Summary of Feed Carcinogenicity Study of *p*-Nitroanisole 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 31 2004.

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

Summary of Feed Carcinogenicity Study of *p*-Nitroanisole in BDF1 Mice

Purpose, materials and methods

p-Nitroanisole (*p*-NA, 1-methoxy-4-nitrobenzene, CAS No. 100-17-4) is a crystalline solid with a melting point of 54°C and a boiling point of 274°C. It is insoluble in water.

The carcinogenicity and chronic toxicity of *p*-NA were examined by feeding groups of 50 Crj:BDF1 mice of both sexes *p*-NA-containing diets for 2 years (104 weeks). The dietary concentration of *p*-NA was 0, 5000, 10000 or 20000 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. *p*-NA was analyzed for purity and stability by both infrared spectrometry and gas chromatography before and after its use. The *p*-NA concentrations in the diet were determined by high performance liquid chromatography at the time of preparation, and on the 8th 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 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. Incidence of neoplastic lesions was 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 10000 and 20000 ppm and the 20000 ppm-fed females were decreased as compared with the respective controls, and the decreased survival rates were attributed to an increased number of deaths due to liver tumors. Yellow urine was observed in all the *p*-NA-fed groups of both sexes. The internal mass was observed more frequently in the 20000 ppm-fed males than in the male control, and the internal mass also occurred dose-dependently in the *p*-NA-fed female groups. Body weights of all the *p*-NA-fed groups of both sexes were decreased dose-dependently. Anemia was suggested in the males as evidenced by decreases in red blood cell counts and hemoglobin concentration. Plasma levels of AST (GOT), ALT (GPT), LDH, and γ-GTP were increased in the males and females, along with the increased liver weight in the males and females.

The incidences of hepatocellular adenomas in the females fed 5000 and 10000 ppm, hepatocellular carcinomas in the 20000 ppm-fed males and in all the *p*-NA-fed female groups, and hepatoblastomas in all the *p*-NA-fed male groups and in the females 10000 and 20000 ppm were significantly increased. The incidence of acidophilic cell foci in the liver was slightly increased in the *p*-NA-fed males and females. Incidences and severities of centrilobular hypertrophy of hepatocytes were increased dose-dependently. Centrilobular hepatocytes with nuclear atypia were observed in the males. Dose-related increases in the incidences of non-neoplastic lesions in the nasal cavity, nasopharynx and lung were noted in the *p*-NA-fed males and/or females. Hemosiderin deposition was observed in the spleen and kidneys of the *p*-NA-fed groups of both sexes.

Conclusions

In mice, there was clear evidence of carcinogenic activity of *p*-NA in males and females, based on the increased incidences of hepatocellular carcinomas and hepatoblastomas. The increased incidence of benign hepatocellular adenomas was also recognized in the females.

TABLES

TABLES (Continued)

TABLE 1 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES OF MALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Week on Study	Control		5000 ppm			10000 ppm			20000 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	23.3 (50)	50 / 50	23.2 (50)	100	50 / 50	23.3 (50)	100	50 / 50	23.3 (50)	100	50 / 50
1	23.8 (50)	50 / 50	23.5 (50)	99	50 / 50	22.9 (50)	96	50 / 50	21.2 (50)	89	50 / 50
2	24.7 (50)	50 / 50	24.5 (50)	99	50 / 50	24.2 (50)	98	50 / 50	23.2 (50)	94	50 / 50
3	25.5 (50)	50 / 50	25.5 (50)	100	50 / 50	25.1 (50)	98	50 / 50	24.6 (50)	96	50 / 50
4	26.6 (50)	50 / 50	26.3 (50)	99	50 / 50	25.9 (50)	97	50 / 50	25.7 (50)	97	50 / 50
5	27.1 (50)	50 / 50	26.8 (50)	99	50 / 50	26.2 (50)	97	50 / 50	26.0 (50)	96	50 / 50
6	27.7 (50)	50 / 50	27.4 (50)	99	50 / 50	27.0 (50)	97	50 / 50	26.6 (50)	96	50 / 50
7	28.8 (50)	50 / 50	28.3 (50)	98	50 / 50	27.0 (50)	94	50 / 50	26.7 (50)	93	50 / 50
8	29.1 (50)	50 / 50	28.8 (50)	99	50 / 50	27.7 (50)	95	50 / 50	27.3 (50)	94	50 / 50
9	29.8 (50)	50 / 50	29.0 (50)	97	50 / 50	28.0 (50)	94	50 / 50	27.6 (50)	93	50 / 50
10	30.9 (50)	50 / 50	29.9 (50)	97	50 / 50	28.7 (50)	93	50 / 50	27.7 (50)	90	50 / 50
11	31.7 (50)	50 / 50	30.5 (50)	96	50 / 50	28.7 (50)	91	50 / 50	27.8 (50)	88	50 / 50
12	32.0 (50)	50 / 50	30.8 (50)	96	50 / 50	28.8 (50)	90	50 / 50	27.8 (50)	87	50 / 50
13	32.7 (50)	50 / 50	31.4 (50)	96	50 / 50	29.4 (50)	90	50 / 50	28.5 (50)	87	50 / 50
14	33.6 (50)	50 / 50	32.1 (50)	96	50 / 50	29.9 (50)	89	50 / 50	28.8 (50)	86	50 / 50
18	36.2 (50)	50 / 50	34.4 (50)	95	50 / 50	31.3 (50)	86	50 / 50	29.7 (50)	82	50 / 50
22	38.7 (50)	50 / 50	36.4 (50)	94	50 / 50	32.6 (50)	84	50 / 50	30.2 (49)	78	49 / 50
26	41.1 (50)	50 / 50	37.8 (50)	92	50 / 50	33.4 (49)	81	49 / 50	30.7 (49)	75	49 / 50
30	43.5 (50)	50 / 50	40.2 (50)	92	50 / 50	34.9 (49)	80	49 / 50	31.1 (49)	71	49 / 50
34	44.7 (50)	50 / 50	41.2 (50)	92	50 / 50	35.0 (48)	78	48 / 50	31.2 (49)	70	49 / 50
38	47.0 (50)	50 / 50	43.4 (50)	92	50 / 50	36.5 (47)	78	47 / 50	31.2 (49)	66	49 / 50
42	48.2 (50)	50 / 50	44.9 (50)	93	50 / 50	37.4 (46)	78	46 / 50	31.9 (49)	66	49 / 50
46	49.9 (50)	50 / 50	46.0 (50)	92	50 / 50	38.2 (45)	77	45 / 50	32.1 (48)	64	48 / 50
50	50.6 (50)	50 / 50	47.4 (50)	94	50 / 50	38.7 (45)	76	45 / 50	32.1 (47)	63	47 / 50
54	51.4 (49)	49 / 50	48.2 (49)	94	49 / 50	39.2 (44)	76	44 / 50	32.4 (47)	63	47 / 50
58	51.6 (49)	49 / 50	48.7 (49)	94	49 / 50	39.5 (44)	77	44 / 50	32.3 (47)	63	47 / 50
62	53.0 (47)	47 / 50	49.8 (49)	94	49 / 50	40.0 (44)	75	44 / 50	32.3 (47)	61	47 / 50
66	53.9 (46)	46 / 50	50.6 (49)	94	49 / 50	41.0 (44)	76	44 / 50	32.3 (44)	60	44 / 50
70	53.7 (46)	46 / 50	51.4 (48)	96	48 / 50	40.4 (44)	75	44 / 50	32.0 (41)	60	41 / 50
74	54.4 (46)	46 / 50	51.6 (48)	95	48 / 50	40.2 (44)	74	44 / 50	31.3 (41)	58	41 / 50
78	54.9 (46)	46 / 50	52.6 (47)	96	47 / 50	40.2 (42)	73	42 / 50	31.4 (39)	57	39 / 50
82	55.4 (44)	44 / 50	52.7 (46)	95	46 / 50	40.7 (40)	73	40 / 50	30.7 (37)	55	37 / 50
86	55.2 (43)	43 / 50	52.1 (46)	94	46 / 50	40.3 (39)	73	39 / 50	30.5 (33)	55	33 / 50
90	55.4 (42)	42 / 50	52.2 (45)	94	45 / 50	40.0 (37)	72	37 / 50	30.0 (31)	54	31 / 50
94	54.1 (41)	41 / 50	51.3 (42)	95	42 / 50	40.0 (36)	74	36 / 50	30.0 (28)	55	28 / 50
98	52.2 (40)	40 / 50	49.2 (40)	94	40 / 50	37.7 (32)	72	32 / 50	30.4 (24)	58	24 / 50
102	50.8 (37)	37 / 50	48.7 (37)	96	37 / 50	37.1 (30)	73	30 / 50	29.8 (16)	59	16 / 50
104	49.6 (36)	36 / 50	47.9 (35)	97	35 / 50	37.4 (27)	75	27 / 50	29.6 (16)	60	16 / 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 *p*-NITROANISOLE

Week on Study	Control		5000 ppm			10000 ppm			20000 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	19.1 (50)	50 / 50	19.1 (50)	100	50 / 50	19.1 (50)	100	50 / 50	19.1 (50)	100	50 / 50
1	19.5 (50)	50 / 50	18.8 (50)	96	50 / 50	19.0 (50)	97	50 / 50	17.4 (50)	89	50 / 50
2	19.8 (50)	50 / 50	19.6 (50)	99	50 / 50	19.5 (50)	98	50 / 50	19.6 (50)	99	50 / 50
3	20.2 (50)	50 / 50	19.8 (50)	98	50 / 50	20.2 (50)	100	50 / 50	20.4 (50)	101	50 / 50
4	20.7 (50)	50 / 50	20.6 (49)	100	50 / 50	20.6 (50)	100	50 / 50	20.9 (50)	101	50 / 50
5	21.3 (50)	50 / 50	21.2 (50)	100	50 / 50	21.3 (50)	100	50 / 50	21.3 (50)	100	50 / 50
6	21.7 (50)	50 / 50	21.5 (50)	99	50 / 50	21.4 (50)	99	50 / 50	21.5 (50)	99	50 / 50
7	22.3 (50)	50 / 50	22.0 (50)	99	50 / 50	22.0 (50)	99	50 / 50	22.0 (50)	99	50 / 50
8	22.6 (50)	50 / 50	22.5 (50)	100	50 / 50	22.3 (50)	99	50 / 50	22.1 (50)	98	50 / 50
9	22.9 (50)	50 / 50	22.7 (50)	99	50 / 50	22.8 (50)	100	50 / 50	22.4 (50)	98	50 / 50
10	23.4 (50)	50 / 50	23.1 (50)	99	50 / 50	23.1 (50)	99	50 / 50	22.8 (50)	97	50 / 50
11	23.7 (50)	50 / 50	23.3 (50)	98	50 / 50	23.3 (50)	98	50 / 50	23.2 (50)	98	50 / 50
12	23.7 (50)	50 / 50	23.3 (50)	98	50 / 50	23.3 (50)	98	50 / 50	23.1 (50)	97	50 / 50
13	24.3 (50)	50 / 50	24.3 (50)	100	50 / 50	23.6 (50)	97	50 / 50	23.3 (50)	96	50 / 50
14	24.7 (50)	50 / 50	24.2 (50)	98	50 / 50	23.6 (50)	96	50 / 50	23.5 (50)	95	50 / 50
18	26.2 (50)	50 / 50	24.9 (50)	95	50 / 50	24.6 (50)	94	50 / 50	24.3 (50)	93	50 / 50
22	27.7 (50)	50 / 50	26.8 (50)	97	50 / 50	25.9 (50)	94	50 / 50	25.1 (50)	91	50 / 50
26	29.7 (50)	50 / 50	27.9 (50)	94	50 / 50	26.7 (50)	90	50 / 50	25.5 (50)	86	50 / 50
30	31.3 (50)	50 / 50	29.2 (50)	93	50 / 50	27.2 (50)	87	50 / 50	26.1 (50)	83	50 / 50
34	32.6 (50)	50 / 50	30.1 (50)	92	50 / 50	27.8 (50)	85	50 / 50	26.8 (50)	82	50 / 50
38	33.9 (50)	50 / 50	31.9 (50)	94	50 / 50	28.7 (50)	85	50 / 50	26.8 (50)	79	50 / 50
42	34.5 (50)	50 / 50	32.4 (50)	94	50 / 50	29.8 (50)	86	50 / 50	27.9 (50)	81	50 / 50
46	35.7 (50)	50 / 50	33.3 (50)	93	50 / 50	30.2 (50)	85	50 / 50	28.3 (49)	79	49 / 50
50	35.8 (50)	50 / 50	33.6 (50)	94	50 / 50	30.4 (50)	85	50 / 50	28.0 (49)	78	49 / 50
54	36.3 (49)	49 / 50	34.2 (49)	94	49 / 50	30.9 (50)	85	50 / 50	28.4 (49)	78	49 / 50
58	37.1 (49)	49 / 50	34.8 (48)	94	48 / 50	31.2 (50)	84	50 / 50	28.6 (49)	77	49 / 50
62	38.0 (49)	49 / 50	35.2 (48)	93	48 / 50	31.1 (50)	82	50 / 50	28.2 (49)	74	49 / 50
66	38.6 (45)	45 / 50	35.7 (48)	92	48 / 50	31.8 (50)	82	50 / 50	28.1 (48)	73	48 / 50
70	38.2 (43)	43 / 50	36.1 (48)	95	48 / 50	31.6 (50)	83	50 / 50	28.0 (47)	73	47 / 50
74	38.7 (43)	43 / 50	36.3 (46)	94	46 / 50	31.5 (49)	81	49 / 50	27.4 (43)	71	43 / 50
78	38.8 (40)	40 / 50	36.2 (46)	93	46 / 50	31.1 (49)	80	49 / 50	27.3 (39)	70	39 / 50
82	37.8 (39)	39 / 50	36.6 (44)	97	44 / 50	31.2 (44)	83	44 / 50	26.9 (38)	71	38 / 50
86	39.7 (34)	34 / 50	36.2 (42)	91	42 / 50	31.0 (42)	78	42 / 50	27.2 (38)	69	38 / 50
90	40.4 (29)	29 / 50	35.6 (42)	88	42 / 50	30.6 (36)	76	36 / 50	26.7 (31)	66	31 / 50
94	40.8 (27)	27 / 50	35.5 (38)	87	38 / 50	30.6 (34)	75	34 / 50	27.0 (28)	66	28 / 50
98	40.4 (25)	25 / 50	35.3 (36)	87	36 / 50	29.7 (33)	74	33 / 50	27.1 (20)	67	20 / 50
102	39.5 (24)	24 / 50	34.7 (30)	88	30 / 50	29.6 (30)	75	30 / 50	27.4 (14)	69	14 / 50
104	38.8 (23)	23 / 50	34.9 (27)	90	27 / 50	29.7 (30)	77	30 / 50	27.1 (13)	70	13 / 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 *p*-NITROANISOLE

Week on Study	Control		5000 ppm			10000 ppm			20000 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	4.0 (50)	50 / 50	3.8 (50)	95	50 / 50	3.7 (50)	93	50 / 50	3.5 (50)	88	50 / 50
2	3.9 (50)	50 / 50	4.0 (50)	103	50 / 50	4.1 (50)	105	50 / 50	4.3 (50)	110	50 / 50
3	4.0 (50)	50 / 50	4.2 (50)	105	50 / 50	4.1 (49)	103	50 / 50	4.2 (50)	105	50 / 50
4	4.1 (50)	50 / 50	4.2 (49)	102	50 / 50	4.0 (50)	98	50 / 50	4.1 (50)	100	50 / 50
5	4.0 (50)	50 / 50	4.1 (50)	103	50 / 50	4.1 (50)	103	50 / 50	4.2 (50)	105	50 / 50
6	3.9 (50)	50 / 50	4.1 (50)	105	50 / 50	4.1 (50)	105	50 / 50	4.1 (49)	105	50 / 50
7	4.1 (50)	50 / 50	4.1 (50)	100	50 / 50	4.0 (50)	98	50 / 50	4.1 (48)	100	50 / 50
8	4.0 (50)	50 / 50	4.2 (50)	105	50 / 50	4.1 (50)	103	50 / 50	4.3 (49)	108	50 / 50
9	4.0 (50)	50 / 50	4.0 (50)	100	50 / 50	3.9 (50)	98	50 / 50	4.0 (49)	100	50 / 50
10	4.1 (50)	50 / 50	4.2 (50)	102	50 / 50	4.0 (50)	98	50 / 50	4.0 (49)	98	50 / 50
11	4.0 (50)	50 / 50	4.0 (50)	100	50 / 50	3.9 (50)	98	50 / 50	4.1 (49)	103	50 / 50
12	4.0 (50)	50 / 50	4.0 (50)	100	50 / 50	3.9 (50)	98	50 / 50	4.2 (50)	105	50 / 50
13	3.9 (50)	50 / 50	3.9 (50)	100	50 / 50	3.9 (50)	100	50 / 50	4.0 (50)	103	50 / 50
14	4.1 (50)	50 / 50	4.1 (50)	100	50 / 50	4.0 (50)	98	50 / 50	4.1 (50)	100	50 / 50
18	4.2 (50)	50 / 50	4.2 (50)	100	50 / 50	4.1 (50)	98	50 / 50	4.3 (50)	102	50 / 50
22	4.2 (50)	50 / 50	4.2 (50)	100	50 / 50	4.2 (50)	100	50 / 50	4.5 (49)	107	49 / 50
26	4.5 (50)	50 / 50	4.3 (50)	96	50 / 50	4.3 (49)	96	49 / 50	4.6 (46)	102	49 / 50
30	4.3 (50)	50 / 50	4.5 (50)	105	50 / 50	4.3 (49)	100	49 / 50	4.5 (47)	105	49 / 50
34	4.4 (50)	50 / 50	4.6 (50)	105	50 / 50	4.5 (48)	102	48 / 50	5.0 (47)	114	49 / 50
38	4.6 (50)	50 / 50	4.6 (48)	100	50 / 50	4.5 (46)	98	47 / 50	5.0 (47)	109	49 / 50
42	4.5 (50)	50 / 50	4.7 (50)	104	50 / 50	4.5 (46)	100	46 / 50	4.6 (49)	102	49 / 50
46	4.6 (50)	50 / 50	4.6 (48)	100	50 / 50	4.6 (44)	100	45 / 50	4.8 (44)	104	48 / 50
50	4.5 (50)	50 / 50	4.7 (49)	104	50 / 50	4.4 (45)	98	45 / 50	4.6 (46)	102	47 / 50
54	4.6 (49)	49 / 50	4.8 (47)	104	49 / 50	4.4 (44)	96	44 / 50	4.9 (45)	107	47 / 50
58	4.8 (49)	49 / 50	4.9 (47)	102	49 / 50	4.9 (44)	102	44 / 50	5.0 (44)	104	47 / 50
62	4.7 (47)	47 / 50	4.9 (49)	104	49 / 50	4.8 (44)	102	44 / 50	5.0 (45)	106	47 / 50
66	4.9 (46)	46 / 50	5.0 (48)	102	49 / 50	4.9 (44)	100	44 / 50	5.0 (39)	102	44 / 50
70	4.9 (46)	46 / 50	5.0 (47)	102	48 / 50	4.8 (44)	98	44 / 50	5.4 (30)	110	41 / 50
74	4.9 (42)	46 / 50	5.0 (39)	102	48 / 50	4.8 (39)	98	44 / 50	5.2 (20)	106	41 / 50
78	5.0 (46)	46 / 50	5.4 (45)	108	47 / 50	5.0 (41)	100	42 / 50	5.5 (20)	110	39 / 50
82	4.8 (44)	44 / 50	5.0 (46)	104	46 / 50	4.8 (39)	100	40 / 50	5.7 (23)	119	37 / 50
86	4.9 (43)	43 / 50	5.2 (46)	106	46 / 50	4.9 (39)	100	39 / 50	6.0 (20)	122	33 / 50
90	4.9 (41)	42 / 50	5.2 (45)	106	45 / 50	4.8 (34)	98	37 / 50	5.3 (11)	108	31 / 50
94	4.8 (40)	41 / 50	5.1 (42)	106	42 / 50	4.9 (33)	102	36 / 50	6.1 (12)	127	28 / 50
98	4.7 (40)	40 / 50	5.2 (40)	111	40 / 50	4.9 (29)	104	32 / 50	6.5 (6)	138	24 / 50
102	4.8 (36)	37 / 50	5.0 (36)	104	37 / 50	4.6 (25)	96	30 / 50	6.5 (3)	135	16 / 50
104	4.6 (35)	36 / 50	4.8 (34)	104	35 / 50	4.8 (23)	104	27 / 50	6.5 (2)	141	16 / 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 *p*-NITROANISOLE

Week on Study	Control		5000 ppm			10000 ppm			20000 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.
	< 50 >		< 50 >			< 50 >			< 50 >		
1	3.6 (50)	50 / 50	3.3 (49)	92	50 / 50	3.5 (49)	97	50 / 50	2.8 (49)	78	50 / 50
2	3.5 (50)	50 / 50	3.4 (50)	97	50 / 50	3.5 (50)	100	50 / 50	3.6 (49)	103	50 / 50
3	3.5 (50)	50 / 50	3.4 (50)	97	50 / 50	3.4 (50)	97	50 / 50	3.4 (49)	97	50 / 50
4	3.8 (50)	50 / 50	3.6 (50)	95	50 / 50	3.6 (50)	95	50 / 50	3.4 (50)	89	50 / 50
5	3.8 (50)	50 / 50	3.7 (50)	97	50 / 50	3.7 (50)	97	50 / 50	3.3 (50)	87	50 / 50
6	3.6 (50)	50 / 50	3.6 (50)	100	50 / 50	3.5 (50)	97	50 / 50	3.2 (50)	89	50 / 50
7	3.8 (50)	50 / 50	3.7 (50)	97	50 / 50	3.6 (50)	95	50 / 50	3.6 (50)	95	50 / 50
8	3.7 (50)	50 / 50	3.8 (50)	103	50 / 50	3.7 (50)	100	50 / 50	3.5 (50)	95	50 / 50
9	3.8 (50)	50 / 50	3.6 (50)	95	50 / 50	3.7 (50)	97	50 / 50	3.5 (50)	92	50 / 50
10	3.8 (50)	50 / 50	3.6 (50)	95	50 / 50	3.6 (50)	95	50 / 50	3.5 (50)	92	50 / 50
11	3.8 (50)	50 / 50	3.7 (50)	97	50 / 50	3.7 (50)	97	50 / 50	3.8 (50)	100	50 / 50
12	3.8 (50)	50 / 50	3.7 (50)	97	50 / 50	3.6 (50)	95	50 / 50	3.6 (50)	95	50 / 50
13	3.9 (50)	50 / 50	3.7 (50)	95	50 / 50	3.7 (50)	95	50 / 50	3.8 (50)	97	50 / 50
14	4.0 (50)	50 / 50	3.7 (50)	93	50 / 50	3.7 (50)	93	50 / 50	3.8 (50)	95	50 / 50
18	3.8 (50)	50 / 50	3.7 (50)	97	50 / 50	4.0 (50)	105	50 / 50	3.8 (50)	100	50 / 50
22	4.0 (50)	50 / 50	3.9 (50)	98	50 / 50	4.2 (50)	105	50 / 50	4.0 (50)	100	50 / 50
26	4.3 (50)	50 / 50	4.1 (50)	95	50 / 50	4.4 (50)	102	50 / 50	4.1 (50)	95	50 / 50
30	4.2 (50)	50 / 50	4.0 (50)	95	50 / 50	4.1 (50)	98	50 / 50	4.1 (50)	98	50 / 50
34	4.5 (50)	50 / 50	4.3 (50)	96	50 / 50	4.4 (50)	98	50 / 50	4.3 (49)	96	50 / 50
38	4.4 (49)	50 / 50	4.4 (50)	100	50 / 50	4.5 (50)	102	50 / 50	4.3 (48)	98	50 / 50
42	4.6 (49)	50 / 50	4.7 (50)	102	50 / 50	4.8 (49)	104	50 / 50	4.7 (47)	102	50 / 50
46	4.7 (50)	50 / 50	4.6 (50)	98	50 / 50	4.9 (50)	104	50 / 50	4.9 (47)	104	49 / 50
50	4.5 (50)	50 / 50	4.3 (50)	96	50 / 50	4.6 (50)	102	50 / 50	4.5 (47)	100	49 / 50
54	4.6 (49)	49 / 50	4.3 (49)	93	49 / 50	4.6 (50)	100	50 / 50	4.8 (47)	104	49 / 50
58	4.8 (47)	49 / 50	4.8 (48)	100	48 / 50	5.2 (48)	108	50 / 50	5.2 (49)	108	49 / 50
62	4.9 (48)	49 / 50	4.8 (47)	98	48 / 50	5.0 (50)	102	50 / 50	4.9 (43)	100	49 / 50
66	4.7 (45)	45 / 50	4.6 (48)	98	48 / 50	5.0 (49)	106	50 / 50	5.2 (45)	111	48 / 50
70	4.7 (42)	43 / 50	4.9 (48)	104	48 / 50	5.0 (48)	106	50 / 50	5.1 (35)	109	47 / 50
74	4.4 (36)	43 / 50	4.6 (41)	105	46 / 50	5.0 (43)	114	49 / 50	4.7 (31)	107	43 / 50
78	4.7 (36)	40 / 50	4.9 (45)	104	46 / 50	5.0 (43)	106	49 / 50	5.1 (24)	109	39 / 50
82	4.6 (38)	39 / 50	4.9 (44)	107	44 / 50	5.2 (41)	113	44 / 50	5.2 (27)	113	38 / 50
86	4.9 (33)	34 / 50	5.0 (42)	102	42 / 50	5.5 (41)	112	42 / 50	5.6 (21)	114	38 / 50
90	5.1 (29)	29 / 50	4.7 (41)	92	42 / 50	5.4 (34)	106	36 / 50	5.4 (15)	106	31 / 50
94	5.1 (27)	27 / 50	4.9 (38)	96	38 / 50	5.6 (29)	110	34 / 50	5.9 (15)	116	28 / 50
98	4.9 (23)	25 / 50	5.0 (35)	102	36 / 50	5.6 (30)	114	33 / 50	6.5 (8)	133	20 / 50
102	4.9 (23)	24 / 50	4.8 (30)	98	30 / 50	5.7 (27)	116	30 / 50	5.7 (5)	116	14 / 50
104	4.9 (21)	23 / 50	5.2 (25)	106	27 / 50	5.7 (25)	116	30 / 50	6.2 (5)	127	13 / 50

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

TABLE 5 INCIDENCES OF EXTERNAL AND INTERNAL MASSES IN CLINICAL OBSERVATION OF MALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Time of mass occurrence (week)	0～13	14～26	27～39	40～52	53～65	66～78	79～91	92～104	0～104
External mass									
Control	0/50	0/50	0/50	0/50	0/50	0/46	0/46	3/41	3/50(1/14)
5000 ppm	0/50	0/50	0/50	1/50	1/50	0/49	0/47	2/42	3/50(3/15)
10000 ppm	0/50	0/50	0/49	0/47	0/45	0/44	0/42	2/36	2/50(1/23)
20000 ppm	0/50	0/50	0/49	1/49	1/47	1/44	1/38	1/29	1/50(1/34)
Internal mass									
Control	1/50	1/50	2/50	1/50	3/50	4/46	7/46	13/41	22/50(7/14)
5000 ppm	2/50	1/50	1/50	3/50	3/50	4/49	8/47	16/42	20/50(9/15)
10000 ppm	1/50	1/50	2/49	2/47	2/45	4/44	8/42	12/36	17/50(10/23)
20000 ppm	1/50	2/50	0/49	1/49	2/47	18/44	28/38	28/29	39/50(24/34)

No. of animals with mass / No. of surviving animals at first week in each period.
(No. of dead and moribund animals with mass / No. of dead and moribund animals)

TABLE 6 INCIDENCES OF EXTERNAL AND INTERNAL MASSES IN CLINICAL OBSERVATION OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Time of mass occurrence (week)	0～13	14～26	27～39	40～52	53～65	66～78	79～91	92～104	0～104
External mass									
Control	0/50	0/50	0/50	0/50	1/49	3/45	4/40	4/28	6/50(3/27)
5000 ppm	0/50	0/50	0/50	0/50	0/49	0/48	2/46	5/39	5/50(3/23)
10000 ppm	0/50	0/50	0/50	0/50	0/50	0/50	4/48	4/35	6/50(5/20)
20000 ppm	0/50	0/50	0/50	0/50	0/49	0/48	1/38	1/29	1/50(1/37)
Internal mass									
Control	0/50	0/50	0/50	0/50	6/49	4/45	8/40	7/28	18/50(16/27)
5000 ppm	0/50	0/50	0/50	2/50	4/49	5/48	12/46	14/39	24/50(16/23)
10000 ppm	0/50	0/50	0/50	1/50	1/50	11/50	13/48	20/35	34/50(16/20)
20000 ppm	0/50	1/50	1/50	1/50	3/49	18/48	30/38	29/29	49/50(36/37)

No. of animals with mass / No. of surviving animals at first week in each period.
(No. of dead and moribund animals with mass / No. of dead and moribund animals)

TABLE 7 HEMATOLOGY OF MALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Group name	Control	5000 ppm	10000 ppm	20000 ppm
No. of examined animals	32	33	26	16
Red blood cell ($10^6/\mu$ L)	9.06 ± 2.12	9.27 ± 1.28	8.77 ± 1.80	7.94 ± 2.48 *
Hemoglobin (g/dL)	12.4 ± 2.7	13.0 ± 1.7	12.6 ± 2.4	10.4 ± 3.2 *
Hematocrit (%)	40.4 ± 8.1	42.2 ± 4.9	40.8 ± 6.3	36.0 ± 9.1 *
MCV (fL)	45.2 ± 4.2	45.7 ± 1.9	47.6 ± 6.3 **	47.2 ± 7.6
MCH (pg)	13.8 ± 1.0	14.0 ± 0.6	14.4 ± 0.6 **	13.3 ± 0.9
MCHC (g/dL)	30.6 ± 1.8	30.7 ± 1.0	30.6 ± 2.5	28.5 ± 2.5 **
Differential WBC (%)				
Eosino	1 ± 1	2 ± 1	2 ± 1	0 ± 0 **

Mean ± S.D.

*) Significant difference, $p<0.05$ (Test of Dunnett)

**) Significant difference, $p<0.01$ (Test of Dunnett)

TABLE 8 HEMATOLOGY OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Group name	Control	5000 ppm	10000 ppm	20000 ppm
No. of examined animals	23	24	29	13
MCH (pg)	14.4 ± 0.3	14.4 ± 0.6	14.0 ± 0.7	13.8 ± 1.4 **
MCHC (g/dL)	31.3 ± 1.0	31.3 ± 0.8	30.7 ± 1.3	28.5 ± 3.5 **
Platelet ($10^3/\mu$ L)	1073 ± 375	1275 ± 344	1321 ± 349	1517 ± 695 *
Differential WBC (%)				
N-band	1 ± 1	1 ± 2	1 ± 2	4 ± 5 **
N-seg	23 ± 13	22 ± 12	26 ± 15	37 ± 19 *

Mean ± S.D.

*) Significant difference, $p<0.05$ (Test of Dunnett)

**) Significant difference, $p<0.01$ (Test of Dunnett)

TABLE 9 BIOCHEMISTRY OF MALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Group name	Control	5000 ppm	10000 ppm	20000 ppm
No. of examined animals	34	34	27	16
Total protein (g/dL)	5.4 ± 0.9	5.3 ± 0.8	5.1 ± 0.5	6.0 ± 0.7 **
Albumin (g/dL)	2.9 ± 0.5	3.0 ± 0.4	2.9 ± 0.3	3.2 ± 0.3 **
Total bilirubin (mg/dL)	0.17 ± 0.10	0.17 ± 0.08	0.18 ± 0.16	0.38 ± 0.37 **
Glucose (mg/dL)	169 ± 56	199 ± 50	212 ± 62 **	177 ± 32
T-cholesterol (mg/dL)	120 ± 64	128 ± 47	124 ± 32	250 ± 59 **
Phospholipid (mg/dL)	215 ± 99	235 ± 73	225 ± 63	450 ± 119 **
GOT (IU/L)	208 ± 302	114 ± 145	162 ± 263	1250 ± 1590 **
GPT (IU/L)	110 ± 134	95 ± 143	134 ± 181	1157 ± 1198 **
LDH (IU/L)	1056 ± 2228	754 ± ####	2087 ± ####	9029 ± 7690 **
ALP (IU/L)	163 ± 111	160 ± 99	296 ± 312 *	923 ± 691 **
γ-GTP (IU/L)	3 ± 4	4 ± 5	2 ± 1	14 ± 15 **
CPK (IU/L)	64 ± 66	51 ± 24	76 ± 85	133 ± 46 **
Urea nitrogen (mg/dL)	24.0 ± 13.9	25.6 ± 5.6 *	28.0 ± 8.6 **	27.6 ± 3.6 **
Chloride (mEq/L)	122 ± 4	123 ± 4	125 ± 4 *	121 ± 3
Calcium (mg/dL)	9.3 ± 0.7	9.3 ± 0.6	9.1 ± 0.5	9.9 ± 0.5 **

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

TABLE 10 BIOCHEMISTRY OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Group name	Control	5000 ppm	10000 ppm	20000 ppm
No. of examined animals	23	25	29	13
Total protein (g/dL)	5.0 ± 0.4	5.0 ± 0.8	5.2 ± 0.8	5.9 ± 0.5 **
Albumin (g/dL)	2.9 ± 0.2	2.8 ± 0.2	2.9 ± 0.3	3.4 ± 0.3 **
Total bilirubin (mg/dL)	0.15 ± 0.03	0.15 ± 0.05	0.16 ± 0.03	0.48 ± 0.59 **
T-cholesterol (mg/dL)	69 ± 22	86 ± 19	127 ± 38 **	225 ± 43 **
Phospholipid (mg/dL)	138 ± 35	165 ± 38	245 ± 68 **	398 ± 77 **
GOT (IU/L)	112 ± 84	85 ± 37	294 ± 366 **	1213 ± 1411 **
GPT (IU/L)	37 ± 23	47 ± 31	324 ± 386 **	1007 ± 1037 **
LDH (IU/L)	685 ± 950	442 ± 573	1387 ± #### *	9917 ± ##### **
ALP (IU/L)	176 ± 70	227 ± 114	495 ± 313 **	943 ± 469 **
γ-GTP (IU/L)	2 ± 1	3 ± 4	5 ± 5 **	14 ± 8 **
CPK (IU/L)	95 ± 85	110 ± 144	108 ± 87	222 ± 200 **
Urea nitrogen (mg/dL)	19.0 ± 5.1	21.8 ± 14.2	31.8 ± 30.1 **	33.5 ± 18.0 **
Chloride (mEq/L)	123 ± 3	124 ± 2	123 ± 4	120 ± 4 *
Calcium (mg/dL)	9.1 ± 0.5	9.0 ± 0.4	9.6 ± 0.5 **	10.1 ± 0.3 **

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

TABLE 11 URINALYSIS OF MALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Group name	Grade	Control	5000 ppm	10000 ppm	20000 ppm
Number of examined animals		36	35	29	16
pH	6.0	6	3	3	10
	6.5	15	10	11	4
	7.0	7	11	4	1
	7.5	6	9	9	1
	8.0	2	2	2	0
	8.5	0	0	0	0
	Chi square test				*
Protein	−	0	0	0	0
	±	4	6	9	10
	+	23	24	19	6
	2+	8	5	1	0
	3+	1	0	0	0
	Chi square test			*	**

Significant difference : * : $p<0.05$ ** : $p<0.01$

TABLE 12 URINALYSIS OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Group name	Grade	Control	5000 ppm	10000 ppm	20000 ppm
Number of examined animals		24	30	30	13
pH	6.0	1	1	2	7
	6.5	4	6	8	6
	7.0	8	7	12	0
	7.5	8	10	5	0
	8.0	2	6	3	0
	8.5	1	0	0	0
	Chi square test				**

Significant difference : * : $p<0.05$ ** : $p<0.01$

TABLE 13 ORGAN WEIGHTS OF MALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Group name	Control	5000 ppm	10000 ppm	20000 ppm
No. of examined animals	36	35	27	16
Body weight (g)	45.8 ± 8.0	45.2 ± 7.5	34.7 ± 4.7 **	27.3 ± 2.8 **
Adrenals (g)	0.012 ± 0.003	0.010 ± 0.002 *	0.010 ± 0.002 **	0.009 ± 0.002 *
Adrenals (%)	0.026 ± 0.009	0.023 ± 0.006	0.029 ± 0.008	0.035 ± 0.007 **
Testes (g)	0.218 ± 0.026	0.219 ± 0.028	0.203 ± 0.041	0.202 ± 0.027
Testes (%)	0.492 ± 0.111	0.498 ± 0.099	0.590 ± 0.126 **	0.743 ± 0.101 **
Heart (g)	0.232 ± 0.042	0.225 ± 0.025	0.196 ± 0.020 **	0.167 ± 0.013 **
Heart (%)	0.532 ± 0.207	0.513 ± 0.112	0.573 ± 0.081 *	0.617 ± 0.063 **
Lungs (g)	0.257 ± 0.104	0.265 ± 0.087	0.252 ± 0.047	0.227 ± 0.025
Lungs (%)	0.590 ± 0.299	0.597 ± 0.177	0.742 ± 0.188 **	0.839 ± 0.120 **
Kidneys (g)	0.649 ± 0.360	0.605 ± 0.163	0.542 ± 0.044 **	0.457 ± 0.068 **
Kidneys (%)	1.463 ± 0.874	1.385 ± 0.524	1.580 ± 0.168 **	1.676 ± 0.187 **
Spleen (g)	0.161 ± 0.399	0.243 ± 0.489	0.103 ± 0.127	0.142 ± 0.118
Spleen (%)	0.375 ± 0.933	0.575 ± 1.141	0.301 ± 0.357	0.525 ± 0.454 **
Liver (g)	2.001 ± 0.931	2.248 ± 0.653 *	2.607 ± 2.501	5.750 ± 2.701 **
Liver (%)	4.529 ± 2.425	5.251 ± 2.298	7.575 ± 7.066 **	20.673 ± 7.440 **
Brain (g)	0.446 ± 0.016	0.450 ± 0.016	0.451 ± 0.020	0.435 ± 0.015
Brain (%)	1.010 ± 0.224	1.024 ± 0.178	1.324 ± 0.176 **	1.607 ± 0.126 **

Mean ± S.D.

*) Significant difference, $p<0.05$ (Test of Dunnett)

**) Significant difference, $p<0.01$ (Test of Dunnett)

TABLE 14 ORGAN WEIGHTS OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Group name	Control	5000 ppm	10000 ppm	20000 ppm
No. of examined animals	23	27	30	13
Body weight (g)	35.8 ± 6.2	32.6 ± 5.1	27.3 ± 5.3 **	25.2 ± 4.1 **
Adrenals (g)	0.013 ± 0.002	0.012 ± 0.002	0.011 ± 0.002 **	0.010 ± 0.002 **
Adrenals (%)	0.036 ± 0.007	0.039 ± 0.010	0.041 ± 0.007	0.039 ± 0.009
Heart (g)	0.173 ± 0.031	0.174 ± 0.033	0.152 ± 0.016 *	0.145 ± 0.025 **
Heart (%)	0.493 ± 0.102	0.541 ± 0.094	0.570 ± 0.086 **	0.580 ± 0.061 *
Lungs (g)	0.218 ± 0.070	0.230 ± 0.028 *	0.253 ± 0.151	0.210 ± 0.043
Lungs (%)	0.642 ± 0.314	0.718 ± 0.103 *	0.963 ± 0.702 **	0.850 ± 0.206 **
Kidneys (g)	0.410 ± 0.043	0.617 ± 1.011	0.490 ± 0.524	0.373 ± 0.082 **
Kidneys (%)	1.170 ± 0.190	1.875 ± 2.845 *	1.885 ± 2.336 **	1.478 ± 0.160 **
Liver (g)	1.499 ± 0.341	1.567 ± 0.413	2.645 ± 0.954 **	6.056 ± 4.245 **
Liver (%)	4.268 ± 1.135	4.867 ± 1.219	9.909 ± 3.902 **	22.727 ± 10.532 **
Brain (g)	0.463 ± 0.014	0.466 ± 0.018	0.458 ± 0.020	0.436 ± 0.021 **
Brain (%)	1.330 ± 0.237	1.467 ± 0.245	1.713 ± 0.204 **	1.775 ± 0.283 **

Mean ± S.D.

*) Significant difference, $p<0.05$ (Test of Dunnett)

**) Significant difference, $p<0.01$ (Test of Dunnett)

TABLE 15
INCIDENCES OF SELECTED NEOPLASTIC LESIONS OF MALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Group name Number of examined animals	Control <50>	5000 ppm <50>	10000 ppm <50>	20000 ppm <50>		
Liver						
Hepatocellular adenoma[1]	12 (24%) [a]	17 (34%)	18 (36%)	3 (6%) *		⇩
Hepatocellular carcinoma[2]	16 (32%)	11 (22%)	14 (28%)	39 (78%) **	⬆⬆	⇧⇧
Hepatoblastoma[3]	1 (2%)	12 (24%) **	18 (36%) **	38 (76%) **	⬆⬆	⇧⇧
1)+2)	22 (44%)	26 (52%)	25 (50%)	40 (80%) **	⬆⬆	⇧⇧
2)+3)	16 (32%)	19 (38%)	28 (56%) *	43 (86%) **	⬆⬆	⇧⇧
1)+2)+3)	22 (44%)	27 (54%)	33 (66%) *	43 (86%) **	⬆⬆	⇧⇧
Hemangioma[4]	7 (14%)	2 (4%)	1 (2%) *	0 (0%) **		⇩⇩
Hemangiosarcoma[5]	1 (2%)	1 (2%)	0 (0%)	0 (0%)		
4)+5)	8 (16%)	3 (6%)	1 (2%) *	0 (0%) **		⇩⇩
Spleen						
Mastcytoma:malignant	0 (0%)	0 (0%)	6 (12%) *	0 (0%)		
Hemangioma[6]	4 (8%)	1 (2%)	0 (0%)	0 (0%)		⇩
Hemangiosarcoma[7]	0 (0%)	1 (2%)	0 (0%)	0 (0%)		
6)+7)	4 (8%)	2 (4%)	0 (0%)	0 (0%)		⇩
Lung						
Bronchiolar-alveolar adenoma[8]	6 (12%)	2 (4%)	1 (2%)	1 (2%)		⇩
Bronchiolar-alveolar carcinoma[9]	3 (6%)	1 (2%)	2 (4%)	1 (2%)		
8)+9)	9 (18%)	3 (6%)	3 (6%)	2 (4%) *		⇩
Lymph node						
Malignant lymphoma	8 (16%)	13 (26%)	6 (12%)	3 (6%)		⇩

[a] : No. of animals with bearing tumor (incidence ; %)
* : Significant difference, $p<0.05$ (Fisher's exact test for neoplastic lesion)
** : Significant difference, $p<0.01$ (Fisher's exact test for neoplastic lesion)
⬆⬆ : Significant difference, $p<0.01$ (Peto test for neoplastic lesion)
⇩⇩and ⇧⇧ : Significant difference, $p<0.01$ (Cochran-Armitage test for neoplastic lesion)
⇩ : Significant difference, $p<0.05$ (Cochran-Armitage test for neoplastic lesion)

TABLE 16
INCIDENCES OF SELECTED NEOPLASTIC LESIONS OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE (SELECTED)

Group name Number of examined animals	Control <50>	5000 ppm <50>	10000 ppm <50>	20000 ppm <50>		
Liver						
Hepatocellular adenoma[1]	5 (10%) [a]	18 (36%) **	13 (26%) *	4 (8%)		
Hepatocellular carcinoma[2]	2 (4%)	12 (24%) **	41 (82%) **	46 (92%) **	⬆⬆	⇧⇧
Hepatoblastoma[3]	0 (0%)	0 (0%)	8 (16%) **	38 (76%) **	⬆⬆	⇧⇧
1)+2)	7 (14%)	24 (48%) **	44 (88%) **	47 (94%) **	⬆⬆	⇧⇧
2)+3)	2 (4%)	12 (24%) **	42 (84%) **	48 (96%) **	⬆⬆	⇧⇧
1)+2)+3)	7 (14%)	24 (48%) **	45 (90%) **	48 (96%) **	⬆⬆	⇧⇧
Histiocytic sarcoma	1 (2%)	0 (0%)	0 (0%)	3 (6%)	⬆	
Hemangioma	3 (6%)	1 (2%)	0 (0%)	0 (0%)		⇩
Spleen						
Malignant lymphoma	7 (14%)	7 (14%)	4 (8%)	1 (2%) *		⇩
Lymph node						
Malignant lymphoma	16 (32%)	16 (32%)	14 (28%)	3 (6%) **		⇩⇩
Uterus						
Endometrial stromal polyp	5 (10%)	1 (2%)	0 (0%) *	0 (0%) *		⇩⇩
All site						
Histiocytic sarcoma	18 (36%)	17 (34%)	15 (30%)	15 (30%)		

[a] : No. of animals with bearing tumor (incidence ; %)
* : Significant difference, $p<0.05$ (Fisher's exact test for neoplastic lesion)
** : Significant difference, $p<0.01$ (Fisher's exact test for neoplastic lesion)
⬆⬆ : Significant difference, $p<0.01$ (Peto test for neoplastic lesion)
⬆ : Significant difference, $p<0.05$ (Peto test for neoplastic lesion)
⇩⇩and ⇧⇧ : Significant difference, $p<0.01$ (Cochran-Armitage test for neoplastic lesion)
⇩ : Significant difference, $p<0.05$ (Cochran-Armitage test for neoplastic lesion)

TABLE 17

INCIDENCES OF SELECTED NON-NEOPLASTIC LESIONS OF MALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Group name	Control				5000 ppm				10000 ppm				20000 ppm			
Number of examined animals	50				50				50				50			
Grade	<1>	<2>	<3>	<4>	<1>	<2>	<3>	<4>	<1>	<2>	<3>	<4>	<1>	<2>	<3>	<4>
Nasal cavity																
Eosinophilic change : respiratory epithelium	12	1	0	0	20	4	0	0	19	7	0	0 *	29	10	0	0 **
Atrophy: olfactory epithelium	0	0	0	0	2	0	0	0	3	3	0	0 *	1	0	0	0
Lung																
Bronchiolar-alveolar cell hyperplasia	3	0	0	0	44	3	0	0 **	40	2	0	0 **	36	5	0	0 **
Bone marrow																
Erythropoiesis:increased	0	0	0	0	3	0	0	0	6	0	0	0 *	14	0	0	0 **
Spleen																
Deposit of hemosiderin	0	0	0	0	0	0	0	0	8	0	0	0 **	13	1	0	0 **
Extramedullary hematopoiesis	9	4	3	0	4	6	6	0	4	4	5	0	5	6	19	2 **
Tooth																
Dysplasia	8	10	8	0	26	11	2	0 **	18	13	6	0	23	5	1	0 **
Liver																
Granulation	13	0	0	0	15	0	0	0	6	1	0	0	0	0	0	0 **
Hepatocellular hypertrophy: central	0	0	0	0	0	15	0	0 **	0	34	1	0 **	0	35	0	0 **
Nuclear atypia:central	0	0	0	0	0	0	0	0	11	2	0	0 **	17	21	0	0 **
Acidophilic cell focus	5	2	0	0	5	2	1	0	3	3	0	0	9	4	0	0
Kidney																
Basophilic change	16	1	0	0	20	0	0	0	14	0	0	0	1	0	0	0 **
Deposit of hemosiderin	0	0	0	0	1	2	1	0	0	2	7	0 **	2	4	17	2 **
Testis																
Mineralization	27	18	2	0	32	9	4	0	23	3	0	0 **	5	0	0	0 **

Grade <1>:Slight, <2>:Moderate, <3>:Marked, <4>:Severe

*) Significant difference, $p<0.05$ (Test of Chi Square)

**) Significant difference, $p<0.01$ (Test of Chi Square)

TABLE 18

INCIDENCES OF SELECTED NON-NEOPLASTIC LESIONS OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Group name	Control				5000 ppm				10000 ppm				20000 ppm			
Number of examined animals	50				50				50				50			
Grade	<1>	<2>	<3>	<4>	<1>	<2>	<3>	<4>	<1>	<2>	<3>	<4>	<1>	<2>	<3>	<4>
Nasal cavity																
Eosinophilic change : olfactory epithelium	4	0	0	0	7	3	0	0	15	2	0	0 **	26	2	0	0 **
Eosinophilic change : respiratory epithelium	26	6	0	0	30	13	1	1 *	29	13	0	0 *	20	24	1	0 **
Respiratory metaplasia:gland	6	0	0	0	4	0	0	0	6	0	0	0	15	0	0	0 *
Nasopharynx																
Eosinophilic change : respiratory epithelium	3	0	0	0	5	0	3	1	7	3	1	0	11	0	0	0 *
Lung																
Bronchiolar-alveolar cell hyperplasia	0	0	0	0	40	2	0	0 **	40	0	0	0 **	41	2	0	0 **
Bone marrow																
Erythropoiesis:increased	2	0	0	0	0	0	0	0	2	0	0	0	17	0	0	0 **
Spleen																
Deposit of hemosiderin	3	0	0	0	13	0	0	0 *	22	0	0	0 **	13	0	0	0 *
Extramedullary hematopoiesis	5	6	9	0	8	1	4	0	11	5	3	1	9	3	29	0 **
Heart																
Mineralization	4	0	0	0	3	0	0	0	6	1	0	0	13	1	0	0 *
Liver																
Hepatocellular hypertrophy: central	0	0	0	0	0	0	0	0	0	1	0	0	0	9	0	0 **
Acidophilic cell focus	2	0	0	0	4	0	0	0	9	0	0	0	7	0	0	0
Kidney																
Deposit of hemosiderin	0	0	0	0	1	0	0	0	1	1	2	0	0	5	24	5 **
Adrenal																
Spindle-cell hyperplasia	5	43	2	0	4	40	6	0	1	41	4	0	21	28	0	0 **

Grade <1>:Slight, <2>:Moderate, <3>:Marked, <4>:Severe
*) Significant difference, $p<0.05$ (Test of Chi Square)
**) Significant difference, $p<0.01$ (Test of Chi Square)

TABLE 19
CAUSE OF DEATH OF MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

	Male				Female			
Group	Control	5000ppm	10000ppm	20000ppm	Control	5000ppm	10000ppm	20000ppm
Number of dead or moribund animals	14	15	23	34	27	23	20	37
No microscopical confirmation	1	0	2	2	1	0	0	0
Urinary retention	2	0	0	2	0	0	0	0
Endocrine system lesion	0	0	0	0	1	0	0	0
Tooth lesion	0	0	2	0	1	0	0	0
Hydronephrosis	2	2	2	0	0	0	1	0
Tumor death :leukemia	4	5	2	1	8	11	7	0
subcutis	0	1	1	1	1	0	1	1
lung	0	0	2	0	0	0	1	0
spleen	1	0	2	0	1	0	0	0
liver	3	7	10	26	2	0	2	31
epididymis	0	0	0	1	—	—	—	—
ovary	—	—	—	—	0	1	0	0
uterus	—	—	—	—	10	11	8	5
brain	0	0	0	1	0	0	0	0
muscle	1	0	0	0	1	0	0	0
peritoneum	0	0	0	0	1	0	0	0

TABLE 20 HISTORICAL CONTROL DATA OF SELECTED NEOPLASTIC LESIONS IN JAPAN BIOASSAY RESEARCH CENTER : Crj:BDF1 MALE MICE

Organs / Tumors	No. of animals examined	No. of animals bearing tumor	Incidence (%)	Min. - Max. (%)
Liver	<1446>			
Hepatocellular adenoma 1)		261	18.0	4 - 34
Hepatocellular carcinoma 2)		291	20.1	2 - 42
Hepatoblastoma 3)		9	0.6	0 - 6
1)+2)		508	35.1	8 - 68
2)+3)		295	20.4	2 - 46
1)+2)+3)		513	35.5	8 - 72
Hemangioma 4)		32	2.2	0 - 12
Hemangiosarcoma 5)		67	4.6	0 - 14
4)+5)				
Spleen	<1445>			
Mastocytoma: malignant		3	0.2	0 - 4
Hemangioma 6)		33	2.3	0 - 10
Hemangiosarcoma 7)		47	3.3	0 - 10
6)+7)				
Lung	<1445>			
Bronchio-alveolar adenoma 8)		113	7.8	2 - 18
Bronchio-alveolar carcinoma 9)		160	11.1	0 - 24
8)+9)		271	18.8	2 - 30
Lymph node	<1446>			
Malignant lymphoma		170	11.8	2 - 28

29 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, 0406

TABLE 21 HISTORICAL CONTROL DATA OF SELECTED NEOPLASTIC LESIONS IN JAPAN BIOASSAY RESEARCH CENTER : Crj:BDF1 FEMALE MICE

Organs / Tumors	No. of animals examined	No. of animals bearing tumor	Incidence (%)	Min. - Max. (%)
Liver	<1448>			
Hepatocellular adenoma 1)		77	5.3	0 - 10
Hepatocellular carcinoma 2)		35	2.4	0 - 12
Hepatoblastoma 3)		0	0.0	0 - 0
1)+2)		108	7.5	4 - 14
2)+3)		35	2.4	0 - 12
1)+2)+3)		108	7.5	2 - 14
Histiocytic sarcoma		19	1.3	0 - 4
Hemangioma		15	1.0	0 - 6
Lung	<1448>			
Bronchio-alveolar adenoma 4)		52	3.6	0 - 10
Bronchio-alveolar carcinoma 5)		43	3.0	0 - 8
4)+5)		94	6.5	0 - 14
Spleen	<1447>			
Malignant lymphoma		94	6.5	0 - 26
Lymph node	<1448>			
Malignant lymphoma		426	29.4	12 - 46
Uterus	<1446>			
Endometrial stromal polyp		44	3.0	0 - 10

29 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, 0406

FIGURES

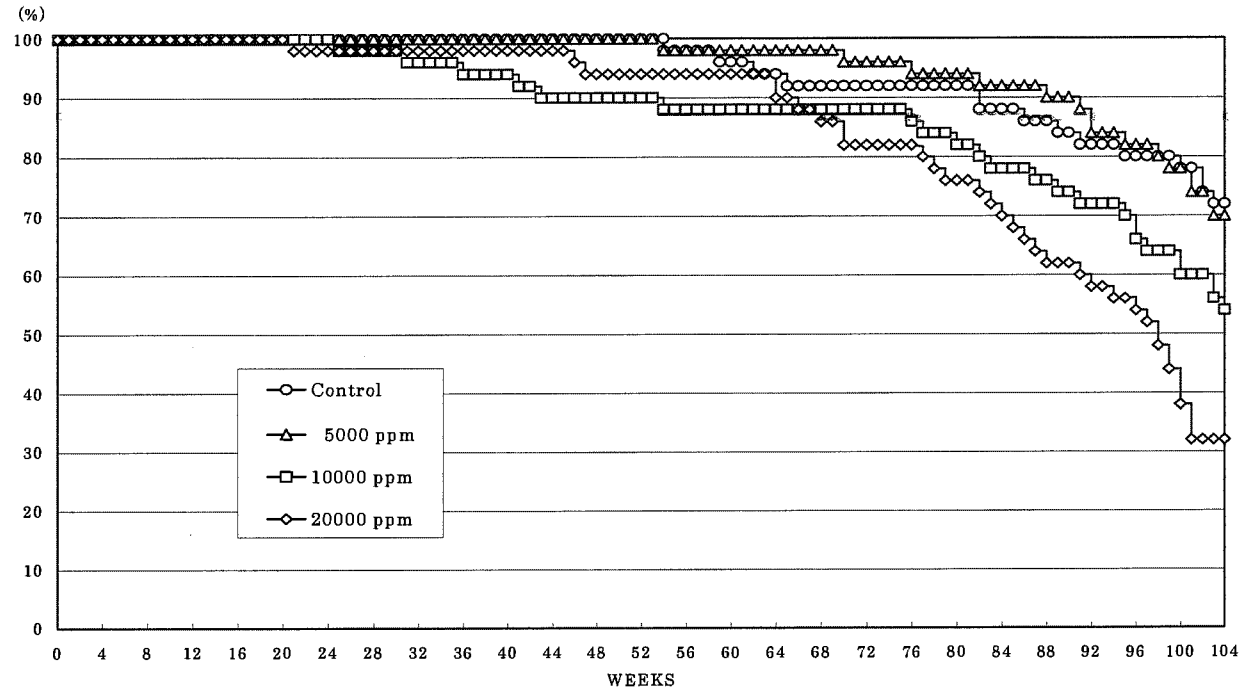


FIGURE 1 SURVIVAL ANIMAL RATE OF MALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

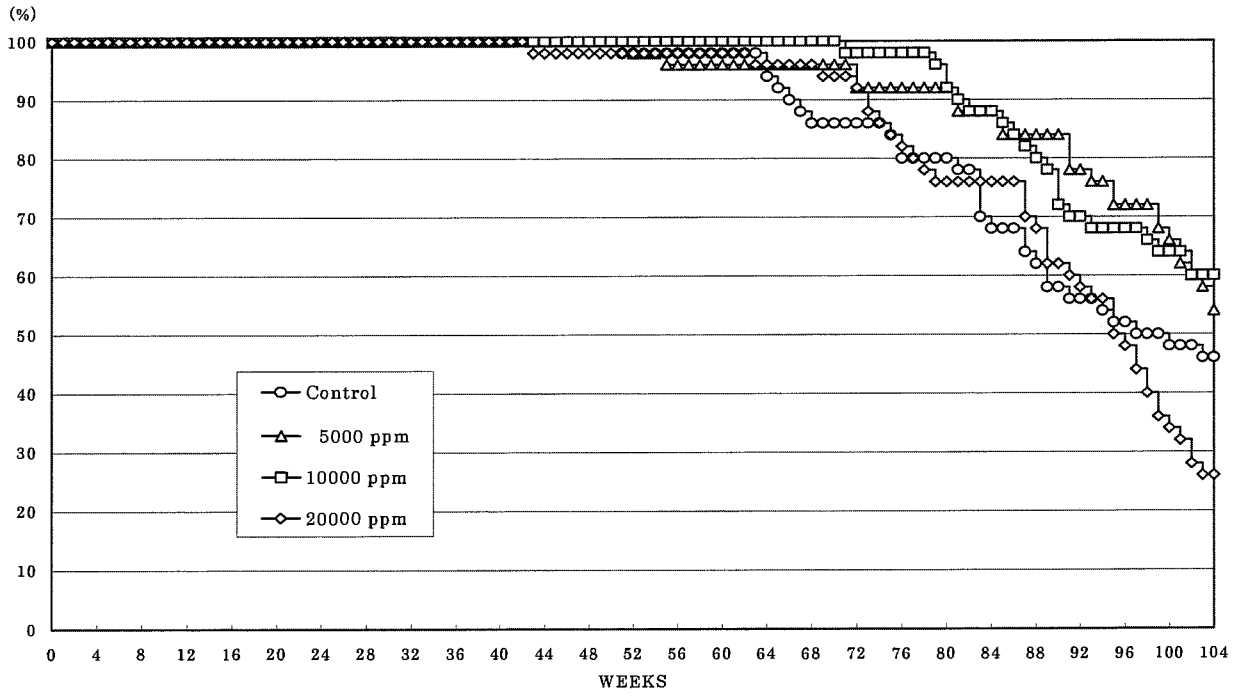


FIGURE 2 SURVIVAL ANIMAL RATE OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

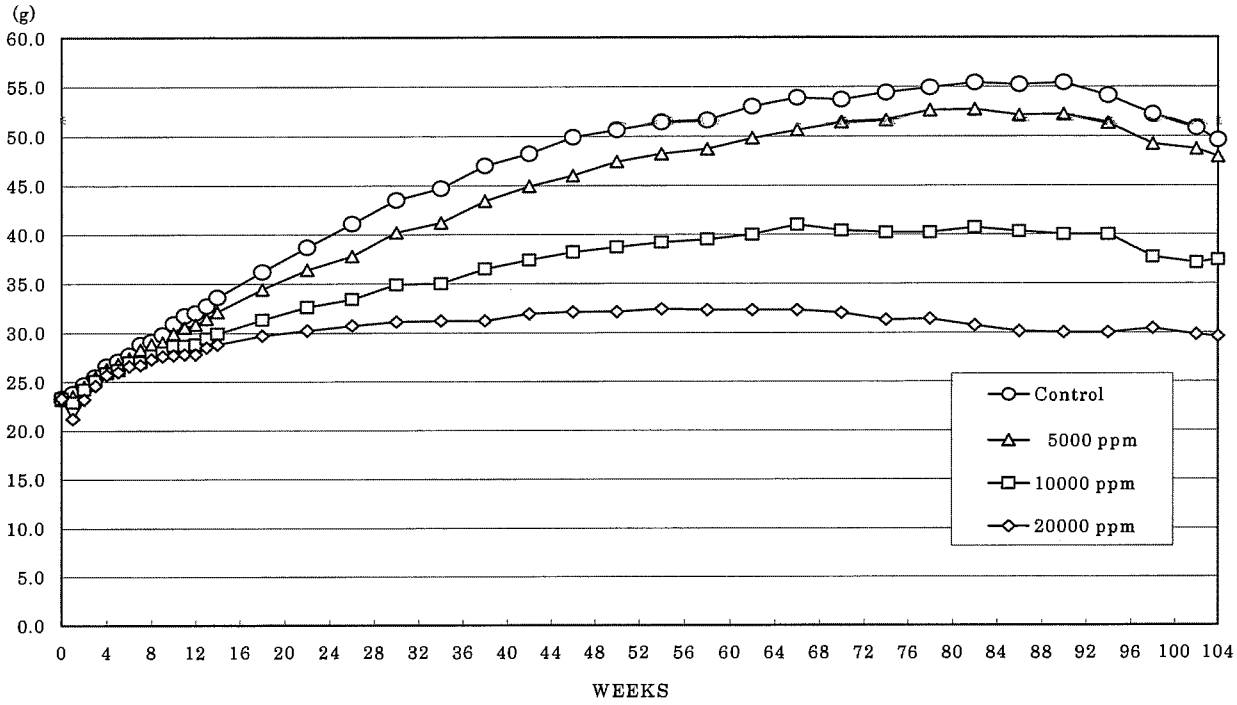


FIGURE 3 BODY WEIGHT CHANGES OF MALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

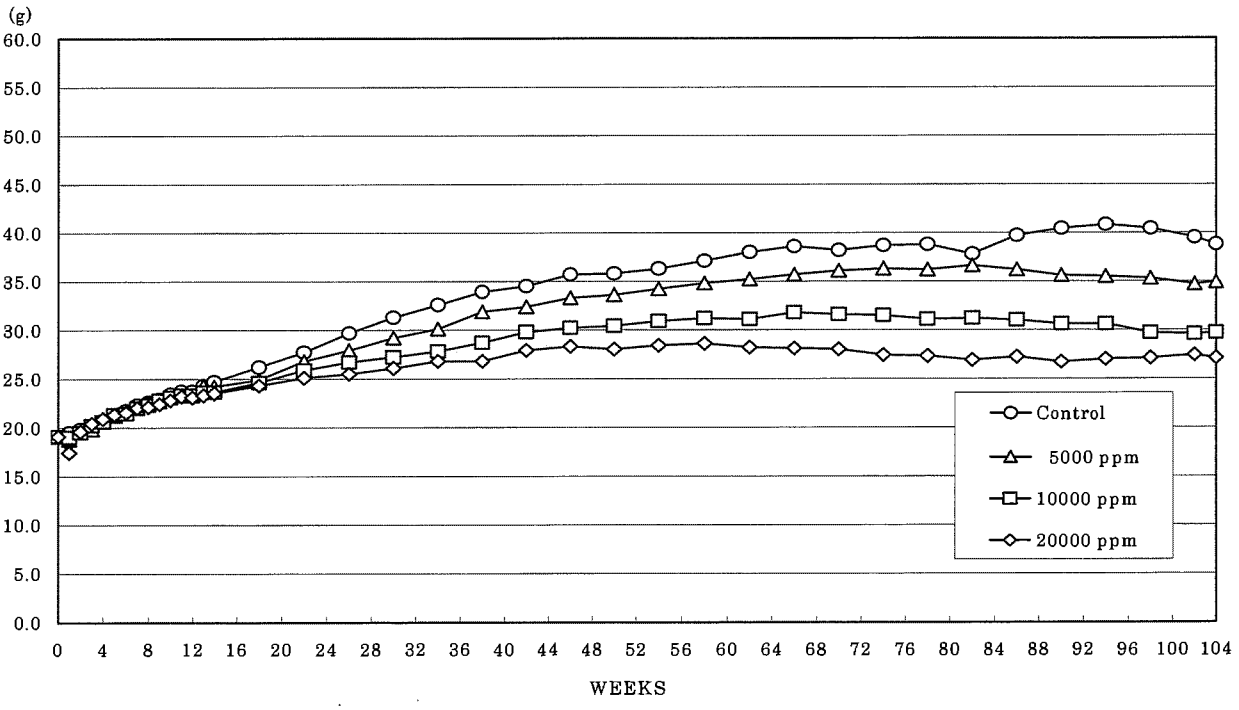


FIGURE 4 BODY WEIGHT CHANGES OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

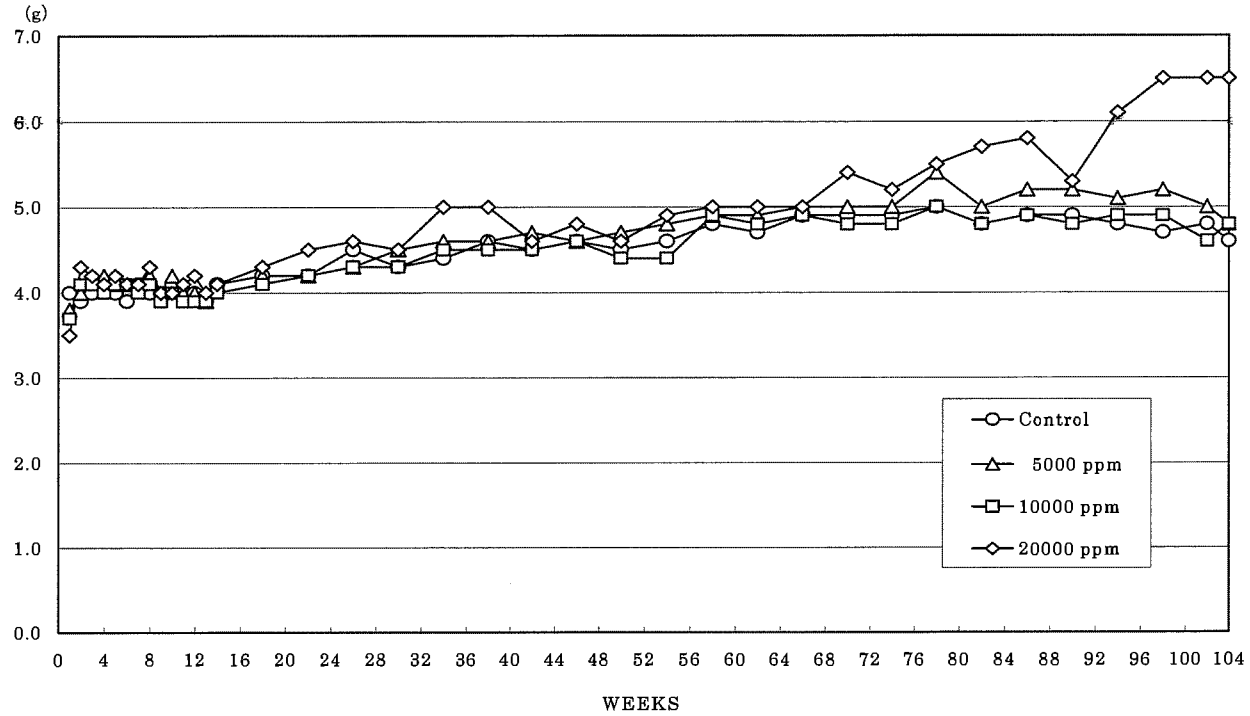


FIGURE 5 FOOD CONSUMPTION CHANGES OF MALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

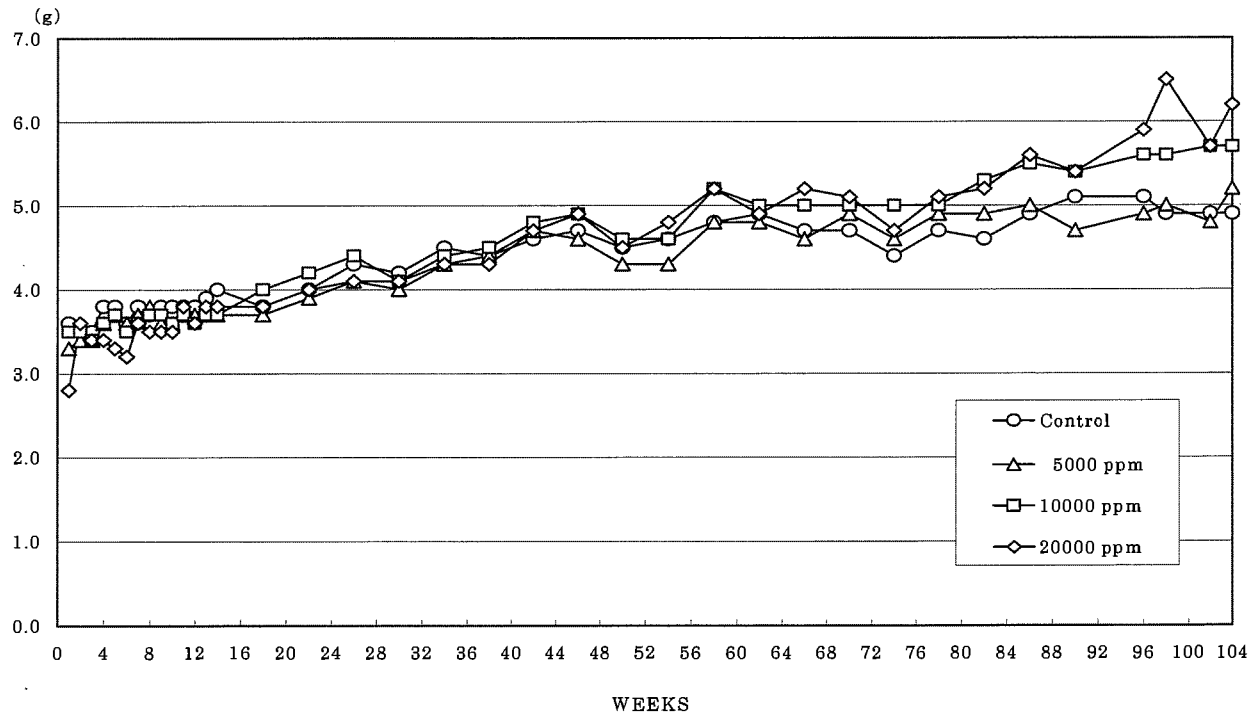
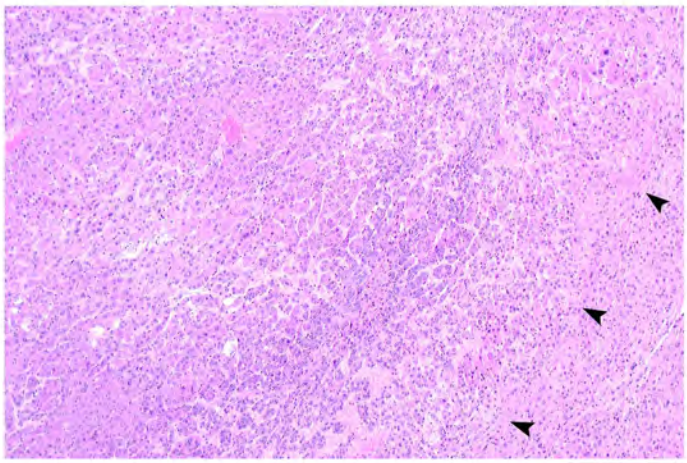
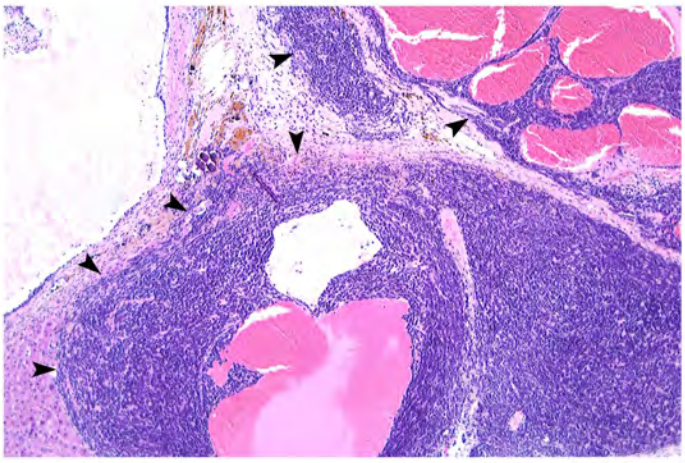


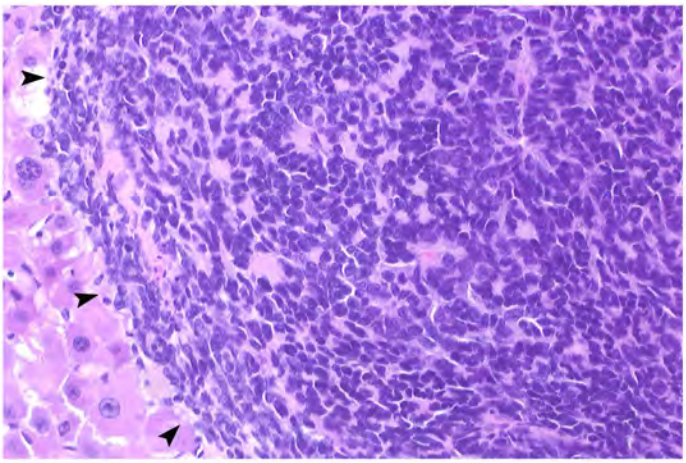
FIGURE 6 FOOD CONSUMPTION CHANGES OF FEMALE MICE IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE



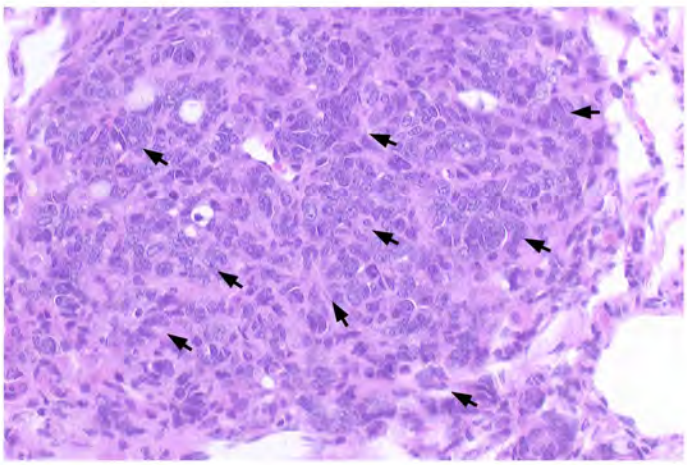
Photograph 1 500 μm
Liver: Hepatocellular carcinoma (arrow heads).
Mouse, Male, 20000 ppm, Animal No. 0402-1337 (H&E)



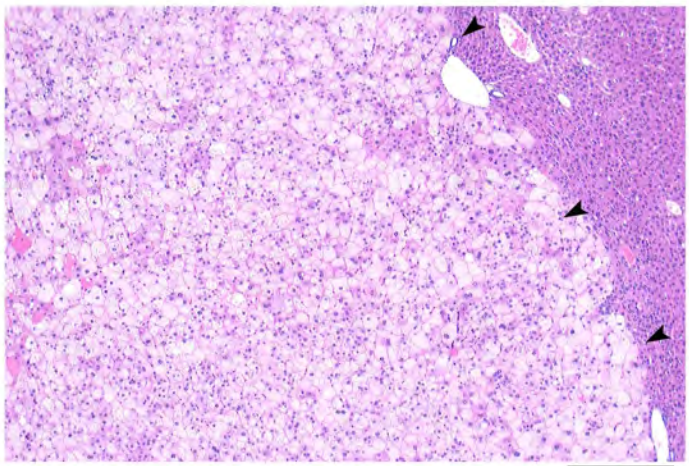
Photograph 2 500 μm
Liver: Hepatoblastoma (arrow heads).
Mouse, Male, 20000 ppm, Animal No. 0402-1306 (H&E)



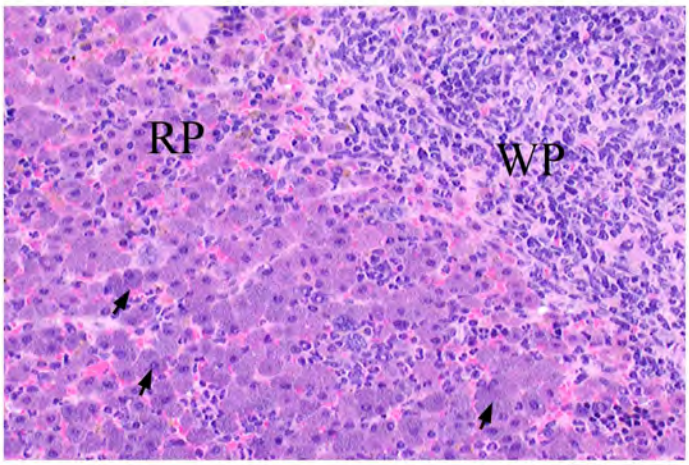
Photograph 3 100 μm
Liver: Hepatoblastoma (arrow heads).
The same animal as that shown in photograph 2 (H&E)



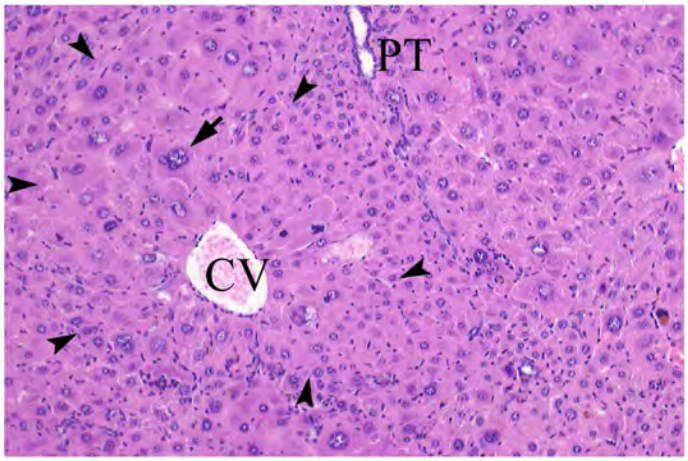
Photograph 4 100 μm
Lung: Metastasis of hepatoblastoma (arrows).
Mouse, Male, 10000 ppm, Animal No. 0402-1219 (H&E)



Photograph 5 500 μm
Liver: Hepatocellular adenoma (arrow heads).
Mouse, Female,5000 ppm, Animal No. 0402-2107 (H&E)

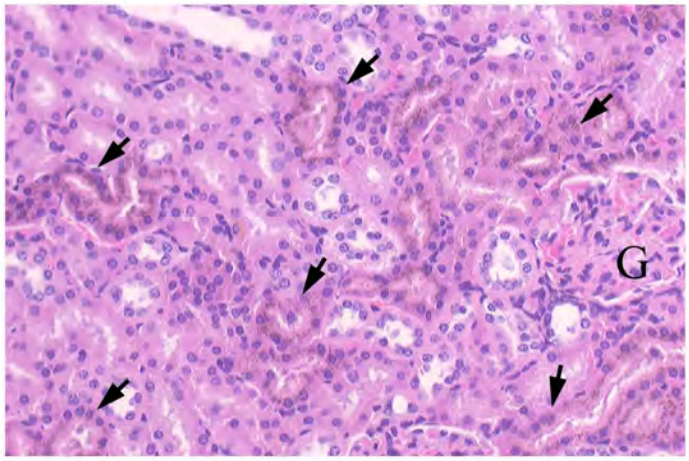


Photograph 6 100 μm
Spleen: Malignant mastcytoma. Tumor cells (arrows) increased in the red pulp(RP). White pulp (WP).
Mouse, Male, 10000 ppm, Animal No. 0402-1217 (H&E)



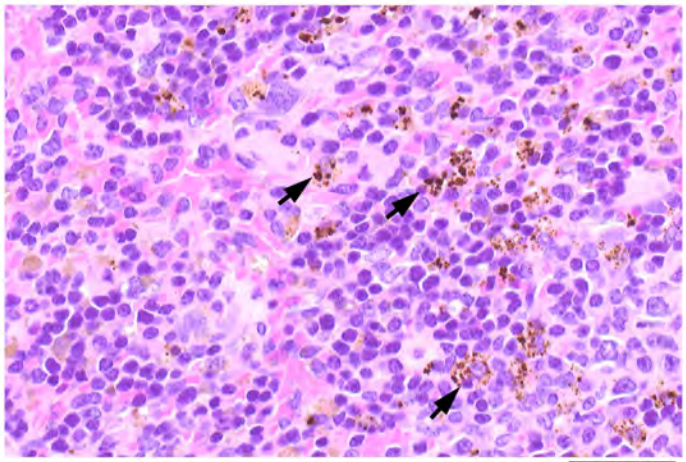
100 μm

Photograph 7
Liver: Hepatocellular hypertrophy in the centrilobular area (arrow heads), and hepatocytes with nuclear atypia in the centrilobular area(arrow). Central vein(CV), Portal triad(PT), Mouse, Male, 20000 ppm, Animal No. 0402-1304 (H&E)



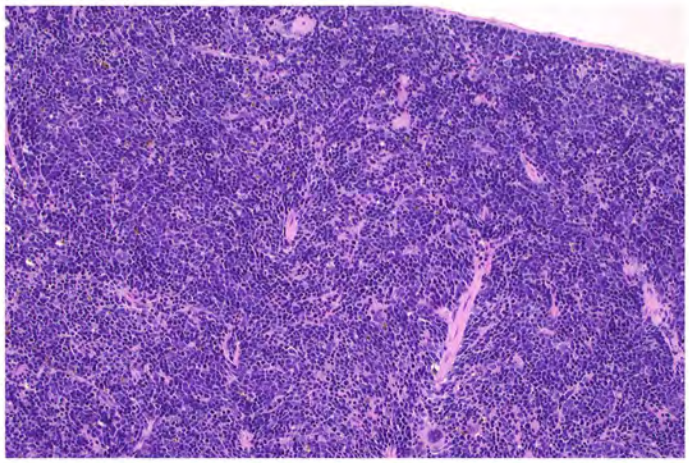
100 μm

Photograph 8
Kidney: Deposit of hemosiderin found in the proximal tubule (arrows). Glomerulus(G),
Mouse, Male, 20000 ppm, Animal No. 0402-1306 (H&E)



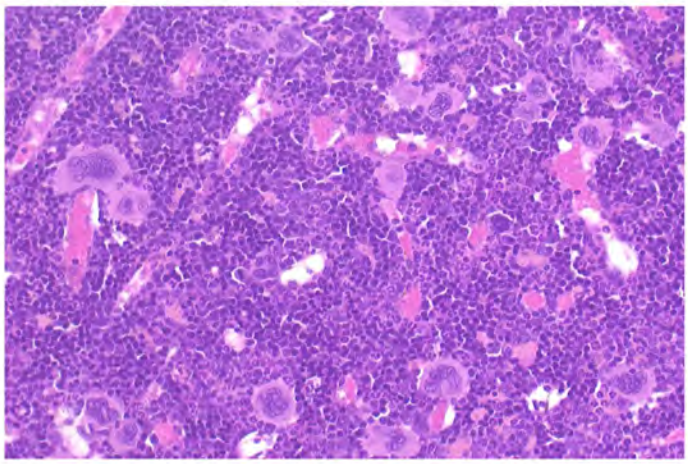
50 μm

Photograph 9
Spleen: Deposit of hemosiderin (arrows).
Mouse, Male, 10000 ppm, Animal No. 0402-1214 (H&E)



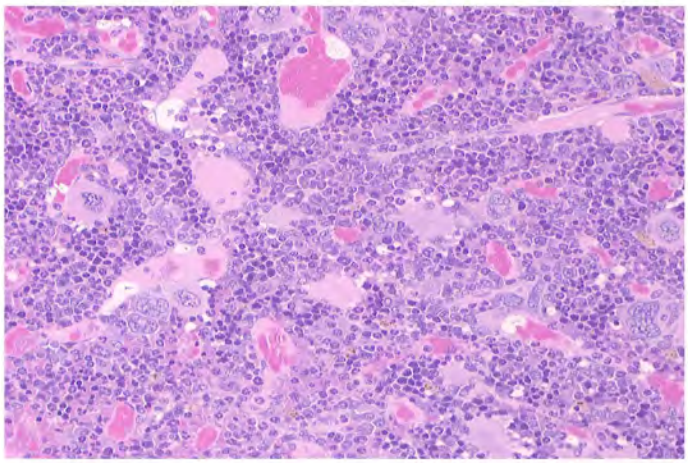
200 μm

Photograph 10
Spleen: Extramedullary hematopoiesis.
Mouse, Male, 20000 ppm, Animal No. 0402-1301 (H&E)



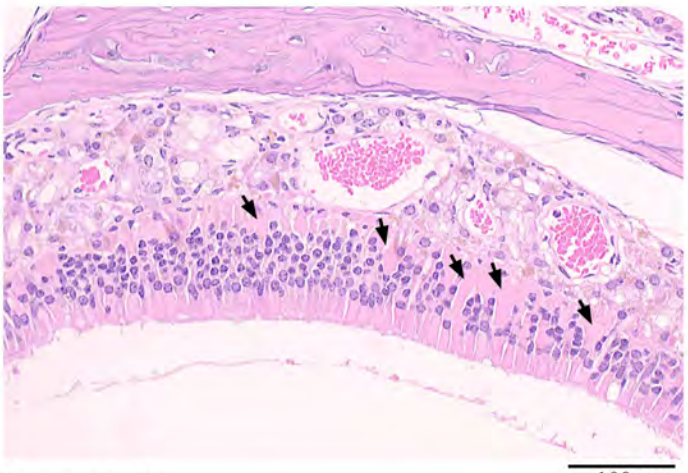
100 μm

Photograph 11
Bone Marrow: Increased erythropoiesis.
Mouse, Male, 20000 ppm, Animal No. 0402-1310 (H&E)

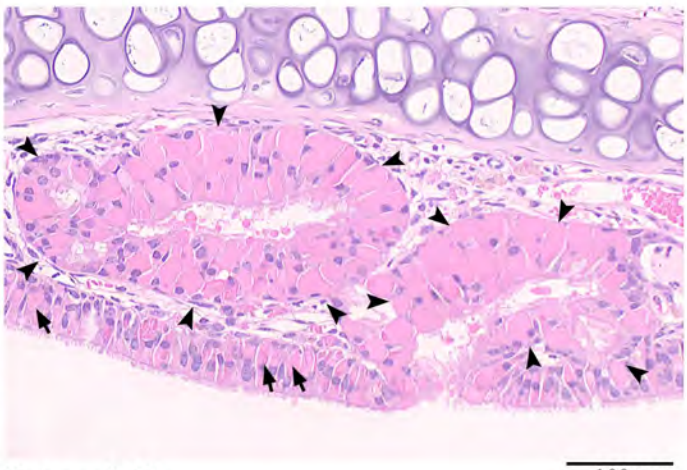


100 μm

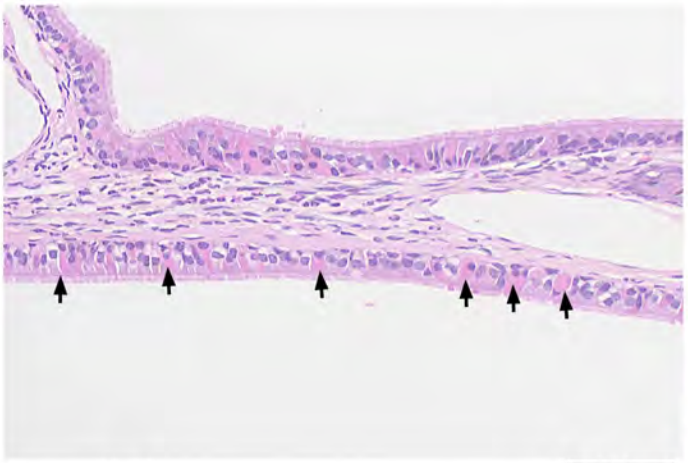
Photograph 12
Bone marrow: Normal.
Mouse, Male, Control, Animal No. 0402-1002 (H&E)



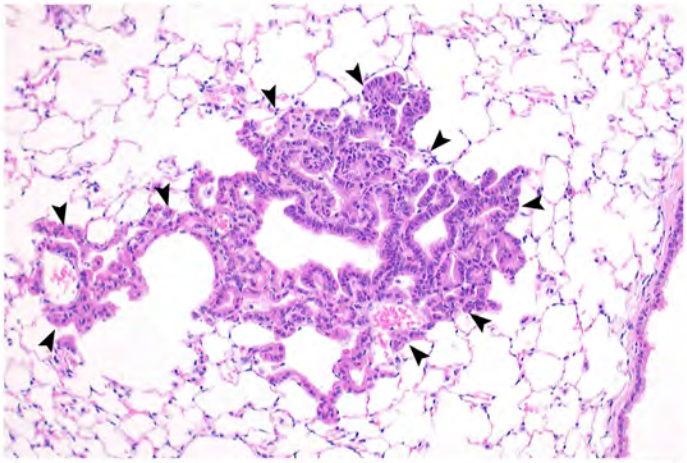
Photograph 13
Nasal cavity: Eosinophilic change of the olfactory epithelium (arrows). Level 2.
Mouse, Female, 20000 ppm, Animal No. 0402-2305 (H&E)



Photograph 14
Nasal cavity: Respiratory metaplasia of the nasal gland (arrow heads) and eosinophilic change of the respiratory epithelium (arrows). Level 2.
Mouse, Female, 20000 ppm, Animal No. 0402-2305 (H&E)



Photograph 15
Nasopharynx: Eosinophilic change of the respitarory epithelium (arrows).
Mouse, Female, 20000 ppm, Animal No. 0402-2305 (H&E)



Photograph 16
Lung: Bronchiolar-alveolar cell hyperplasia (arrow heads).
Mouse, Male, 5000 ppm, Animal No. 0402-1102 (H&E)