grade 0)	6	9	4	2
Minimal (Grade 1)	17	14	19	1
Mild (Grade 2)	26	25	22	29
Moderate (Grade 3)	1	2	5	18
Marked (Grade 4)	0	0	0	0
Group average severity grade	1.4	1.4	1.6	2.3**

** Significantly different ($P \leq 0.01$) from the control group by the Mann-Whitney U test
[a] Number of animals with kidney examined microscopically
[b] Number of animals with lesion

Thyroid gland: The incidence of C-cell adenoma or carcinoma (combined) occurred with a significant positive trend in female rats and was slightly, but not significantly, increased in the 1,000 and 2,500 ppm groups at the end of the 2-year study (0 ppm, 3/49; 500 ppm, 4/49; 1,000 ppm, 8/50; 2,500 ppm, 9/50; Table B3). This positive trend was not considered chemical related because the incidence in 2,500 ppm females was only slightly above the historical average of 15% and well within the range of 6% to 31% for historical controls (Table B4b). Further, C-cell hyperplasia was decreased in females (28/49, 24/49, 27/50, 18/50; Table B5), although the decrease in 2,500 ppm females was not statistically significant by pairwise comparison.

Uterus: Stromal polyps occurred with a significant positive trend (0 ppm, 2/50; 500 ppm, 5/50; 1,000 ppm, 9/50; 2,500 ppm, 9/50; Table B3) in the uteri of female rats exposed to TBBC. Increased incidences of stromal polyps in females exposed to 1,000 or 2,500 ppm were significant; however, the incidences are only slightly above the historical control average of 16% and are well within the historical control range of 2% to 30% (Table B4c). The incidence in controls is unusually low compared to that in historical controls. Stromal sarcoma was also present in one 500 ppm and one 2,500 ppm female.

Mammary gland: The incidence of fibroadenoma occurred with a statistically significant negative trend in female rats (29/50, 24/50, 11/50, 16/50; Table B3), and the decreases were significant in the 1,000 and 2,500 ppm groups. There was also a significant negative trend in the incidence of mammary gland fibroadenoma, adenoma, or carcinoma (combined) in females (32/50, 24/50, 11/50, 16/50; Table B3).

MICE

15-DAY STUDY

All 10,000 and 25,000 ppm male and female mice and eight males and eight females receiving 5,000 ppm TBBC died (Table 11). The two surviving 5,000 ppm males had a mean body weight loss of 25% and a final mean body weight 35% lower than that of the controls; the final mean body weight of 2,500 ppm males was similar to that of the controls. The two surviving 5,000 ppm females had a mean body weight loss of 10% and a final mean body weight 27% lower than that of the controls; the final mean body weight of 2,500 ppm females was 13% lower than that of the controls. Male and female mice receiving 1,000 ppm TBBC had final mean body weights similar to those of the controls. Feed consumption by 5,000, 10,000, and 25,000 ppm males and females was markedly lower than that by controls. Mice exposed to 1,000, 2,500, or 5,000 ppm received approximate doses of 285, 585, or 475 mg TBBC per kilogram body weight per day (males) or 360, 950, or 1,030 mg/kg per day (females). Approximate doses for mice exposed to 10,000 or

TABLE 11
Survival, Body Weights, and Feed Consumption of Mice in the 15-Day Feed Study of 4,4'-Thiobis(6-t-Butyl-m-Cresol)

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	10/10	21.3 ± 0.4	24.2 ± 0.7	3.0 ± 0.6		6.7	9.1
1,000	10/10	21.6 ± 0.5	26.1 ± 0.5	4.5 ± 0.2	108	5.9	7.7
2,500	10/10	21.9 ± 0.2	23.8 ± 0.4	2.0 ± 0.5	98	4.0	6.7
5,000	2/10[d]	21.0 ± 0.6	15.9 ± 0.4**	−5.3 ± 0.3**	65	1.2	2.3
10,000	0/10[e]	21.7 ± 0.5	–	–	–	1.0	1.4
25,000	0/10[f]	22.0 ± 0.4	–	–	–	1.7	–[g]
Female							
0	10/10	15.7 ± 0.3	18.9 ± 0.4	3.1 ± 0.3	–	6.1	13.1
1,000	10/10	15.5 ± 0.3	19.3 ± 0.2	3.8 ± 0.4	103	4.8	7.8
2,500	10/10	16.2 ± 0.4	16.5 ± 0.5**	0.3 ± 0.4**	87	4.2	8.2
5,000	2/10[h]	15.3 ± 0.2	13.8 ± 0.1**	−1.2 ± 0.7**	73	2.2	3.8
10,000	0/10[i]	16.4 ± 0.3	–	–	–	1.3	–[g]
25,000	0/10[j]	16.8 ± 0.2*	–	–	–	0.9	–[g]

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** (P≤0.01)
a Number of animals surviving at 15 days/number initially in group
b Weights are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies. No final mean body weights or body weight changes were calculated for groups with 100% mortality.
c Feed consumption is expressed as grams per animal per day.
d Day of death: 10, 12, 12, 12, 13, 14, 15, 15
e Day of death: 8, 8, 9, 10, 10, 10, 11, 11, 12, 12
f Day of death: 4, 4, 4, 5, 5, 5, 6, 6, 6, 6
g All animals in these exposure groups died prior to the second week of the study
h Day of death: 9, 10, 10, 10, 11, 11, 11, 15
i Day of death: 6, 7, 7, 7, 7, 8, 8, 8, 8, 8
j Day of death: 4, 4, 4, 4, 5, 5, 5, 5, 5, 5

to 10,000 or 25,000 ppm cannot be calculated due to early deaths. Reduced feed consumption by exposed groups was seen as early as the first day of the study. The reduction in feed consumption was attributed to poor feed palatability.

Diarrhea was observed in 25,000 ppm mice beginning on either day 2 or day 3 of the study. Diarrhea was also present in most 10,000 ppm males (beginning on day 8) and females (beginning on day 2). Five 5,000 ppm males exhibited diarrhea (beginning on day 9), as did nine 5,000 ppm females (beginning on day 2).

Significantly different absolute or relative organ weights in exposed groups of mice were associated with lower mean body weights or were attributed to severe debilitation and stress (thymus, spleen) and were not considered to be the result of organ-specific toxicity (Table F4).

Because all 10,000 and 25,000 ppm male and female mice died and because of morbidity in surviving 5,000 ppm males, hematology parameters were measured only in males and females receiving 1,000 or 2,500 ppm and in 5,000 ppm females (Table G7). Segmented neutrophil counts were significantly higher in 2,500 and 5,000 ppm females. The increases were modest and were not accompanied by an increase in the number of immature cells, suggesting that these increases were not an inflammatory response. The increased numbers of circulating mature neutrophils may have been related to a shift in the total blood pool distribution without an absolute increase.

Significant increases in mean erythrocyte hemoglobin concentration values occurred in all surviving exposed male and female mice. Increased mean erythrocyte hemoglobin concentration is not a physiologic possibility and is usually an artifact caused by sample handling or analytical error. However, any condition that would cause increased erythrocyte fragility leading to increased post-sampling hemolysis could cause an increase in mean erythrocyte hemoglobin concentration values.

Microscopic examination was not performed on tissues from mice in the 10,000 or 25,000 ppm groups because they died before the end of the study. Kidneys were examined microscopically in the 2,500 and 5,000 ppm groups. The principal lesion caused by the ingestion of TBBC was minimal focal renal tubule necrosis in eight males and three females that received 5,000 ppm. Most of the affected mice also had a few protein casts within tubule lumens. Depletion of cells from the bone marrow and lymphoid organs was observed in many mice in the 5,000 ppm group. Bone marrow depletion was attributed to nutrient deficiency accompanying weight loss; depletion of lymphoid organs is commonly associated with low body weight, debilitation, and stress.

13-WEEK STUDY

All animals survived to the end of the study (Table 12). The final mean body weight of 2,500 ppm males was 15% lower than that of the controls. Female mice receiving 500, 1,000, or 2,500 ppm TBBC had final mean body weights 11%, 15%, and 22% lower than that of the controls, respectively. Final mean body weights of mice in other exposure groups were similar to those of the controls. Due to spillage and scattering, there were limitations in measuring feed consumption by mice and the data were difficult to interpret. Feed consumption by 2,500 ppm males averaged 24% less than that by the controls through week 3 of the study and was similar to that by the controls throughout the remainder of the study. No conclusions can be drawn from the slight variations in feed consumption observed in the male control group in the latter part of the study. Feed consumption by 2,500 ppm females averaged 27% less than that by the controls during most of the study. Mice exposed to 100, 250, 500, 1,000, or 2,500 ppm received approximate doses of 15, 30, 65, 145, or 345 mg TBBC per kilogram body weight per day (males) or 10, 35, 60, 165, or 340 mg/kg per day (females). Variations in feed consumption by males or females at other exposure levels did not appear to be chemical related. Since no clinical findings related to TBBC administration were observed in the present study, the reduction in feed consumption by 2,500 ppm females was probably due to poor feed palatability.

TABLE 12
Survival, Body Weights, and Feed Consumption of Mice in the 13-Week Feed Study of 4,4'-Thiobis(6-t-Butyl-m-Cresol)

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)	Feed Consumption[c] Week 1	Week 13
Male							
0	9/9	21.3 ± 0.4	30.8 ± 1.1	9.5 ± 0.8		3.3	2.8
100	10/10	21.5 ± 0.5	30.6 ± 1.0	9.0 ± 0.6	99	3.6	2.9
250	10/10	21.8 ± 0.4	31.7 ± 0.6	9.8 ± 0.6	103	3.1	3.5
500	10/10	21.6 ± 0.6	30.5 ± 0.9	8.9 ± 0.6	99	3.7	3.2
1,000	10/10	22.2 ± 0.4	30.8 ± 0.6	8.7 ± 0.6	100	–[d]	3.8
2,500	10/10	21.6 ± 0.4	26.3 ± 0.4**	4.7 ± 0.3**	85	2.6	4.0
Female							
0	10/10	17.7 ± 0.3	30.7 ± 0.8	13.0 ± 0.8		3.0	3.4
100	10/10	17.7 ± 0.3	28.1 ± 0.7	10.4 ± 0.6**	91	2.2	2.6
250	10/10	17.9 ± 0.3	29.2 ± 0.7	11.3 ± 0.6**	95	3.1	3.4
500	10/10	17.9 ± 0.4	27.3 ± 0.7**	9.4 ± 0.4**	89	2.8	3.4
1,000	10/10	17.7 ± 0.3	26.0 ± 0.4**	8.3 ± 0.3**	85	2.9	4.2
2,500	10/10	17.9 ± 0.3	23.8 ± 0.5**	5.9 ± 0.4**	78	2.0	3.7

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams per animal per day.
[d] Feed consumption values were invalid due to technical error.

Absolute and relative liver weights of 2,500 ppm males and females were slightly but significantly greater than those of the controls (Table F5). Males exposed to 500, 1,000, or 2,500 ppm and females exposed to 2,500 ppm had significantly increased absolute and relative spleen weights. Differences in the absolute or relative weights of other organs were related to reductions in mean body weights.

The erythrocyte counts, hematocrit and hemoglobin concentrations, and mean erythrocyte volume values of 2,500 ppm males and females were significantly less than those of the controls (Table G8). The hematocrit and erythrocyte counts of 1,000 ppm males and females were also significantly reduced. These differences were consistent with a developing mild microcytic, normochromic, nonresponsive anemia similar to differences observed in male rats in the 13-week study.

The principal lesions associated with the administration of TBBC to mice for 13 weeks occurred in the liver and were similar to those observed in rats (Table 13). The lesions were only observed in 2,500 ppm mice. The lesions in the liver consisted of individual or aggregates of enlarged Kupffer cells with abundant yellow-tan, pigmented cytoplasm (Kupffer cell hypertrophy), focal accumulations of similar macrophages in or adjacent to the portal areas, and a slight increase in small bile ductules in the portal areas (bile duct hyperplasia) (Plates 5 and 6). As in rats, the mesenteric lymph nodes of the 2,500 ppm mice contained increased numbers of enlarged macrophages.

Dose selection rationale: Because of the reduction in mean body weights, the increase in liver and spleen weights, and the accompanying histopathologic changes of the liver in 2,500 ppm males and females, the exposures selected for the 2-year study in mice were 250, 500, and 1,000 ppm.

TABLE 13
Incidences of Selected Nonneoplastic Lesions in Mice in the 13-Week Feed Study of 4,4′-Thiobis(6-*t*-Butyl-*m*-Cresol)

Dose (ppm)	0	100	250	500	1,000	2,500
Male						
Liver[a]	9	–[c]	–	–	10	10
Bile Duct Hyperplasia[b]	0	–	–	–	0	10**(1.0)[d]
Kupffer Cell Hypertrophy	0	–	–	–	0	10**(4.0)
Lymph Node, Mesenteric	9	–	–	–	10	10
Macrophage, Hyperplasia	0	–	–	–	0	5* (1.0)
Female						
Liver	10	–	–	–	10	10
Bile Duct Hyperplasia	0	–	–	–	0	6**(1.0)
Kupffer Cell Hypertrophy	0	–	–	–	0	10**(3.4)
Lymph Node, Mesenteric	10	–	–	–	10	10
Macrophage, Hyperplasia	0	–	–	–	1 (1.0)	1 (2.0)

* Significantly different (P≤0.05) from the control group by Fisher's exact test
** P≤0.01
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Organ not examined microscopically
[d] Average severity grade of lesions in affected animals (1=minimal; 2=mild; 3=moderate; 4=marked)

2-YEAR STUDY

Survival

Estimates of survival probabilities for male and female mice administered TBBC in feed for 2 years are presented in Table 14 and in Kaplan-Meier survival curves (Figure 3). Survival rates of exposed males and females were similar to those of the controls.

Body Weights, Feed Consumption, and Clinical Findings

The mean body weight of male mice receiving 1,000 ppm TBBC was approximately 10% lower than that of the controls from week 45 through the end of the study (Table 15). The mean body weight of males receiving 500 ppm TBBC was slightly lower than that of the controls throughout the study. The mean body weight of 250 ppm males was similar to that of the controls throughout the study. The mean body weight of 1,000 ppm females was 11% lower than that of the controls by week 45 and was 18% lower by the end of the study (Table 16 and Figure 4). Final mean body weights of 250 and 500 ppm females were approximately 9% lower than that of the controls. Exposure levels of 250, 500, or 1,000 ppm resulted in a daily ingestion of TBBC of 30, 60, or 145 mg/kg body weight for males or 45, 110, or 255 mg/kg for females. Feed consumption by exposed male mice was similar to that by the controls (Tables J3 and J4). No clinical findings were attributed to TBBC administration.

TABLE 14
Survival of Mice in the 2-Year Feed Study of 4,4'-Thiobis(6-t-Butyl-m-Cresol)

	0 ppm	250 ppm	500 ppm	1,000 ppm
Male				
Animals initially in study	60	60	60	60
15-month interim evaluation[a]	10	10	10	10
Natural deaths	6	6	1	4
Moribund	2	2	0	1
Animals surviving to study termination	42[c]	42[c]	49	45
Percent probability of survival at end of study[b]	84	84	98	90
Mean survival (days)[c]	673	667	683	678
Survival analysis[d]	P=0.242N	P=0.859	P=0.036N	P=0.536N
Female				
Animals initially in study	60	60	60	60
15-month interim evaluation[a]	9	9	10	10
Natural deaths	7	9	11	11
Moribund	4	3	3	4
Missing[a]		1		
Animals surviving to study termination	40[e]	38	36	35
Percent probability of survival at end of study	79	76	72	71
Mean survival (days)	658	660	654	644
Survival analysis	P=0.346	P=1.000	P=0.651	P=0.468

a Censored from survival analyses
b Kaplan-Meier determinations based on the number of animals alive on the first day of terminal sacrifice
c Mean of all deaths (uncensored, censored, and terminal sacrifice)
d The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed columns. A negative trend or lower mortality in an exposure group is indicated by N.
e Includes one animal that died the last week of the study

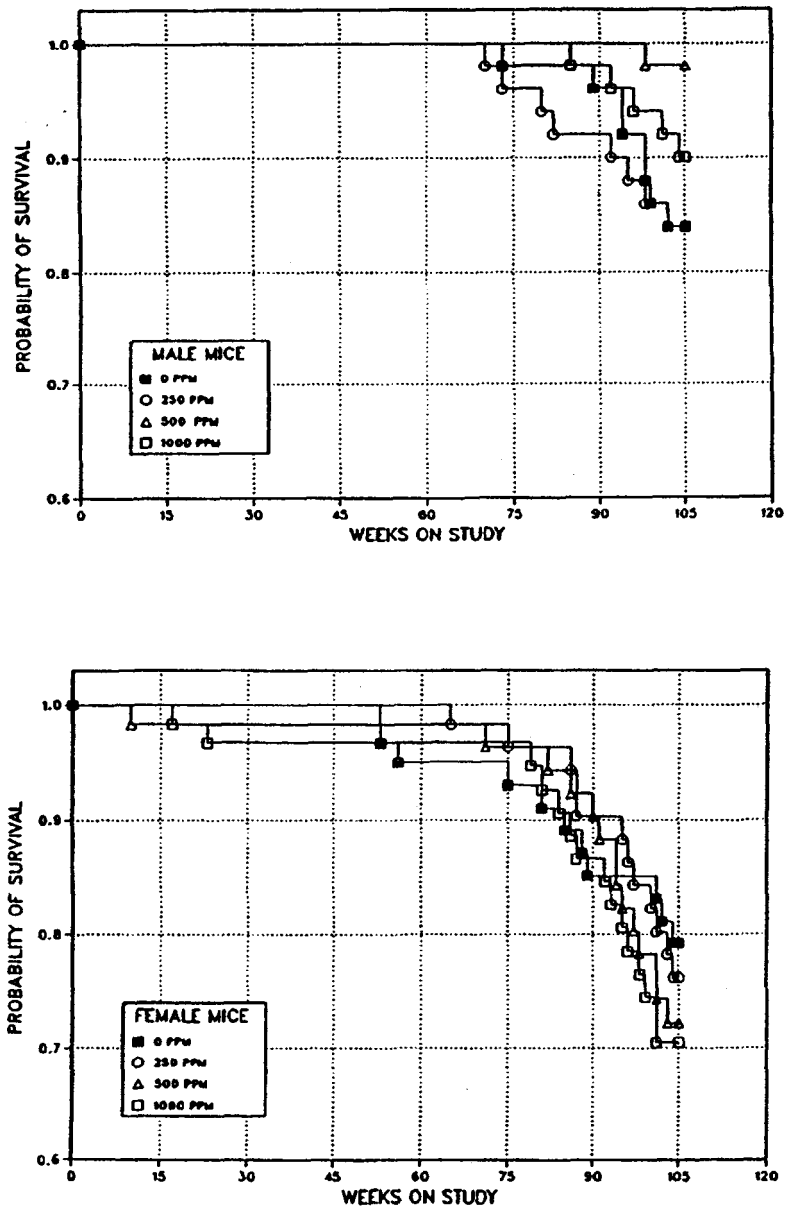

FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Mice Administered 4,4'-Thiobis(6-*t*-Butyl-*m*-Cresol) in Feed for 2 Years

TABLE 15
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of 4,4′-Thiobis(6-*t*-Butyl-*m*-Cresol)

Weeks on Study	0 ppm		250 ppm			500 ppm			1,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	22.1	60	22.2	101	60	22.2	101	60	22.4	101	60
2	23.5	60	23.8	101	60	23.9	102	60	24.4	104	60
3	24.7	60	24.8	100	60	25.1	102	60	25.2	102	60
4	25.4	60	25.5	100	60	25.9	102	60	25.9	102	60
5	26.5	60	26.2	99	60	26.6	100	60	26.4	100	60
6	27.3	60	27.2	100	60	27.4	100	60	27.3	100	60
7	27.8	60	27.8	100	60	27.8	100	60	28.0	101	60
8	28.8	60	28.5	99	60	28.6	99	60	28.4	99	60
9	29.2	60	29.1	100	60	28.8	99	60	28.8	99	60
10	30.2	60	30.1	100	60	29.6	98	60	29.3	97	60
11	30.6	60	30.4	99	60	30.2	99	60	29.9	98	60
12	31.6	60	31.2	99	60	31.0	98	60	30.5	97	60
13	32.0	60	31.5	98	60	31.1	97	60	30.9	97	60
17	35.1	60	34.5	98	60	33.8	96	60	33.3	95	60
21	37.0	60	36.4	98	60	35.7	97	60	34.8	94	60
25	38.0	60	37.2	98	60	36.2	95	60	35.3	93	60
29	38.9	60	37.8	97	60	36.7	94	60	35.8	92	60
33	41.1	60	40.1	98	60	39.3	96	60	37.6	92	60
37	41.5	60	42.0	101	60	40.6	98	60	37.9	91	60
41	42.3	60	42.2	100	60	41.1	97	60	38.5	91	60
45	44.2	60	43.5	98	60	42.2	96	60	39.9	90	60
49	45.6	60	44.7	98	60	43.6	96	60	41.3	91	60
53	46.8	60	46.1	99	60	44.5	95	60	42.3	90	60
57	47.5	60	46.9	99	60	45.6	96	60	43.3	91	60
61	48.0	60	46.9	98	60	45.8	95	60	43.2	90	60
65[a]	48.3	60	47.5	98	60	45.9	95	60	44.1	91	60
69	47.7	50	47.1	99	50	46.0	96	50	43.7	92	50
73	47.8	50	47.5	99	49	46.0	96	50	43.4	91	50
77	48.8	49	49.0	100	48	47.5	97	50	44.9	92	50
81	48.3	49	48.8	101	47	47.5	98	50	43.9	91	50
85	47.5	49	48.5	102	46	45.8	96	50	42.8	90	50
89	46.9	49	47.2	101	46	45.3	97	50	42.8	91	49
93	46.4	48	47.4	102	45	44.5	96	50	42.3	91	48
97	46.5	46	49.2	106	44	45.2	97	50	42.6	92	47
101	46.0	43	48.3	105	43	45.0	98	49	42.8	93	46
104	47.0	42	49.5	105	42	46.2	98	49	43.2	92	45
Mean for weeks											
1-13	27.7		27.6	96		27.6	100		27.5	99	
14-52	40.4		39.8	99		38.8	96		36.2	90	
53-104	47.4		47.9	101		45.7	96		43.2	91	

[a] Interim evaluation occurred.

TABLE 16
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of 4,4′-Thiobis(6-*t*-Butyl-*m*-Cresol)

Weeks on Study	0 ppm		250 ppm			500 ppm			1,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.2	60	18.2	100	60	18.5	102	60	18.6	102	60
2	20.2	60	20.5	102	60	20.5	102	60	20.7	103	60
3	21.3	60	21.7	102	60	21.8	102	60	21.9	103	60
4	22.6	60	22.6	100	60	22.5	100	60	22.7	100	60
5	23.6	60	23.6	100	60	23.5	100	60	23.6	100	60
6	24.6	60	24.5	100	60	24.3	99	60	24.5	100	60
7	25.2	60	25.3	100	60	25.1	100	60	25.3	100	60
8	26.1	60	25.8	99	60	25.8	99	60	25.8	99	60
9	27.0	60	26.6	99	60	26.5	98	60	26.4	98	60
10	28.2	60	27.8	99	60	27.2	97	60	27.2	97	60
11	28.6	60	28.3	99	60	27.8	97	59	27.8	97	60
12	29.4	60	29.0	99	60	28.4	97	59	28.4	97	60
13	30.3	60	29.8	98	60	28.7	95	59	28.9	95	60
17	33.3	60	32.8	99	60	32.1	96	59	31.3	94	59
21	35.8	60	34.9	98	60	34.2	96	59	33.5	94	59
25	36.2	60	35.3	98	59	34.4	95	59	33.6	93	58
29	37.8	60	35.9	95	59	35.2	93	59	34.3	91	58
33	40.6	60	39.1	96	59	38.5	95	59	36.8	91	58
37	41.1	60	40.6	99	59	40.0	97	59	37.3	91	58
41	41.9	60	40.8	97	59	40.0	96	59	38.0	91	58
45	43.9	60	42.7	97	59	41.7	95	59	39.2	89	58
49	45.1	60	44.1	98	59	43.0	95	59	40.3	89	58
53	46.8	60	45.8	98	59	44.6	95	59	42.1	90	58
57	49.1	57	47.0	96	59	45.8	93	59	42.7	87	58
61	49.8	57	47.5	95	59	46.8	94	59	43.0	86	58
65[a]	50.5	57	48.1	95	58	48.1	95	59	43.5	86	58
69	49.9	48	48.3	97	49	47.3	95	49	43.1	86	48
73	51.2	48	48.4	95	49	47.6	93	48	43.4	85	48
77	53.2	47	50.2	94	48	48.7	92	48	44.4	84	48
81	52.5	47	50.1	95	48	47.8	91	48	43.2	82	47
85	51.7	46	49.0	95	48	46.8	91	47	42.5	82	45
89	51.2	44	49.3	96	45	46.6	91	46	42.5	83	43
93	51.0	43	48.3	95	45	45.2	89	44	42.0	82	41
97	50.9	43	49.7	98	42	45.9	90	41	42.0	83	39
101	50.2	43	46.7	93	41	45.0	90	38	41.1	82	36
104	50.7	40	46.4	92	38	46.0	91	36	41.6	82	35
Mean for weeks											
1-13	25.0		24.9	100		24.7	99		24.8	99	
14-52	39.5		38.4	97		37.7	95		36.0	91	
53-104	50.6		48.2	95		46.6	92		42.7	84	

[a] Interim evaluation occurred.

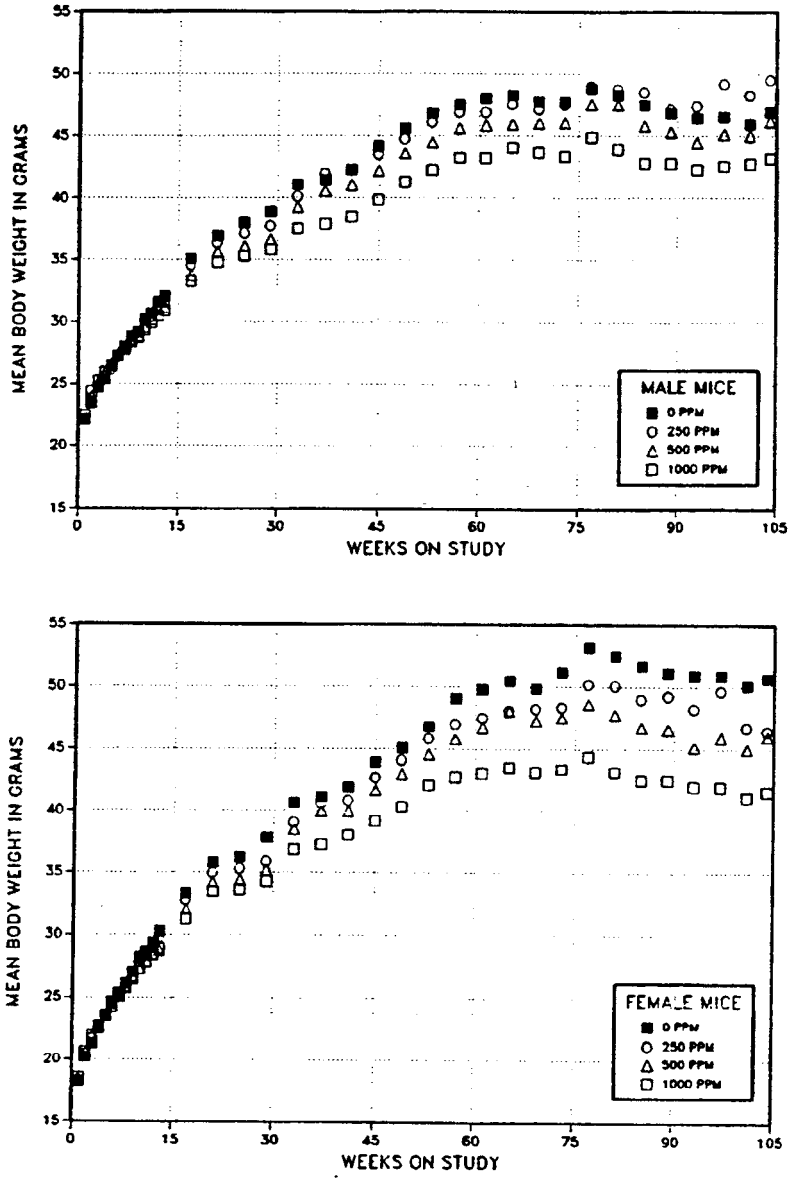

FIGURE 4
Growth Curves for Male and Female Mice
Administered 4,4′-Thiobis(6-*t*-Butyl-*m*-Cresol) in Feed for 2 Years

Hematology and Clinical Chemistry

Significantly lower hematocrit level, hemoglobin concentration, and erythrocyte count in 1,000 ppm males at 15 months were considered evidence of a mild normocytic normochromic nonresponsive anemia (Table G11). These decreases were similar to those that occurred in rats. Significantly decreased total leukocyte counts occurred in 500 and 1,000 ppm male mice at the 15-month interim evaluation.

Serum alkaline phosphatase (ALP) activities in 1,000 ppm males were slightly but significantly greater than those of the controls at 3 and 9 months (Tables G9 and G10). While ALP activity in 1,000 ppm males was numerically greater than that in controls at 15 months, the difference was not statistically significant. The ALP activity in 1,000 ppm females at 9 months was significantly greater than that in controls. Serum levels of total bilirubin in 250, 500, and 1,000 ppm males were significantly greater than those in controls at 9 and 15 months. At 9 months, the serum total bilirubin level in 250 ppm males was also significantly greater. These findings are consistent with hepatocellular damage.

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions in the liver and bone marrow. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Liver: At the 15-month interim evaluation, the relative liver weight of 1,000 ppm females was greater than that of controls due to a decrease in mean body weight in this group (Table F6). Absolute and relative liver weights of all other exposed male and female mice were similar to those of the controls. The incidence and severity of cytoplasmic vacuolization occurred with a negative trend in male mice (lipid accumulation was characterized as cytoplasmic vacuolization at 15 months, and as fatty change at 2 years, based on criteria discussed previously on page 41 in the rat study) (Tables 17, C3, and C5). An eosinophilic focus was present in one 500 ppm male at 15 months. At the end of the study, the incidences of fatty change, clear cell and eosinophilic foci, and hepatocellular adenoma or carcinoma (combined) all occurred with negative trends in male mice. Most of the negative trends were statistically significant and most occurrences in 1,000 ppm males were significant by pairwise comparison. A basophilic focus was present in one 500 ppm male.

Bone marrow: Myelofibrosis was present in all groups of females with a significant positive trend (0 ppm, 21/51; 250 ppm, 18/50; 500 ppm, 23/50; 1,000 ppm, 34/50; Table D4) and the incidence in 1,000 ppm females was significant by pairwise comparison.

GENETIC TOXICOLOGY

TBBC (33 to 10,000 μg/plate) was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 when tested in a pre-incubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Table E1; Zeiger *et al.*, 1987). A precipitate was observed on plates treated with 333 μg or greater TBBC. In cytogenetic tests with cultured Chinese hamster ovary cells, TBBC induced sister chromatid exchanges with and without S9, at doses which induced cell cycle delay (Table E2). No induction of chromosomal aberrations was observed in these cells, with or without S9 (Table E3). Because of TBBC-induced cell cycle delay, cultures analyzed for chromosomal aberrations were incubated for 20.5 hours, rather than the usual 12 hours, to allow sufficient cells to accumulate for harvest.

TABLE 17
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Male Mice in the 2-Year Feed Study of 4,4′-Thiobis(6-*t*-Butyl-*m*-Cresol)

Dose (ppm)	0	250	500	1,000
15-Month Interim Evaluation				
Liver[a]	10	10	10	10
Cytoplasmic Vacuolization[b]	6 (2.7)[c]	2 (2.0)	3 (2.3)	1* (1.0)
Eosinophilic Focus	0	0	1	0
Hepatocellular Adenoma	0	2	4	2
2-Year Study				
Liver	50	50	50	50
Fatty Change	19 (1.9)	17 (2.0)	5**(2.0)	6**(1.0)
Clear Cell Focus	6	5	2	0*
Eosinophilic Focus	2	3	2	0
Basophilic Focus	0	0	1	0
Focus, Any Type	8	8	5	0**
Hepatocellular Adenoma or Carcinoma[d]				
Overall rate[e]	25/50 (50%)	30/50 (60%)	27/50 (54%)	16/50 (32%)
Adjusted rate[f]	55.4%	62.4%	54.0%	34.7%
Terminal rate[g]	22/42 (52%)	24/42 (57%)	26/49 (53%)	15/45 (33%)
First incidence (days)	620	489	682	638
Logistic regression test[h]	P=0.018N	P=0.221	P=0.471	P=0.046N

* Significantly different (P≤0.05) from the control group by the Fisher exact test (15-month interim evaluation) or the logistic regression test (2-year study)
** P≤0.01
a Number of animals with liver examined microscopically
b Number of animals with lesion
c Average severity grade of lesions in affected animals (1=minimal; 2=mild; 3=moderate; 4=marked)
d Historical incidence for 2-year feed studies with untreated control groups (mean ± standard deviation): 485/1,366 (35.5% ± 14.3%); range 10%-68%
e Number of animals with neoplasm per number of animals with liver examined microscopically
f Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
g Observed incidence at terminal kill
h Beneath the control incidence are the P values associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The logistic regression test regards these neoplasms as nonfatal. A negative trend or lower incidence in an exposed group is indicated by N.

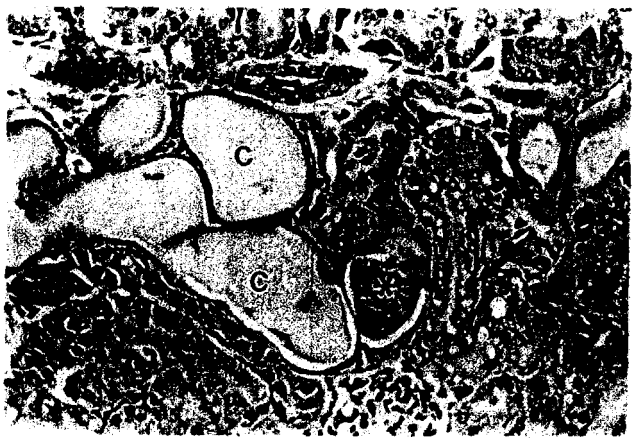

PLATE 1
Kidney of a female F344/N rat receiving 10,000 ppm 4,4′-thiobis(6-*t*-butyl-*m*-cresol) in the 15-day feed study. A segment of a proximal convoluted tubule with flattened epithelium is distended by a hyaline cast (C). Note the adjacent tubule filled with exfoliated necrotic cells (*) and other tubules with vacuolated epithelial cells and pyknotic nuclei (arrows). H&E, 80×

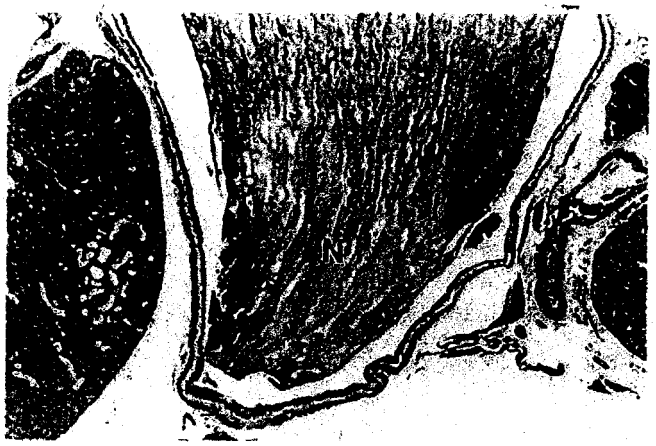

PLATE 2
Kidney of another female F344/N rat receiving 10,000 ppm 4,4′-thiobis(6-*t*-butyl-*m*-cresol) in the 15-day feed study. Note the coagulation necrosis of the renal papilla (N). H&E, 10×

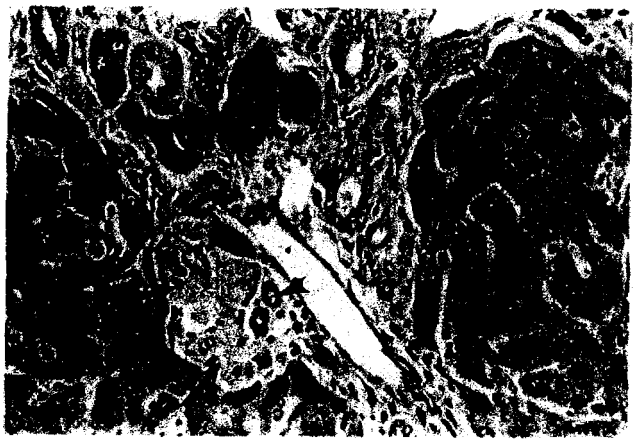

PLATE 3
Liver of a male F344/N rat receiving 5,000 ppm 4,4′-thiobis(6-*t*-butyl-*m*-cresol) in the 13-week feed study. Note the accumulation of enlarged Kupffer cells in the hepatic sinusoids and portal area (arrows) and proliferation of small bile ductules (arrow heads). H&E, 80×

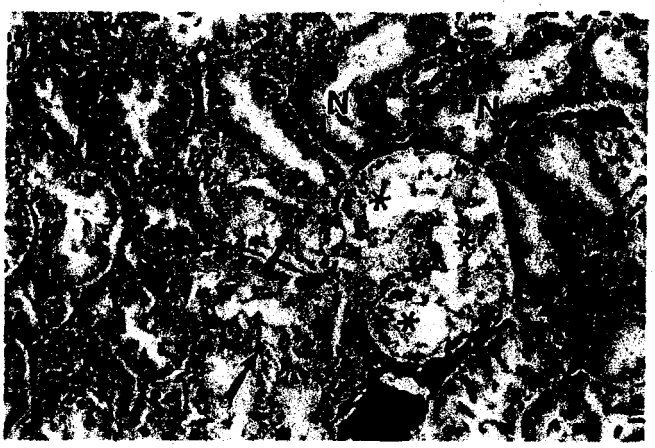

PLATE 4
Kidney of a male F344/N rat receiving 5,000 ppm 4,4′-thiobis(6-*t*-butyl-*m*-cresol) in the 13-week feed study. The segment of proximal convoluted tubule in the center of the field exhibits complete necrosis of the epithelium (*). Adjacent tubules exhibit necrosis of individual cells which have pyknotic nuclei (arrows). Compare with normal tubule epithelium (N). H&E, 80×

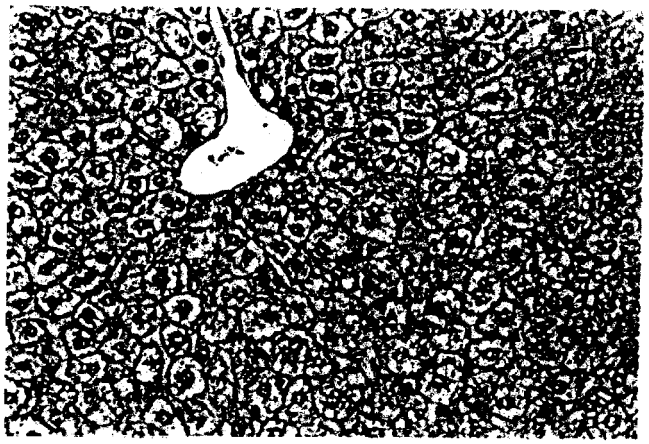

PLATE 5
Liver of a control male B6C3F$_1$ mouse in the 13-week feed study of 4,4′-thiobis(6-*t*-butyl-*m*-cresol). Compare with Plate 6. H&E, 80×

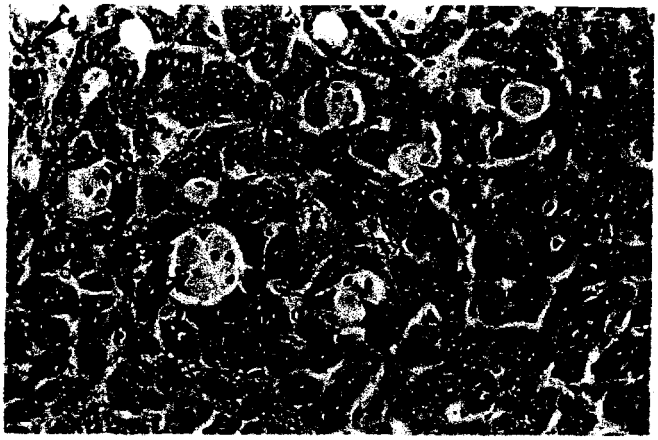

PLATE 6
Liver of a male B6C3F$_1$ mouse receiving 2,500 ppm 4,4′-thiobis(6-*t*-butyl-*m*-cresol) in the 13-week feed study. Note the scattered individual and small clusters of enlarged Kupffer cells (arrows) and the proliferation of small bile ductules (arrow heads). The hepatocyte nuclei are larger than normal and the cytoplasm contains an increased amount of basophilic material (rough endoplasmic reticulum). H&E, 80×

DISCUSSION AND CONCLUSIONS

4,4'-Thiobis(6-*t*-butyl-*m*-cresol) (TBBC) is used in the rubber and plastics industries as an antioxidant and as a stabilizer in polyethylene and polyolefin food packaging materials. Because of concern regarding the elevated cancer risk of workers in the rubber industry, the National Cancer Institute nominated TBBC for toxicology and carcinogenesis studies as a representative of the sulfur-containing class of antioxidants used in rubber processing. Because food packaging appeared to represent the most widespread potential for human exposure, the oral route of administration was chosen for the 15-day, 13-week, and 2-year studies in F344/N rats and B6C3F$_1$ mice.

The principal toxic effects associated with the administration of TBBC in the present studies occurred in the liver and kidney of rats and mice. With the exception of the renal lesions observed in the 15-day and 13-week studies, these findings are in agreement with the few studies reported in the literature. Birnbaum *et al.* (1983) reported that the liver was the major site of metabolism of TBBC in rats and that the compound was excreted primarily in the bile. In a 30-day feed study in rats, 2,500 ppm TBBC produced increased liver weight and growth retardation; rats fed diets containing 500 ppm for 90 days displayed only reduced feed consumption and slight growth retardation (Lefaux, 1968). A dose-related increase in liver weight accompanied by a slight increase in the number of Kupffer cells was reported in females exposed to 200 mg/kg in a study in which mice were administered 10, 100, or 200 mg/kg daily by gavage for 14 days (Munson *et al.*, 1988). In the NTP 15-day studies in rats or mice receiving TBBC in feed at doses ranging from 1,000 to 25,000 ppm, liver toxicity was not observed in surviving animals. However, in the NTP 13-week studies in rats, absolute and relative liver weights were significantly greater in females receiving 5,000 ppm than in controls. Males and females in the 2,500 and 5,000 ppm groups exhibited Kupffer cell hypertrophy, hepatocyte necrosis, and bile duct hyperplasia. In addition, males and females exposed to 5,000 ppm TBBC also exhibited centrilobular hepatocyte hypertrophy. Consistent with these histopathologic findings in the 13-week rat studies, there were significant elevations in serum levels of alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Increased levels of ALT are usually associated with damage to hepatocytes; increases in ALP are usually associated with biliary disease. Male and female rats receiving 5,000 ppm in these studies exhibited a significant increase in size and number of macrophages in the mesenteric lymph nodes; a lesser, but similar response occurred in 2,500 ppm rats.

The 13-week NTP study in mice also elicited hepatotoxicity in 2,500 ppm males and females as exhibited by slight but significant increases in absolute and relative liver weights and the presence of Kupffer cell hypertrophy and bile duct hyperplasia. The response in rats at the same exposure level (2,500 ppm) was similar, except that liver weights in 2,500 ppm rats were unaffected and necrosis and centrilobular hypertrophy were observed in rats but not in mice. Based on average daily feed consumption, 2,500 ppm rats ingested roughly one-third as much TBBC on a body weight basis as mice. Thus, the liver of rats may be more sensitive than that of mice to the effects of this chemical. Additionally, there was a mild increase in size and number of macrophages in mesenteric lymph nodes of male and female mice administered 2,500 ppm; this response was similar to that observed in 2,500 ppm rats.

In the 2-year rat study, the highest exposure level (2,500 ppm TBBC) produced liver toxicity. At this exposure level, males and females exhibited increases in liver weights, Kupffer cell hypertrophy, cytoplasmic vacuolization, and basophilic and mixed cell foci at the 15-month interim evaluation and at the end of the 2-year study. In addition, marked significant increases in serum ALT and sorbitol dehydrogenase activities (SDH) occurred in males and females at the 15-month evaluation; these cytoplasmic enzymes are released into the blood following hepatocellular injury. The mild but significant increases in ALP which occurred in males in various exposure groups at the 3-, 9-, and 15-month evaluations are indicative of disturbances involving the hepatobiliary system. This increase did not occur in females. Although certain liver responses occurred in males and females, liver weight increase was more pronounced in females, there was a strong significant increase in the incidence of cytoplasmic vacuolization

in females but not in males, and mixed cell foci occurred in twice as many 2,500 ppm females as 2,500 ppm males. Thus, the preponderance of these responses occurred in females.

The incidence of hepatocellular adenoma or carcinoma (combined) was slightly increased in male rats administered 2,500 ppm TBBC (0 ppm, 1/50; 500 ppm, 3/50; 1,000 ppm, 3/50; 2,500 ppm, 5/49), but the increased incidence was not significant and did not exceed the range of 0% to 10% in historical control male rats. Furthermore, the incidences of these neoplasms were not increased in females, despite the fact that females demonstrated a greater number of different nonneoplastic responses. Therefore, the incidence of hepatocellular adenoma or carcinoma (combined) in male rats is not considered a carcinogenic response to TBBC.

In contrast to the findings in the 13-week study at 2,500 ppm, liver weights of mice were unaffected and there were no microscopic findings of hepatotoxicity in mice exposed to 1,000 ppm TBBC in feed for 2 years. Since 1,000 ppm male and female mice actually had a greater average daily ingestion of TBBC on a mg/kg body weight basis than did rats exposed to 2,500 ppm TBBC, the lack of microscopic findings in mice may indicate (as appeared to be the case in the 13-week studies) a higher degree of liver sensitivity in rats. This conclusion is strengthened by the marked significant increase in ALT and SDH found in rats but not mice. Total bilirubin in 1,000 ppm male mice was slightly but significantly greater than that in controls at 9 and 15 months. This response did not occur in female mice or in rats. In addition, the serum activity of ALP was significantly higher in male and female mice at various exposure levels and time points; these increases were milder in degree but similar to those that occurred in the rats. Increases in serum levels of total bilirubin would be consistent with either cholestasis or a liver function disorder in which circulating bilirubin could not be removed by the liver for conjugation and excretion. Increases in both ALP activity and total bilirubin concentration would be consistent with cholestasis. However, increases in total bilirubin concentration related to cholestasis are usually accompanied by increases in direct bilirubin, which did not occur in the present studies. In males, liver lesions which occurred with a significant negative trend included fatty change, clear cell foci, and hepatocellular adenoma or carcinoma (combined). The significant negative trends were considered to be related to the administration of TBBC. In 1,000 ppm male mice, the incidence of hepatocellular adenoma or carcinoma (combined) was significantly lower than that of controls by pairwise comparison. This result may be due to the reduction in mean body weight, since a significant positive association has been found between liver neoplasm prevalence and body weight in male $B6C3F_1$ mice (Rao *et al.*, 1990).

Evidence of kidney toxicity was present in rats and mice in the NTP 15-day studies and in rats in the 13-week study. In 10,000 ppm rats in the 15-day study, necrosis of the papilla was observed in one female and two males and focal necrosis of the tubules was observed in four males and seven females. Eight male mice and three female mice receiving 5,000 ppm in the 15-day study had tubule necrosis. Following 13 weeks of exposure, pigmentation and degeneration of the renal cortical tubule epithelial cells were present in male and female rats receiving 2,500 or 5,000 ppm; mild to moderate cortical tubule necrosis was also found in 5,000 ppm males and females. These lesions appear to be related to the administration of TBBC. Kidney lesions were not reported in the feed studies summarized by Lefaux (1968) in which rats were exposed to 500 or 2,500 ppm for 30 days and 50 or 500 ppm for 90 days. In the present NTP 2-year rat study, chronic nephropathy common in aging rats was found in nearly all animals. However, the severity of nephropathy in 2,500 ppm females was significantly greater than that in the control group, and the increase was attributed to the administration of TBBC. In remaining female exposure groups and in all exposed males, the severity of nephropathy was similar to that of the controls.

In the 13-week NTP studies, TBBC again affected hematology parameters in rats and mice. Significant decreases in hemoglobin and hematocrit values occurred in male rats and male and female mice; mean erythrocyte volume values were significantly lower in rats and mice; erythrocyte counts were significantly lower in mice but not in rats; and neutrophil counts were significantly higher in rats but not in mice.

In the 2-year study, results of hematocrit and hemoglobin analyses performed on two sets of male rats evaluated at 15 months were conflicting. However,

the results in each set of females indicated significant decreases; male mice also had a significant decrease in these parameters and in erythrocyte counts.

The significant increases in platelets which occurred mainly in 2,500 ppm male and female rats in the 2-year study are consistent with a reactive thrombocytosis. This condition has been observed with inflammations, trauma, surgery, hyposplenic or asplenic states, malignancies, acute blood loss, and hyperadrenocorticism.

The neurotoxicity evaluation in the 13-week study demonstrated statistically significant increases in grip strength in exposed rats, which did not occur in the 2-year study. While these evaluations were performed on animals of the same strain and age using the same methodology, they were conducted at two different laboratories. Therefore, the toxicologic significance of the positive findings in the 13-week study is uncertain. Further, no significant effects of TBBC were found on motor nerve excitability or conduction, neuromuscular transmission, muscle contractility, or neuropathology.

Although the rate of survival was less than 50% in 1,000 ppm male rats (42%) and 2,500 ppm male rats (36%), the survival rate for the control group was only 36% and reduced survival does not appear to be chemical related. Further, 50% of the 2,500 ppm males survived until week 97 and 50% of the 1,000 ppm male rats survived until week 101, allowing adequate time for the possible development of neoplasms. Some degree of chemical-related toxicity in 2,500 ppm rats was observed; mean body weights of rats in this group were slightly but consistently reduced, despite the fact that feed consumption by this group was similar to that by the controls. The final mean body weight of 2,500 ppm males was 5% less than that of the controls; the mean body weight of females exposed to 2,500 ppm TBBC dropped to 14% below that of the controls at week 65 and was 6% lower than that of the controls at the end of the study. There was also enough evidence of liver toxicity in the 2,500 ppm male and female rats in the 2-year study to indicate that a greater exposure level would have compromised the sensitivity of the study to detect neoplasia. In addition, exposure to 5,000 ppm TBBC in the 13-week study resulted in a significant increase in absolute and relative liver weight in females, marked reductions in final mean body weights and feed consumption in both males and females, and liver and kidney toxicity in males and females, as mentioned earlier. These observations indicate that rats could not have tolerated an exposure level much higher than 2,500 ppm.

Although no overt organ toxicity was observed in mice in the highest exposure group in the 2-year study (1,000 ppm), the reductions in final mean body weights were indicative of a toxic response to TBBC. The final mean body weights of 1,000 ppm male and female mice were 8% and 18% lower than that of the controls, respectively; feed consumption by the 1,000 ppm males was similar to that by the controls. In the 13-week study, 2,500 ppm males had a final mean body weight 15% lower than that of the controls and the final mean body weight of 2,500 ppm females was 22% lower than that of the controls. This exposure level also produced Kupffer cell hypertrophy and bile duct hyperplasia in males and females. At 15 months, males had a significant increase in total bilirubin at all exposure levels and 500 and 1,000 ppm females had a significant elevation in ALP. It is probable that an exposure level greater than 1,000 ppm for 2 years would have caused severe weight loss and liver toxicity in mice.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of 4,4′-thiobis(6-*t*-butyl-*m*-cresol) in male or female F344/N rats administered 500, 1,000, or 2,500 ppm or in male or female B6C3F$_1$ mice administered 250, 500, or 1,000 ppm.

Nonneoplastic lesions associated with exposure to TBBC included: Kupffer cell hypertrophy, cytoplasmic vacuolization, and mixed cell foci in the liver of male and female rats, fatty change in the liver of female rats, and an increase in the severity of nephropathy in the kidney of female rats. In addition, decreased incidences of fibroadenoma, adenoma, or carcinoma (combined) were observed in the mammary gland of female rats. Decreases also occurred in the incidences of fatty change, clear cell foci, and adenoma or carcinoma (combined) in the liver of male mice.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

REFERENCES

Armitage, P. (1971). *Statistical Methods in Medical Research*, pp. 362-365. John Wiley and Sons, New York.

Ashby, J., and Tennant, R.W. (1991). Definitive relationships among chemical structure, carcinogenicity, and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* **257**, 229-306.

Birnbaum, L.S., Eastin, W.C., Jr., Johnson, L., and Matthews, H.B. (1983). Disposition of 4,4′-thiobis(6-*t*-butyl-*m*-cresol) in rats. *Drug Metab. Dispos.* **11**, 537-543.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Borghoff, S.J., Stefanski, S.A., and Birnbaum, L.S. (1988). The effect of age on the glucuronidation and toxicity of 4,4′-thiobis(6-*t*-butyl-*m*-cresol). *Toxicol. Appl. Pharmacol.* **92**, 453-466.

Code of Federal Regulations (CFR) **21**, Part 58.

Cox, D.R. (1972). Regression models and life-tables. *J. R. Stat. Soc.* **B34**, 187-220.

Crawford, B.D. (1985). Perspectives on the somatic mutation model of carcinogenesis. In *Advances in Modern Environmental Toxicology: Mechanisms and Toxicity of Chemical Carcinogens and Mutagens* (M.A. Mehlman, W.G. Flamm and R.J. Lorentzen, Eds.), pp. 13-59, Princeton Scientific Publishing Co., Inc., Princeton, NJ.

Dinse, G.E., and Haseman, J.K. (1986). Logistic regression analysis of incidental-tumor data from animal carcinogenicity experiments. *Fundam. Appl. Toxicol.* **6**, 44-52.

Dinse, G.E., and Lagakos, S.W. (1983). Regression analysis of tumour prevalence data. *Appl. Statist.* **32**, 236-248.

Draganov, I., Radeva, M., and Yanev, E. (1974). Effect of the antioxidant Santonox on the growth of the Yoshida sarcoma [in Russian, English summary]. *Med.-Biol. Probl.* **2**, 269-272.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1095-1121.

Edwards, P.M., and Parker, V.H. (1977). A simple, sensitive and objective method for early assessment of acrylamide neuropathy in rats. *Toxicol. Appl. Pharmacol.* **40**, 589-591.

Environmental Health Research & Testing, Inc. (EHRT) (1989). Screening of priority chemicals for reproductive hazards. Project No. 6 ETOX-85-1002. EHRT, Cincinnati, OH.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Mol. Mutagen.* **10** (Suppl. 10), 1-175.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *J. Natl. Cancer Inst.* **62**, 957-974.

Haseman, J.K. (1984). Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. *Environ. Health Perspect.* **58**, 385-392.

Haseman, J.K., Huff, J., and Boorman, G.A. (1984). Use of historical control data in carcinogenicity studies in rodents. *Toxicol. Pathol.* **12**, 126-135.

Haseman, J.K., Huff, J.E., Rao, G.N., Arnold, J.E., Boorman, G.A., and McConnell, E.E. (1985). Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N × C3H/HeN)F_1 (B6C3F_1) mice. *JNCI* **75**, 975-984.

Hejtmankova, N., Simanek, V., Holcik, J., Hejtmanek, M., and Santavy, F. (1979). Part II. Antifungal and mutagenic activity of phenolic substances with different alkyl groups: A study of the relationship between the biological activity and the constitution of the investigated compounds. *Acta Univ. Palacki. Olomuc. Fac. Med.* **90**, 75-87.

Hollander, M., and Wolfe, D.A. (1973). *Nonparametric Statistical Methods*, pp. 120-123. John Wiley and Sons, New York.

Holsapple, M.P., White, K.L., Jr., McCay, J.S., Bradley, S.G., and Munson, A.E. (1988). An immunotoxicological evaluation of 4,4′-thiobis-(6-*t*-butyl-*m*-cresol) in female B6C3F_1 mice: 2. Humoral and cell-mediated immunity, macrophage function, and host resistance. *Fundam. Appl. Toxicol.* **10**, 701-716.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation of incomplete observations. *J. Am. Stat. Assoc.* **53**, 457-481.

Lefaux, R. (1968). Monomers and additives. In *Practical Toxicology of Plastics* (P.P. Hopf, Ed.), pp. 399-400. CRC Press, Cleveland, OH.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.

McCormick, W.E. (1972). Environmental health control for the rubber industry, Part II: Antioxidants and antiozonants. *J. of Rubber Chemistry and Technology* **45**, 627-637.

McKnight, B., and Crowley, J. (1984). Tests for differences in tumor incidence based on animal carcinogenesis experiments. *J. Am. Stat. Assoc.* **79**, 639-648.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

Meyer, O.A., Tilson, H.A., Byrd, W.C., and Riley, M.T. (1979). A method for the routine assessment of fore- and hindlimb grip strength of rats and mice. *Neurobehav. Toxicol.* **1**, 233-236.

Miller, J.A., and Miller, E.C. (1977). Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer* (H.H. Hiatt, J.D. Watson, and J.A. Winsten, Eds.), pp. 605-628. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Monson, R.R., and Fine, L.J. (1978). Cancer mortality and morbidity among rubber workers. *J. Natl. Cancer Inst.* **61**, 1047-1053.

Munson, A.E., White, K.L., Jr., Barnes, D.W., Musgrove, D.L., Lysy, H.H., and Holsapple, M.P. (1988). An immunotoxicological evaluation of 4,4′-thiobis(6-*t*-butyl-*m*-cresol) in female B6C3F_1 mice: 1. Body and organ weight, hematology, serum chemistries, bone marrow cellularity, and hepatic microsomal parameters. *Fundam. Appl. Toxicol.* **10**, 691-700.

National Cancer Institute (NCI) (1976). Guidelines for Carcinogen Bioassay in Small Rodents. Technical Report Series No. 1. NIH Publication No. 76-801. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.