Summary of Feed Carcinogenicity Study

of 2,4-Dichloro-1-Nitrobenzene

in BDF1 Mice

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Japan Bioassay Research Center

Japan Industrial Safety and Health Association

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 March 25 2005.

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

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Purpose, materials and methods

2,4-Dichloro-1-nitrobenzene (2,4DCNB, CAS No. 611-06-3) is a light yellow crystal with a melting point of 33°C and a boiling point of 258.5°C, and it is insoluble in water.

The carcinogenicity and chronic toxicity of 2,4DCNB were examined by feeding groups of 50 Crj:BDF1 mice of both sexes 2,4DCNB-containing diets for 2 years (104 weeks). The dietary concentration of 2,4DCNB was 0, 750, 1500 or 3000 ppm (w/w) for male mice and 0, 1500, 3000 or 6000 ppm for female mice. The highest dose levels were 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. 2,4DCNB was analyzed for purity and stability by both infrared spectrometry and gas chromatography before and after its use. The 2,4DCNB concentrations in the diet were determined by gas chromatography at the time of preparation, and on the 9th 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 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 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 males fed 1500 and 3000 ppm and the females fed 3000 and 6000 ppm were decreased, and the decreased survival rates were causally related to the increased number of deaths due to malignant liver tumors in the males and due to both malignant liver tumors and peritoneal tumors in the females. Body weights were significantly decreased dose-dependently in the males fed 1500 and 3000 ppm and in all the 2,4DCNB-fed female groups. Growth rates of the 2,4DCNB-fed groups of both sexes were suppressed earlier with an increase in the dose levels. Food consumption was decreased in the 3000 ppm-fed males and in the 6000 ppm-fed females during the early and middle periods of 2-year administration. The yellow urine, which was be colored by a metabolite of 2,4DCNB, was observed in all the 2,4DCNB-fed groups of both sexes throughout the administration period.

The incidences of hepatic tumors (hepatoblastomas, and hepatocellular adenomas and carcinomas) and peritoneal tumors (hemangiosarcomas) were increased dose-dependently in males and females. The malignant liver tumors metastasized to the lung, while the hemangiosarcoma metastasized to the uterus and urinary bladder. The significantly increased incidences of hepatic tumors were noted even in the males fed 750 ppm and above and in the females fed 1500 ppm and above. The increased incidences of peritoneal hemangiosarcomas were observed in the 3000 ppm-fed males and in the females fed 1500 ppm and above. As a pre-neoplastic lesion, the incidence of acidophilic hepatocellular foci was increased in the females fed 3000 ppm and above. As non-neoplastic lesions, the incidence of hepatic lesions (centrilobular hypertrophy of hepatocytes) was increased in all the 2,4DCNB-fed male groups and in the 6000 ppm-fed females. In the nasal cavity, the incidences of pigment deposit and respiratory metaplasia in the olfactory epithelium and submucosal gland were increased in males and females, and incidences of eosinophilic change in the olfactory and respiratory epithelia were increased in females. The incidence of eosinophilic change in the nasopharynx was increased in males and females. Those histopathological changes in the upper respiratory tract appeared in the males fed 750 ppm and above and in the females fed 1500 ppm and above.

Conclusions

In mice, there was clear evidence of carcinogenic activity of 2,4DCNB in males and females, based on the increased incidences of hepatoblastomas, hepatocellular adenomas and carcinomas in the liver and hemangiosarcoma in the peritoneum.

TABLES

TABLES (CONTINUED)

TABLE 1 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES OF MALE MICE IN THE 2-YEAR FEED STUDY OF 2,4-DICHLORO-1-NITROBENZENE

Week on Study	Control		750 ppm			1500 ppm			3000 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	23.7 (50)	50 / 50	23.7 (50)	100	50 / 50	23.7 (50)	100	50 / 50	23.7 (50)	100	50 / 50
1	24.9 (50)	50 / 50	24.7 (50)	99	50 / 50	24.3 (50)	98	50 / 50	23.7 (50)	95	50 / 50
2	25.7 (50)	50 / 50	25.2 (50)	98	50 / 50	24.9 (50)	97	50 / 50	24.3 (50)	95	50 / 50
3	26.0 (50)	50 / 50	25.8 (50)	99	50 / 50	25.7 (50)	99	50 / 50	25.1 (50)	97	50 / 50
4	27.0 (50)	50 / 50	26.6 (50)	99	50 / 50	26.1 (50)	97	50 / 50	25.7 (50)	95	50 / 50
5	27.9 (50)	50 / 50	27.6 (50)	99	50 / 50	27.3 (50)	98	50 / 50	26.5 (50)	95	50 / 50
6	28.7 (50)	50 / 50	28.2 (50)	98	50 / 50	27.7 (50)	97	50 / 50	26.9 (50)	94	50 / 50
7	29.5 (50)	50 / 50	29.0 (50)	98	50 / 50	28.6 (50)	97	50 / 50	27.6 (50)	94	50 / 50
8	30.2 (50)	50 / 50	30.0 (50)	99	50 / 50	29.3 (50)	97	50 / 50	28.2 (50)	93	50 / 50
9	31.2 (50)	50 / 50	30.8 (50)	99	50 / 50	30.1 (50)	96	50 / 50	28.6 (50)	92	50 / 50
10	31.9 (50)	50 / 50	31.5 (50)	99	50 / 50	30.9 (50)	97	50 / 50	29.3 (50)	92	50 / 50
11	32.6 (50)	50 / 50	32.1 (50)	98	50 / 50	31.6 (50)	97	50 / 50	29.8 (50)	91	50 / 50
12	33.2 (50)	50 / 50	32.7 (50)	98	50 / 50	31.9 (50)	96	50 / 50	30.1 (50)	91	50 / 50
13	34.0 (50)	50 / 50	33.4 (50)	98	50 / 50	32.8 (50)	96	50 / 50	30.9 (50)	91	50 / 50
14	35.0 (50)	50 / 50	34.4 (50)	98	50 / 50	33.8 (50)	97	50 / 50	31.7 (50)	91	50 / 50
18	38.0 (50)	50 / 50	36.9 (50)	97	50 / 50	36.5 (50)	96	50 / 50	33.9 (50)	89	50 / 50
22	40.4 (50)	50 / 50	39.7 (50)	98	50 / 50	38.9 (50)	96	50 / 50	36.1 (50)	89	50 / 50
26	43.0 (50)	50 / 50	42.0 (50)	98	50 / 50	40.6 (50)	94	50 / 50	37.7 (50)	88	50 / 50
30	45.2 (50)	50 / 50	44.0 (50)	97	50 / 50	42.2 (50)	93	50 / 50	39.1 (50)	87	50 / 50
34	47.4 (50)	50 / 50	45.9 (50)	97	50 / 50	43.7 (50)	92	50 / 50	40.5 (50)	85	50 / 50
38	48.6 (50)	50 / 50	47.2 (50)	97	50 / 50	45.0 (50)	93	50 / 50	41.4 (50)	85	50 / 50
42	49.9 (50)	50 / 50	48.5 (50)	97	50 / 50	46.9 (50)	94	50 / 50	42.5 (50)	85	50 / 50
46	50.8 (50)	50 / 50	49.8 (50)	98	50 / 50	48.0 (50)	94	50 / 50	43.6 (50)	86	50 / 50
50	51.6 (50)	50 / 50	50.5 (50)	98	50 / 50	48.7 (49)	94	49 / 50	44.1 (50)	85	50 / 50
54	52.6 (49)	49 / 50	51.3 (50)	98	50 / 50	49.2 (49)	94	49 / 50	45.2 (49)	86	49 / 50
58	54.0 (49)	49 / 50	51.4 (50)	95	50 / 50	49.3 (49)	91	49 / 50	45.7 (49)	85	49 / 50
62	53.8 (49)	49 / 50	53.5 (49)	99	49 / 50	50.3 (48)	93	48 / 50	47.2 (48)	88	48 / 50
66	53.4 (48)	48 / 50	53.7 (49)	101	49 / 50	51.5 (47)	96	47 / 50	47.0 (48)	88	48 / 50
70	54.5 (46)	46 / 50	54.8 (49)	101	49 / 50	51.6 (47)	95	47 / 50	47.3 (48)	87	48 / 50
74	54.4 (45)	45 / 50	54.3 (48)	100	48 / 50	51.3 (46)	94	46 / 50	46.9 (46)	86	46 / 50
78	54.9 (44)	44 / 50	55.3 (48)	101	48 / 50	51.9 (46)	95	46 / 50	46.4 (42)	85	42 / 50
82	54.4 (44)	44 / 50	55.8 (48)	103	48 / 50	52.3 (45)	96	45 / 50	47.4 (35)	87	35 / 50
86	53.8 (42)	42 / 50	54.7 (47)	102	47 / 50	51.5 (43)	96	43 / 50	45.0 (32)	84	32 / 50
90	53.8 (40)	40 / 50	55.1 (45)	102	45 / 50	49.7 (43)	92	43 / 50	43.5 (31)	81	31 / 50
94	53.0 (40)	40 / 50	53.8 (45)	102	45 / 50	49.5 (39)	93	39 / 50	42.1 (27)	79	27 / 50
98	51.7 (38)	38 / 50	52.1 (41)	101	41 / 50	46.4 (37)	90	37 / 50	40.2 (24)	78	24 / 50
102	50.9 (37)	37 / 50	50.7 (39)	100	39 / 50	46.4 (31)	91	31 / 50	37.5 (23)	74	23 / 50
104	50.9 (35)	35 / 50	50.8 (38)	100	38 / 50	45.9 (29)	90	29 / 50	37.1 (23)	73	23 / 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 2,4-DICHLORO-1-NITROBENZENE

Week on Study	Control		1500 ppm			3000 ppm			6000 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.
	<49>		<50>			<50>			<50>		
0	19.4 (49)	49 / 49	19.3 (50)	99	50 / 50	19.3 (50)	99	50 / 50	19.3 (50)	99	50 / 50
1	19.9 (49)	49 / 49	19.5 (50)	98	50 / 50	19.4 (50)	97	50 / 50	15.6 (50)	78	50 / 50
2	20.0 (49)	49 / 49	19.8 (50)	99	50 / 50	19.8 (50)	99	50 / 50	18.7 (50)	94	50 / 50
3	20.2 (49)	49 / 49	20.3 (50)	100	50 / 50	20.3 (50)	100	50 / 50	19.4 (50)	96	50 / 50
4	20.8 (49)	49 / 49	20.5 (50)	99	50 / 50	20.6 (50)	99	50 / 50	20.2 (50)	97	50 / 50
5	21.3 (49)	49 / 49	21.3 (50)	100	50 / 50	21.2 (50)	100	50 / 50	20.5 (50)	96	50 / 50
6	21.7 (49)	49 / 49	21.6 (49)	100	49 / 50	21.5 (50)	99	50 / 50	20.4 (50)	94	50 / 50
7	22.1 (49)	49 / 49	22.2 (49)	100	49 / 50	22.0 (50)	100	50 / 50	20.6 (50)	93	50 / 50
8	22.9 (49)	49 / 49	22.5 (49)	98	49 / 50	22.6 (50)	99	50 / 50	20.8 (50)	91	50 / 50
9	23.4 (49)	49 / 49	23.1 (49)	99	49 / 50	23.0 (50)	98	50 / 50	20.7 (50)	88	50 / 50
10	23.7 (49)	49 / 49	23.4 (49)	99	49 / 50	23.4 (50)	99	50 / 50	20.9 (50)	88	50 / 50
11	23.9 (49)	49 / 49	23.7 (49)	99	49 / 50	23.5 (50)	98	50 / 50	21.3 (50)	89	50 / 50
12	23.9 (49)	49 / 49	23.5 (49)	98	49 / 50	23.7 (50)	99	50 / 50	21.4 (50)	90	50 / 50
13	24.8 (49)	49 / 49	24.2 (49)	98	49 / 50	24.2 (50)	98	50 / 50	21.7 (50)	88	50 / 50
14	25.0 (49)	49 / 49	24.9 (49)	100	49 / 50	24.6 (50)	98	50 / 50	21.9 (50)	88	50 / 50
18	27.0 (49)	49 / 49	26.0 (49)	96	49 / 50	25.9 (50)	96	50 / 50	22.6 (50)	84	50 / 50
22	29.0 (49)	49 / 49	27.9 (49)	96	49 / 50	27.1 (50)	93	50 / 50	23.4 (50)	81	50 / 50
26	29.9 (49)	49 / 49	29.1 (49)	97	49 / 50	28.1 (50)	94	50 / 50	24.0 (50)	80	50 / 50
30	31.5 (49)	49 / 49	30.6 (48)	97	48 / 50	28.6 (50)	91	50 / 50	24.7 (50)	78	50 / 50
34	33.2 (49)	49 / 49	31.6 (48)	95	48 / 50	29.8 (50)	90	50 / 50	25.1 (50)	76	50 / 50
38	34.7 (49)	49 / 49	32.8 (48)	95	48 / 50	30.7 (50)	88	50 / 50	25.4 (50)	73	50 / 50
42	36.2 (49)	49 / 49	34.1 (48)	94	48 / 50	31.7 (50)	88	50 / 50	26.0 (50)	72	50 / 50
46	37.3 (48)	48 / 49	36.0 (47)	97	47 / 50	32.4 (50)	87	50 / 50	26.5 (49)	71	49 / 50
50	38.2 (48)	48 / 49	36.7 (47)	96	47 / 50	33.0 (50)	86	50 / 50	26.2 (49)	69	49 / 50
54	38.5 (48)	48 / 49	37.7 (46)	98	46 / 50	33.8 (50)	88	50 / 50	26.8 (48)	70	48 / 50
58	39.6 (47)	47 / 49	37.6 (46)	95	46 / 50	34.1 (50)	86	50 / 50	27.0 (48)	68	48 / 50
62	40.4 (47)	47 / 49	38.7 (46)	96	46 / 50	34.3 (50)	85	50 / 50	27.3 (47)	68	47 / 50
66	40.6 (46)	46 / 49	39.3 (46)	97	46 / 50	34.7 (49)	85	49 / 50	27.2 (47)	67	47 / 50
70	40.7 (46)	46 / 49	39.0 (46)	96	46 / 50	35.2 (47)	86	47 / 50	27.2 (46)	67	46 / 50
74	41.3 (46)	46 / 49	39.0 (45)	94	45 / 50	35.4 (46)	86	46 / 50	27.1 (46)	66	46 / 50
78	42.2 (44)	44 / 49	39.2 (45)	93	45 / 50	35.2 (46)	83	46 / 50	26.6 (42)	63	42 / 50
82	41.7 (44)	44 / 49	38.9 (44)	93	44 / 50	34.6 (45)	83	45 / 50	26.5 (36)	64	36 / 50
86	41.0 (44)	44 / 49	38.4 (42)	94	42 / 50	33.7 (42)	82	42 / 50	26.1 (31)	64	31 / 50
90	40.2 (43)	43 / 49	37.9 (40)	94	40 / 50	32.6 (37)	81	37 / 50	25.7 (28)	64	28 / 50
94	40.4 (39)	39 / 49	37.8 (37)	94	37 / 50	32.6 (31)	81	31 / 50	25.3 (25)	63	25 / 50
98	40.7 (34)	34 / 49	37.3 (32)	92	32 / 50	31.4 (24)	77	24 / 50	24.8 (21)	61	21 / 50
102	40.5 (32)	32 / 49	36.7 (29)	91	29 / 50	31.3 (20)	77	20 / 50	24.6 (20)	61	20 / 50
104	40.3 (28)	28 / 49	36.9 (28)	92	28 / 50	30.8 (18)	76	18 / 50	24.2 (19)	60	19 / 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 2,4-DICHLORO-1-NITROBENZENE

Week on Study	Control		750 ppm			1500 ppm			3000 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	4.1 (49)	50 / 50	4.1 (50)	100	50 / 50	3.9 (50)	95	50 / 50	3.8 (49)	93	50 / 50
2	3.9 (50)	50 / 50	3.7 (50)	95	50 / 50	3.7 (50)	95	50 / 50	3.8 (48)	97	50 / 50
3	3.8 (48)	50 / 50	3.8 (50)	100	50 / 50	3.9 (49)	103	50 / 50	3.8 (47)	100	50 / 50
4	3.9 (50)	50 / 50	3.8 (50)	97	50 / 50	3.8 (50)	97	50 / 50	3.9 (50)	100	50 / 50
5	4.0 (50)	50 / 50	4.0 (50)	100	50 / 50	4.1 (50)	103	50 / 50	3.9 (49)	98	50 / 50
6	4.2 (49)	50 / 50	4.0 (50)	95	50 / 50	3.9 (49)	93	50 / 50	3.8 (48)	90	50 / 50
7	4.2 (50)	50 / 50	4.1 (50)	98	50 / 50	4.1 (49)	98	50 / 50	3.9 (49)	93	50 / 50
8	4.1 (50)	50 / 50	4.0 (50)	98	50 / 50	4.0 (50)	98	50 / 50	3.8 (50)	93	50 / 50
9	4.2 (50)	50 / 50	4.0 (50)	95	50 / 50	4.2 (50)	100	50 / 50	3.8 (50)	90	50 / 50
10	4.2 (49)	50 / 50	4.1 (50)	98	50 / 50	4.2 (50)	100	50 / 50	3.9 (50)	93	50 / 50
11	4.1 (50)	50 / 50	3.9 (50)	95	50 / 50	4.0 (50)	98	50 / 50	3.8 (50)	93	50 / 50
12	4.2 (50)	50 / 50	4.0 (50)	95	50 / 50	4.1 (50)	98	50 / 50	3.8 (50)	90	50 / 50
13	4.1 (50)	50 / 50	3.9 (50)	95	50 / 50	4.1 (50)	100	50 / 50	3.9 (50)	95	50 / 50
14	4.3 (50)	50 / 50	4.0 (50)	93	50 / 50	4.1 (50)	95	50 / 50	3.9 (50)	91	50 / 50
18	4.2 (50)	50 / 50	3.9 (50)	93	50 / 50	4.0 (50)	95	50 / 50	3.7 (50)	88	50 / 50
22	4.1 (49)	50 / 50	3.9 (50)	95	50 / 50	4.1 (49)	100	50 / 50	3.9 (50)	95	50 / 50
26	4.3 (50)	50 / 50	4.1 (50)	95	50 / 50	4.1 (50)	95	50 / 50	4.0 (50)	93	50 / 50
30	4.2 (50)	50 / 50	4.0 (50)	95	50 / 50	4.1 (50)	98	50 / 50	3.9 (50)	93	50 / 50
34	4.6 (50)	50 / 50	4.2 (50)	91	50 / 50	4.2 (49)	91	50 / 50	4.1 (50)	89	50 / 50
38	4.7 (50)	50 / 50	4.3 (50)	91	50 / 50	4.2 (50)	89	50 / 50	4.1 (50)	87	50 / 50
42	4.6 (45)	50 / 50	4.3 (48)	93	50 / 50	4.3 (50)	93	50 / 50	4.0 (50)	87	50 / 50
46	4.4 (50)	50 / 50	4.2 (50)	95	50 / 50	4.4 (47)	100	50 / 50	4.1 (50)	93	50 / 50
50	4.7 (49)	50 / 50	4.5 (50)	96	50 / 50	4.5 (49)	96	49 / 50	4.3 (50)	91	50 / 50
54	4.7 (42)	49 / 50	4.5 (46)	96	50 / 50	4.4 (43)	94	49 / 50	4.5 (46)	96	49 / 50
58	5.0 (48)	49 / 50	4.5 (50)	90	50 / 50	4.4 (49)	88	49 / 50	4.4 (48)	88	49 / 50
62	4.9 (49)	49 / 50	4.9 (49)	100	49 / 50	4.7 (48)	96	48 / 50	4.6 (46)	94	48 / 50
66	4.8 (45)	48 / 50	4.9 (48)	102	49 / 50	4.9 (47)	102	47 / 50	4.7 (47)	98	48 / 50
70	5.0 (42)	46 / 50	4.9 (48)	98	49 / 50	4.7 (44)	94	47 / 50	4.7 (47)	94	48 / 50
74	5.0 (44)	45 / 50	5.0 (48)	100	48 / 50	5.0 (46)	100	46 / 50	4.6 (45)	92	46 / 50
78	5.1 (43)	44 / 50	4.9 (45)	96	48 / 50	4.8 (42)	94	46 / 50	4.5 (42)	88	42 / 50
82	5.2 (43)	44 / 50	5.0 (48)	96	48 / 50	5.0 (43)	96	45 / 50	4.6 (35)	88	35 / 50
86	5.1 (41)	42 / 50	4.8 (45)	94	47 / 50	4.9 (43)	96	43 / 50	4.6 (31)	90	32 / 50
90	5.1 (38)	40 / 50	4.7 (45)	92	45 / 50	4.6 (41)	90	43 / 50	4.8 (31)	94	31 / 50
94	5.2 (38)	40 / 50	4.8 (44)	92	45 / 50	4.9 (39)	94	39 / 50	4.9 (26)	94	27 / 50
98	5.0 (32)	38 / 50	4.8 (40)	96	41 / 50	4.7 (34)	94	37 / 50	5.0 (22)	100	24 / 50
102	5.1 (36)	37 / 50	4.8 (39)	94	39 / 50	4.8 (29)	94	31 / 50	4.7 (20)	92	23 / 50
104	5.4 (32)	35 / 50	5.0 (37)	93	38 / 50	5.2 (29)	96	29 / 50	5.3 (20)	98	23 / 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 2,4-DICHLORO-1-NITROBENZENE

Week on Study	Control		1500 ppm			3000 ppm			6000 ppm		
	Av. FC.	No. of Surviv.	Av. FC.	% of cont.	No. of Surviv.	Av. FC.	% of cont.	No. of Surviv.	Av. FC.	% of cont.	No. of Surviv.
	<49>		<50>			<50>			<50>		
1	3.6 (49)	49 / 49	3.5 (50)	97	50 / 50	3.5 (49)	97	50 / 50	2.7 (45)	75	50 / 50
2	3.4 (49)	49 / 49	3.3 (50)	97	50 / 50	3.5 (50)	103	50 / 50	4.5 (37)	132	50 / 50
3	3.4 (49)	49 / 49	3.4 (50)	100	50 / 50	3.3 (50)	97	50 / 50	3.3 (42)	97	50 / 50
4	3.5 (49)	49 / 49	3.4 (50)	97	50 / 50	3.2 (50)	91	50 / 50	3.4 (50)	97	50 / 50
5	3.5 (49)	49 / 49	3.6 (50)	103	50 / 50	3.4 (50)	97	50 / 50	3.2 (48)	91	50 / 50
6	3.5 (49)	49 / 49	3.5 (48)	100	49 / 50	3.3 (50)	94	50 / 50	3.0 (44)	86	50 / 50
7	3.6 (49)	49 / 49	3.6 (49)	100	49 / 50	3.5 (48)	97	50 / 50	3.4 (47)	94	50 / 50
8	3.5 (49)	49 / 49	3.6 (49)	103	49 / 50	3.4 (50)	97	50 / 50	3.2 (50)	91	50 / 50
9	3.6 (49)	49 / 49	3.6 (49)	100	49 / 50	3.5 (50)	97	50 / 50	3.3 (49)	92	50 / 50
10	3.6 (49)	49 / 49	3.7 (49)	103	49 / 50	3.5 (50)	97	50 / 50	3.1 (49)	86	50 / 50
11	3.5 (49)	49 / 49	3.6 (49)	103	49 / 50	3.3 (50)	94	50 / 50	3.1 (49)	89	50 / 50
12	3.7 (49)	49 / 49	3.5 (49)	95	49 / 50	3.4 (50)	92	50 / 50	3.1 (50)	84	50 / 50
13	3.6 (47)	49 / 49	3.6 (49)	100	49 / 50	3.4 (50)	94	50 / 50	3.2 (50)	89	50 / 50
14	3.6 (49)	49 / 49	3.7 (49)	103	49 / 50	3.5 (50)	97	50 / 50	3.1 (50)	86	50 / 50
18	3.6 (49)	49 / 49	3.5 (49)	97	49 / 50	3.5 (50)	97	50 / 50	3.2 (50)	89	50 / 50
22	4.0 (49)	49 / 49	3.9 (49)	98	49 / 50	3.6 (50)	90	50 / 50	3.2 (50)	80	50 / 50
26	3.9 (49)	49 / 49	4.0 (49)	103	49 / 50	3.7 (50)	95	50 / 50	3.4 (47)	87	50 / 50
30	3.9 (49)	49 / 49	4.0 (48)	103	48 / 50	3.6 (50)	92	50 / 50	3.4 (50)	87	50 / 50
34	4.2 (49)	49 / 49	4.1 (48)	98	48 / 50	3.9 (50)	93	50 / 50	3.7 (50)	88	50 / 50
38	4.1 (49)	49 / 49	3.9 (48)	95	48 / 50	3.9 (50)	95	50 / 50	3.5 (50)	85	50 / 50
42	4.0 (49)	49 / 49	3.9 (48)	98	48 / 50	3.8 (50)	95	50 / 50	3.8 (48)	95	50 / 50
46	3.9 (48)	48 / 49	4.2 (47)	108	47 / 50	3.9 (50)	100	50 / 50	3.7 (49)	95	49 / 50
50	4.2 (48)	48 / 49	4.2 (47)	100	47 / 50	3.9 (50)	93	50 / 50	3.8 (47)	90	49 / 50
54	4.2 (48)	48 / 49	4.2 (44)	100	46 / 50	4.0 (46)	95	50 / 50	3.8 (47)	90	48 / 50
58	4.1 (47)	47 / 49	4.0 (46)	98	46 / 50	4.1 (50)	100	50 / 50	4.0 (46)	98	48 / 50
62	4.1 (47)	47 / 49	4.0 (46)	98	46 / 50	3.8 (50)	93	50 / 50	4.2 (46)	102	47 / 50
66	4.5 (46)	46 / 49	4.2 (46)	93	46 / 50	4.1 (49)	91	49 / 50	4.3 (44)	96	47 / 50
70	4.4 (46)	46 / 49	4.3 (46)	98	46 / 50	4.2 (47)	95	47 / 50	4.4 (42)	100	46 / 50
74	4.3 (46)	46 / 49	4.2 (45)	98	45 / 50	4.2 (46)	98	46 / 50	4.6 (43)	107	46 / 50
78	4.6 (44)	44 / 49	4.4 (45)	96	45 / 50	4.5 (46)	98	46 / 50	4.5 (34)	98	42 / 50
82	4.4 (44)	44 / 49	4.1 (44)	93	44 / 50	4.2 (44)	95	45 / 50	4.4 (27)	100	36 / 50
86	4.2 (43)	44 / 49	4.2 (42)	100	42 / 50	4.3 (40)	102	42 / 50	4.8 (27)	114	31 / 50
90	4.3 (43)	43 / 49	4.2 (40)	98	40 / 50	3.9 (37)	91	37 / 50	4.5 (21)	105	28 / 50
94	4.4 (39)	39 / 49	4.3 (37)	98	37 / 50	4.6 (31)	105	31 / 50	4.8 (16)	109	25 / 50
98	4.7 (33)	34 / 49	4.2 (32)	89	32 / 50	4.6 (23)	98	24 / 50	5.4 (14)	115	21 / 50
102	4.7 (32)	32 / 49	4.2 (29)	89	29 / 50	5.4 (19)	115	20 / 50	5.4 (9)	115	20 / 50
104	4.6 (27)	28 / 49	4.8 (28)	104	28 / 50	5.3 (16)	115	18 / 50	6.2 (13)	135	19 / 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 2,4-DICHLORO-1-NITROBENZENE

Group Name	Control	750 ppm	1500 ppm	3000 ppm
No. of examined animals	33	36	29	20
RED BLOOD CELL (10^6/μL)	9.68 ± 0.87	9.69 ± 1.19	9.28 ± 2.07	8.22 ± 1.92 *
HEMOGLOBIN (g/dL)	13.4 ± 1.2	13.2 ± 1.7	12.4 ± 2.8	11.3 ± 2.5 *
MCV (fL)	42.4 ± 1.6	42.5 ± 3.0	42.3 ± 3.1	44.7 ± 4.1 *
MCHC (g/dL)	32.7 ± 0.7	32.1 ± 1.0 *	31.6 ± 1.2 **	31.1 ± 2.0 **
Differential WBC (%)				
N-BAND	1 ± 1	1 ± 1	2 ± 4 *	3 ± 5 *

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 2,4-DICHLORO-1-NITROBENZENE

Group Name	Control	1500 ppm	3000 ppm	6000 ppm
No. of examined animals	27	26	17	18
MCV (fL)	43.5 ± 3.6	43.7 ± 4.6	44.8 ± 7.9	41.7 ± 1.5 **
MCH (pg)	14.0 ± 0.6	13.7 ± 0.7	13.7 ± 1.6 *	13.3 ± 0.3 **
MCHC (g/dL)	32.3 ± 1.8	31.6 ± 2.4	30.9 ± 1.8 **	31.9 ± 1.1
Differential WBC (%)				
N-SEG	23 ± 10	20 ± 10	28 ± 11	35 ± 15 **

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 2,4-DICHLORO-1-NITROBENZENE

Group Name	Control	750 ppm	1500 ppm	3000 ppm
No. of examined animals	34	36	29	21
ALBUMIN (g/dL)	2.8 ± 0.4	2.8 ± 0.4	3.0 ± 0.5	3.1 ± 0.4 *
T-BILIRUBIN (mg/dL)	0.11 ± 0.02	0.13 ± 0.06	0.14 ± 0.05 *	0.24 ± 0.17 **
GLUCOSE (mg/dL)	200 ± 26	187 ± 54	183 ± 47	166 ± 45 **
T-CHOLESTEROL (mg/dL)	119 ± 59	133 ± 44 *	149 ± 75 *	179 ± 78 **
TRIGLYCERIDE (mg/dL)	40 ± 18	43 ± 25	38 ± 26	26 ± 14 *
PHOSPHOLIPID (mg/dL)	213 ± 78	231 ± 67	267 ± 120 *	342 ± 148 **
GOT (IU/L)	60 ± 20	134 ± 179 *	470 ± 1254 **	568 ± 789 **
GPT (IU/L)	47 ± 51	100 ± 117 *	545 ± 1593 **	575 ± 856 **
LDH (IU/L)	258 ± 98	851 ± 2586	1932 ± 3818 **	6313 ± 8684 **
ALP (IU/L)	143 ± 154	179 ± 142 *	468 ± 727 **	952 ± 1025 **
G-GTP (IU/L)	2 ± 1	2 ± 1	3 ± 6	5 ± 5 **
CPK (IU/L)	48 ± 28	70 ± 91	87 ± 149 **	95 ± 99 **
SODIUM (mEq/L)	152 ± 1	153 ± 2	153 ± 2	154 ± 2 **

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 2,4-DICHLORO-1-NITROBENZENE

Group Name	Control	1500 ppm	3000 ppm	6000 ppm
No. of examined animals	27	27	17	18
TOTAL PROTEIN (g/dL)	4.8 ± 0.8	4.9 ± 0.6	5.1 ± 0.9 *	5.8 ± 0.4 **
ALBUMIN (g/dL)	2.7 ± 0.3	2.9 ± 0.4	3.1 ± 0.5 **	3.5 ± 0.2 **
A/G RATIO	1.3 ± 0.3	1.5 ± 0.3 *	1.6 ± 0.2 **	1.5 ± 0.2
T-BILIRUBIN (mg/dL)	0.13 ± 0.10	0.17 ± 0.11	0.19 ± 0.11	0.24 ± 0.09 **
GLUCOSE (mg/dL)	163 ± 35	158 ± 38	152 ± 31	132 ± 33 *
T-CHOLESTEROL (mg/dL)	74 ± 16	105 ± 29 *	154 ± 70 **	269 ± 89 **
TRIGLYCERIDE (mg/dL)	32 ± 15	37 ± 23	24 ± 11	20 ± 10 *
PHOSPHOLIPID (mg/dL)	138 ± 26	201 ± 57 **	299 ± 137 **	466 ± 152 **
GOT (IU/L)	124 ± 208	203 ± 294	352 ± 384 *	342 ± 284 *
GPT (IU/L)	59 ± 105	174 ± 352	356 ± 531 **	696 ± 659 **
LDH (IU/L)	1208 ± 4813	1647 ± 4070 *	3378 ± 5731 **	2268 ± 1957 **
ALP (IU/L)	179 ± 43	500 ± 629 **	972 ± 1441 **	1592 ± 1056 **
G-GTP (IU/L)	1 ± 1	3 ± 5	11 ± 12 **	58 ± 59 **
CPK (IU/L)	150 ± 345	157 ± 173 *	186 ± 155 **	139 ± 76 **
UREA NITROGEN (mg/dL)	18.7 ± 13.5	24.6 ± 22.1	23.8 ± 14.0 **	23.7 ± 5.4 **
CALCIUM (mg/dL)	8.8 ± 0.4	9.0 ± 0.5	9.2 ± 0.5 *	9.9 ± 0.6 **

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 2,4-DICHLORO-1-NITROBENZENE

Group name		Control	750 ppm	1500 ppm	3000 ppm
Number of examined animals		37	38	30	23
	Grade				
pH	5.0	0	0	0	0
	6.0	5	3	5	2
	6.5	14	11	2	3
	7.0	10	14	6	7
	7.5	8	7	10	7
	8.0	0	1	7	3
	8.5	0	2	0	1
	Chi square test			**	
Protein	−	0	1	1	1
	±	10	12	18	17
	+	19	22	7	5
	2+	7	3	4	0
	3+	1	0	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 2,4-DICHLORO-1-NITROBENZENE

Group name		Control	1500 ppm	3000 ppm	6000 ppm
Number of examined animals		29	29	18	19
	Grade				
Protein	−	0	0	2	7
	±	10	16	11	11
	+	15	7	5	1
	2+	4	5	0	0
	3+	0	1	0	0
	4+	0	0	0	0
	Chi square test			*	**
Ketone body	−	7	3	2	3
	±	19	10	7	8
	+	2	10	6	6
	2+	1	6	3	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 2,4-DICHLORO-1-NITROBENZENE

Group Name	Control	750 ppm	1500 ppm	3000 ppm
No. of examined animals	35	38	29	23
Body weight (g)	48.0 ± 9.2	47.5 ± 8.2	43.2 ± 8.3	34.2 ± 4.7 **
Adrenals (g)	0.014 ± 0.004	0.013 ± 0.004	0.012 ± 0.004	0.014 ± 0.005
Adrenals (%)	0.031 ± 0.011	0.029 ± 0.012	0.028 ± 0.011	0.040 ± 0.012 *
Testes (g)	0.236 ± 0.039	0.228 ± 0.030	0.230 ± 0.020	0.220 ± 0.031
Testes (%)	0.510 ± 0.135	0.497 ± 0.134	0.554 ± 0.121	0.654 ± 0.113 **
Heart (g)	0.222 ± 0.023	0.231 ± 0.043	0.223 ± 0.023	0.213 ± 0.029
Heart (%)	0.476 ± 0.081	0.502 ± 0.137	0.531 ± 0.102 *	0.630 ± 0.091 **
Lungs (g)	0.226 ± 0.042	0.264 ± 0.172	0.223 ± 0.042	0.213 ± 0.029
Lungs (%)	0.498 ± 0.174	0.636 ± 0.799	0.542 ± 0.179	0.632 ± 0.109 **
Kidneys (g)	0.619 ± 0.098	0.631 ± 0.080	1.154 ± 2.863	0.629 ± 0.120
Kidneys (%)	1.328 ± 0.304	1.368 ± 0.307	2.703 ± 6.545 *	1.850 ± 0.280 **
Spleen (g)	0.092 ± 0.064	0.108 ± 0.098	0.092 ± 0.034	0.113 ± 0.074
Spleen (%)	0.196 ± 0.137	0.248 ± 0.257	0.226 ± 0.116	0.331 ± 0.201 **
Liver (g)	1.858 ± 0.768	2.150 ± 0.736	3.238 ± 2.168 **	5.249 ± 3.279 **
Liver (%)	4.117 ± 2.669	4.680 ± 1.994	8.252 ± 6.564 **	15.710 ± 9.670 **
Brain (g)	0.452 ± 0.016	0.448 ± 0.021	0.446 ± 0.021	0.439 ± 0.021
Brain (%)	0.984 ± 0.244	0.981 ± 0.234	1.069 ± 0.201	1.303 ± 0.149 **

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 2,4-DICHLORO-1-NITROBENZENE

Group Name	Control	1500 ppm		3000 ppm		6000 ppm	
No. of examined animals	28	28		18		19	
Body weight (g)	38.0 ± 7.1	34.6 ± 4.8		28.5 ± 3.2	**	22.5 ± 2.7	**
Adrenals (g)	0.015 ± 0.005	0.014 ± 0.004		0.013 ± 0.003		0.011 ± 0.003	**
Adrenals (%)	0.040 ± 0.014	0.042 ± 0.011		0.046 ± 0.013		0.052 ± 0.014	*
Ovaries (g)	0.060 ± 0.043	0.276 ± 1.148		0.081 ± 0.098		0.022 ± 0.009	**
Ovaries (%)	0.163 ± 0.116	0.856 ± 3.600		0.276 ± 0.304		0.097 ± 0.037	
Heart (g)	0.180 ± 0.038	0.171 ± 0.023		0.156 ± 0.015	*	0.143 ± 0.019	**
Heart (%)	0.490 ± 0.149	0.507 ± 0.127		0.552 ± 0.073	*	0.635 ± 0.051	**
Lungs (g)	0.246 ± 0.178	0.223 ± 0.053		0.213 ± 0.059		0.203 ± 0.055	
Lungs (%)	0.763 ± 1.014	0.657 ± 0.177		0.759 ± 0.255	**	0.909 ± 0.261	**
Kidneys (g)	0.430 ± 0.058	0.541 ± 0.574		0.422 ± 0.060		0.374 ± 0.073	**
Kidneys (%)	1.167 ± 0.261	1.628 ± 1.905		1.490 ± 0.234	**	1.661 ± 0.221	**
Spleen (g)	0.154 ± 0.113	0.312 ± 0.493		0.233 ± 0.338		0.089 ± 0.078	*
Spleen (%)	0.428 ± 0.350	0.926 ± 1.572		0.764 ± 0.956		0.379 ± 0.269	
Liver (g)	1.536 ± 0.686	2.257 ± 1.296	**	3.276 ± 1.525	**	3.790 ± 1.324	**
Liver (%)	4.213 ± 2.167	6.734 ± 4.371	**	11.616 ± 5.557	**	17.271 ± 6.650	**
Brain (g)	0.469 ± 0.018	0.461 ± 0.022		0.453 ± 0.018	*	0.429 ± 0.018	**
Brain (%)	1.290 ± 0.320	1.354 ± 0.180		1.601 ± 0.152	**	1.927 ± 0.206	**

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

TABLE 13 INCIDENCES OF SELECTED NEOPLASTIC LESIONS OF MALE MICE IN THE 2-YEAR FEED STUDY OF 2,4-DICHLORO-1-NITROBENZENE

Group Name	Control	750 ppm	1500 ppm	3000 ppm	Peto	Cochran-
Number of examined animals	50	50	50	50	test	Armitage test
liver	<50>	<50>	<50>	<50>		
hepatocellular adenoma	18 (36 %)	34 (68 %)**	30 (60 %)*	43 (86 %)**	↑↑	↑↑
hepatocellular carcinoma	7 (14 %)	7 (14 %)	11 (22 %)	15 (30 %)*	↑↑	↑
hepatoblastoma	1 (2 %)	5 (10 %)	16 (32 %)**	27 (54 %)**	↑↑	↑↑
hemangioma	3 (6 %)	3 (6 %)	5 (10 %)	1 (2 %)		
histiocytic sarcoma	2 (4 %)	3 (6 %)	3 (6 %)	1 (2 %)		
peritoneum	<50>	<50>	<50>	<50>		
hemangioma	0 (0 %)	1 (2 %)	0 (0 %)	0 (0 %)		
hemangiosarcoma	1 (2 %)	0 (0 %)	2 (4 %)	5 (10 %)	↑↑	↑
epididymis	<50>	<50>	<50>	<50>		
histiocytic sarcoma	0 (0 %)	1 (2 %)	1 (2 %)	4 (8 %)	↑↑	↑

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 2,4-DICHLORO-1-NITROBENZENE

Group Name	Control	1500 ppm	3000 ppm	6000 ppm	Peto	Cochran-
Number of examined animals	49	50	50	50	test	Armitage test
liver	<49>	<50>	<50>	<50>		
hepatocellular adenoma	8 (16 %)	25 (50 %)**	42 (84 %)**	45 (90 %)**	↑↑	↑↑
hepatocellular carcinoma	1 (2 %)	2 (4 %)	11 (22 %)**	21 (42 %)**	↑↑	↑↑
hepatoblastoma	0 (0 %)	2 (4 %)	7 (14 %)**	7 (14 %)**	↑↑	↑↑
hemangioma	3 (6 %)	2 (4 %)	3 (6 %)	0 (0 %)		
histiocytic sarcoma	0 (0 %)	0 (0 %)	1 (2 %)	2 (4 %)		
peritoneum	<49>	<50>	<50>	<50>		
hemangioma	0 (0 %)	0 (0 %)	1 (2 %)	0 (0 %)		
hemangiosarcoma	0 (0 %)	3 (6 %)	7 (14 %)**	17 (34 %)**	↑↑	↑↑

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 2,4-DICHLORO-1-NITROBENZENE

Group Name	Control				750 ppm				1500 ppm				3000 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
liver	<50>				<50>				<50>				<50>			
clear cell focus	3	1	0	0	5	0	0	0	8	0	0	0	0	0	0	0
acidophilic cell focus	1	0	0	0	4	0	0	0	4	1	0	0	1	0	0	0
basophilic cell focus	2	0	0	0	3	0	0	0	1	0	1	0	0	0	0	0
hepatocellular hypertrophy:central	0	0	0	0	7	0	0	0 *	22	0	0	0 **	7	22	0	0 **
nasal cavity	<50>				<50>				<50>				<50>			
deposit of pigment	0	0	0	0	44	0	0	0 **	40	0	0	0 **	39	0	0	0 **
eosinophilic change:olfactory epithelium	17	2	0	0	16	3	0	0	9	2	0	0	17	7	1	0
eosinophilic change:respiratory epithelium	21	7	1	0	23	11	1	0	25	14	1	0	22	5	2	0
respiratory metaplasia:olfactory epithelium	20	0	0	0	15	0	0	0	26	23	0	0 **	1	46	3	0 **
respiratory metaplasia:gland	14	0	0	0	19	5	1	0 *	14	19	14	0 **	0	6	43	0 **
nasopharynx	<50>				<50>				<50>				<50>			
eosinophilic change	3	0	0	0	2	0	1	0	4	0	2	0	27	1	2	0 **
bone marrow	<50>				<50>				<50>				<50>			
erythropoiesis:increased	0	0	0	0	3	1	0	0	13	5	0	0 **	24	0	0	0 **
spleen	<50>				<50>				<50>				<50>			
deposit of hemosiderin	0	0	0	0	2	0	0	0	7	2	0	0 **	5	0	0	0
extramedullary hematopoiesis	7	1	1	0	5	3	1	0	13	7	10	0 **	10	13	12	0 **
kidney	<50>				<50>				<50>				<50>			
deposit of hemosiderin	0	0	0	0	0	1	1	0	0	5	6	0 **	0	3	15	0 **

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

TABLE 16 INCIDENCES OF SELECTED NON-NEOPLASTIC LESIONS OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF 2,4-DICHLORO-1-NITROBENZENE

Group Name	Control				1500 ppm				3000 ppm				6000 ppm			
Number of examined animals	49				50				50				50			
Grade of non-neoplastic lesion	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
liver	<49>				<50>				<50>				<50>			
clear cell focus	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
acidophilic cell focus	0	0	0	0	2	1	0	0	5	1	0	0 *	8	0	0	0 *
basophilic cell focus	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
hepatocellular hypertrophy:central	0	0	0	0	0	0	0	0	2	0	0	0	6	17	0	0 **
nasal cavity	<49>				<50>				<50>				<50>			
deposit of pigment	0	0	0	0	43	0	0	0 **	38	0	0	0 **	47	0	0	0 **
eosinophilic change:olfactory epithelium	6	1	0	0	14	3	0	0	23	1	2	0 **	22	22	1	0 **
eosinophilic change:respiratory epithelium	28	4	1	0	11	34	3	0 **	25	21	4	0 **	44	4	0	0 **
respiratory metaplasia:olfactory epithelium	9	0	0	0	7	40	1	0 **	0	42	8	0 **	0	6	44	0 **
respiratory metaplasia:gland	6	0	0	0	4	28	15	0 **	0	2	47	0 **	0	0	50	0 **
nasopharynx	<49>				<50>				<50>				<50>			
eosinophilic change	0	0	3	0	20	6	7	2 **	23	6	6	1 **	19	14	3	0 **
bone marrow	<49>				<50>				<50>				<50>			
erythropoiesis:increased	2	0	0	0	5	2	0	0	7	3	0	0 *	10	0	0	0 *
spleen	<49>				<50>				<50>				<50>			
deposit of hemosiderin	0	0	0	0	1	0	0	0	9	1	0	0 **	7	1	0	0 *
extramedullary hematopoiesis	4	6	5	0	3	3	8	0	11	10	13	0 **	10	10	13	0 **
kidney	<49>				<50>				<50>				<50>			
deposit of hemosiderin	0	0	0	0	0	1	2	0	2	1	4	0	1	3	3	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 : Crj:BDF_1 MALE MICE

Organs Tumors	No. of animals examined	No. of animals bearing tumor	Incidence (%)	Min. - Max. (%)
Liver	1496			
hepato cellular adenoma		273	18.2	4 - 34
hepato cellular carcinoma		307	20.5	2 - 42
hepatoblastoma		10	0.7	0 - 6
Peritoneum	1496			
hemangioma[a]		9	0.6	0 - 12
hemangiosarcoma[b]		3	0.2	0 - 4
Epididymis	1496			
histiocytic sarcoma		20	1.3	0 - 6

30 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

a: The tumors include one case of hemangioma in the retroperitoneum.
b: The tumors include one case of hemangioendothelioma in the peritoneum and one case of hemangiosarcoma in the retroperitoneum.

TABLE 18 HISTORICAL CONTROL DATA OF SELECTED NEOPLASTIC LESIONS IN JAPAN BIOASSAY RESEARCH CENTER : Crj:BDF_1 FEMALE MICE

Organs Tumors	No. of animals examined	No. of animals bearing tumor	Incidence (%)	Min. - Max. (%)
Liver	1498			
hepato cellular adenoma		82	5.5	0 - 12
hepato cellular carcinoma		37	2.5	0 - 8
hepatoblastoma		0	0	0 - 0
Peritoneum	1498			
hemangioma		1	0.1	0 - 2
hemangiosarcoma[a]		6	0.4	0 - 4

30 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

a: The tumors include three cases of hemangiosarcoma in the retroperitoneum.

TABLE 19 CAUSE OF DEATH OF MICE IN THE 2-YEAR FEED STUDY OF 2,4-DICHLORO-1-NITROBENZENE

	Male				Female			
Group name	Control	750 ppm	1500 ppm	3000 ppm	Control	1500 ppm	3000 ppm	6000 ppm
Number of dead or moribund animals	15	12	21	27	21	22	32	31
No microscopical confirmation	1	2	1	2	1	1	0	0
Respiratory system lesion	0	0	0	0	0	0	0	1
Cardiovascular lesion	0	0	1	1	0	0	0	1
Renal lesion	0	1	0	0	0	1	0	0
Urinary retention	5	0	2	0	0	0	0	0
Hydronephrosis	1	0	1	1	0	0	0	0
Tumor death ：leukemia	1	0	0	0	8	10	5	1
subcutis	1	1	0	0	2	0	1	1
lung	1	1	0	2	0	0	0	0
spleen	0	0	0	1	0	0	0	1
tooth	0	0	1	0	0	0	0	0
salivary gland	0	0	0	0	0	1	0	0
stomach	0	0	0	0	0	2	0	0
liver	3	5	14	15	0	2	10	9
ovary	—	—	—	—	0	0	1	0
uterus	—	—	—	—	9	5	9	5
epididymis	0	0	0	3	—	—	—	—
mammary gland	1	0	0	0	1	0	0	0
peripheral nerves	0	1	0	1	0	0	0	0
bone	0	0	0	0	0	0	0	1
peritoneum	1	0	1	1	0	0	6	11
retroperitoneum	0	1	0	0	0	0	0	0

FIGURES

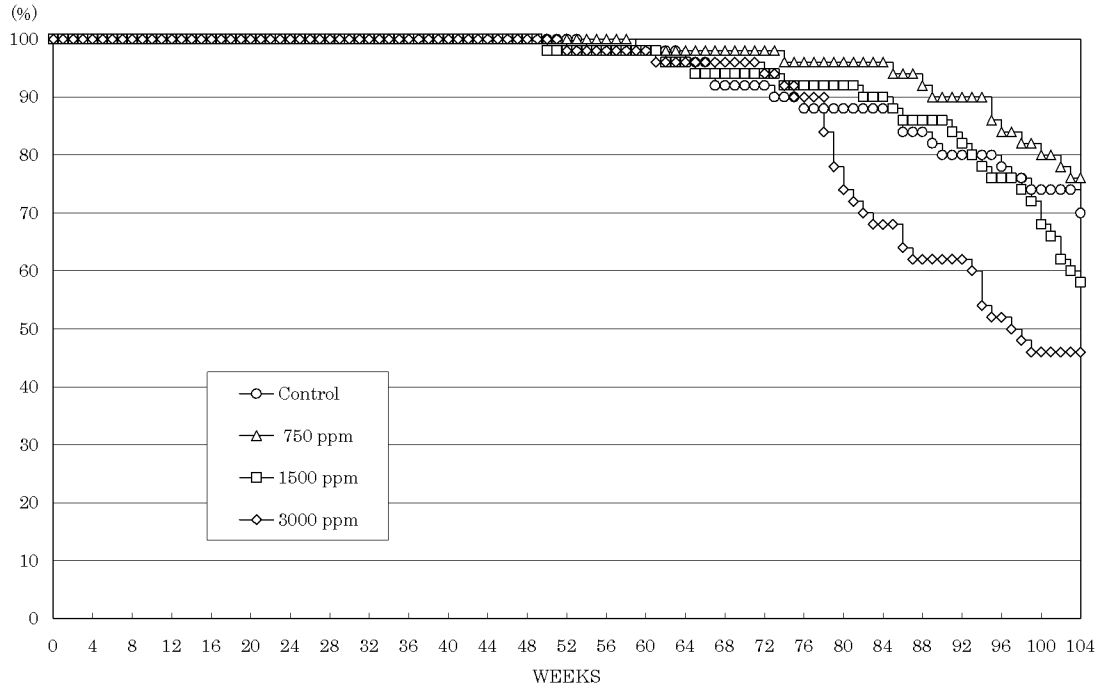


FIGURE 1 SURVIVAL ANIMAL RATE OF MALE MICE IN THE 2-YEAR FEED STUDY OF 2,4-DICHLORO-1-NITROBENZENE

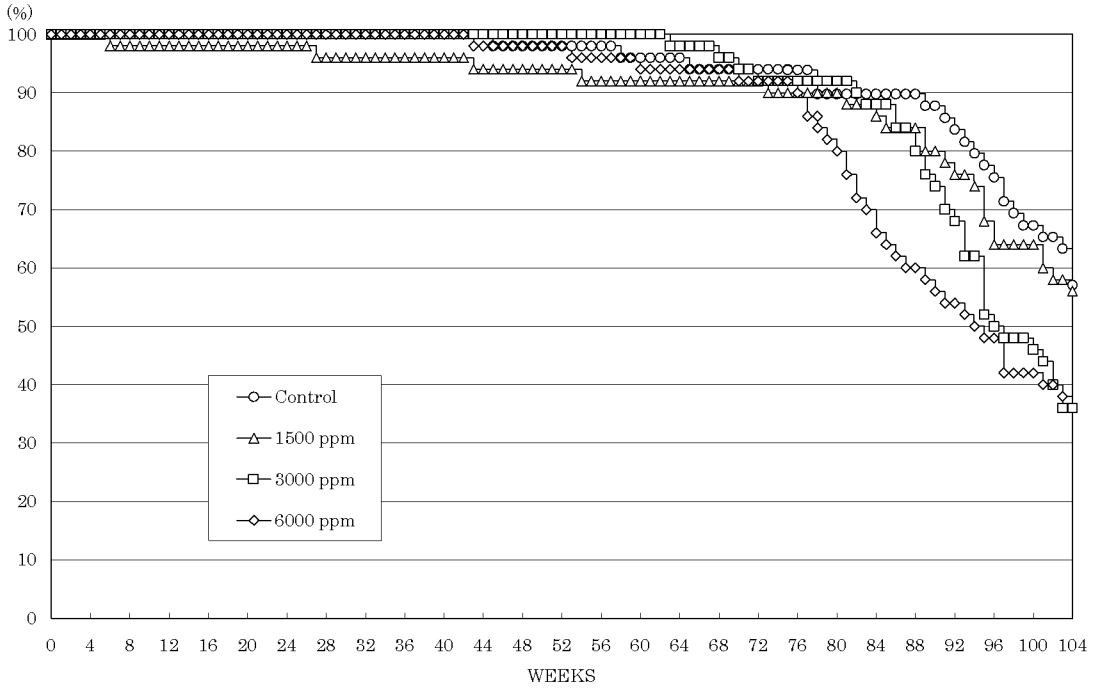


FIGURE 2 SURVIVAL ANIMAL RATE OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF 2,4-DICHLORO-1-NITROBENZENE

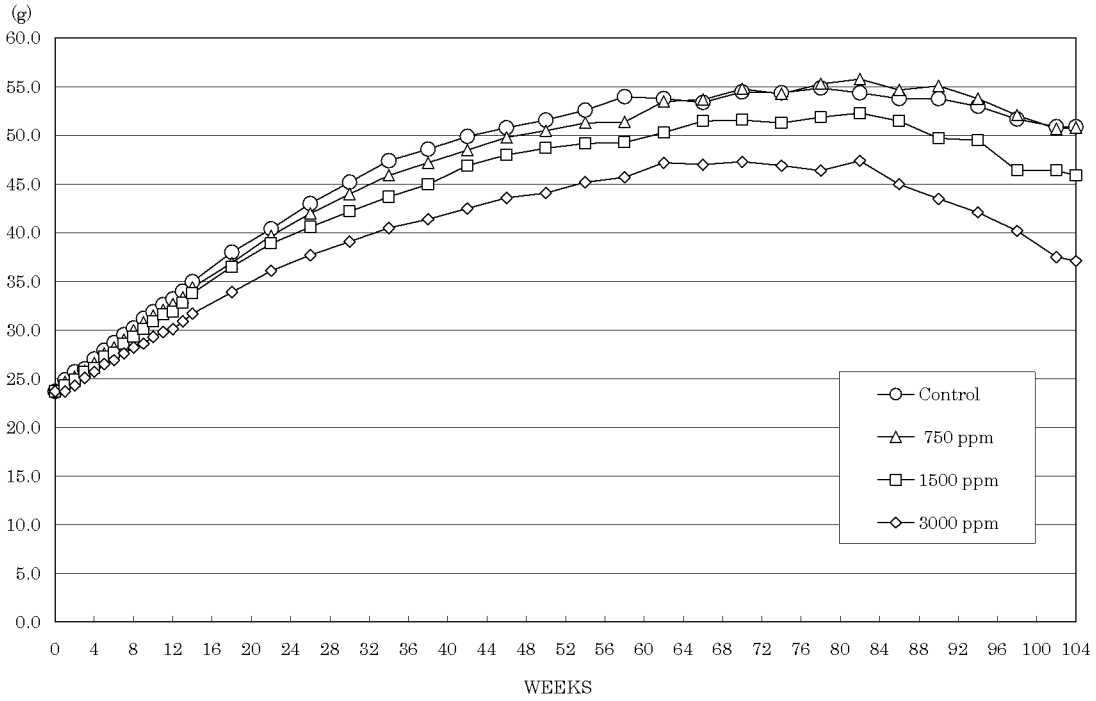


FIGURE 3 BODY WEIGHT CHANGES OF MALE MICE IN THE 2-YEAR FEED STUDY OF 2,4-DICHLORO-1-NITROBENZENE

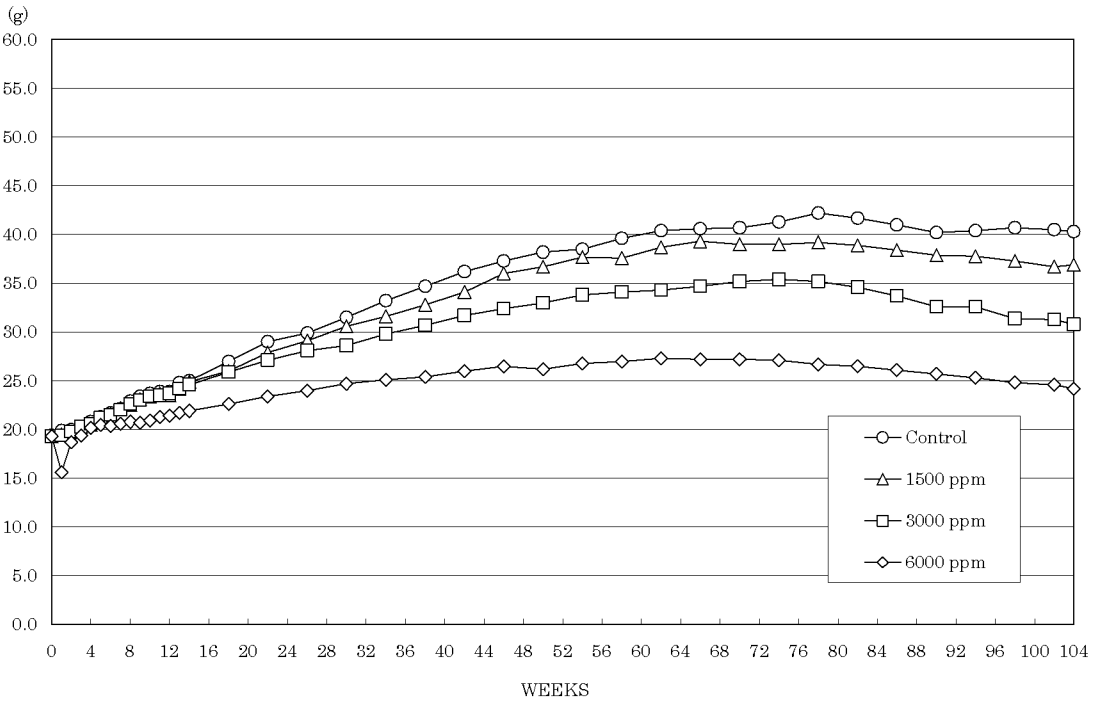


FIGURE 4 BODY WEIGHT CHANGES OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF 2,4-DICHLORO-1-NITROBENZENE

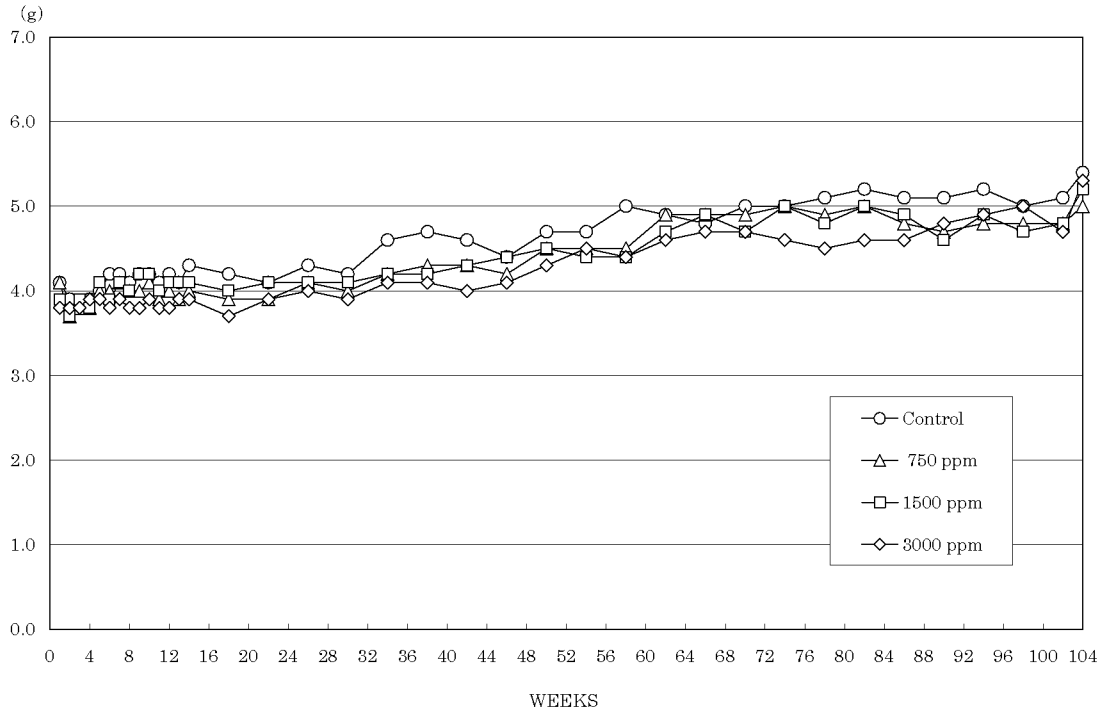


FIGURE 5 FOOD CONSUMPTION CHANGES OF MALE MICE IN THE 2-YEAR FEED STUDY OF 2,4-DICHLORO-1-NITROBENZENE

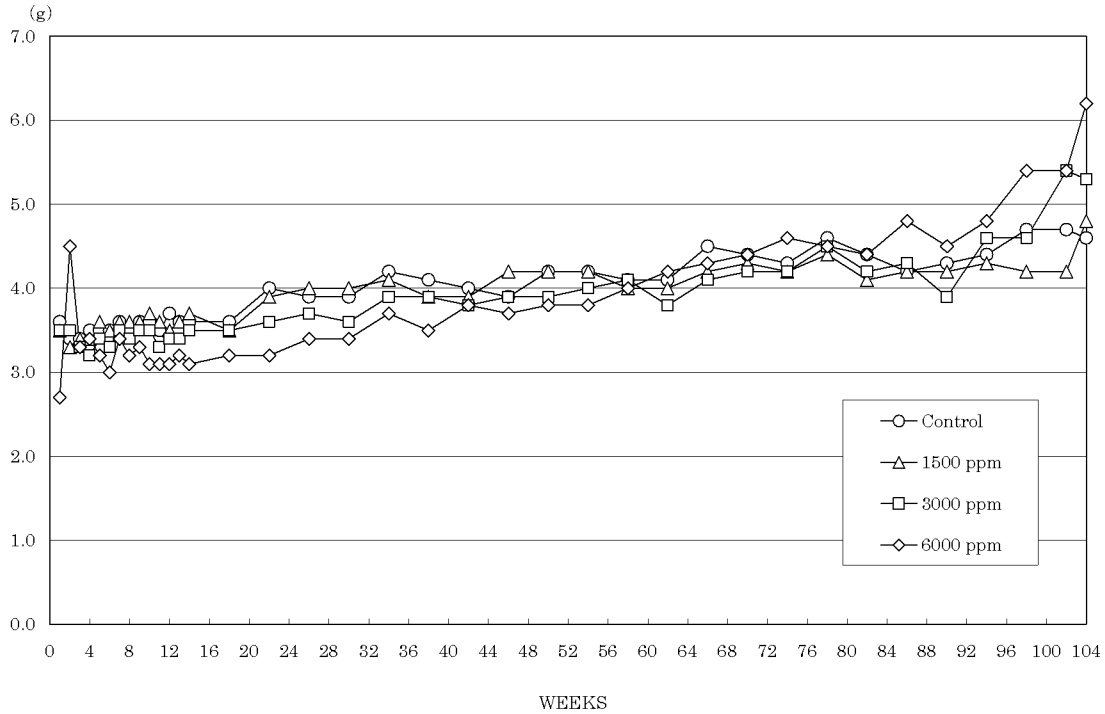
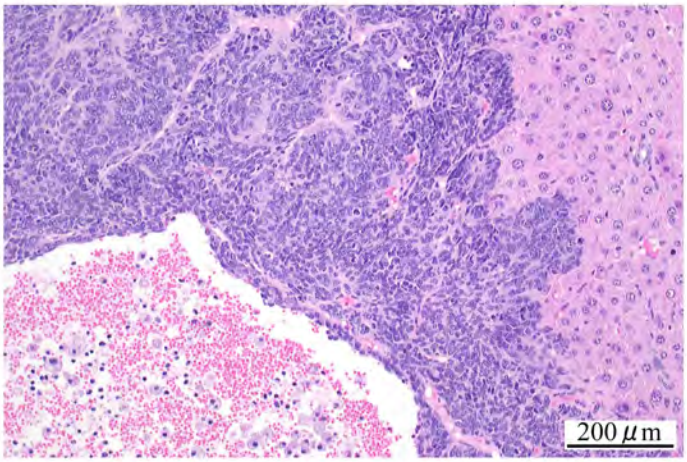
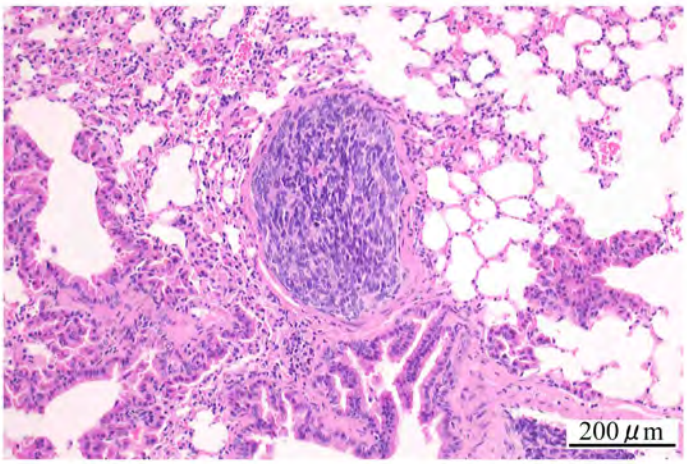


FIGURE 6 FOOD CONSUMPTION CHANGES OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF 2,4-DICHLORO-1-NITROBENZENE

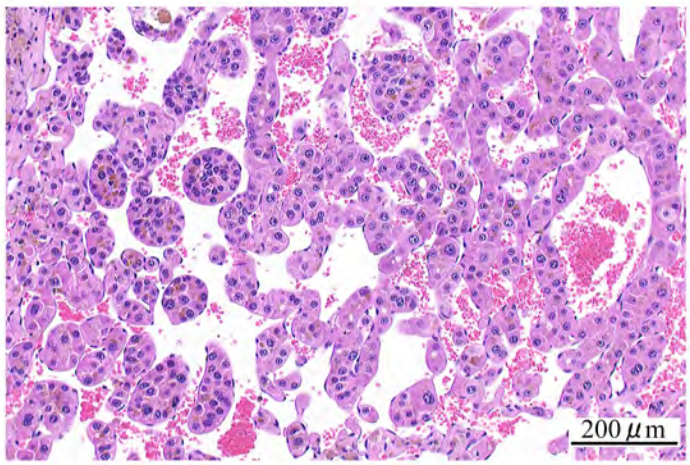
Photograph 1
Liver: Nodule (Hepatoblastoma)
Mouse, Male, 3000 ppm, Animal No. 0422-1320



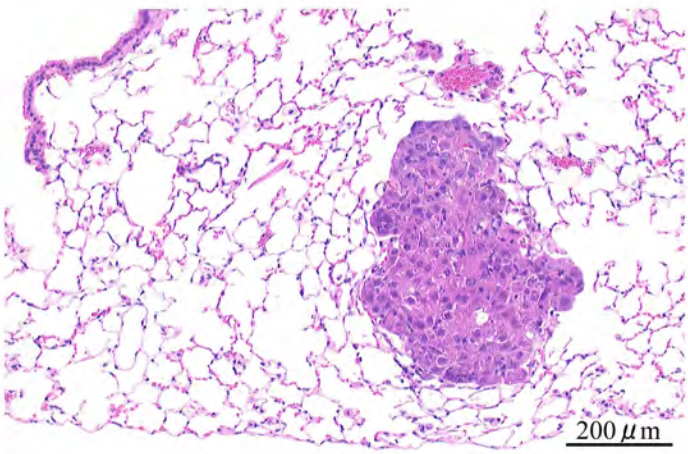
Photograph 2
Liver: Hepatoblastoma
Mouse, Male, 3000ppm, Animal No. 0422-1302 (H&E)



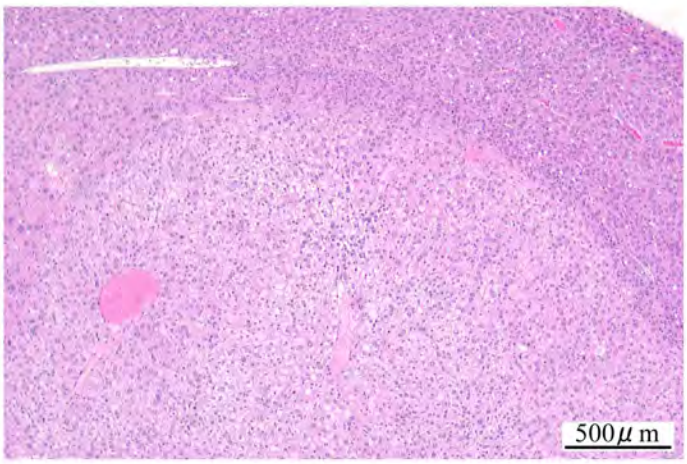
Photograph 3
Lung: Metastasis of hepatoblastoma
Mouse, Male, 3000 ppm, Animal No. 0422-1302 (H&E)



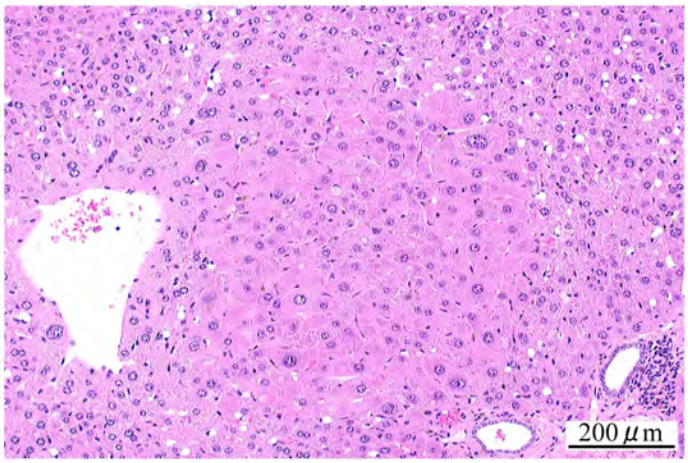
Photograph 4
Liver: Hepatocellular carcinoma
Mouse, Female, 6000 ppm, Animal No. 0422-2324 (H&E)



Photograph 5
Lung: Metastasis of hepatocellular carcinoma
Mouse, Female, 6000 ppm, Animal No. 0422-2324 (H&E)

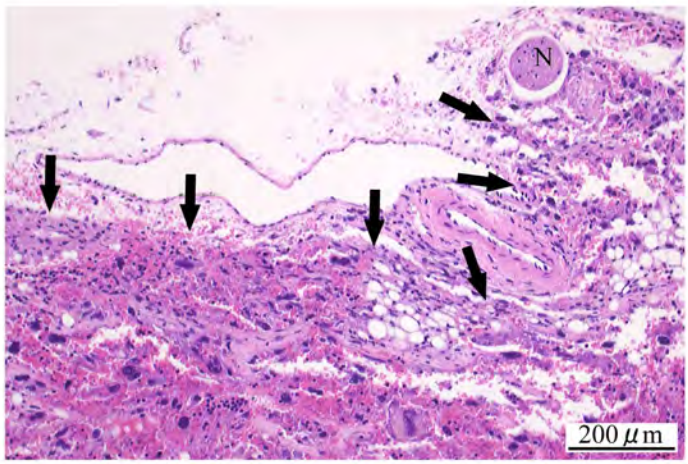


Photograph 6
Liver: Hepatocellular adenoma
Mouse, Male, 750 ppm, Animal No. 0422-1129 (H&E)



Photograph 7
Liver: Acidophilic cell focus
Mouse, Female, 6000 ppm, Animal No. 0422-2324 (H&E)

Photograph 8
Peritoneum: Nodule
Mouse, Female, 6000 ppm, Animal No. 0422-2322
Note a reddish nodule in the pelvic cavity (open arrow) and hemorrhagic fluid (filled arrow).



Photograph 9
Peritoneum: Hemangiosarcoma (Arrows)
Mouse, Female, 6000 ppm, Animal No. 0422-2322 (H&E)
N: Nerve bundle