Summary of Feeding Carcinogenicity Study of 1-Chloro-2,4-Dinitrobenzene in BDF_1 Mice

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Japan Bioassay Laboratory

Japan Industrial Safety and Health Association

PREFACE

The tests were contracted and supported by the Ministry of Labour of Japan. The tests were conducted by Japan Bioassay Laboratory (JBL) and the report was prepared by JBL and peer reviewed by outside expert pathologist. Complete report was submitted to Ministry of Labour of Japan on August 19, 1992.

This English Summary was translated by JBL from Japanese complete report.

Summary of Feed Carcinogenicity Study of 1-Chloro-2,4-Dinitrobenzene in BDF_1 Mice

Purpose, materials and methods

1-Chloro-2,4-dinitrobenzene (CDNB : CAS No. 97-00-7) is a yellow crystal with a melting point of 51°C. It is insoluble in water and soluble in ether, benzene, carbon disulfide, and hot ethanol.

The carcinogenicity and chronic toxicity of CDNB were examined in Crj:BDF_1 mice. Groups of test animals were administered CDNB in their diets for 2 years (104 weeks). Each group consisted of either 50 male or 50 female mice. The dietary concentrations of CDNB were 0, 320, 800 or 2000 ppm (w/w). Both sexes were administered each concentration of CDNB. The highest dose level was chosen so as not to exceed the maximum tolerated dose (MTD), based on both growth rate and toxicity in a previous 13-week toxicity study. The identity of the CDNB used in these experiments was confirmed by mass spectrometry. It was analyzed by infrared spectrometry, ultraviolet spectrometry and gas chromatography before and after its use to affirm its stability. The concentrations of CDNB in the diet were determined by gas chromatography at the time of preparation and on the 7th day after preparation while stored at room temperature. The animals were observed daily for clinical signs and mortality. Body weight and food consumption were measured once a week for the first 14 weeks and every 2 weeks thereafter. Animals found dead, or in a moribund state, or surviving to the end of the 2-year administration period underwent complete necropsy. Urinalysis was performed near the end of the administration period. Hematology and blood biochemistry analysis were performed at the terminal necropsy: surviving animals were fasted overnight and bled under deep ether anesthesia. Organs and tissues were removed, weighed and examined for macroscopic lesions at necropsy. The organs and tissues were then fixed and embedded in paraffin. Five μm thick tissue sections were prepared and stained with hematoxylin and eosin and examined microscopically. Incidences of neoplastic lesions were statistically analyzed by Fisher's exact test. Positive dose-response trends of CDNB induction of neoplastic lesions were analyzed by Peto's test. Incidences of non-neoplastic lesions and urinalysis were analyzed by the Chi-square test. Changes in body weight, food consumption, hematological and blood biochemical parameters, and organ weights were analyzed by Dunnett's test. The present study was conducted with reference to the Organisation for Economic Co-operation and Development

(OECD) Good Laboratory Practice and the OECD Guideline for Testing of Chemicals 451 “Carcinogenicity Studies”.

Results

No significant differences in survival rates were found between any of the groups administered CDNB and their respective controls. Yellow urine and yellow coloration of the fur were observed in all the CDNB-fed groups of both sexes after the 75th week of administration. Body weights were decreased in first half of the administration period in males fed 2000 ppm CDNB and throughout most of the administration period in females fed 2000 ppm CDNB. Food consumption by the 2000 ppm-fed males and females decreased in approximate accordance with the decrease in body weight. Hemoglobin concentration, hematocrit value and mean corpuscular hemoglobin were decreased in females fed 2000 ppm CDNB. Plasma levels of glutamic oxaloacetic transaminase (GOT), lactate dehydrogenase and creatine phosphokinase were increased in males fed 2000 ppm and GOT and chlorine were increased in females fed 2000 ppm CDNB. There were no CDNB related changes in urinalysis parameters or organ weights in any of the CDNB administered groups.

No significant increases in the incidence of neoplastic or tumor-related lesions were found in any of the CDNB-administered groups of either sex. Induction of several non-neoplastic lesions in the CDNB-administered groups was noted: The incidences of hemosiderin deposition and extramedullary hematopoiesis in the spleen were significantly increased in 2000 ppm CDNB fed males and hemosiderin deposition in the spleen was significantly increased in 2000 ppm CDNB fed females. Deposition of pigment in the thyroid was significantly increased in both males and females fed 800 and 2000 ppm CDNB. Hyperplasia in the forestomach was significantly increased in males fed 2000 ppm CDNB and hyperplasia in the glandular stomach was significantly decreased in females fed 2000 ppm CDNB.

Conclusions

There was no evidence for carcinogenicity of 1-chloro-2,4-dinitrobenzene in male or female mice.

Incidences of selected neoplastic lesions of male mice in the 2-year feed carcinogenicity study of 1-chloro-2,4-dinitrobenzene

Dose (ppm)		0	320	800	2000	Peto test	Cochran-Armitage test
Number of examined animals		50	50	50	50		
benign tumor							
lung	bronchiolar-alveolar adenoma	3	3	5	4		
liver	hepatocellular adenoma	4	8	5	7		
Harderian gland	adenoma	3	0	0	3		
malignant tumor							
lung	bronchiolar-alveolar carcinoma	6	6	6	3		
lymph node	malignant lymphoma	7	8	10	7		
spleen	malignant lymphoma	2	3	0	3		
	hemangioendothelioma	3	1	3	3		
liver	hepatocellular carcinoma	7	1 *	9	3		
	histiocytic sarcoma	3	0	1	2		
	hemangioendothelioma	4	0	2	2		

Incidences of selected neoplastic lesions of female mice in the 2-year feed carcinogenicity study of 1-chloro-2,4-dinitrobenzene

Dose (ppm)		0	320	800	2000	Peto test	Cochran-Armitage test
Number of examined animals		50	50	50	50		
benign tumor							
lung	bronchiolar-alveolar adenoma	0	3	0	2		
liver	hepatocellular adenoma	1	3	1	1		
pituitary	adenoma	1	3	4	3		
malignant tumor							
lung	bronchiolar-alveolar carcinoma	3	2	2	0		
lymph node	malignant lymphoma	14	13	13	16		
uterus	histiocytic sarcoma	15	10	8	8		

Significant difference

* : p≦0.05		** : p≦0.01		(Fisher test)
↑ : p≦0.05	increase	↑ ↑ : p≦0.01	increase	(Peto, Cochran-Armitage test)
↓ : p≦0.05	decrease	↓ ↓ : p≦0.01	decrease	(Cochran-Armitage test)

SELECTED TABLES

TABLE 27 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN MALE MOUSE

(TWO-YEAR STUDIES)

Week-Day on Study	Control Av.Wt.	Control No.of Surviv. <50>	320 ppm Av.Wt.	320 ppm % of cont. <50>	320 ppm No.of Surviv.	800 ppm Av.Wt.	800 ppm % of cont. <50>	800 ppm No.of Surviv.	2000 ppm Av.Wt.	2000 ppm % of cont. <50>	2000 ppm No.of Surviv.
0-0	22.6 (50)	50/50	22.6 (50)	100	50/50	22.6 (50)	100	50/50	22.6 (50)	100	50/50
1-7	24.4 (50)	50/50	24.2 (50)	99	50/50	24.2 (50)	99	50/50	23.5 (50)	96	50/50
2-7	24.8 (50)	50/50	24.7 (50)	100	50/50	25.0 (50)	101	50/50	23.4 (50)	94	50/50
3-7	24.5 (50)	50/50	24.3 (50)	99	50/50	24.3 (50)	99	50/50	23.9 (50)	98	50/50
4-7	25.7 (50)	50/50	25.9 (50)	101	50/50	25.9 (50)	101	50/50	25.4 (50)	99	50/50
5-7	25.6 (50)	50/50	25.8 (50)	101	50/50	26.3 (50)	103	50/50	24.9 (50)	97	50/50
6-7	27.1 (50)	50/50	27.0 (50)	100	50/50	27.3 (50)	101	50/50	26.0 (50)	96	50/50
7-7	28.6 (50)	50/50	28.8 (50)	101	50/50	28.1 (50)	98	50/50	26.7 (50)	93	50/50
8-7	30.2 (50)	50/50	30.3 (50)	100	50/50	30.2 (50)	100	50/50	27.9 (50)	92	50/50
9-7	30.9 (50)	50/50	30.4 (50)	98	50/50	30.4 (50)	98	50/50	28.3 (50)	92	50/50
10-7	32.3 (50)	50/50	31.6 (50)	98	50/50	30.6 (50)	95	50/50	29.0 (50)	90	50/50
11-7	33.0 (50)	50/50	32.2 (50)	98	50/50	31.9 (50)	97	50/50	30.4 (50)	92	50/50
12-7	33.4 (50)	50/50	33.2 (50)	99	50/50	33.3 (50)	100	50/50	31.1 (50)	93	50/50
13-7	35.2 (50)	50/50	33.9 (50)	96	50/50	34.7 (50)	99	50/50	32.0 (50)	91	50/50
14-7	33.8 (50)	50/50	32.7 (50)	97	50/50	32.9 (50)	97	50/50	30.6 (50)	91	50/50
16-7	35.4 (50)	50/50	34.7 (50)	98	50/50	34.6 (49)	98	49/50	32.3 (50)	91	50/50
18-7	36.8 (50)	50/50	35.8 (50)	97	50/50	36.6 (49)	99	49/50	33.8 (50)	92	50/50
20-7	37.5 (50)	50/50	36.2 (50)	97	50/50	37.2 (49)	99	49/50	34.1 (50)	91	50/50
22-7	38.7 (50)	50/50	37.1 (50)	96	50/50	37.0 (49)	96	49/50	34.9 (50)	90	50/50
24-7	39.5 (50)	50/50	38.3 (50)	97	50/50	38.7 (49)	98	49/50	35.4 (50)	90	50/50
26-7	39.8 (50)	50/50	39.1 (49)	98	49/50	38.8 (49)	97	49/50	36.2 (50)	91	50/50
28-7	41.0 (50)	50/50	40.0 (49)	98	49/50	40.1 (49)	98	49/50	37.1 (50)	90	50/50
30-7	43.0 (50)	50/50	41.0 (49)	95	49/50	41.0 (49)	95	49/50	38.2 (50)	89	50/50
32-7	43.3 (50)	50/50	41.9 (49)	97	49/50	42.1 (49)	97	49/50	38.3 (50)	88	50/50
34-7	42.2 (50)	50/50	41.9 (49)	99	49/50	42.0 (49)	100	49/50	38.6 (50)	91	50/50
36-7	44.2 (50)	50/50	43.2 (49)	98	49/50	43.6 (49)	99	49/50	39.9 (50)	90	50/50
38-7	44.1 (50)	50/50	43.4 (49)	98	49/50	44.2 (49)	100	49/50	40.4 (50)	92	50/50
40-7	44.7 (50)	50/50	44.5 (49)	100	49/50	44.7 (49)	100	49/50	40.7 (50)	91	50/50
42-7	44.3 (50)	50/50	43.4 (49)	98	49/50	44.2 (49)	100	49/50	39.6 (50)	89	50/50
44-7	44.5 (50)	50/50	43.8 (49)	98	49/50	43.8 (49)	98	49/50	40.4 (50)	91	50/50
46-7	45.0 (50)	50/50	44.1 (49)	98	49/50	44.4 (49)	99	49/50	40.8 (50)	91	50/50
48-7	44.2 (50)	50/50	43.0 (49)	97	49/50	43.6 (49)	99	49/50	40.7 (50)	92	50/50
50-7	43.8 (50)	50/50	42.6 (48)	97	48/50	44.1 (49)	101	49/50	40.1 (50)	92	50/50
52-7	43.7 (50)	50/50	43.3 (48)	99	48/50	44.7 (49)	102	49/50	40.8 (50)	93	50/50
54-7	43.7 (50)	50/50	43.0 (48)	98	48/50	43.3 (49)	99	49/50	39.8 (50)	91	50/50
56-7	44.1 (50)	50/50	44.5 (48)	101	48/50	44.6 (49)	101	49/50	40.7 (50)	92	50/50
58-7	43.8 (50)	50/50	44.6 (48)	102	48/50	44.3 (49)	101	48/50	40.9 (50)	93	50/50
60-7	44.5 (50)	50/50	44.9 (48)	101	48/50	45.6 (48)	102	48/50	42.1 (50)	95	50/50
62-7	45.1 (50)	50/50	45.2 (48)	100	48/50	46.0 (48)	102	48/50	42.9 (50)	95	50/50
64-7	45.2 (50)	50/50	44.7 (47)	99	47/50	45.4 (48)	100	48/50	42.9 (50)	95	50/50
66-7	44.2 (50)	50/50	43.4 (47)	98	47/50	44.2 (48)	100	48/50	42.6 (48)	96	48/50
68-7	44.2 (50)	50/50	44.5 (47)	101	47/50	44.9 (48)	102	48/50	41.7 (47)	94	47/50
70-7	44.3 (50)	50/50	44.9 (47)	101	47/50	45.5 (47)	103	48/50	42.1 (47)	95	47/50
72-7	43.7 (49)	49/50	43.6 (47)	100	47/50	45.6 (48)	104	48/50	42.1 (47)	96	47/50
74-7	43.7 (48)	48/50	43.8 (47)	100	47/50	45.4 (48)	104	48/50	41.8 (47)	96	47/50
76-7	42.4 (48)	48/50	43.0 (46)	101	46/50	44.0 (48)	104	48/50	39.9 (46)	94	46/50
78-7	43.1 (48)	48/50	42.8 (46)	99	46/50	43.8 (48)	102	48/50	40.9 (46)	95	45/50
80-7	41.7 (48)	47/50	42.3 (46)	101	46/50	44.4 (48)	106	48/50	40.8 (45)	98	45/50
82-7	42.9 (46)	46/50	42.2 (46)	98	46/50	43.6 (48)	102	48/50	41.2 (44)	96	44/50
84-7	42.7 (45)	45/50	42.0 (46)	98	46/50	43.8 (47)	103	47/50	40.3 (44)	94	44/50
86-7	42.9 (44)	44/50	42.4 (46)	99	46/50	43.4 (47)	101	47/50	40.4 (44)	94	44/50
88-7	42.4 (43)	43/50	42.2 (46)	100	46/50	43.5 (47)	103	47/50	40.5 (43)	96	43/50
90-7	42.1 (43)	43/50	42.8 (45)	102	45/50	44.2 (46)	105	46/50	41.4 (43)	98	43/50
92-7	42.5 (42)	42/50	43.1 (45)	101	45/50	43.8 (46)	103	46/50	40.8 (42)	96	42/50
94-7	42.3 (41)	41/50	43.0 (45)	102	45/50	42.4 (45)	100	46/50	40.6 (41)	96	41/50
96-7	41.9 (40)	40/50	42.5 (45)	101	45/50	42.7 (43)	102	43/50	40.2 (41)	96	41/50
98-7	42.4 (40)	40/50	43.2 (45)	102	45/50	42.6 (42)	100	42/50	41.0 (41)	97	41/50
100-7	41.0 (40)	40/50	41.9 (44)	102	44/50	41.0 (42)	100	42/50	39.5 (41)	96	41/50
102-7	40.6 (39)	39/50	40.3 (44)	99	44/50	41.5 (41)	102	41/50	40.2 (40)	99	40/50
104-7	41.8 (36)	36/50	42.3 (43)	101	43/50	41.1 (40)	98	40/50	40.6 (40)	97	40/50

< >:No.of effective animals, ( ):No.of measured animals Av.Wt.: g

TABLE 28 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN FEMALE MOUSE

(TWO-YEAR STUDIES)

Week-Day on Study	Control Av.Wt.	Control No.of Surviv. <50>	320 ppm Av.Wt.	320 ppm % of cont. <50>	320 ppm No.of Surviv.	800 ppm Av.Wt.	800 ppm % of cont. <50>	800 ppm No.of Surviv.	2000 ppm Av.Wt.	2000 ppm % of cont. <50>	2000 ppm No.of Surviv.
0-0	18.3 (50)	50/50	18.3 (50)	100	50/50	18.3 (50)	100	50/50	18.3 (50)	100	50/50
1-7	18.7 (50)	50/50	18.7 (50)	100	50/50	18.3 (50)	98	50/50	18.1 (50)	97	50/50
2-7	19.2 (50)	50/50	19.2 (50)	100	50/50	19.3 (50)	101	50/50	18.9 (50)	98	50/50
3-7	19.1 (50)	50/50	19.6 (50)	103	50/50	19.5 (50)	102	50/50	19.2 (50)	101	50/50
4-7	20.0 (50)	50/50	20.5 (50)	103	50/50	20.2 (50)	101	50/50	19.6 (50)	98	50/50
5-7	20.9 (50)	50/50	21.1 (50)	101	50/50	20.5 (50)	98	50/50	19.6 (50)	94	50/50
6-7	21.7 (50)	50/50	21.7 (50)	100	50/50	21.3 (50)	98	50/50	20.4 (49)	94	50/50
7-7	21.8 (50)	50/50	22.0 (50)	101	50/50	21.3 (50)	98	50/50	20.4 (50)	94	50/50
8-7	22.5 (50)	50/50	22.8 (50)	101	50/50	21.9 (50)	97	50/50	20.8 (50)	92	50/50
9-7	23.1 (50)	50/50	23.5 (50)	102	50/50	22.8 (50)	99	50/50	21.8 (50)	94	50/50
10-7	23.4 (50)	50/50	24.1 (50)	103	50/50	22.9 (50)	98	50/50	22.2 (50)	95	50/50
11-7	23.8 (50)	50/50	24.4 (50)	103	50/50	23.4 (50)	98	50/50	22.0 (50)	92	50/50
12-7	23.4 (50)	50/50	24.2 (50)	103	50/50	23.4 (50)	100	50/50	22.2 (50)	95	50/50
13-7	24.4 (50)	50/50	25.1 (50)	103	50/50	24.2 (50)	99	50/50	22.8 (50)	93	50/50
14-7	24.5 (50)	50/50	25.3 (50)	103	50/50	23.8 (50)	97	50/50	22.2 (50)	91	50/50
16-7	25.1 (50)	50/50	25.3 (50)	101	50/50	24.3 (50)	97	50/50	22.5 (50)	90	50/50
18-7	26.0 (50)	50/50	26.6 (50)	102	50/50	25.4 (50)	98	50/50	23.7 (50)	91	50/50
20-7	26.2 (50)	50/50	26.8 (50)	102	50/50	26.2 (50)	100	50/50	23.8 (50)	91	50/50
22-7	26.7 (50)	50/50	27.2 (50)	102	50/50	26.0 (50)	97	50/50	24.4 (50)	91	50/50
24-7	27.6 (50)	50/50	28.0 (50)	101	50/50	27.5 (50)	100	50/50	24.7 (50)	89	50/50
26-7	27.9 (50)	50/50	28.6 (49)	103	49/50	27.4 (50)	98	50/50	25.1 (50)	90	50/50
28-7	28.4 (50)	50/50	28.8 (49)	101	49/50	27.8 (50)	98	50/50	25.2 (50)	89	50/50
30-7	29.1 (49)	49/50	29.6 (49)	102	49/50	27.7 (49)	95	49/50	25.6 (49)	88	49/50
32-7	29.3 (49)	49/50	30.1 (49)	103	49/50	29.2 (49)	100	49/50	26.3 (49)	90	49/50
34-7	29.3 (49)	49/50	30.4 (49)	104	49/50	29.5 (49)	101	49/50	26.1 (49)	89	49/50
36-7	30.3 (49)	49/50	31.5 (49)	104	49/50	30.6 (49)	101	49/50	26.8 (49)	88	49/50
38-7	30.7 (49)	49/50	31.7 (49)	103	49/50	31.1 (49)	101	49/50	26.8 (49)	87	49/50
40-7	30.5 (49)	49/50	31.8 (49)	104	49/50	30.8 (49)	101	49/50	27.2 (49)	89	49/50
42-7	30.8 (49)	49/50	31.5 (49)	102	49/50	29.7 (49)	96	49/50	27.2 (49)	88	49/50
44-7	30.9 (49)	49/50	32.2 (49)	104	49/50	31.0 (49)	100	49/50	27.5 (49)	89	49/50
46-7	31.5 (49)	49/50	32.6 (49)	103	49/50	31.6 (49)	100	49/50	28.3 (49)	90	49/50
48-7	30.8 (49)	49/50	31.9 (49)	104	49/50	30.6 (49)	99	49/50	27.8 (49)	90	49/50
50-7	30.5 (49)	49/50	32.0 (49)	105	49/50	30.8 (49)	101	49/50	27.9 (49)	91	49/50
52-7	31.2 (49)	49/50	32.6 (49)	104	49/50	31.3 (49)	100	49/50	28.6 (49)	92	49/50
54-7	31.3 (49)	49/50	32.6 (49)	104	49/50	31.2 (49)	100	49/50	28.3 (49)	90	49/50
56-7	31.1 (49)	49/50	33.1 (49)	106	49/50	31.7 (49)	102	49/50	28.2 (49)	91	49/50
58-7	30.9 (49)	49/50	32.5 (49)	105	49/50	31.3 (49)	101	49/50	28.7 (49)	93	49/50
60-7	31.7 (49)	49/50	33.2 (49)	105	49/50	33.0 (49)	104	49/50	29.9 (49)	94	49/50
62-7	32.9 (49)	49/50	34.3 (49)	104	49/50	33.1 (49)	101	49/50	29.9 (49)	91	49/50
64-7	32.5 (48)	48/50	33.7 (49)	104	49/50	32.8 (49)	101	49/50	29.2 (49)	90	49/50
66-7	32.0 (48)	47/50	33.4 (49)	104	49/50	32.0 (49)	100	49/50	29.7 (49)	93	49/50
68-7	32.1 (47)	47/50	33.7 (49)	105	49/50	32.2 (49)	100	49/50	29.5 (49)	92	49/50
70-7	32.5 (47)	47/50	34.3 (49)	106	49/50	33.4 (49)	103	49/50	29.2 (49)	90	49/50
72-7	33.1 (47)	46/50	34.8 (47)	105	47/50	33.6 (49)	102	49/50	30.7 (49)	93	49/50
74-7	33.1 (46)	46/50	34.6 (46)	105	46/50	33.4 (49)	101	49/50	30.5 (49)	92	49/50
76-7	31.8 (46)	46/50	33.3 (46)	105	46/50	32.5 (49)	102	49/50	29.2 (49)	92	49/50
78-7	32.8 (46)	46/50	33.1 (46)	101	46/50	32.0 (48)	98	48/50	30.2 (49)	92	49/50
80-7	32.5 (44)	44/50	33.3 (46)	102	46/50	33.0 (48)	102	48/50	30.5 (49)	94	49/50
82-7	33.1 (44)	44/50	34.0 (46)	103	46/50	32.8 (47)	99	47/50	30.3 (49)	92	49/50
84-7	33.1 (44)	44/50	34.2 (46)	103	46/50	32.9 (47)	99	47/50	29.9 (49)	90	49/50
86-7	32.5 (43)	42/50	33.9 (46)	104	46/50	32.5 (46)	100	46/50	29.4 (49)	90	49/50
88-7	32.9 (38)	38/50	34.1 (45)	104	45/50	31.7 (46)	96	46/50	29.2 (49)	89	49/50
90-7	33.8 (36)	36/50	33.8 (43)	100	43/50	32.9 (44)	97	44/50	29.8 (47)	88	47/50
92-7	32.9 (35)	35/50	32.7 (43)	99	43/50	32.6 (44)	99	43/50	29.6 (46)	90	46/50
94-7	32.7 (35)	35/50	32.9 (43)	101	43/50	32.2 (42)	98	42/50	29.9 (46)	91	45/50
96-7	32.1 (33)	33/50	32.2 (41)	100	41/50	32.0 (42)	100	42/50	29.9 (43)	93	43/50
98-7	32.3 (33)	33/50	32.9 (40)	102	40/50	31.9 (41)	99	41/50	29.9 (42)	93	42/50
100-7	32.2 (32)	32/50	32.7 (35)	102	35/50	31.2 (40)	97	40/50	29.8 (38)	93	38/50
102-7	31.5 (32)	32/50	32.2 (33)	102	33/50	31.2 (37)	99	37/50	30.4 (37)	97	37/50
104-7	32.9 (31)	31/50	33.1 (30)	101	30/50	32.5 (35)	99	35/50	29.8 (36)	91	35/50

< >:No.of effective animals,( ):No.of measured animals Av.Wt.: g

TABLE 29 FOOD CONSUMPTION IN MALE MOUSE

(TWO-YEAR STUDIES)

Week-Day on Study	Control Av.FC.	Control No.of Surviv. <50>	320 ppm Av.FC.	320 ppm % of cont. <50>	320 ppm No.of Surviv.	800 ppm Av.FC.	800 ppm % of cont. <50>	800 ppm No.of Surviv.	2000 ppm Av.FC.	2000 ppm % of cont. <50>	2000 ppm No.of Surviv.
1-7	4.2 (50)	50/50	4.2 (50)	100	50/50	4.1 (50)	98	50/50	4.2 (50)	100	50/50
2-7	3.8 (50)	50/50	3.8 (50)	100	50/50	3.9 (50)	103	50/50	3.7 (50)	97	50/50
3-7	3.4 (50)	50/50	3.4 (50)	100	50/50	3.4 (50)	100	50/50	3.6 (50)	106	50/50
4-7	3.7 (50)	50/50	3.9 (50)	105	50/50	3.7 (50)	100	50/50	4.0 (50)	108	50/50
5-7	3.5 (50)	50/50	3.4 (50)	97	50/50	3.6 (50)	103	50/50	3.4 (50)	97	50/50
6-7	4.0 (50)	50/50	3.8 (50)	95	50/50	3.9 (50)	98	50/50	3.7 (50)	93	50/50
7-7	4.2 (50)	50/50	4.3 (50)	102	50/50	4.0 (50)	95	50/50	3.7 (50)	88	50/50
8-7	4.5 (50)	50/50	4.3 (48)	96	50/50	4.4 (50)	98	50/50	4.1 (50)	91	50/50
9-7	4.2 (50)	50/50	4.0 (50)	95	50/50	4.0 (50)	95	50/50	4.0 (50)	95	50/50
10-7	4.4 (50)	50/50	4.3 (50)	98	50/50	4.0 (50)	91	50/50	4.0 (50)	91	50/50
11-7	4.2 (50)	50/50	4.2 (50)	100	50/50	4.4 (50)	105	50/50	4.4 (50)	105	50/50
12-7	4.2 (50)	50/50	4.3 (50)	102	50/50	4.6 (50)	110	50/50	4.2 (49)	100	50/50
13-7	4.6 (50)	50/50	4.5 (50)	98	50/50	4.7 (50)	102	50/50	4.5 (50)	98	50/50
14-7	3.7 (50)	50/50	3.8 (50)	103	50/50	3.7 (50)	100	50/50	3.6 (50)	97	50/50
16-7	4.3 (50)	50/50	4.3 (50)	100	50/50	4.1 (49)	95	49/50	4.1 (50)	95	50/50
18-7	4.6 (50)	50/50	4.4 (50)	96	50/50	4.6 (49)	100	49/50	4.4 (50)	96	50/50
20-7	4.4 (50)	50/50	4.3 (50)	98	50/50	4.2 (49)	95	49/50	4.2 (50)	95	50/50
22-7	4.6 (50)	50/50	4.5 (50)	98	50/50	4.3 (49)	93	49/50	4.5 (50)	98	50/50
24-7	4.8 (50)	50/50	4.9 (50)	102	50/50	5.0 (49)	104	49/50	4.7 (50)	98	50/50
26-7	5.1 (50)	50/50	5.2 (49)	102	49/50	4.9 (49)	96	49/50	4.9 (50)	96	50/50
28-7	4.9 (50)	50/50	4.7 (49)	96	49/50	4.6 (49)	94	49/50	4.4 (50)	90	50/50
30-7	5.2 (50)	50/50	4.7 (49)	90	49/50	4.7 (49)	90	49/50	4.8 (50)	92	50/50
32-7	4.7 (50)	50/50	4.5 (49)	96	49/50	4.5 (49)	96	49/50	4.4 (50)	94	50/50
34-7	5.1 (50)	50/50	5.0 (49)	98	49/50	4.7 (49)	92	49/50	4.7 (50)	92	50/50
36-7	4.7 (50)	50/50	4.6 (49)	98	49/50	4.5 (49)	96	49/50	4.4 (50)	94	50/50
38-7	4.7 (50)	50/50	4.7 (49)	100	49/50	4.8 (49)	102	49/50	4.8 (50)	102	50/50
40-7	5.1 (50)	50/50	5.1 (49)	100	49/50	4.9 (49)	96	49/50	4.7 (50)	92	50/50
42-7	4.9 (50)	50/50	4.7 (49)	96	49/50	4.9 (49)	100	49/50	4.3 (50)	88	50/50
44-7	5.0 (50)	50/50	5.1 (49)	102	49/50	5.1 (49)	102	49/50	5.0 (50)	100	50/50
46-7	5.0 (50)	50/50	5.0 (49)	100	49/50	5.0 (49)	100	49/50	4.7 (50)	94	50/50
48-7	4.7 (50)	50/50	4.7 (49)	100	49/50	4.5 (49)	96	49/50	4.5 (50)	96	50/50
50-7	4.2 (50)	50/50	4.2 (48)	100	48/50	4.4 (49)	105	49/50	3.9 (50)	93	50/50
52-7	5.2 (50)	50/50	5.0 (48)	96	48/50	5.3 (49)	102	49/50	4.8 (50)	92	50/50
54-7	4.7 (50)	50/50	4.8 (48)	102	48/50	4.4 (49)	94	49/50	4.2 (50)	89	50/50
56-7	4.3 (50)	50/50	4.7 (48)	109	48/50	4.6 (49)	107	49/50	4.6 (50)	107	50/50
58-7	4.8 (50)	50/50	4.8 (48)	100	48/50	4.6 (49)	96	48/50	4.5 (50)	94	50/50
60-7	5.0 (50)	50/50	4.8 (48)	96	48/50	5.1 (48)	102	48/50	4.9 (50)	98	50/50
62-7	4.9 (50)	50/50	4.9 (48)	100	48/50	5.0 (48)	102	48/50	4.6 (50)	94	50/50
64-7	4.8 (50)	50/50	4.5 (48)	94	47/50	4.4 (48)	92	48/50	4.3 (50)	90	50/50
66-7	4.2 (50)	50/50	4.2 (47)	100	47/50	4.1 (48)	98	48/50	4.5 (48)	107	48/50
68-7	4.5 (50)	50/50	4.5 (47)	100	47/50	4.6 (48)	102	48/50	4.4 (47)	98	47/50
70-7	4.9 (50)	50/50	4.9 (47)	100	47/50	5.1 (48)	104	48/50	4.6 (47)	94	47/50
72-7	4.4 (49)	49/50	4.9 (47)	111	47/50	5.0 (48)	114	48/50	4.8 (47)	109	47/50
74-7	4.7 (48)	48/50	4.6 (47)	98	47/50	4.7 (48)	100	48/50	4.7 (46)	100	47/50
76-7	4.7 (48)	48/50	4.7 (46)	100	46/50	4.6 (48)	98	48/50	4.5 (46)	96	46/50
78-7	5.0 (48)	48/50	4.8 (46)	96	46/50	4.8 (48)	96	48/50	5.0 (46)	100	45/50
80-7	4.5 (48)	47/50	4.9 (46)	109	46/50	5.3 (48)	118	48/50	5.1 (45)	113	45/50
82-7	5.3 (47)	46/50	4.8 (46)	91	46/50	5.2 (48)	98	48/50	5.1 (44)	96	44/50
84-7	4.8 (45)	45/50	4.8 (46)	100	46/50	4.7 (47)	98	47/50	4.6 (44)	96	44/50
86-7	4.7 (45)	44/50	4.7 (46)	100	46/50	4.6 (47)	98	47/50	4.7 (44)	100	44/50
88-7	4.9 (44)	43/50	4.8 (46)	98	46/50	5.0 (47)	102	47/50	4.8 (43)	98	43/50
90-7	4.9 (43)	43/50	4.9 (45)	100	45/50	4.7 (47)	96	46/50	4.8 (43)	98	43/50
92-7	4.9 (43)	42/50	4.8 (45)	98	45/50	4.5 (46)	92	46/50	4.5 (42)	92	42/50
94-7	4.9 (42)	41/50	4.6 (45)	94	45/50	4.6 (46)	94	46/50	4.9 (41)	100	41/50
96-7	5.0 (40)	40/50	5.0 (45)	100	45/50	4.8 (43)	96	43/50	4.7 (41)	94	41/50
98-7	4.9 (40)	40/50	5.0 (45)	102	45/50	4.3 (43)	88	42/50	4.7 (41)	96	41/50
100-7	5.2 (40)	40/50	4.9 (45)	94	44/50	4.5 (42)	87	42/50	5.0 (41)	96	41/50
102-7	5.0 (40)	39/50	4.2 (44)	84	44/50	5.3 (42)	106	41/50	5.2 (41)	104	40/50
104-7	5.5 (36)	36/50	5.7 (43)	104	43/50	5.2 (40)	95	40/50	5.2 (40)	95	40/50

< >:No.of effective animals, ( ):No.of measured animals Av.FC.: g

TABLE 30 FOOD CONSUMPTION IN FEMALE MOUSE

(TWO-YEAR STUDIES)

Week-Day on Study	Control Av.FC.	Control No.of Surviv. <50>	320 ppm Av.FC.	320 ppm % of cont. <50>	320 ppm No.of Surviv.	800 ppm Av.FC.	800 ppm % of cont. <50>	800 ppm No.of Surviv.	2000 ppm Av.FC.	2000 ppm % of cont. <50>	2000 ppm No.of Surviv.
1-7	3.5 (50)	50/50	3.3 (50)	94	50/50	3.2 (50)	91	50/50	3.6 (50)	103	50/50
2-7	3.3 (50)	50/50	3.2 (50)	97	50/50	3.2 (50)	97	50/50	3.5 (50)	106	50/50
3-7	3.3 (50)	50/50	3.2 (50)	97	50/50	3.1 (50)	94	50/50	3.2 (50)	97	50/50
4-7	3.6 (50)	50/50	3.5 (50)	97	50/50	3.4 (50)	94	50/50	3.3 (50)	92	50/50
5-7	3.7 (50)	50/50	3.7 (50)	100	50/50	3.7 (50)	100	50/50	3.5 (50)	95	50/50
6-7	3.9 (50)	50/50	3.8 (50)	97	50/50	3.8 (50)	97	50/50	3.7 (50)	95	50/50
7-7	4.0 (50)	50/50	3.8 (50)	95	50/50	3.8 (50)	95	50/50	3.6 (50)	90	50/50
8-7	4.3 (50)	50/50	4.0 (50)	93	50/50	4.0 (50)	93	50/50	3.9 (50)	91	50/50
9-7	4.4 (50)	50/50	4.1 (50)	93	50/50	4.3 (50)	98	50/50	4.0 (50)	91	50/50
10-7	4.2 (50)	50/50	4.1 (50)	98	50/50	4.1 (50)	98	50/50	3.9 (50)	93	50/50
11-7	4.4 (50)	50/50	4.3 (50)	98	50/50	4.1 (50)	93	50/50	3.8 (50)	86	50/50
12-7	4.3 (50)	50/50	4.2 (50)	98	50/50	4.1 (50)	95	50/50	4.1 (50)	95	50/50
13-7	4.8 (50)	50/50	4.5 (50)	94	50/50	4.5 (50)	94	50/50	4.4 (50)	92	50/50
14-7	4.5 (50)	50/50	4.2 (50)	93	50/50	4.1 (50)	91	50/50	4.0 (50)	89	50/50
16-7	4.4 (50)	50/50	4.1 (50)	93	50/50	3.9 (50)	89	50/50	3.8 (50)	86	50/50
18-7	4.6 (50)	50/50	4.7 (50)	102	50/50	4.5 (50)	98	50/50	4.3 (50)	93	50/50
20-7	4.3 (50)	50/50	4.1 (50)	95	50/50	4.2 (50)	98	50/50	4.0 (50)	93	50/50
22-7	4.7 (50)	50/50	4.5 (50)	96	50/50	4.4 (50)	94	50/50	4.4 (50)	94	50/50
24-7	5.0 (50)	50/50	4.7 (50)	94	50/50	5.0 (50)	100	50/50	4.5 (50)	90	50/50
26-7	4.7 (50)	50/50	4.7 (49)	100	49/50	4.5 (50)	96	50/50	4.4 (50)	94	50/50
28-7	4.7 (50)	50/50	4.3 (49)	91	49/50	4.3 (50)	91	50/50	4.1 (50)	87	50/50
30-7	4.9 (49)	49/50	4.5 (49)	92	49/50	4.3 (49)	88	49/50	4.1 (49)	84	49/50
32-7	4.8 (49)	49/50	4.3 (49)	90	49/50	4.4 (49)	92	49/50	4.2 (49)	88	49/50
34-7	5.1 (49)	49/50	4.8 (49)	94	49/50	4.8 (49)	94	49/50	4.3 (49)	84	49/50
36-7	4.9 (49)	49/50	5.0 (49)	102	49/50	4.8 (49)	98	49/50	4.5 (49)	92	49/50
38-7	5.1 (49)	49/50	4.9 (49)	96	49/50	5.1 (49)	100	49/50	4.7 (49)	92	49/50
40-7	4.6 (49)	49/50	4.4 (49)	96	49/50	4.3 (49)	93	49/50	4.3 (49)	93	49/50
42-7	5.2 (49)	49/50	4.9 (49)	94	49/50	4.4 (49)	85	49/50	4.7 (49)	90	49/50
44-7	5.6 (49)	49/50	5.4 (49)	96	49/50	5.3 (49)	95	49/50	5.0 (49)	89	49/50
46-7	5.1 (49)	49/50	4.8 (49)	94	49/50	4.8 (49)	94	49/50	4.8 (49)	94	49/50
48-7	5.0 (49)	49/50	4.8 (49)	96	49/50	4.6 (49)	92	49/50	4.7 (49)	94	49/50
50-7	4.7 (49)	49/50	4.5 (49)	96	49/50	4.2 (49)	89	49/50	3.8 (49)	81	49/50
52-7	5.1 (49)	49/50	4.9 (49)	96	49/50	5.0 (49)	98	49/50	4.9 (49)	96	49/50
54-7	4.7 (49)	49/50	4.6 (49)	98	49/50	4.7 (49)	100	49/50	4.4 (49)	94	49/50
56-7	4.2 (49)	49/50	4.6 (49)	110	49/50	4.7 (49)	112	49/50	4.0 (49)	95	49/50
58-7	5.0 (49)	49/50	5.0 (49)	100	49/50	4.6 (49)	92	49/50	4.5 (49)	90	49/50
60-7	4.8 (48)	49/50	4.8 (49)	100	49/50	5.2 (49)	108	49/50	4.7 (49)	98	49/50
62-7	5.7 (47)	49/50	5.3 (49)	93	49/50	5.0 (49)	88	49/50	4.8 (49)	84	49/50
64-7	4.7 (49)	48/50	4.4 (49)	94	49/50	4.3 (49)	91	49/50	4.1 (49)	87	49/50
66-7	4.5 (48)	47/50	4.4 (49)	98	49/50	4.1 (49)	91	49/50	4.4 (49)	98	49/50
68-7	4.8 (23)	47/50	5.0 (49)	104	49/50	4.8 (49)	100	49/50	4.4 (49)	92	49/50
70-7	5.1 (47)	47/50	5.0 (49)	98	49/50	5.0 (49)	98	49/50	4.5 (49)	88	49/50
72-7	5.0 (47)	46/50	4.8 (48)	96	47/50	4.7 (49)	94	49/50	4.7 (49)	94	49/50
74-7	4.8 (45)	46/50	4.5 (46)	94	46/50	4.7 (49)	98	49/50	4.4 (49)	92	49/50
76-7	4.8 (46)	46/50	4.8 (46)	100	46/50	4.6 (49)	96	49/50	4.5 (49)	94	49/50
78-7	5.4 (46)	46/50	4.9 (46)	91	46/50	4.7 (48)	87	48/50	5.0 (49)	93	49/50
80-7	5.0 (46)	44/50	4.7 (46)	94	46/50	5.1 (48)	102	48/50	5.0 (49)	100	49/50
82-7	5.5 (44)	44/50	5.3 (46)	96	46/50	4.9 (47)	89	47/50	4.9 (49)	89	49/50
84-7	5.0 (44)	44/50	5.0 (46)	100	46/50	4.7 (47)	94	47/50	4.7 (49)	94	49/50
86-7	4.8 (43)	42/50	4.9 (46)	102	46/50	4.8 (46)	100	46/50	4.6 (49)	96	49/50
88-7	4.5 (39)	38/50	4.8 (45)	107	45/50	4.9 (46)	109	46/50	4.7 (49)	104	49/50
90-7	5.1 (36)	36/50	4.7 (44)	92	43/50	4.9 (44)	96	44/50	4.8 (47)	94	47/50
92-7	4.7 (36)	35/50	4.5 (43)	96	43/50	4.6 (44)	98	43/50	4.4 (46)	94	46/50
94-7	5.1 (35)	35/50	4.7 (43)	92	43/50	4.8 (42)	94	42/50	4.8 (46)	94	45/50
96-7	4.9 (34)	33/50	4.7 (41)	96	41/50	5.1 (42)	104	42/50	5.0 (43)	102	43/50
98-7	5.0 (33)	33/50	5.0 (40)	100	40/50	4.6 (42)	92	41/50	4.5 (43)	90	42/50
100-7	4.7 (33)	32/50	4.8 (36)	102	35/50	4.5 (40)	96	40/50	4.5 (41)	96	38/50
102-7	4.8 (32)	32/50	4.5 (35)	94	33/50	4.4 (39)	92	37/50	4.7 (37)	98	37/50
104-7	5.5 (31)	31/50	5.5 (32)	100	30/50	5.0 (35)	91	35/50	5.0 (36)	91	35/50

< >:No.of effective animals,( ):No.of measured animals Av.FC.: g

Table 31 CLINICAL OBSERVATION (104W-SUMMRY) -MICE-

Findings	Male				Female			
	2000ppm S(DM)*	800ppm S(DM)	320ppm S(DM)	0ppm S(DM)	2000ppm S(DM)	800ppm S(DM)	320ppm S(DM)	0ppm S(DM)
COLORED(着色)	40(6)	40(8)	39(3)	21(3)	35(14)	35(7)	15(5)	7(3)
YELLOW URINE(黄色尿)	40(6)	40(8)	43(4)	0(0)	35(14)	35(13)	30(16)	0(0)
INTERNAL MASS(内部腫瘤)	3(1)	2(5)	1(1)	5(5)	6(7)	5(5)	5(10)	5(5)
EXTERNAL MASS(外部腫瘤)								
M.EYE(眼腫瘤)	1(1)	0(0)	0(0)	0(1)	0(0)	0(0)	0(1)	0(0)
M.NOSE(鼻腫瘤)	0(1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
M.PERI MOUTH(口周囲腫瘤)	0(0)	0(0)	0(0)	0(1)	0(0)	0(0)	0(0)	0(0)
M.MANDIBULAR(下顎部腫瘤)	0(0)	0(0)	0(1)	0(0)	0(0)	0(0)	0(0)	0(0)
M.EAR(耳介部腫瘤)	0(0)	0(0)	1(0)	0(0)	1(0)	0(0)	0(0)	0(0)
M.NECK(頸部腫瘤)	0(1)	0(0)	0(0)	0(1)	1(0)	0(1)	0(1)	0(1)
M.BREAST(胸部腫瘤)	0(0)	0(0)	1(0)	1(0)	0(0)	0(1)	0(0)	1(1)
M.ABDOMEN(腹部腫瘤)	1(1)	4(0)	5(0)	3(3)	0(0)	0(1)	0(0)	0(0)
M.ANTERIOR.DORSUM(背側前部腫瘤)	0(0)	0(0)	0(0)	0(0)	1(0)	0(1)	0(0)	0(0)
M.INTERSCAPULUM(肩甲間部腫瘤)	0(0)	2(0)	1(0)	0(0)	1(0)	0(0)	0(0)	0(0)
M.POSTERIOR DORSUM(背側後部腫瘤)	0(0)	0(0)	0(0)	0(0)	0(0)	0(1)	0(0)	0(0)
M.HINDLIMB(後肢腫瘤)	0(0)	1(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(1)
M.GENITALIA(外陰部腫瘤)	2(0)	2(1)	0(0)	1(0)	0(1)	0(1)	0(1)	0(1)
M.ANUS(肛門腫瘤)	0(1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
M.TAIL(尾腫瘤)	0(0)	1(0)	0(0)	1(0)	0(0)	0(0)	0(0)	0(0)
NO.of Animals with EXTERNAL MASS	4(3)	9(1)	5(1)	6(3)	3(1)	0(4)	0(3)	1(1)
NO.of Survival Amimals (Dead & Moribund Animals)	40(10)	40(10)	43(7)	36(14)	35(15)	35(15)	30(20)	31(19)
NO.of Observed Animals	50	50	50	50	50	50	50	50

* : S=Survival Amimals (DM=Dead & Moribund Animals)

TABLE 32 C A U S E O F D E A T H : M O U S E

Sex	Male				Female			
Group Name	Control	320ppm	800ppm	2000ppm	Control	320ppm	800ppm	2000ppm
Number of Dead/Moribund Animal	14	7	10	10	19	20	15	15
no microscopic comfirmation		1	1		2	2		2
urinary system lesion							1	
renal lesion	1		1			1		
endocrine system lesion		1						
urinary retention				1	1			
amyloidosis				1				
tooth lesion						1		1
Tumor death : leukemia	5	2	4	3	5	6	8	7
: subcutis						1	1	1
: skin/apendage		1						
: lung	1	1	1		2	1	1	
: liver	7		3	3	1		1	
: urin bladd		1		1				
: peripheral nerves				1				
: ovary						1		
: uterus					8	7	3	4

SELECTED FIGURES

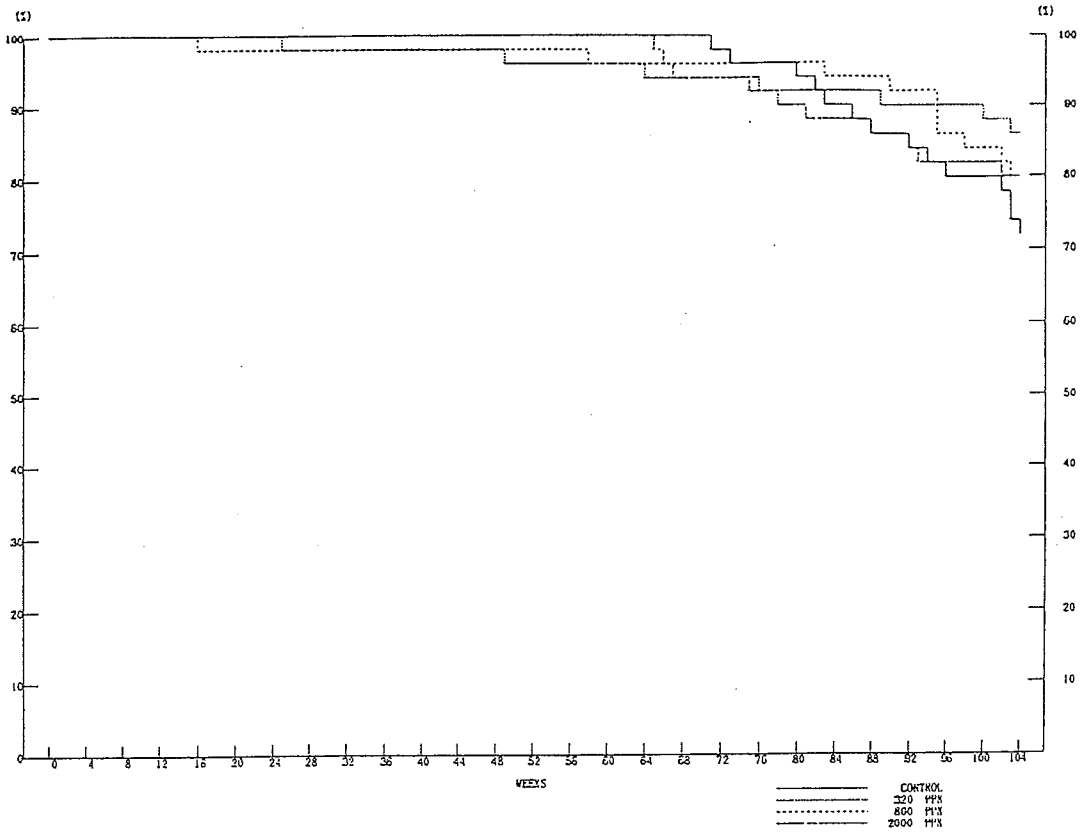


FIGURE 8 SURVIVAL ANIMAL RATE : MOUSE:MALE(TWO-YEAR STUDIES)

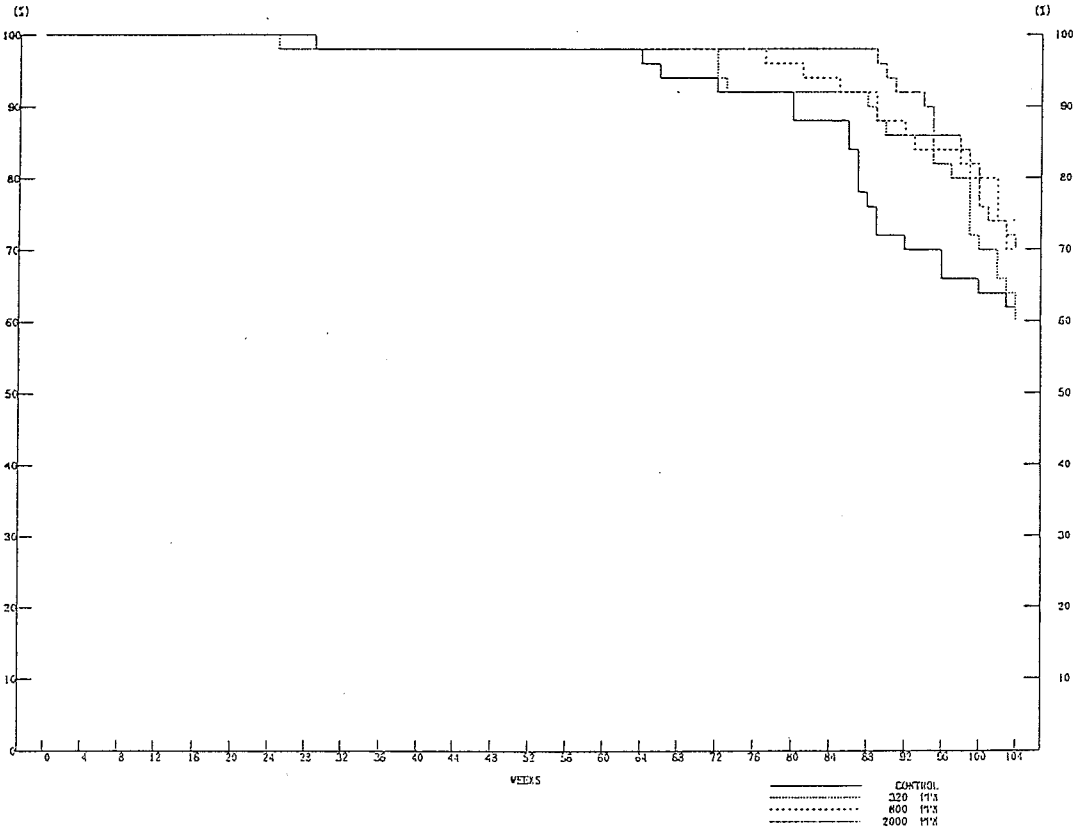


FIGURE 9 SURVIVAL ANIMAL RATE : MOUSE:FEMALE(TWO-YEAR STUDIES)

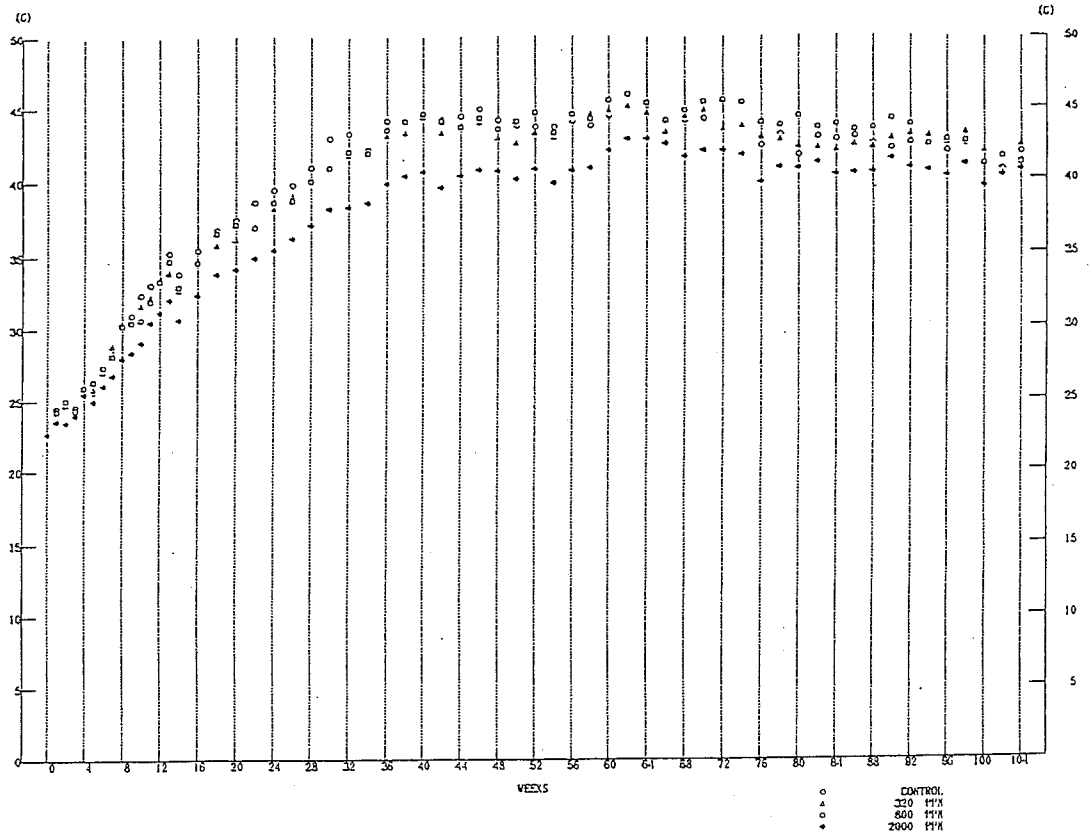


FIGURE 10 BODY WEIGHT CHANGES : MOUSE:MALE(TWO-YEAR STUDIES)

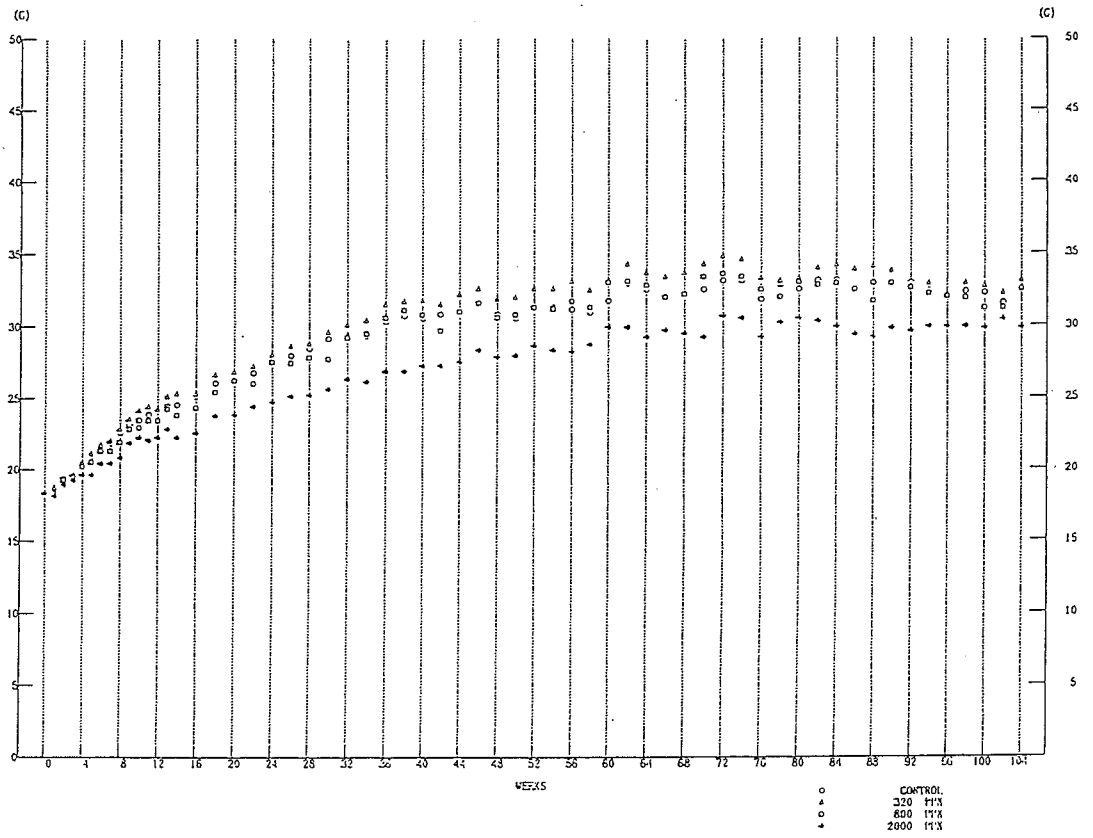


FIGURE 11 BODY WEIGHT CHANGES : MOUSE:FEMALE(TWO-YEAR STUDIES)

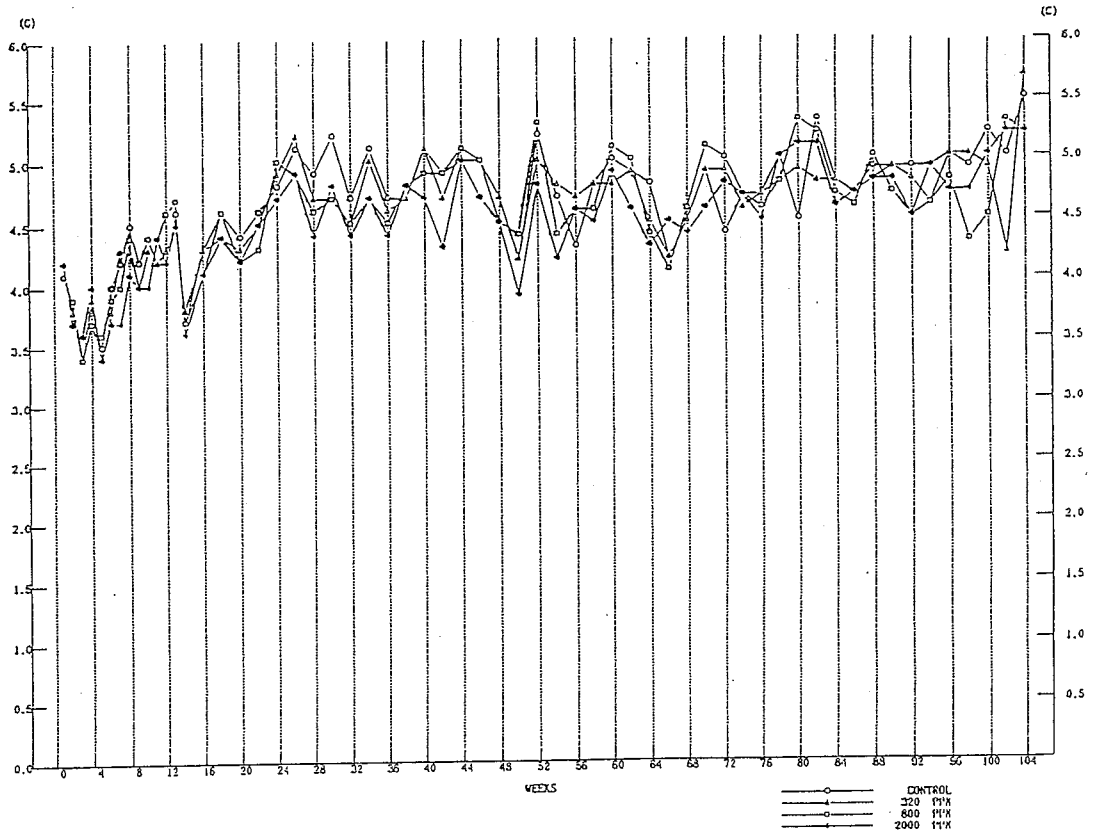


FIGURE 12 FOOD CONSUMPTION : MOUSE:MALE(TWO-YEAR STUDIES)

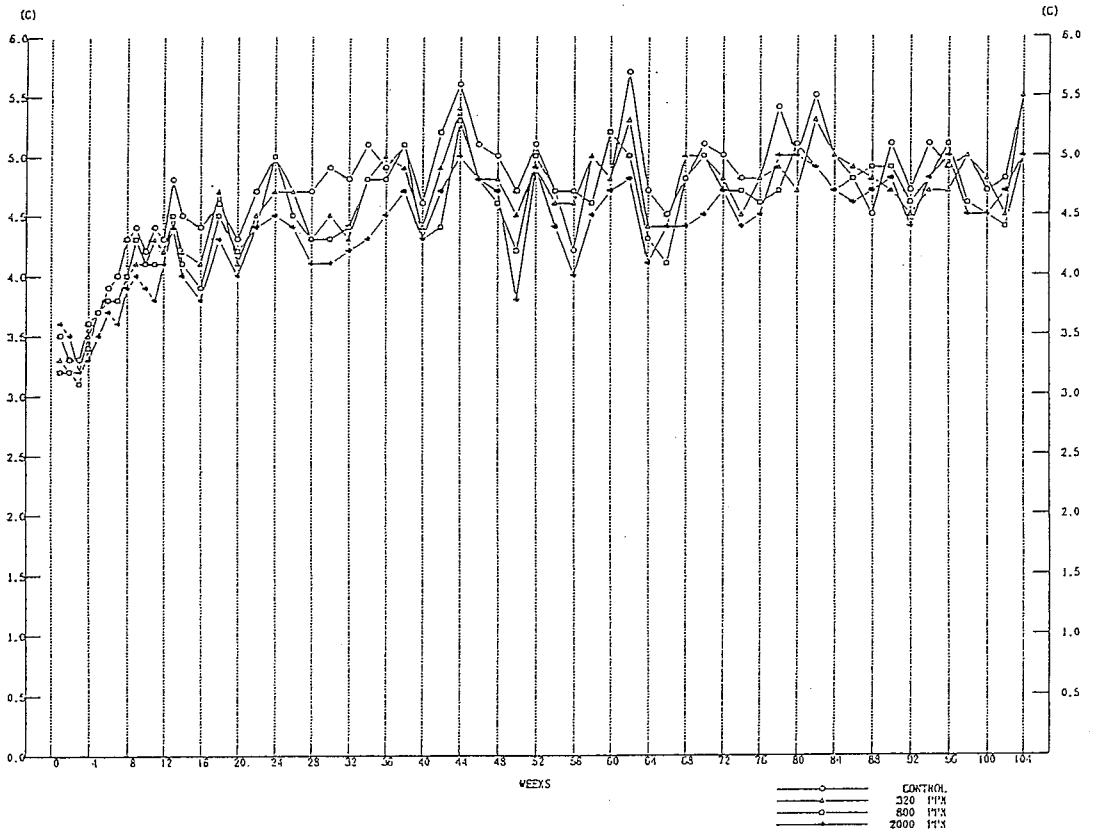
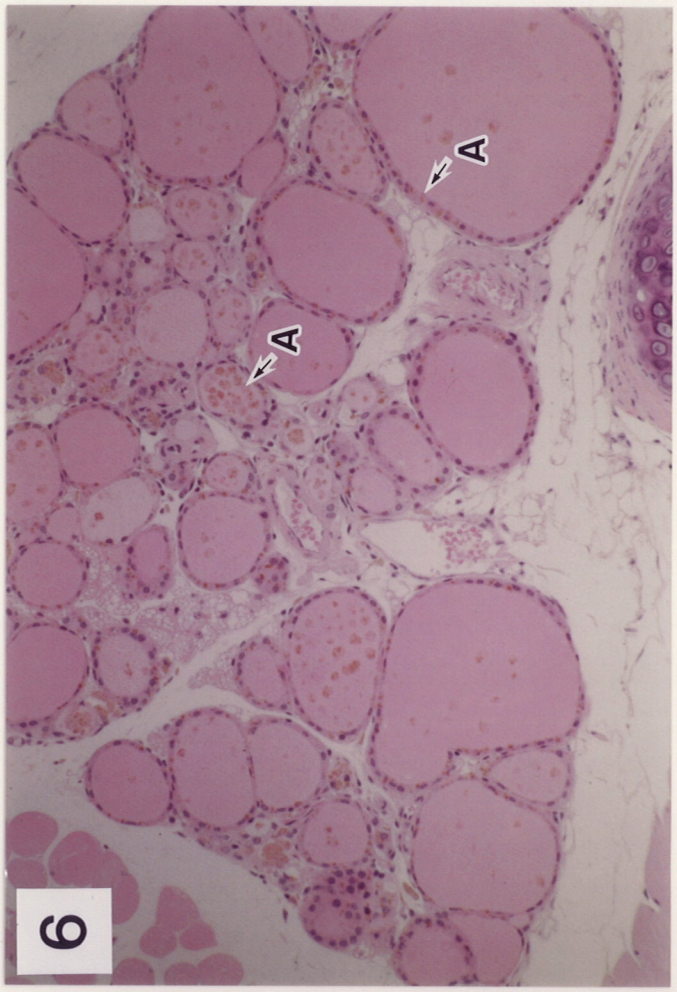


FIGURE 13 FOOD CONSUMPTION : MOUSE:FEMALE(TWO-YEAR STUDIES)

PHOTOGRAPHS

PHOTOGRAPH 6 THYROID, DEPOSIT OF PIGMENNT:A
104-WEEK STUDY, MOUSE, MALE, 2000ppm, ANIMAL No. 0096-1304
(H. E., X150)

PHOTOGRAPH 7 STOMACH, HYPERPLASIA;FORESTOMACH:A
104-WEEK STUDY, MOUSE, MALE, 2000ppm, ANIMAL No. 0096-1316
(H. E., X60)


A
A
6


A
7