Summary of Feed Carcinogenicity Study of 1-Chloro-2-Nitrobenzene in B6D2F1 Mice

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Japan Bioassay Research Center

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PREFACE

The tests were contracted and supported by the Ministry of Health, Labour and Welfare of Japan. The tests were conducted by Japan Bioassay Research Center (JBRC) and the report was prepared by JBRC and peer reviewed by outside expert pathologist. Complete report was submitted to Ministry of Health, Labour and Welfare of Japan on April 13, 2006.

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

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Purpose, materials and methods

1-Chloro-2-nitrobenzene (*o*-CNB, *o*-chloronitrobenzene, CAS No. 88-73-3) is a yellow monoclinic needle with a melting point of 33°C and a boiling point of 245°C. It is insoluble in water.

The carcinogenicity and chronic toxicity of *o*-CNB were examined by feeding groups of 50 B6D2F1/Crlj mice of both sexes *o*-CNB-containing diets for 2 years (104 weeks). The dietary concentration of *o*-CNB was 0, 100, 500 or 2500 ppm (w/w). The highest dose level was chosen so as not to exceed the maximum tolerated dose (MTD), based on both growth rate and toxicity in the previous 13-week toxicity study. *o*-CNB was analyzed for purity and stability by both infrared spectrometry and high performance liquid chromatography before and after its use. The *o*-CNB concentrations in the diet were determined by high performance liquid chromatography at the time of preparation, and on the 8th day after preparation 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 4 weeks thereafter. Animals found dead, 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. For hematology and blood biochemistry, the surviving animals were bled under ether anesthesia, after they were fasted overnight, at the terminal necropsy. Organs and tissues were removed, weighed and examined for macroscopic lesions at necropsy. The organs and tissues were fixed and embedded in paraffin. Tissue sections of 5 μm thick were prepared and stained with hematoxylin and eosin and examined for histopathology. Incidences of neoplastic lesions were statistically analyzed by Fisher's exact test. A positive trend of the dose-response relation for the neoplastic incidence was analyzed by Peto's test. Incidences of non-neoplastic lesions and urinalysis were analyzed by 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 in accordance with the Organisation for Economic Co-operation and Development (OECD) Good Laboratory Practice and with reference to the OECD Guideline for Testing of Chemicals 451 "Carcinogenicity Studies".

Results

Survival rates of the 2500 ppm-fed males and females and the 500 ppm-fed males were significantly decreased, and the decreased survival rates were causally related to the increased death rate due to liver tumors. The yellow urine, which may have been colored by a metabolite of *o*-CNB, was observed in all the 2500 ppm-fed males and females throughout the 2-year administration period. The internal mass was observed frequently in both the *o*-CNB-fed males and females. Body weights of the 2500 ppm-fed males and females were significantly decreased through the administration period, and their terminal body weights were suppressed to 60% and 71%, respectively, as compared with the controls. The 500 ppm-fed males and females significantly decreased their body weights to 78% and 88% at the end of the 2-year administration period as compared with the controls, respectively. There was no significant difference in growth rate between any of the 100 ppm-fed groups of both sexes and the respective control.

The incidences of hepatocellular adenomas and carcinomas, and hepatoblastomas were increased dose-dependently in males and females, and the increased incidences of those hepatic tumors were recognized in the males and females fed the lowest dose level of 100 ppm. As non-neoplastic lesions, centrilobular hypertrophy of hepatocytes was observed in the *o*-CNB-fed males and females, while nuclear enlargement of hepatocytes was observed in the *o*-CNB-fed males. The significantly increased incidences of hemosiderin deposition and extramedullary hematopoiesis in the spleen and hemosiderin deposition in the kidney and increased erythropoiesis in the bone marrow were noted in both the *o*-CNB-fed males and females. In the nasal cavity, the incidences of eosinophilic change in the olfactory epithelium and respiratory metaplasia in both the olfactory epithelium and gland were increased in the *o*-CNB-fed females.

Conclusions

In mice, there was clear evidence of carcinogenic activity of *o*-CNB in males and females, based on the increased incidences of hepatocellular adenomas and carcinomas, and hepatoblastomas.

TABLES

TABLES (CONTINUED)

TABLE 1 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES OF MALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Week on Study	Control		100 ppm			500 ppm			2500 ppm		
	Av. Wt.	No. of Surviv. <50>	Av. Wt.	% of cont.	No. of Surviv. <50>	Av. Wt.	% of cont.	No. of Surviv. <50>	Av. Wt.	% of cont.	No. of Surviv. <50>
0	23.0 (50)	50 / 50	23.0 (50)	100	50 / 50	23.0 (50)	100	50 / 50	23.0 (50)	100	50 / 50
1	24.2 (50)	50 / 50	23.9 (50)	99	50 / 50	24.0 (50)	99	50 / 50	23.4 (50)	97	50 / 50
2	25.3 (50)	50 / 50	24.9 (50)	98	50 / 50	24.9 (50)	98	50 / 50	25.1 (50)	99	50 / 50
3	25.8 (50)	50 / 50	25.6 (50)	99	50 / 50	25.7 (50)	100	50 / 50	26.1 (50)	101	50 / 50
4	26.5 (50)	50 / 50	26.4 (50)	100	50 / 50	26.6 (50)	100	50 / 50	27.1 (50)	102	50 / 50
5	27.5 (50)	50 / 50	27.3 (50)	99	50 / 50	27.4 (50)	100	50 / 50	27.7 (50)	101	50 / 50
6	28.1 (50)	50 / 50	27.8 (50)	99	50 / 50	28.1 (50)	100	50 / 50	28.1 (50)	100	50 / 50
7	29.0 (50)	50 / 50	28.6 (50)	99	50 / 50	29.1 (50)	100	50 / 50	28.6 (50)	99	50 / 50
8	29.4 (50)	50 / 50	29.2 (50)	99	50 / 50	29.6 (50)	101	50 / 50	29.1 (50)	99	50 / 50
9	30.3 (50)	50 / 50	29.7 (50)	98	50 / 50	30.1 (50)	99	50 / 50	29.4 (50)	97	50 / 50
10	31.1 (50)	50 / 50	30.5 (50)	98	50 / 50	31.1 (50)	100	50 / 50	30.2 (49)	97	49 / 50
11	31.8 (50)	50 / 50	31.4 (50)	99	50 / 50	31.8 (50)	100	50 / 50	31.0 (49)	97	49 / 50
12	32.3 (50)	50 / 50	31.8 (50)	98	50 / 50	32.2 (50)	100	50 / 50	31.1 (49)	96	49 / 50
13	33.3 (50)	50 / 50	32.4 (50)	97	50 / 50	33.1 (50)	99	50 / 50	31.8 (49)	95	49 / 50
14	34.1 (50)	50 / 50	33.3 (50)	98	50 / 50	34.0 (50)	100	50 / 50	32.6 (49)	96	49 / 50
18	36.6 (50)	50 / 50	35.8 (50)	98	50 / 50	36.5 (50)	100	50 / 50	34.2 (49)	93	49 / 50
22	38.8 (50)	50 / 50	38.1 (50)	98	50 / 50	38.8 (50)	100	50 / 50	35.4 (49)	91	49 / 50
26	41.2 (50)	50 / 50	40.4 (50)	98	50 / 50	40.9 (50)	99	50 / 50	36.5 (49)	89	49 / 50
30	43.6 (50)	50 / 50	42.9 (50)	98	50 / 50	43.1 (50)	99	50 / 50	37.4 (49)	86	49 / 50
34	45.7 (50)	50 / 50	45.3 (50)	99	50 / 50	45.2 (50)	99	50 / 50	38.4 (49)	84	49 / 50
38	47.0 (50)	50 / 50	46.8 (50)	100	50 / 50	47.1 (50)	100	50 / 50	39.5 (49)	84	49 / 50
42	48.3 (50)	50 / 50	48.5 (50)	100	50 / 50	48.3 (50)	100	50 / 50	40.0 (49)	83	49 / 50
46	50.2 (49)	49 / 50	49.6 (50)	99	50 / 50	50.1 (50)	100	50 / 50	40.7 (49)	81	49 / 50
50	51.2 (49)	49 / 50	51.1 (50)	100	50 / 50	51.7 (49)	101	49 / 50	41.1 (49)	80	49 / 50
54	51.8 (49)	49 / 50	52.0 (50)	100	50 / 50	53.2 (49)	103	49 / 50	41.4 (49)	80	49 / 50
58	52.5 (49)	49 / 50	52.8 (50)	101	50 / 50	53.6 (49)	102	49 / 50	40.6 (49)	77	49 / 50
62	53.7 (48)	48 / 50	54.3 (50)	101	50 / 50	54.7 (49)	102	49 / 50	40.5 (47)	75	47 / 50
66	53.8 (47)	47 / 50	54.5 (48)	101	48 / 50	55.6 (48)	103	48 / 50	39.5 (47)	73	47 / 50
70	53.6 (47)	47 / 50	54.7 (47)	102	47 / 50	55.3 (48)	103	48 / 50	37.6 (43)	70	43 / 50
74	54.1 (47)	47 / 50	55.2 (46)	102	46 / 50	55.2 (45)	102	45 / 50	36.1 (39)	67	39 / 50
78	54.3 (45)	45 / 50	54.9 (45)	101	45 / 50	54.0 (42)	99	42 / 50	35.8 (35)	66	35 / 50
82	54.4 (45)	45 / 50	55.4 (45)	102	45 / 50	52.5 (40)	97	40 / 50	33.5 (30)	62	30 / 50
86	53.0 (45)	45 / 50	54.8 (45)	103	45 / 50	50.9 (38)	96	38 / 50	33.7 (23)	64	23 / 50
90	52.4 (40)	40 / 50	53.1 (45)	101	45 / 50	47.6 (33)	91	33 / 50	32.0 (18)	61	18 / 50
94	52.8 (39)	39 / 50	51.8 (44)	98	44 / 50	45.0 (29)	85	29 / 50	32.1 (14)	61	14 / 50
98	51.6 (38)	38 / 50	50.6 (40)	98	40 / 50	42.2 (25)	82	25 / 50	30.6 (11)	59	11 / 50
102	51.2 (37)	37 / 50	50.2 (37)	98	37 / 50	40.6 (22)	79	22 / 50	30.7 (10)	60	10 / 50
104	51.0 (35)	35 / 50	49.2 (35)	96	35 / 50	40.0 (17)	78	17 / 50	30.6 (8)	60	8 / 50

< > : No. of effective animals, ( ) : No. of measured animals, Av. Wt. : Averaged body weight (Unit : g).

TABLE 2 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Week on Study	Control		100 ppm			500 ppm			2500 ppm		
	Av. Wt.	No. of Surviv.	Av. Wt.	% of cont.	No. of Surviv.	Av. Wt.	% of cont.	No. of Surviv.	Av. Wt.	% of cont.	No. of Surviv.
	<50>		<50>			<50>			<50>		
0	18.6 (50)	50 / 50	18.6 (50)	100	50 / 50	18.6 (50)	100	50 / 50	18.6 (50)	100	50 / 50
1	19.0 (50)	50 / 50	19.1 (50)	101	50 / 50	19.3 (50)	102	50 / 50	19.6 (50)	103	50 / 50
2	19.5 (50)	50 / 50	19.6 (50)	101	50 / 50	19.9 (50)	102	50 / 50	20.3 (50)	104	50 / 50
3	20.1 (50)	50 / 50	20.3 (50)	101	50 / 50	20.6 (50)	102	50 / 50	21.0 (50)	104	50 / 50
4	20.5 (50)	50 / 50	20.8 (50)	101	50 / 50	21.3 (50)	104	50 / 50	21.7 (50)	106	50 / 50
5	21.4 (50)	50 / 50	21.4 (50)	100	50 / 50	22.1 (50)	103	50 / 50	22.5 (50)	105	50 / 50
6	21.9 (50)	50 / 50	21.8 (50)	100	50 / 50	22.7 (50)	104	50 / 50	23.1 (50)	105	50 / 50
7	22.4 (50)	50 / 50	22.7 (50)	101	50 / 50	23.2 (50)	104	50 / 50	24.0 (50)	107	50 / 50
8	23.0 (50)	50 / 50	22.9 (50)	100	50 / 50	23.7 (50)	103	50 / 50	24.0 (50)	104	50 / 50
9	23.3 (50)	50 / 50	23.1 (50)	99	50 / 50	24.0 (50)	103	50 / 50	24.7 (50)	106	50 / 50
10	23.9 (50)	50 / 50	24.2 (50)	101	50 / 50	24.8 (50)	104	50 / 50	25.3 (50)	106	50 / 50
11	24.4 (50)	50 / 50	24.3 (50)	100	50 / 50	25.4 (50)	104	50 / 50	25.7 (50)	105	50 / 50
12	24.5 (50)	50 / 50	24.4 (50)	100	50 / 50	25.3 (50)	103	50 / 50	25.7 (50)	105	50 / 50
13	24.8 (50)	50 / 50	25.1 (50)	101	50 / 50	25.8 (50)	104	50 / 50	25.9 (50)	104	50 / 50
14	25.7 (50)	50 / 50	25.8 (50)	100	50 / 50	26.7 (50)	104	50 / 50	26.3 (50)	102	50 / 50
18	27.4 (50)	50 / 50	27.5 (50)	100	50 / 50	28.1 (50)	103	50 / 50	27.6 (50)	101	50 / 50
22	28.8 (50)	50 / 50	29.2 (50)	101	50 / 50	30.0 (50)	104	50 / 50	29.2 (50)	101	50 / 50
26	30.4 (50)	50 / 50	30.1 (50)	99	50 / 50	31.2 (50)	103	50 / 50	29.7 (50)	98	50 / 50
30	32.0 (50)	50 / 50	31.8 (50)	99	50 / 50	32.9 (50)	103	50 / 50	30.8 (50)	96	50 / 50
34	33.0 (50)	50 / 50	33.3 (50)	101	50 / 50	34.0 (50)	103	50 / 50	31.7 (50)	96	50 / 50
38	34.2 (50)	50 / 50	33.9 (50)	99	50 / 50	34.9 (50)	102	50 / 50	31.8 (50)	93	50 / 50
42	35.4 (50)	50 / 50	35.1 (50)	99	50 / 50	36.4 (50)	103	50 / 50	32.3 (50)	91	50 / 50
46	36.3 (50)	50 / 50	36.3 (50)	100	50 / 50	37.4 (49)	103	49 / 50	32.2 (49)	89	49 / 50
50	36.9 (49)	49 / 50	36.7 (50)	99	50 / 50	38.1 (49)	103	49 / 50	31.8 (49)	86	49 / 50
54	37.9 (49)	49 / 50	38.2 (50)	101	50 / 50	38.8 (49)	102	49 / 50	31.3 (49)	83	49 / 50
58	38.6 (49)	49 / 50	38.9 (50)	101	50 / 50	39.4 (49)	102	49 / 50	30.6 (48)	79	48 / 50
62	38.5 (49)	49 / 50	39.2 (50)	102	50 / 50	39.6 (49)	103	49 / 50	30.2 (47)	78	47 / 50
66	39.4 (49)	49 / 50	40.0 (50)	102	50 / 50	40.3 (48)	102	48 / 50	29.4 (46)	75	46 / 50
70	40.1 (49)	49 / 50	40.2 (50)	100	50 / 50	40.8 (48)	102	48 / 50	28.7 (46)	72	46 / 50
74	39.8 (49)	49 / 50	40.0 (49)	101	49 / 50	40.0 (48)	101	48 / 50	27.7 (44)	70	44 / 50
78	39.8 (49)	49 / 50	39.5 (48)	99	48 / 50	38.7 (47)	97	47 / 50	27.5 (36)	69	36 / 50
82	39.7 (42)	42 / 50	39.9 (45)	101	45 / 50	38.2 (40)	96	40 / 50	27.2 (31)	69	31 / 50
86	39.2 (39)	39 / 50	39.3 (43)	100	43 / 50	37.4 (37)	95	37 / 50	26.7 (27)	68	27 / 50
90	38.8 (38)	38 / 50	39.2 (40)	101	40 / 50	36.8 (35)	95	35 / 50	26.2 (22)	68	22 / 50
94	38.9 (36)	36 / 50	39.1 (39)	101	39 / 50	35.3 (33)	91	33 / 50	26.0 (15)	67	15 / 50
98	38.3 (31)	31 / 50	39.1 (35)	102	35 / 50	34.8 (27)	91	27 / 50	26.1 (12)	68	12 / 50
102	38.2 (30)	30 / 50	38.4 (35)	101	35 / 50	33.8 (26)	88	26 / 50	25.8 (8)	68	8 / 50
104	37.4 (29)	29 / 50	37.9 (34)	101	34 / 50	33.0 (26)	88	26 / 50	26.5 (5)	71	5 / 50

< > : No. of effective animals, ( ) : No. of measured animals, Av. Wt. : Averaged body weight (Unit : g).

TABLE 3 FOOD CONSUMPTION CHANGES OF MALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Week on Study	Control		100 ppm			500 ppm			2500 ppm		
	Av. FC.	No. of Surviv. <50>	Av. FC.	% of cont.	No. of Surviv. <50>	Av. FC.	% of cont.	No. of Surviv. <50>	Av. FC.	% of cont.	No. of Surviv. <50>
1	3.9 (50)	50 / 50	3.8 (50)	97	50 / 50	3.9 (50)	100	50 / 50	4.0 (50)	103	50 / 50
2	3.7 (50)	50 / 50	3.7 (50)	100	50 / 50	3.8 (50)	103	50 / 50	4.3 (50)	116	50 / 50
3	3.6 (50)	50 / 50	3.7 (50)	103	50 / 50	3.7 (50)	103	50 / 50	3.8 (50)	106	50 / 50
4	3.8 (50)	50 / 50	3.7 (50)	97	50 / 50	3.7 (50)	97	50 / 50	3.9 (50)	103	50 / 50
5	3.8 (50)	50 / 50	3.8 (50)	100	50 / 50	3.8 (50)	100	50 / 50	3.9 (50)	103	50 / 50
6	3.7 (50)	50 / 50	3.6 (50)	97	50 / 50	3.7 (50)	100	50 / 50	3.7 (50)	100	50 / 50
7	3.9 (50)	50 / 50	3.8 (50)	97	50 / 50	3.9 (50)	100	50 / 50	3.9 (50)	100	50 / 50
8	3.9 (50)	50 / 50	3.8 (50)	97	50 / 50	3.9 (50)	100	50 / 50	3.8 (50)	97	50 / 50
9	4.0 (50)	50 / 50	3.8 (50)	95	50 / 50	3.9 (50)	98	50 / 50	3.9 (50)	98	50 / 50
10	4.1 (50)	50 / 50	3.9 (50)	95	50 / 50	3.9 (50)	95	50 / 50	3.9 (49)	95	49 / 50
11	4.0 (50)	50 / 50	3.9 (50)	98	50 / 50	3.9 (50)	98	50 / 50	3.9 (49)	98	49 / 50
12	4.0 (50)	50 / 50	4.0 (50)	100	50 / 50	4.0 (50)	100	50 / 50	3.9 (49)	98	49 / 50
13	4.0 (50)	50 / 50	3.9 (50)	98	50 / 50	3.9 (50)	98	50 / 50	3.9 (49)	98	49 / 50
14	4.0 (50)	50 / 50	3.9 (50)	98	50 / 50	4.0 (50)	100	50 / 50	4.0 (49)	100	49 / 50
18	4.0 (50)	50 / 50	3.9 (50)	98	50 / 50	3.8 (50)	95	50 / 50	3.8 (49)	95	49 / 50
22	4.2 (50)	50 / 50	4.1 (50)	98	50 / 50	4.0 (50)	95	50 / 50	3.9 (49)	93	49 / 50
26	4.3 (50)	50 / 50	4.2 (50)	98	50 / 50	4.1 (50)	95	50 / 50	4.1 (49)	95	49 / 50
30	4.4 (50)	50 / 50	4.2 (50)	95	50 / 50	4.3 (50)	98	50 / 50	4.1 (49)	93	49 / 50
34	4.4 (50)	50 / 50	4.2 (50)	95	50 / 50	4.3 (50)	98	50 / 50	4.2 (49)	95	49 / 50
38	4.3 (50)	50 / 50	4.1 (50)	95	50 / 50	4.2 (50)	98	50 / 50	4.2 (49)	98	49 / 50
42	4.4 (50)	50 / 50	4.4 (50)	100	50 / 50	4.3 (49)	98	50 / 50	4.2 (49)	95	49 / 50
46	4.5 (49)	49 / 50	4.3 (50)	96	50 / 50	4.4 (50)	98	50 / 50	4.2 (48)	93	49 / 50
50	4.5 (49)	49 / 50	4.5 (50)	100	50 / 50	4.5 (49)	100	49 / 50	4.4 (49)	98	49 / 50
54	4.5 (49)	49 / 50	4.5 (50)	100	50 / 50	4.6 (49)	102	49 / 50	4.5 (49)	100	49 / 50
58	4.5 (49)	49 / 50	4.5 (50)	100	50 / 50	4.4 (49)	98	49 / 50	4.2 (49)	93	49 / 50
62	4.5 (48)	48 / 50	4.4 (50)	98	50 / 50	4.6 (49)	102	49 / 50	4.5 (47)	100	47 / 50
66	4.7 (47)	47 / 50	4.5 (48)	96	48 / 50	4.6 (48)	98	48 / 50	4.8 (46)	102	47 / 50
70	4.7 (47)	47 / 50	4.5 (47)	96	47 / 50	4.5 (48)	96	48 / 50	4.7 (41)	100	43 / 50
74	4.8 (45)	47 / 50	4.7 (46)	98	46 / 50	4.7 (45)	98	45 / 50	4.8 (38)	100	39 / 50
78	5.1 (44)	45 / 50	4.9 (45)	96	45 / 50	4.7 (42)	92	42 / 50	5.2 (24)	102	35 / 50
82	5.0 (45)	45 / 50	4.8 (43)	96	45 / 50	4.6 (40)	92	40 / 50	5.3 (20)	106	30 / 50
86	4.7 (42)	45 / 50	5.0 (44)	106	45 / 50	4.9 (37)	104	38 / 50	5.8 (16)	123	23 / 50
90	4.7 (36)	40 / 50	4.6 (45)	98	45 / 50	4.3 (33)	91	33 / 50	4.9 (10)	104	18 / 50
94	4.7 (37)	39 / 50	4.6 (41)	98	44 / 50	4.3 (25)	91	29 / 50	4.6 (4)	98	14 / 50
98	4.7 (34)	38 / 50	4.7 (37)	100	40 / 50	4.2 (23)	89	25 / 50	4.7 (4)	100	11 / 50
102	4.8 (36)	37 / 50	4.8 (37)	100	37 / 50	4.6 (22)	96	22 / 50	5.6 (5)	117	10 / 50
104	4.7 (34)	35 / 50	4.7 (34)	100	35 / 50	4.8 (17)	102	17 / 50	5.0 (3)	106	8 / 50

< > : No. of effective animals, ( ) : No. of measured animals, Av. FC. : Averaged food consumption (Unit : g).

TABLE 4 FOOD CONSUMPTION CHANGES OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Week on Study	Control		100 ppm			500 ppm			2500 ppm		
	Av. FC.	No. of Surviv. <50>	Av. FC.	% of cont.	No. of Surviv. <50>	Av. FC.	% of cont.	No. of Surviv. <50>	Av. FC.	% of cont.	No. of Surviv. <50>
1	3.5 (50)	50 / 50	3.8 (50)	109	50 / 50	3.7 (50)	106	50 / 50	3.7 (50)	106	50 / 50
2	3.2 (50)	50 / 50	3.5 (50)	109	50 / 50	3.6 (50)	113	50 / 50	3.6 (50)	113	50 / 50
3	3.4 (50)	50 / 50	3.5 (50)	103	50 / 50	3.4 (50)	100	50 / 50	3.4 (50)	100	50 / 50
4	3.3 (50)	50 / 50	3.4 (50)	103	50 / 50	3.5 (50)	106	50 / 50	3.4 (50)	103	50 / 50
5	3.5 (50)	50 / 50	3.6 (50)	103	50 / 50	3.7 (48)	106	50 / 50	3.6 (50)	103	50 / 50
6	3.5 (50)	50 / 50	3.5 (50)	100	50 / 50	3.6 (50)	103	50 / 50	3.6 (50)	103	50 / 50
7	3.7 (50)	50 / 50	3.9 (50)	105	50 / 50	3.9 (50)	105	50 / 50	4.0 (50)	108	50 / 50
8	3.6 (50)	50 / 50	3.6 (50)	100	50 / 50	3.7 (50)	103	50 / 50	3.8 (50)	106	50 / 50
9	3.7 (50)	50 / 50	3.6 (50)	97	50 / 50	3.9 (50)	105	50 / 50	3.9 (50)	105	50 / 50
10	3.8 (50)	50 / 50	3.9 (50)	103	50 / 50	3.9 (50)	103	50 / 50	3.8 (50)	100	50 / 50
11	3.7 (50)	50 / 50	3.9 (50)	105	50 / 50	3.9 (50)	105	50 / 50	3.9 (50)	105	50 / 50
12	3.8 (50)	50 / 50	3.9 (50)	103	50 / 50	3.9 (50)	103	50 / 50	4.0 (50)	105	50 / 50
13	3.7 (50)	50 / 50	3.9 (50)	105	50 / 50	3.7 (50)	100	50 / 50	3.7 (50)	100	50 / 50
14	3.9 (50)	50 / 50	3.9 (50)	100	50 / 50	3.9 (50)	100	50 / 50	3.8 (50)	97	50 / 50
18	3.7 (50)	50 / 50	3.8 (50)	103	50 / 50	3.8 (50)	103	50 / 50	3.8 (50)	103	50 / 50
22	3.8 (50)	50 / 50	4.0 (50)	105	50 / 50	4.0 (50)	105	50 / 50	4.1 (50)	108	50 / 50
26	4.0 (50)	50 / 50	4.1 (50)	103	50 / 50	4.1 (50)	103	50 / 50	4.1 (50)	103	50 / 50
30	4.2 (50)	50 / 50	4.2 (50)	100	50 / 50	4.2 (50)	100	50 / 50	4.2 (50)	100	50 / 50
34	4.2 (50)	50 / 50	4.4 (50)	105	50 / 50	4.1 (50)	98	50 / 50	4.3 (50)	102	50 / 50
38	4.1 (49)	50 / 50	4.2 (50)	102	50 / 50	4.1 (47)	100	50 / 50	4.1 (50)	100	50 / 50
42	4.3 (50)	50 / 50	4.3 (50)	100	50 / 50	4.3 (50)	100	50 / 50	4.3 (50)	100	50 / 50
46	4.3 (50)	50 / 50	4.5 (49)	105	50 / 50	4.4 (49)	102	49 / 50	4.4 (49)	102	49 / 50
50	4.4 (49)	49 / 50	4.4 (49)	100	50 / 50	4.5 (49)	102	49 / 50	4.4 (49)	100	49 / 50
54	4.4 (49)	49 / 50	4.6 (49)	105	50 / 50	4.5 (49)	102	49 / 50	4.6 (46)	105	49 / 50
58	4.5 (48)	49 / 50	4.6 (50)	102	50 / 50	4.5 (49)	100	49 / 50	4.8 (45)	107	48 / 50
62	4.3 (49)	49 / 50	4.5 (50)	105	50 / 50	4.5 (49)	105	49 / 50	4.8 (41)	112	47 / 50
66	4.5 (48)	49 / 50	4.6 (48)	102	50 / 50	4.5 (48)	100	48 / 50	4.6 (41)	102	46 / 50
70	4.4 (47)	49 / 50	4.5 (48)	102	50 / 50	4.5 (45)	102	48 / 50	4.5 (40)	102	46 / 50
74	4.5 (49)	49 / 50	4.6 (49)	102	49 / 50	4.5 (48)	100	48 / 50	4.7 (39)	104	44 / 50
78	4.7 (46)	49 / 50	4.5 (43)	96	48 / 50	4.5 (45)	96	47 / 50	4.6 (24)	98	36 / 50
82	4.8 (41)	42 / 50	4.6 (40)	96	45 / 50	4.6 (39)	96	40 / 50	4.6 (23)	96	31 / 50
86	4.7 (38)	39 / 50	5.0 (41)	106	43 / 50	4.9 (34)	104	37 / 50	4.9 (18)	104	27 / 50
90	4.5 (36)	38 / 50	4.5 (33)	100	40 / 50	4.6 (32)	102	35 / 50	4.6 (16)	102	22 / 50
94	4.4 (33)	36 / 50	4.4 (34)	100	39 / 50	4.2 (29)	95	33 / 50	4.8 (9)	109	15 / 50
98	4.8 (28)	31 / 50	4.7 (33)	98	35 / 50	4.8 (24)	100	27 / 50	5.0 (7)	104	12 / 50
102	4.8 (30)	30 / 50	4.6 (35)	96	35 / 50	4.9 (23)	102	26 / 50	5.5 (6)	115	8 / 50
104	4.4 (26)	29 / 50	4.4 (31)	100	34 / 50	4.7 (22)	107	26 / 50	4.3 (1)	98	5 / 50

< > : No. of effective animals, ( ) : No. of measured animals, Av. FC. : Averaged food consumption (Unit : g).

TABLE 5 HEMATOLOGY OF MALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Group Name	Control	100 ppm	500 ppm	2500 ppm
No. of examined animals	33	33	14	8
RETICULOCYTE (%)	2.4 ± 1.6	2.8 ± 2.3	9.4 ± 12.7 **	8.0 ± 5.4 **
Differential WBC (%)				
N-SEG	26 ± 9	28 ± 12	33 ± 12	57 ± 12 **
EOSINO	2 ± 2	1 ± 1	1 ± 1 *	0 ± 0 **
LYMPHO	67 ± 10	64 ± 17	61 ± 14	35 ± 14 **

Mean ± S.D.
Significant difference: * : p≦0.05 ** : p≦0.01 Test of Dunnett

TABLE 6 HEMATOLOGY OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Group Name	Control	100 ppm	500 ppm	2500 ppm
No. of examined animals	29	34	25	4
MCH (pg)	14.8 ± 0.6	14.7 ± 0.5	14.4 ± 0.6 *	15.3 ± 0.5
RETICULOCYTE (%)	4.7 ± 8.0	3.1 ± 2.7	5.2 ± 3.9 **	5.3 ± 1.0 *
WBC ($10^3/\mu$ L)	3.31 ± 3.44	3.33 ± 4.78	3.08 ± 1.56	1.12 ± 0.41 *
Differential WBC (%)				
N-SEG	27 ± 14	22 ± 11	28 ± 14	69 ± 9 **
EOSINO	3 ± 3	2 ± 2	2 ± 6 *	0 ± 1 *
LYMPHO	62 ± 18	69 ± 16	61 ± 19	25 ± 6 **

Mean ± S.D.
Significant difference: * : p≦0.05 ** : p≦0.01 Test of Dunnett

TABLE 7 BIOCHEMISTRY OF MALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Group Name	Control	100 ppm	500 ppm		2500 ppm	
No. of examined animals	34	34	14		8	
T-BILIRUBIN (mg/dL)	0.15 ± 0.07	0.14 ± 0.03	0.29 ± 0.26	*	0.38 ± 0.20	**
GLUCOSE (mg/dL)	197 ± 48	199 ± 54	167 ± 58		138 ± 22	*
T-CHOLESTEROL (mg/dL)	128 ± 59	151 ± 64	200 ± 79	**	339 ± 78	**
PHOSPHOLIPID (mg/dL)	217 ± 86	247 ± 79	354 ± 140	**	576 ± 120	**
AST (IU/L)	306 ± 787	156 ± 209	549 ± 590	**	3136 ± 3412	**
ALT (IU/L)	234 ± 579	120 ± 173	610 ± 759	**	2400 ± 2502	**
LDH (IU/L)	929 ± 2145	495 ± 693	7530 ± 10481	**	10515 ± 10479	**
ALP (IU/L)	145 ± 52	215 ± 186	1013 ± 715	**	2448 ± 1025	**
G-GTP (IU/L)	2 ± 1	1 ± 1	3 ± 2	*	74 ± 29	**
CK (IU/L)	54 ± 32	58 ± 34	202 ± 395	**	139 ± 38	**
POTASSIUM (mEq/L)	4.1 ± 0.4	4.1 ± 0.5	4.2 ± 0.9		5.0 ± 0.8	**
CALCIUM (mg/dL)	9.2 ± 0.6	9.3 ± 0.5	9.8 ± 1.3	*	10.2 ± 0.4	**
INORGANIC PHOSPHORUS (mg/dL)	6.3 ± 0.8	6.4 ± 0.9	6.2 ± 1.3		7.4 ± 0.8	**

Mean ± S.D.
Significant difference: * : p≦0.05 ** : p≦0.01 Test of Dunnett

TABLE 8 BIOCHEMISTRY OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Group Name	Control	100 ppm	500 ppm		2500 ppm	
No. of examined animals	29	34	26		4	
TOTAL PROTEIN (g/dL)	5.1 ± 0.9	4.9 ± 0.4	6.0 ± 1.1	**	6.6 ± 0.2	**
ALBUMIN (g/dL)	2.7 ± 0.3	2.8 ± 0.2	3.0 ± 0.2	**	3.7 ± 0.2	**
T-BILIRUBIN (mg/dL)	0.14 ± 0.03	0.16 ± 0.07	0.24 ± 0.16	**	0.58 ± 0.12	**
GLUCOSE (mg/dL)	170 ± 35	160 ± 40	165 ± 46		61 ± 44	**
T-CHOLESTEROL (mg/dL)	76 ± 21	89 ± 23	166 ± 73	**	558 ± 245	**
PHOSPHOLIPID (mg/dL)	137 ± 32	164 ± 35	312 ± 154	**	867 ± 307	**
AST (IU/L)	94 ± 45	105 ± 122	449 ± 824	**	1432 ± 796	**
ALT (IU/L)	36 ± 27	51 ± 62	480 ± 816	**	2115 ± 779	**
LDH (IU/L)	409 ± 395	393 ± 528	2078 ± 4212	**	6228 ± 2802	**
ALP (IU/L)	170 ± 56	200 ± 95	805 ± 738	**	4432 ± 1221	**
G-GTP (IU/L)	1 ± 1	1 ± 1	5 ± 8	**	250 ± 30	**
UREA NITROGEN (mg/dL)	17.5 ± 5.2	15.2 ± 3.0	21.3 ± 9.8		35.5 ± 15.1	*
POTASSIUM (mEq/L)	4.2 ± 0.3	4.2 ± 0.6	4.2 ± 0.6		6.2 ± 1.8	*
CALCIUM (mg/dL)	9.0 ± 0.5	9.0 ± 0.4	9.8 ± 0.7	**	10.3 ± 0.6	**
INORGANIC PHOSPHORUS (mg/dL)	5.7 ± 0.8	5.7 ± 0.9	6.1 ± 1.2		7.9 ± 1.3	**

Mean ± S.D.
Significant difference: * : p≦0.05 ** : p≦0.01 Test of Dunnett

TABLE 9 URINALYSIS OF MALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Group Name		Control	100 ppm	500 ppm	2500 ppm
No. of examined animals		36	36	20	10
	Grade				
Protein	−	0	0	0	1
	±	3	2	5	4
	+	21	18	9	5
	2+	11	14	6	0
	3+	1	2	0	0
	4+	0	0	0	0
	Chi square test				*

Significant difference: * : p≦0.05 ** : p≦0.01

TABLE 10 URINALYSIS OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Group Name		Control	100 ppm	500 ppm	2500 ppm
No. of examined animals		29	34	26	8
	Grade				
Protein	−	0	0	5	1
	±	7	4	7	4
	+	15	19	13	2
	2+	7	11	1	1
	3+	0	0	0	0
	4+	0	0	0	0
	Chi square test			*	
Ketone body	−	3	0	4	0
	±	21	25	17	2
	+	3	8	4	4
	2+	2	1	1	2
	3+	0	0	0	0
	4+	0	0	0	0
	Chi square test				*

Significant difference: * : p≦0.05 ** : p≦0.01

TABLE 11 ORGAN WEIGHTS OF MALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Group Name	Control	100 ppm	500 ppm		2500 ppm	
No. of examined animals	35	35	17		8	
Body weight (g)	47.7 ± 8.5	46.1 ± 9.8	37.2 ± 5.6	**	28.2 ± 1.9	**
Adrenals (g)	0.011 ± 0.002	0.011 ± 0.002	0.013 ± 0.003		0.011 ± 0.002	
Adrenals (%)	0.024 ± 0.008	0.026 ± 0.010	0.035 ± 0.007	**	0.037 ± 0.008	**
Testes (g)	0.227 ± 0.021	0.255 ± 0.130	0.224 ± 0.021		0.199 ± 0.019	*
Testes (%)	0.491 ± 0.102	0.584 ± 0.374	0.614 ± 0.106	**	0.706 ± 0.046	**
Heart (g)	0.219 ± 0.024	0.215 ± 0.027	0.205 ± 0.028		0.179 ± 0.021	**
Heart (%)	0.473 ± 0.107	0.487 ± 0.119	0.557 ± 0.079	*	0.637 ± 0.073	**
Lungs (g)	0.211 ± 0.087	0.220 ± 0.106	0.190 ± 0.020		0.175 ± 0.014	
Lungs (%)	0.459 ± 0.212	0.528 ± 0.387	0.521 ± 0.084	*	0.624 ± 0.068	**
Kidneys (g)	0.595 ± 0.056	0.659 ± 0.270	0.606 ± 0.060		0.545 ± 0.059	
Kidneys (%)	1.281 ± 0.222	1.510 ± 0.841	1.650 ± 0.180	**	1.936 ± 0.160	**
Spleen (g)	0.110 ± 0.083	0.129 ± 0.123	0.270 ± 0.336		0.110 ± 0.042	
Spleen (%)	0.247 ± 0.205	0.308 ± 0.299	0.737 ± 0.888	**	0.393 ± 0.158	*
Liver (g)	2.055 ± 1.074	2.195 ± 0.623	4.652 ± 2.197	**	7.974 ± 1.338	**
Liver (%)	4.682 ± 3.366	5.101 ± 2.348	12.890 ± 6.586	**	28.286 ± 4.120	**
Brain (g)	0.449 ± 0.012	0.449 ± 0.017	0.448 ± 0.013		0.421 ± 0.019	**
Brain (%)	0.975 ± 0.201	1.025 ± 0.260	1.230 ± 0.182	**	1.500 ± 0.127	**

Mean ± S.D.
Significant difference: * : p≦0.05 ** : p≦0.01 Test of Dunnett

TABLE 12 ORGAN WEIGHTS OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Group Name	Control	100 ppm	500 ppm		2500 ppm	
No. of examined animals	29	34	26		5	
Body weight (g)	34.9 ± 5.5	35.5 ± 4.5	30.3 ± 3.5	**	24.4 ± 2.4	**
Ovaries (g)	0.072 ± 0.078	0.069 ± 0.051	0.069 ± 0.066		0.022 ± 0.013	*
Ovaries (%)	0.205 ± 0.220	0.197 ± 0.140	0.223 ± 0.203		0.092 ± 0.053	
Heart (g)	0.164 ± 0.019	0.164 ± 0.024	0.158 ± 0.015		0.166 ± 0.055	
Heart (%)	0.480 ± 0.099	0.471 ± 0.093	0.529 ± 0.076		0.670 ± 0.148	**
Lungs (g)	0.186 ± 0.063	0.208 ± 0.126	0.215 ± 0.168		0.197 ± 0.027	
Lungs (%)	0.562 ± 0.316	0.611 ± 0.446	0.773 ± 0.891	**	0.807 ± 0.104	**
Kidneys (g)	0.417 ± 0.065	0.425 ± 0.074	0.467 ± 0.136		0.422 ± 0.068	
Kidneys (%)	1.220 ± 0.279	1.221 ± 0.291	1.569 ± 0.533	**	1.721 ± 0.142	**
Spleen (g)	0.157 ± 0.108	0.216 ± 0.310	0.260 ± 0.253		0.139 ± 0.073	
Spleen (%)	0.477 ± 0.386	0.616 ± 0.883	0.874 ± 0.901	*	0.550 ± 0.226	
Liver (g)	1.413 ± 0.294	1.599 ± 0.745	3.601 ± 2.252	**	8.152 ± 1.387	**
Liver (%)	4.147 ± 1.235	4.614 ± 2.557	12.174 ± 7.567	**	33.269 ± 3.223	**
Brain (g)	0.461 ± 0.011	0.464 ± 0.014	0.450 ± 0.018	**	0.409 ± 0.006	**
Brain (%)	1.353 ± 0.228	1.329 ± 0.180	1.505 ± 0.183	*	1.688 ± 0.153	**

Mean ± S.D.
Significant difference: * : $p \leqq 0.05$ ** : $p \leqq 0.01$ Test of Dunnett

TABLE 13 INCIDENCES OF SELECTED NEOPLASTIC LESIONS OF MALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Group Name	Control	100 ppm	500 ppm	2500 ppm	Peto	Cochran-
Number of examined animals	50	50	50	50	test	Armitage test
liver	<50>	<50>	<50>	<50>		
hepatocellular adenoma	19 (38 %)	29 (58 %) *	30 (60 %) *	34 (68 %) **	↑ ↑	↑
hepatocellular carcinoma	15 (30 %)	14 (28 %)	20 (40 %)	35 (70 %) **	↑ ↑	↑ ↑
hepatoblastoma	1 (2 %)	6 (12 %)	35 (70 %) **	44 (88 %) **	↑ ↑	↑ ↑
lung	<50>	<50>	<50>	<50>		
bronchiolar-alveolar adenoma	9 (18 %)	8 (16 %)	3 (6 %)	0 (0 %) **		↓ ↓
bronchiolar-alveolar carcinoma	4 (8 %)	10 (20 %)	3 (6 %)	1 (2 %)		↓
lymph node	<50>	<50>	<50>	<50>		
malignant lymphoma	5 (10 %)	7 (14 %)	4 (8 %)	1 (2 %)		↓

Significant difference * : p≦0.05 ** : p≦0.01 Fisher's exact test for neoplastic lesion
↑(↓) : p≦0.05 ↑ ↑(↓ ↓) : p≦0.01 Peto or Cochran-Armitage test for neoplastic lesion
< > : Number of animals examined at the site

TABLE 14 INCIDENCES OF SELECTED NEOPLASTIC LESIONS OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Group Name	Control	100 ppm	500 ppm	2500 ppm	Peto	Cochran-
Number of examined animals	50	50	50	50	test	Armitage test
liver	<50>	<50>	<50>	<50>		
hepatocellular adenoma	8 (16 %)	22 (44 %) **	48 (96 %) **	38 (76 %) **	↑ ↑	↑ ↑
hepatocellular carcinoma	0 (0 %)	3 (6 %)	14 (28 %) **	48 (96 %) **	↑ ↑	↑ ↑
hepatoblastoma	0 (0 %)	0 (0 %)	9 (18 %) **	28 (56 %) **	↑ ↑	↑ ↑
lung	<50>	<50>	<50>	<50>		
bronchiolar-alveolar adenoma	4 (8 %)	4 (8 %)	0 (0 %)	0 (0 %)		↓
pituitary gland	<49>	<50>	<50>	<50>		
adenoma	4 (8 %)	6 (12 %)	2 (4 %)	0 (0 %)		↓
uterus	<50>	<50>	<50>	<50>		
histiocytic sarcoma	15 (30 %)	8 (16 %)	12 (24 %)	6 (12 %) *		
lymph node	<50>	<50>	<50>	<50>		
malignant lymphoma	16 (32 %)	17 (34 %)	17 (34 %)	3 (6 %) **		↓ ↓

Significant difference * : p≦0.05 ** : p≦0.01 Fisher's exact test for neoplastic lesion
↑(↓) : p≦0.05 ↑ ↑(↓ ↓) : p≦0.01 Peto or Cochran-Armitage test for neoplastic lesion
< > : Number of animals examined at the site

TABLE 15 INCIDENCES OF SELECTED NON-NEOPLASTIC LESIONS OF MALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Group Name	Control				100 ppm				500 ppm				2500 ppm			
Number of examined animals	50				50				50				50			
Grade of non-neoplastic lesion	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
lung	<50>				<50>				<50>				<50>			
inflammatory infiltration	0	0	0	0	0	1	0	0	6	0	0	0 *	1	0	0	0
bronchiolar-alveolar cell hyperplasia	0	0	0	0	4	0	0	0	15	0	0	0 **	14	1	0	0 **
bone marrow	<50>				<50>				<50>				<50>			
erythropoiesis:increased	6	0	0	0	3	0	0	0	20	8	0	0 **	24	14	0	0 **
spleen	<50>				<50>				<50>				<50>			
deposit of hemosiderin	7	2	0	0	20	0	0	0 **	18	3	0	0 *	34	6	0	0 **
extramedullary hematopoiesis	12	6	0	0	8	4	2	0	6	22	9	0 **	10	25	4	0 **
salivary gland	<50>				<50>				<50>				<50>			
lymphocytic infiltration	8	0	0	0	3	0	0	0	0	0	0	0 **	1	0	0	0 *
liver	<50>				<50>				<50>				<50>			
granulation	26	1	0	0	11	0	0	0 **	4	5	0	0 **	2	0	0	0 **
hepatocellular hypertrophy:central	0	0	0	0	29	3	0	0 **	2	39	1	0 **	1	22	19	0 **
nuclear enlargement:central	0	0	0	0	0	0	0	0	18	0	0	0 **	6	0	0	0 *
kidney	<50>				<50>				<50>				<50>			
deposit of hemosiderin	0	1	0	0	1	0	2	0	0	9	17	0 **	4	1	27	0 **
adrenal gland	<50>				<50>				<50>				<50>			
spindle-cell hyperplasia	31	0	0	0	29	2	0	0	34	0	0	0	20	0	0	0 *

Grade 1: Slight 2 : Moderate 3 : Marked 4 :Severe
< > : Number of animals examined at the site
Significant difference ; * : $p \leqq 0.05$ ** : $p \leqq 0.01$ Test of Chi Square

TABLE 16 INCIDENCES OF SELECTED NON-NEOPLASTIC LESIONS OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

Group Name	Control				100 ppm				500 ppm				2500 ppm			
Number of examined animals	50				50				50				50			
Grade of non-neoplastic lesion	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
nasal cavity	<50>				<50>				<50>				<50>			
eosinophilic change:olfactory epithelium	8	1	0	0	7	0	0	0	9	2	0	0	24	3	0	0 **
eosinophilic change:respiratoryepithelium	19	10	0	0	22	9	0	0	23	17	0	0 *	21	15	1	0
respiratory metaplasia:olfactory epithelium	3	0	0	0	2	0	0	0	8	0	0	0	20	0	0	0 **
respiratory metaplasia:gland	17	0	0	0	12	1	0	0	19	2	0	0	28	9	0	0 **
lung	<50>				<50>				<50>				<50>			
bronchiolar-alveolar cell hyperplasia	8	0	0	0	11	0	0	0	21	0	0	0 **	17	0	0	0
bone marrow	<50>				<50>				<50>				<50>			
erythropoiesis:increased	3	0	0	0	4	0	0	0	15	1	0	0 **	29	9	0	0 **
spleen	<50>				<50>				<50>				<50>			
deposit of hemosiderin	12	5	0	0	19	4	0	0	23	4	0	0	41	4	0	0 **
extramedullary hematopoiesis	10	3	10	0	7	3	3	0	13	13	8	0 *	9	26	8	0 **
stomach	<50>				<50>				<50>				<50>			
hyperplasia:glandular stomach	11	26	9	0	17	25	7	0	16	24	4	0	20	20	0	0 **
liver	<50>				<50>				<50>				<50>			
granulation	10	14	0	0	11	16	0	0	7	5	0	0 *	0	0	0	0 **
hepatocellular hypertrophy:central	0	0	0	0	0	0	0	0	15	14	0	0 **	1	32	4	0 **
kidney	<50>				<50>				<50>				<50>			
deposit of hemosiderin	0	0	0	0	0	0	0	0	1	0	3	0	2	3	12	0 **
pituitary gland	<50>				<50>				<50>				<50>			
hyperplasia	2	0	0	0	3	0	0	0	0	4	0	0 *	0	0	0	0
adrenal gland	<50>				<50>				<50>				<50>			
spindle-cell hyperplasia	7	39	4	0	9	37	3	0	8	38	1	0	37	10	0	0 **
ovary	<50>				<50>				<50>				<50>			
cyst	8	2	0	0	9	0	0	0	4	0	0	0	0	0	1	0 **
uterus	<50>				<50>				<50>				<50>			
cystic endometrial hyperplasia	18	12	0	0	23	14	1	0	20	7	2	0	4	1	0	0 **

Grade 1: Slight 2 : Moderate 3 : Marked 4 :Severe
< > : Number of animals examined at the site
Significant difference ; * : $p \leqq 0.05$ ** : $p \leqq 0.01$ Test of Chi Square

TABLE 17 HISTORICAL CONTROL DATA OF SELECTED NEOPLASTIC LESIONS IN JAPAN BIOASSAY RESEARCH CENTER : B6D2F1/Crlj MALE MICE

Organs Tumors	No. of animals examined	No. of animals bearing tumor	Incidence (%)	Min. - Max. (%)
Liver	1696			
Hepatocellular adenoma		315	18.6	4 - 36
Hepatocellular carcinoma		326	19.2	2 - 42
Hepatoblastoma		13	0.8	0 - 6

34 carcinogenicity studies examined in Japan Bioassay Research Center were used.
Study No. : 0044, 0060, 0062, 0064, 0066, 0068, 0096, 0105, 0116, 0140, 0159, 0163, 0190, 0206, 0211, 0225, 0243, 0268, 0270, 0279, 0285, 0297, 0319, 0329, 0343, 0348, 0366, 0372, 0402, 0406, 0418, 0422, 0438, 0449

TABLE 18 HISTORICAL CONTROL DATA OF SELECTED NEOPLASTIC LESIONS IN JAPAN BIOASSAY RESEARCH CENTER : B6D2F1/Crlj FEMALE MICE

Organs Tumors	No. of animals examined	No. of animals bearing tumor	Incidence (%)	Min. - Max. (%)
Liver	1697			
Hepatocellular adenoma		102	6.0	0 - 16
Hepatocellular carcinoma		40	2.4	0 - 8

34 carcinogenicity studies examined in Japan Bioassay Research Center were used.
Study No. : 0044, 0060, 0062, 0064, 0066, 0068, 0096, 0105, 0116, 0140, 0159, 0163, 0190, 0206, 0211, 0225, 0243, 0268, 0270, 0279, 0285, 0297, 0319, 0329, 0343, 0348, 0366, 0372, 0402, 0406, 0418, 0422, 0438, 0449

TABLE 19 CAUSE OF DEATH OF MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

	Male				Female			
Group name	Control	100 ppm	500 ppm	2500 ppm	Control	100 ppm	500 ppm	2500 ppm
Number of dead or moribund animals	15	15	33	42	21	16	24	45
No microscopical confirmation	0	1	0	2	0	0	0	0
integumentary system lesion	0	0	1	0	0	0	0	0
cardiovascular lesion	0	1	0	0	0	0	0	0
digestive system lesion	0	0	0	0	0	0	1	0
hemorrhage	0	0	1	0	0	0	0	0
urinary retention	1	3	0	0	0	0	0	0
hydronephrosis	0	1	0	0	0	0	0	0
renal lesion	0	0	0	0	0	1	0	0
Tumor death ： leukemia	3	3	1	0	8	8	7	2
subcutis	1	0	1	0	1	0	1	0
lung	0	1	0	0	0	0	0	0
spleen	0	2	0	0	0	0	0	0
salivary gland	1	0	0	0	0	0	0	0
small intestine	0	0	0	0	1	0	0	0
liver	7	2	29	40	1	1	7	39
adrenal gland	1	0	0	0	0	1	0	0
epididymis	1	1	0	0	0	0	0	0
ovary	—	—	—	—	0	0	0	1
uterus	—	—	—	—	9	5	8	3
peripheral nerves	0	0	0	0	1	0	0	0

FIGURES

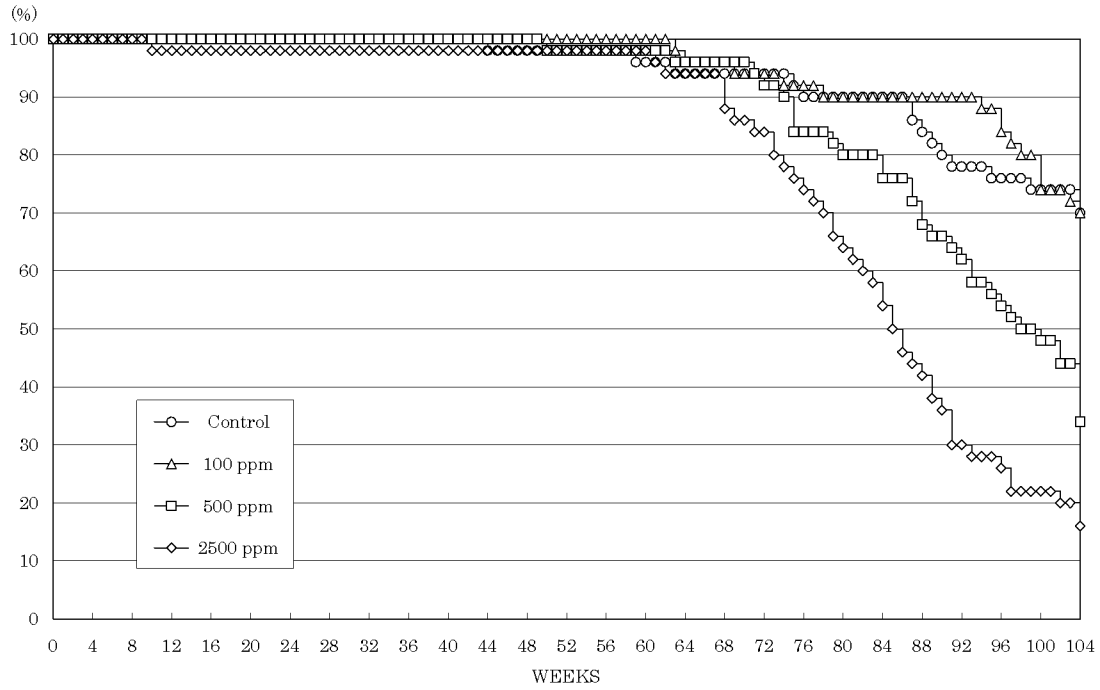


FIGURE 1 SURVIVAL ANIMAL RATE OF MALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

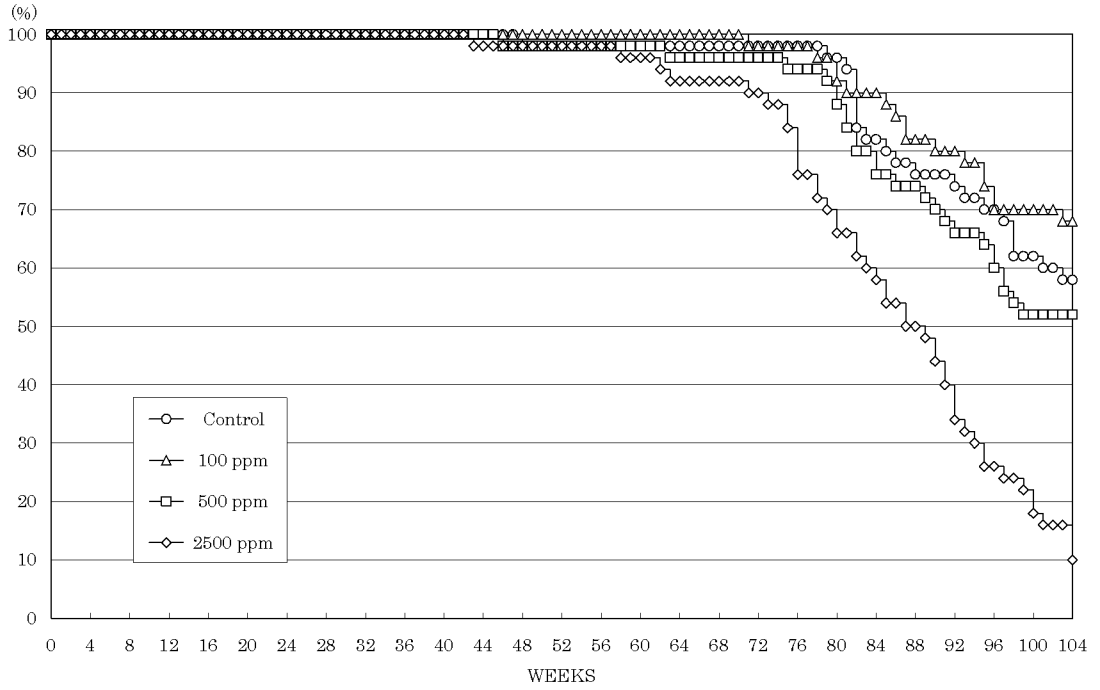


FIGURE 2 SURVIVAL ANIMAL RATE OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

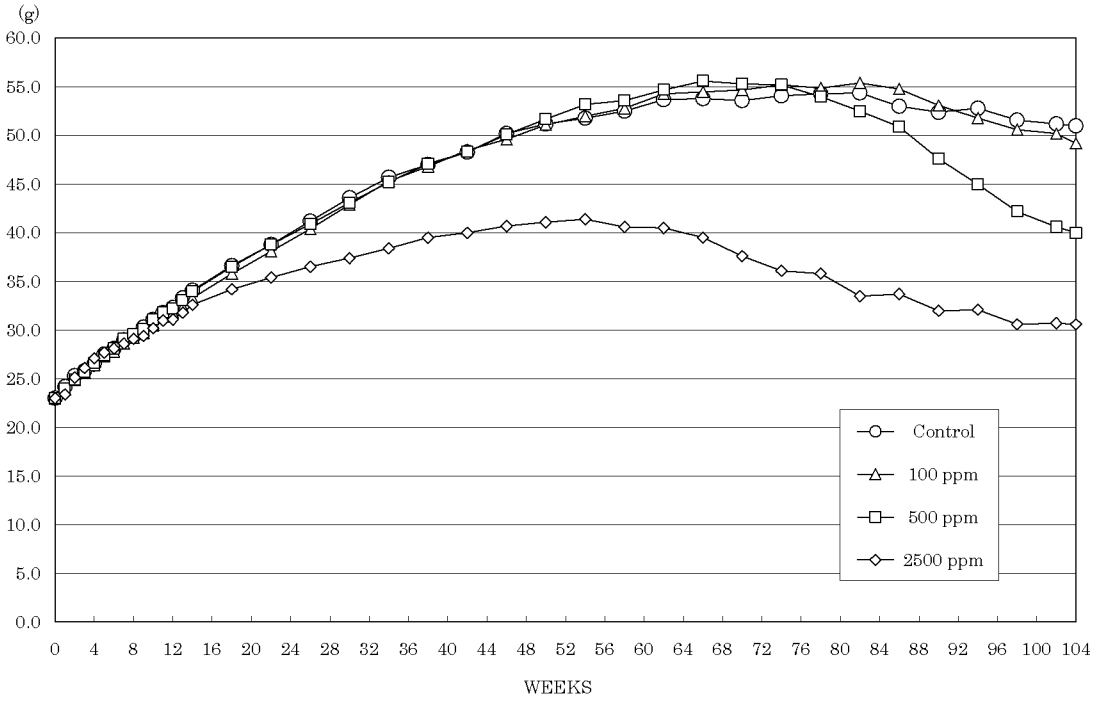


FIGURE 3 BODY WEIGHT CHANGES OF MALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

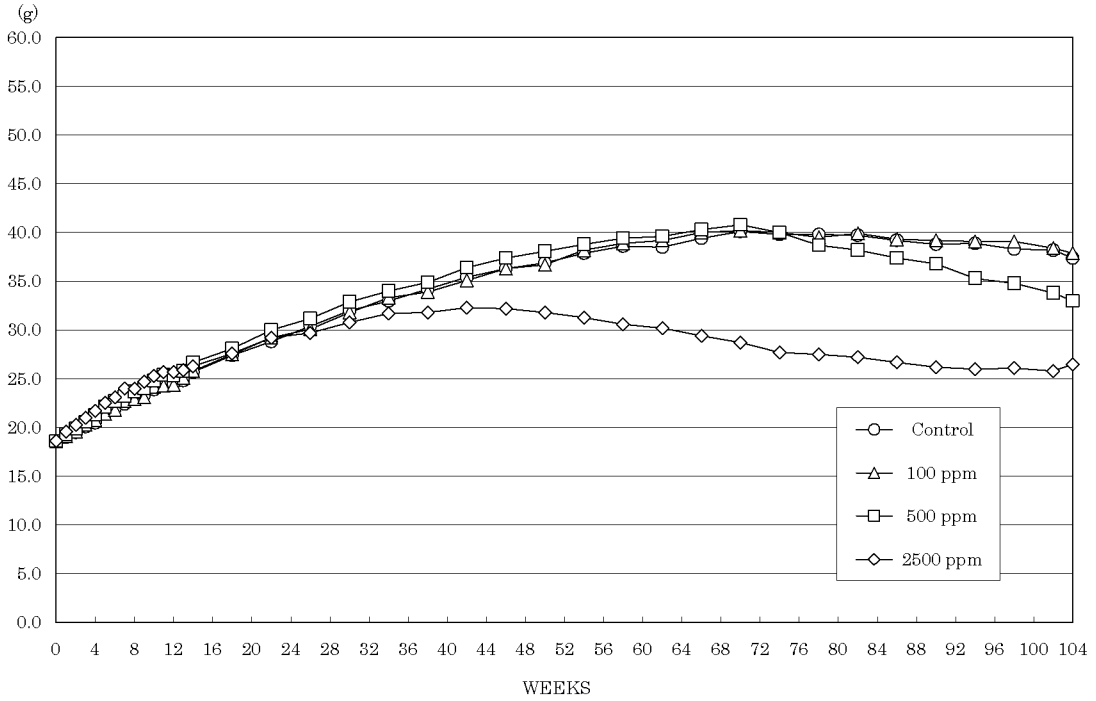


FIGURE 4 BODY WEIGHT CHANGES OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

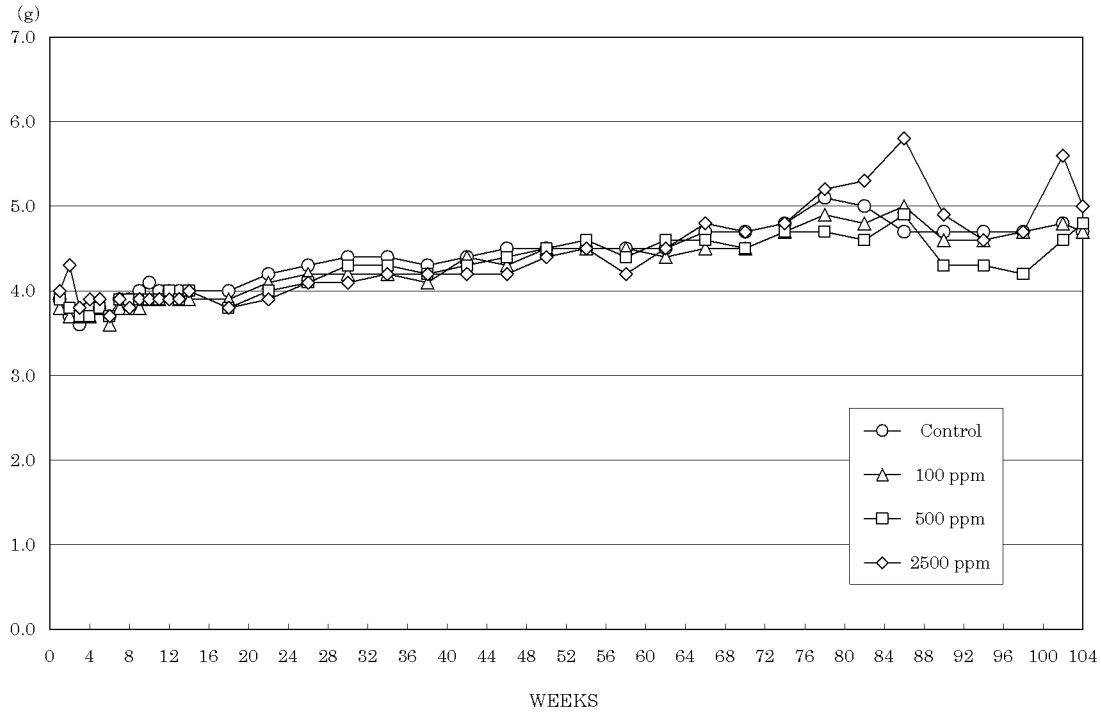


FIGURE 5 FOOD CONSUMPTION CHANGES OF MALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

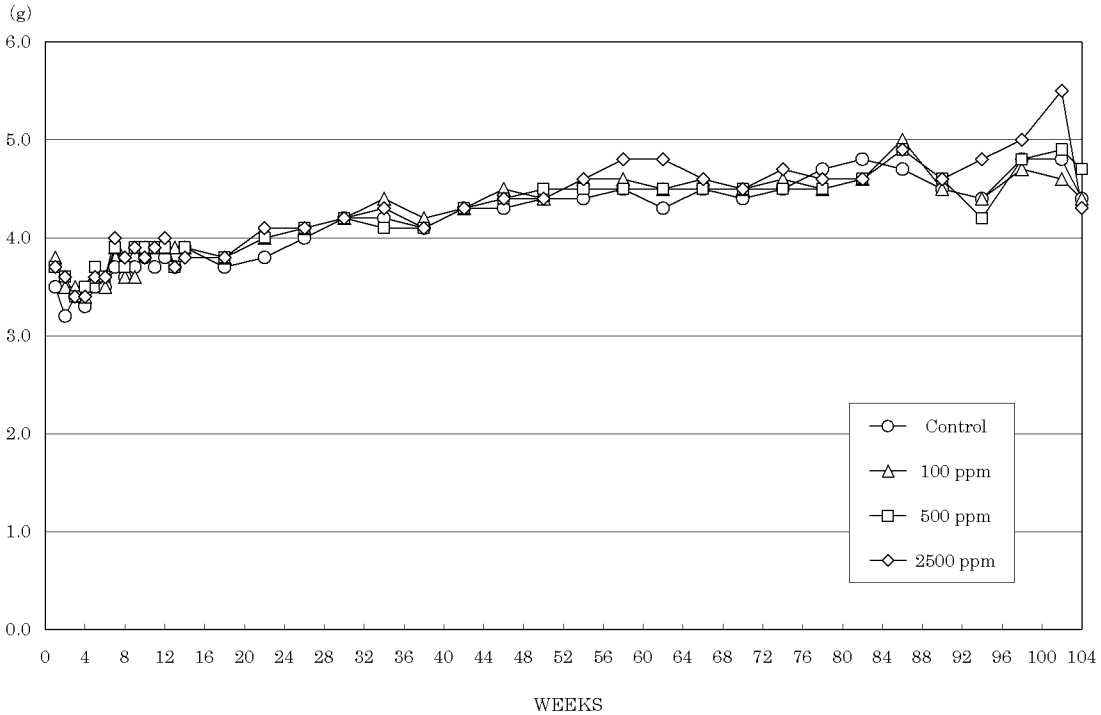
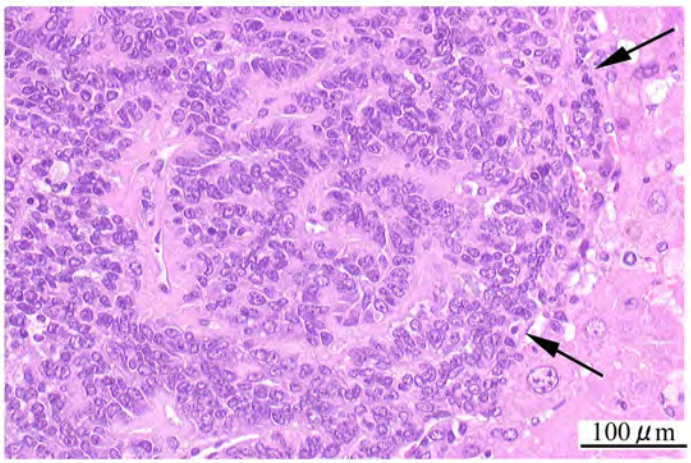
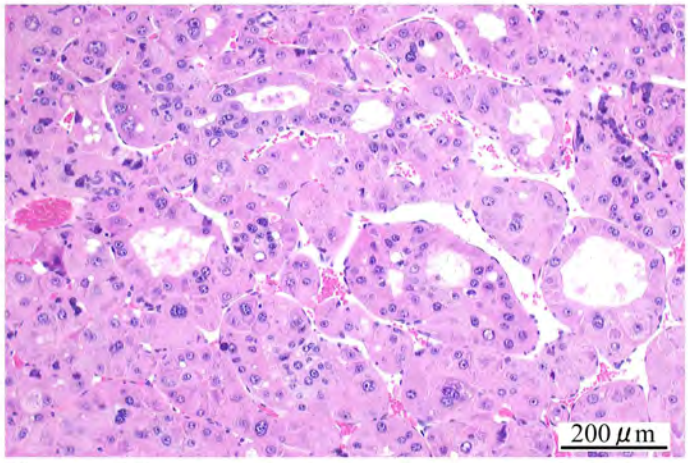


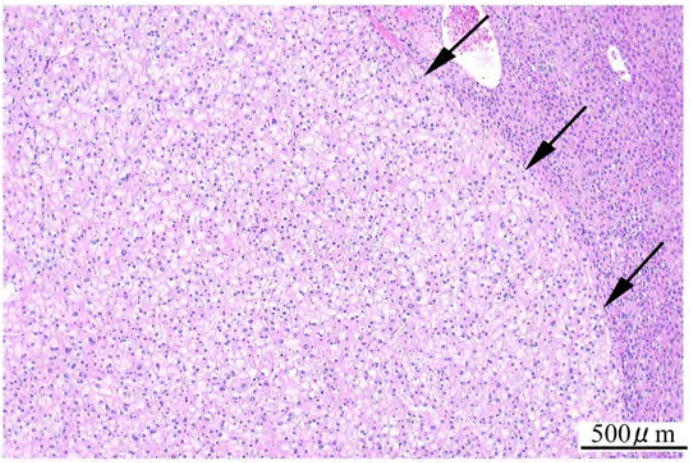
FIGURE 6 FOOD CONSUMPTION CHANGES OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE



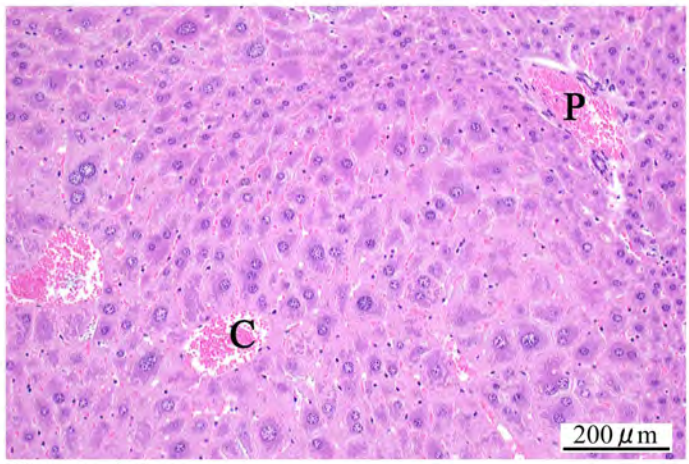
Photograph 1
Liver: Hepatoblostoma (Arrows)
Mouse, Male, 2500 ppm, Animal No. 0462-1302 (H&E)



Photograph 2
Liver: Hepatocellular carcinoma
Mouse, Male, 2500 ppm, Animal No. 0462-1316 (H&E)



Photograph 3
Liver: Hepatocellular adenoma (Arrows)
Mouse, Male, 100 ppm, Animal No. 0462-1118 (H&E)



Photograph 4
Liver: Hepatocellular hypertrophy and nuclear enlargement
Central vein(C), Portal triad(P).
Mouse, Male, 2500 ppm, Animal No. 0462-1307 (H&E)