Summary of Drinking Water Carcinogenicity Study of *o*-Phenylenediamine Dihydrochloride in BDF1 Mice

February 2004

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 February 26 2004.

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

Summary of Drinking Water Carcinogenicity Study of *o*-Phenylenediamine Dihydrochloride in BDF1 Mice

Purpose, materials and methods

o-Phenylenediamine dihydrochloride (*o*-PD2HCl, 1,2-benzenediamine dihydrochloride, CAS No. 615-28-1) is a light red crystalline powder with a melting point of 258°C and is soluble in water.

The carcinogenicity and chronic toxicity of *o*-PD2HCl were examined in groups of 50 Crj:BDF1 mice of both sexes administered *o*-PD2HCl in drinking water for 2 years (104 weeks). The drinking water concentration of *o*-PD2HCl was 0, 500, 1000 or 2000 ppm (w/w) for male mice and 0, 1000, 2000 or 4000 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. *o*-PD2HCl was analyzed for purity and stability by both infrared spectrometry and high performance liquid chromatography before and after its use. The concentrations of *o*-PD2HCl in drinking water were determined by high performance liquid chromatography at the time of preparation, and on 8th day after preparation, while stored at room temperature. The animals were observed daily for clinical signs and mortality. Body weight, water consumption 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 dose-response relationship 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, water consumption, food consumption, hematological and blood biochemical parameters and organ weights were analyzed by Dunnett's test. The present studies were 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 *o*-PD2HCl-administered females were slightly higher than those of the control. Body weights, food consumption and water consumption of all the *o*-PD2HCl-administered groups of both sexes were significantly decreased, but water consumption of the 2000 and 4000 ppm-administered females was recovered to the control level during the late period of 2-year administration.

The incidences of hepatocellular adenomas in males and hepatocellular adenomas and carcinomas in females were increased dose-dependently. The significantly increased incidence of hepatocellular adenomas in the males and females was noted even at the lowest dose level, whereas the incidence of hepatocellular carcinomas was significantly increased in the females administered 2000 ppm and above. The incidences of the hepatocellular tumors exceeded the respective maximum incidences of the Japan Bioassay Research Center (JBRC) historical control data. As pre-neoplastic lesions, the incidences of altered cell foci in the liver, including basophilic, acidophilic and clear cell foci, were increased in the 4000 ppm-administered females. In addition to hepatic tumors, blood biochemical parameters (ALP and ALT (GPT)) were increased. Moreover, the incidence of benign papillary adenomas in the gall bladder was increased in both male and females administered 2000 ppm. Since the gall bladder papillary adenoma has not been observed in the JBRC historical control data, the gall bladder tumor was judged to be induced by the *o*-PD2HCl administration. In addition, histopathological findings relating to the *o*-PD2HCl administration were noted in the nasal cavity, nasopharynx and kidneys.

Since the incidence of hepatocellular adenomas was increased at the lowest dose level, the lower confidence limit of the benchmark dose yielding a response with 10% extra risk ($BMDL_{10}$) was calculated instead of the no-observed-adverse-effect-level (NOAEL). The $BMDL_{10}$ value for the endpoint of hepatocellular adenomas in males was 134 ppm, while the $BMDL_{10}$ value for the endpoint of gall bladder papillary adenomas in males was 1014 ppm.

Conclusions

In mice, there was some evidence of carcinogenic activity of *o*-PD2HCl in males, based on the increased incidences of hepatocellular adenomas and gall bladder papillary adenomas, and there was clear evidence of carcinogenic activity of *o*-PD2HCl in females, based on the increased incidences of hepatocellular adenomas and carcinomas and gall bladder papillary adenomas.

TABLES

TABLES (CONTINUED)

TABLES (CONTINUED)

TABLE 1 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES OF MALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Week on Study	Control		500 ppm			1000 ppm			2000 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	22.8 (50)	50 / 50	22.8 (50)	100	50 / 50	22.8 (50)	100	50 / 50	22.8 (50)	100	50 / 50
1	23.5 (50)	50 / 50	23.4 (50)	100	50 / 50	22.9 (50)	97	50 / 50	22.2 (50)	94	50 / 50
2	24.8 (50)	50 / 50	24.5 (50)	99	50 / 50	24.3 (50)	98	50 / 50	23.5 (50)	95	50 / 50
3	25.4 (50)	50 / 50	25.3 (50)	100	50 / 50	24.9 (50)	98	50 / 50	23.8 (50)	94	50 / 50
4	25.9 (50)	50 / 50	26.0 (50)	100	50 / 50	25.7 (50)	99	50 / 50	24.9 (50)	96	50 / 50
5	26.9 (50)	50 / 50	26.3 (50)	98	50 / 50	26.1 (50)	97	50 / 50	25.1 (50)	93	50 / 50
6	27.6 (50)	50 / 50	27.2 (50)	99	50 / 50	26.7 (50)	97	50 / 50	26.2 (50)	95	50 / 50
7	28.2 (50)	50 / 50	27.5 (50)	98	50 / 50	26.7 (50)	95	50 / 50	26.5 (50)	94	50 / 50
8	29.6 (50)	50 / 50	28.9 (50)	98	50 / 50	28.0 (50)	95	50 / 50	27.3 (50)	92	50 / 50
9	29.9 (50)	50 / 50	29.2 (50)	98	50 / 50	27.8 (50)	93	50 / 50	27.2 (50)	91	50 / 50
10	30.8 (50)	50 / 50	29.5 (50)	96	50 / 50	28.8 (50)	94	50 / 50	28.1 (50)	91	50 / 50
11	31.1 (50)	50 / 50	29.9 (50)	96	50 / 50	29.0 (50)	93	50 / 50	28.0 (50)	90	50 / 50
12	32.0 (50)	50 / 50	30.9 (50)	97	50 / 50	29.6 (50)	93	50 / 50	28.6 (50)	89	50 / 50
13	32.7 (50)	50 / 50	31.3 (50)	96	50 / 50	30.1 (50)	92	50 / 50	28.7 (50)	88	50 / 50
14	33.7 (50)	50 / 50	32.3 (50)	96	50 / 50	30.8 (50)	91	50 / 50	29.4 (50)	87	50 / 50
18	36.2 (50)	50 / 50	34.0 (50)	94	50 / 50	32.4 (50)	90	50 / 50	30.8 (50)	85	50 / 50
22	38.5 (50)	50 / 50	35.9 (50)	93	50 / 50	33.6 (50)	87	50 / 50	31.8 (50)	83	50 / 50
26	40.8 (50)	50 / 50	37.1 (50)	91	50 / 50	35.1 (50)	86	50 / 50	33.2 (50)	81	50 / 50
30	42.9 (50)	50 / 50	38.3 (50)	89	50 / 50	36.1 (50)	84	50 / 50	33.9 (50)	79	50 / 50
34	44.4 (50)	50 / 50	39.0 (50)	88	50 / 50	36.5 (50)	82	50 / 50	34.5 (50)	78	50 / 50
38	45.7 (50)	50 / 50	39.7 (50)	87	50 / 50	37.4 (50)	82	50 / 50	35.1 (50)	77	50 / 50
42	46.8 (50)	50 / 50	41.0 (49)	88	49 / 50	38.2 (50)	82	50 / 50	35.9 (49)	77	49 / 50
46	47.8 (50)	50 / 50	41.9 (49)	88	49 / 50	38.9 (50)	81	50 / 50	36.4 (49)	76	49 / 50
50	48.7 (50)	50 / 50	42.7 (49)	88	49 / 50	39.5 (50)	81	50 / 50	37.2 (49)	76	49 / 50
54	49.5 (50)	50 / 50	43.9 (49)	89	49 / 50	40.3 (50)	81	50 / 50	37.4 (49)	76	49 / 50
58	49.8 (50)	50 / 50	43.4 (48)	87	48 / 50	40.1 (50)	81	50 / 50	37.0 (49)	74	49 / 50
62	50.5 (50)	50 / 50	43.3 (48)	86	48 / 50	40.0 (49)	79	49 / 50	36.7 (49)	73	49 / 50
66	51.6 (50)	50 / 50	45.0 (47)	87	47 / 50	41.0 (49)	79	49 / 50	38.0 (49)	74	49 / 50
70	52.1 (50)	50 / 50	45.1 (46)	87	46 / 50	41.4 (49)	79	49 / 50	38.4 (49)	74	49 / 50
74	52.0 (50)	50 / 50	45.6 (46)	88	46 / 50	41.5 (49)	80	49 / 50	37.6 (49)	72	49 / 50
78	52.1 (50)	50 / 50	45.3 (45)	87	45 / 50	41.7 (49)	80	49 / 50	38.1 (48)	73	48 / 50
82	52.6 (48)	48 / 50	46.0 (44)	87	44 / 50	41.4 (49)	79	49 / 50	37.7 (48)	72	48 / 50
86	52.4 (48)	48 / 50	46.1 (43)	88	43 / 50	41.1 (49)	78	49 / 50	36.5 (47)	70	47 / 50
90	51.6 (47)	47 / 50	44.9 (43)	87	43 / 50	39.4 (48)	76	48 / 50	35.9 (43)	70	43 / 50
94	50.5 (45)	45 / 50	44.6 (41)	88	41 / 50	39.1 (47)	77	47 / 50	35.7 (41)	71	41 / 50
98	51.2 (41)	41 / 50	44.1 (40)	86	40 / 50	38.6 (46)	75	46 / 50	35.2 (40)	69	40 / 50
102	51.8 (39)	39 / 50	43.5 (39)	84	39 / 50	38.5 (42)	74	42 / 50	34.2 (40)	66	40 / 50
104	52.6 (38)	38 / 50	44.4 (38)	84	38 / 50	38.8 (42)	74	42 / 50	34.5 (39)	66	39 / 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 DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Week on Study	Control		1000 ppm			2000 ppm			4000 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	18.9 (50)	50 / 50	18.9 (50)	100	50 / 50	18.9 (50)	100	50 / 50	18.9 (50)	100	50 / 50
1	18.7 (50)	50 / 50	18.9 (50)	101	50 / 50	18.3 (50)	98	50 / 50	16.3 (50)	87	50 / 50
2	19.9 (50)	50 / 50	19.8 (50)	99	50 / 50	19.3 (50)	97	50 / 50	18.1 (50)	91	50 / 50
3	20.5 (50)	50 / 50	20.5 (50)	100	50 / 50	19.8 (50)	97	50 / 50	19.1 (50)	93	50 / 50
4	21.2 (50)	50 / 50	20.8 (50)	98	50 / 50	20.0 (50)	94	50 / 50	19.3 (50)	91	50 / 50
5	21.6 (50)	50 / 50	20.7 (50)	96	50 / 50	20.0 (50)	93	50 / 50	19.7 (50)	91	50 / 50
6	22.2 (50)	50 / 50	22.3 (50)	100	50 / 50	21.0 (50)	95	50 / 50	20.2 (50)	91	50 / 50
7	22.7 (50)	50 / 50	22.2 (50)	98	50 / 50	21.5 (50)	95	50 / 50	20.6 (50)	91	50 / 50
8	23.2 (50)	50 / 50	22.4 (50)	97	50 / 50	21.5 (50)	93	50 / 50	21.1 (50)	91	50 / 50
9	23.3 (50)	50 / 50	23.0 (50)	99	50 / 50	22.2 (50)	95	50 / 50	21.4 (50)	92	50 / 50
10	23.9 (50)	50 / 50	23.5 (50)	98	50 / 50	22.5 (50)	94	50 / 50	21.8 (50)	91	50 / 50
11	24.1 (50)	50 / 50	23.4 (50)	97	50 / 50	22.6 (50)	94	50 / 50	21.8 (50)	90	50 / 50
12	24.1 (50)	50 / 50	23.5 (50)	98	50 / 50	22.8 (50)	95	50 / 50	22.0 (50)	91	50 / 50
13	24.7 (50)	50 / 50	23.8 (50)	96	50 / 50	23.3 (50)	94	50 / 50	22.3 (50)	90	50 / 50
14	24.9 (50)	50 / 50	24.0 (50)	96	50 / 50	23.2 (50)	93	50 / 50	22.4 (50)	90	50 / 50
18	26.0 (50)	50 / 50	25.1 (50)	97	50 / 50	24.2 (50)	93	50 / 50	23.3 (50)	90	50 / 50
22	27.3 (50)	50 / 50	25.8 (50)	95	50 / 50	24.7 (50)	90	50 / 50	23.7 (50)	87	50 / 50
26	28.6 (50)	50 / 50	26.5 (50)	93	50 / 50	25.5 (50)	89	50 / 50	24.4 (50)	85	50 / 50
30	29.7 (50)	50 / 50	27.1 (50)	91	50 / 50	25.5 (50)	86	50 / 50	24.5 (50)	82	50 / 50
34	30.5 (50)	50 / 50	27.6 (50)	90	50 / 50	26.1 (50)	86	50 / 50	24.6 (50)	81	50 / 50
38	31.3 (50)	50 / 50	28.3 (50)	90	50 / 50	26.7 (50)	85	50 / 50	25.1 (50)	80	50 / 50
42	31.8 (50)	50 / 50	28.6 (50)	90	50 / 50	27.1 (50)	85	50 / 50	25.3 (50)	80	50 / 50
46	32.6 (49)	49 / 50	29.6 (50)	91	50 / 50	26.8 (50)	82	50 / 50	25.7 (50)	79	50 / 50
50	33.2 (49)	49 / 50	29.7 (49)	89	49 / 50	27.6 (50)	83	50 / 50	25.9 (49)	78	49 / 50
54	33.8 (48)	48 / 50	30.0 (49)	89	49 / 50	27.4 (50)	81	50 / 50	26.0 (49)	77	49 / 50
58	33.8 (48)	48 / 50	29.7 (49)	88	49 / 50	27.3 (49)	81	49 / 50	25.7 (49)	76	49 / 50
62	33.4 (48)	48 / 50	30.4 (49)	91	49 / 50	27.2 (49)	81	49 / 50	25.6 (49)	77	49 / 50
66	34.5 (48)	48 / 50	31.3 (48)	91	48 / 50	27.9 (49)	81	49 / 50	26.1 (48)	76	48 / 50
70	35.1 (48)	48 / 50	31.8 (48)	91	48 / 50	28.3 (49)	81	49 / 50	25.7 (48)	73	48 / 50
74	35.5 (48)	48 / 50	32.1 (46)	90	46 / 50	28.3 (48)	80	48 / 50	25.5 (47)	72	47 / 50
78	35.4 (47)	47 / 50	32.1 (45)	91	45 / 50	28.4 (46)	80	46 / 50	25.4 (46)	72	46 / 50
82	35.7 (41)	41 / 50	32.5 (43)	91	43 / 50	29.1 (45)	82	45 / 50	24.7 (45)	69	45 / 50
86	35.6 (39)	39 / 50	32.0 (42)	90	42 / 50	28.7 (42)	81	42 / 50	24.0 (44)	67	44 / 50
90	34.7 (39)	39 / 50	31.6 (40)	91	40 / 50	28.7 (39)	83	39 / 50	23.7 (43)	68	43 / 50
94	34.2 (36)	36 / 50	31.3 (36)	92	36 / 50	28.6 (34)	84	34 / 50	23.6 (40)	69	40 / 50
98	34.0 (35)	35 / 50	31.3 (33)	92	33 / 50	28.4 (33)	84	33 / 50	23.5 (40)	69	40 / 50
102	33.4 (30)	30 / 50	32.0 (30)	96	30 / 50	28.5 (28)	85	28 / 50	23.5 (35)	70	35 / 50
104	35.8 (24)	24 / 50	31.5 (29)	88	29 / 50	29.3 (28)	82	28 / 50	23.9 (34)	67	34 / 50

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

TABLE 3 WATER CONSUMPTION CHANGES OF MALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Week on Study	Control		500 ppm			1000 ppm			2000 ppm		
	Av. WC.	No. of Surviv. <50>	Av. WC.	% of cont.	No. of Surviv. <50>	Av. WC.	% of cont.	No. of Surviv. <50>	Av. WC.	% of cont.	No. of Surviv. <50>
1	4.7 (47)	50 / 50	4.4 (50)	94	50 / 50	3.4 (50)	72	50 / 50	2.3 (50)	49	50 / 50
2	4.7 (43)	50 / 50	4.8 (44)	102	50 / 50	3.7 (50)	79	50 / 50	2.7 (50)	57	50 / 50
3	4.4 (44)	50 / 50	4.9 (48)	111	50 / 50	3.8 (50)	86	50 / 50	2.7 (50)	61	50 / 50
4	4.6 (47)	50 / 50	4.5 (48)	98	50 / 50	3.5 (50)	76	50 / 50	2.8 (50)	61	50 / 50
5	4.5 (48)	50 / 50	4.0 (50)	89	50 / 50	3.4 (50)	76	50 / 50	2.7 (50)	60	50 / 50
6	4.8 (46)	50 / 50	4.6 (49)	96	50 / 50	3.8 (50)	79	50 / 50	3.2 (50)	67	50 / 50
7	4.4 (49)	50 / 50	4.1 (50)	93	50 / 50	3.5 (50)	80	50 / 50	3.0 (50)	68	50 / 50
8	4.5 (44)	50 / 50	4.3 (47)	96	50 / 50	3.6 (49)	80	50 / 50	3.0 (50)	67	50 / 50
9	4.6 (46)	50 / 50	4.2 (48)	91	50 / 50	3.4 (48)	74	50 / 50	3.2 (50)	70	50 / 50
10	4.8 (50)	50 / 50	4.5 (48)	94	50 / 50	3.6 (50)	75	50 / 50	3.1 (50)	65	50 / 50
11	4.3 (49)	50 / 50	4.0 (49)	93	50 / 50	3.5 (50)	81	50 / 50	2.9 (50)	67	50 / 50
12	4.2 (50)	50 / 50	4.1 (50)	98	50 / 50	3.4 (50)	81	50 / 50	2.9 (50)	69	50 / 50
13	4.4 (49)	50 / 50	4.1 (50)	93	50 / 50	3.6 (49)	82	50 / 50	3.0 (50)	68	50 / 50
14	4.3 (43)	50 / 50	4.1 (49)	95	50 / 50	3.3 (49)	77	50 / 50	3.0 (50)	70	50 / 50
18	3.8 (50)	50 / 50	3.6 (50)	95	50 / 50	3.2 (50)	84	50 / 50	2.8 (50)	74	50 / 50
22	3.7 (50)	50 / 50	3.4 (50)	92	50 / 50	3.0 (50)	81	50 / 50	2.7 (50)	73	50 / 50
26	3.7 (50)	50 / 50	3.5 (50)	95	50 / 50	3.1 (50)	84	50 / 50	2.7 (50)	73	50 / 50
30	3.7 (50)	50 / 50	3.5 (50)	95	50 / 50	3.0 (50)	81	50 / 50	2.8 (50)	76	50 / 50
34	4.0 (50)	50 / 50	3.6 (50)	90	50 / 50	3.2 (50)	80	50 / 50	2.9 (50)	73	50 / 50
38	4.1 (50)	50 / 50	3.9 (50)	95	50 / 50	3.3 (50)	80	50 / 50	3.0 (50)	73	50 / 50
42	4.1 (50)	50 / 50	3.7 (49)	90	49 / 50	3.3 (50)	80	50 / 50	3.1 (49)	76	49 / 50
46	4.1 (50)	50 / 50	3.7 (49)	90	49 / 50	3.4 (50)	83	50 / 50	3.2 (49)	78	49 / 50
50	3.9 (50)	50 / 50	3.7 (49)	95	49 / 50	3.3 (50)	85	50 / 50	3.0 (49)	77	49 / 50
54	4.0 (50)	50 / 50	3.6 (49)	90	49 / 50	3.3 (50)	83	50 / 50	3.1 (49)	78	49 / 50
58	4.0 (50)	50 / 50	3.9 (48)	97	48 / 50	3.5 (50)	88	50 / 50	3.1 (49)	78	49 / 50
62	4.1 (50)	50 / 50	3.4 (48)	83	48 / 50	3.4 (49)	83	49 / 50	3.1 (49)	76	49 / 50
66	4.2 (50)	50 / 50	3.7 (47)	88	47 / 50	3.5 (49)	83	49 / 50	3.4 (49)	81	49 / 50
70	4.4 (50)	50 / 50	3.9 (46)	89	46 / 50	3.6 (49)	82	49 / 50	3.5 (49)	80	49 / 50
74	4.4 (50)	50 / 50	3.8 (46)	86	46 / 50	3.6 (49)	82	49 / 50	3.4 (49)	77	49 / 50
78	4.5 (50)	50 / 50	4.0 (45)	89	45 / 50	3.7 (49)	82	49 / 50	3.4 (47)	76	48 / 50
82	4.6 (48)	48 / 50	4.0 (44)	87	44 / 50	3.7 (49)	80	49 / 50	3.5 (48)	76	48 / 50
86	4.4 (47)	48 / 50	3.9 (43)	89	43 / 50	3.6 (49)	82	49 / 50	3.3 (47)	75	47 / 50
90	4.5 (45)	47 / 50	4.2 (42)	93	43 / 50	3.9 (48)	87	48 / 50	3.6 (43)	80	43 / 50
94	4.2 (43)	45 / 50	4.0 (40)	95	41 / 50	3.9 (47)	93	47 / 50	3.8 (41)	90	41 / 50
98	4.7 (39)	41 / 50	4.0 (40)	85	40 / 50	3.9 (46)	83	46 / 50	3.9 (40)	83	40 / 50
102	4.8 (38)	39 / 50	4.0 (39)	83	39 / 50	4.1 (42)	85	42 / 50	4.0 (40)	83	40 / 50
104	4.6 (36)	38 / 50	4.0 (38)	87	38 / 50	4.0 (41)	87	42 / 50	4.1 (38)	89	39 / 50

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

TABLE 4 WATER CONSUMPTION CHANGES OF FEMALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Week on Study	Control		1000 ppm			2000 ppm			4000 ppm		
	Av. WC.	No. of Surviv. <50>	Av. WC.	% of cont.	No. of Surviv. <50>	Av. WC.	% of cont.	No. of Surviv. <50>	Av. WC.	% of cont.	No. of Surviv. <50>
1	4.2 (50)	50 / 50	3.3 (50)	79	50 / 50	2.2 (50)	52	50 / 50	1.6 (50)	38	50 / 50
2	4.4 (49)	50 / 50	3.3 (49)	75	50 / 50	2.4 (50)	55	50 / 50	1.8 (50)	41	50 / 50
3	4.2 (50)	50 / 50	3.3 (50)	79	50 / 50	2.3 (50)	55	50 / 50	1.8 (50)	43	50 / 50
4	4.3 (50)	50 / 50	3.2 (50)	74	50 / 50	2.4 (50)	56	50 / 50	1.9 (50)	44	50 / 50
5	4.2 (50)	50 / 50	3.1 (50)	74	50 / 50	2.3 (50)	55	50 / 50	1.9 (50)	45	50 / 50
6	4.4 (50)	50 / 50	3.5 (50)	80	50 / 50	2.7 (50)	61	50 / 50	2.1 (50)	48	50 / 50
7	4.2 (50)	50 / 50	3.4 (50)	81	50 / 50	2.6 (50)	62	50 / 50	2.0 (50)	48	50 / 50
8	4.3 (50)	50 / 50	3.5 (49)	81	50 / 50	2.6 (50)	60	50 / 50	2.0 (50)	47	50 / 50
9	4.3 (50)	50 / 50	3.6 (50)	84	50 / 50	2.7 (50)	63	50 / 50	2.1 (50)	49	50 / 50
10	4.2 (50)	50 / 50	3.5 (49)	83	50 / 50	2.7 (50)	64	50 / 50	2.0 (50)	48	50 / 50
11	4.2 (50)	50 / 50	3.5 (50)	83	50 / 50	2.7 (50)	64	50 / 50	2.2 (50)	52	50 / 50
12	4.3 (50)	50 / 50	3.5 (50)	81	50 / 50	2.7 (49)	63	50 / 50	2.2 (50)	51	50 / 50
13	4.3 (48)	50 / 50	3.8 (48)	88	50 / 50	2.9 (50)	67	50 / 50	2.3 (50)	53	50 / 50
14	4.3 (50)	50 / 50	3.6 (50)	84	50 / 50	3.0 (49)	70	50 / 50	2.2 (50)	51	50 / 50
18	4.1 (50)	50 / 50	3.4 (50)	83	50 / 50	2.7 (50)	66	50 / 50	2.2 (50)	54	50 / 50
22	4.1 (50)	50 / 50	3.3 (50)	80	50 / 50	2.7 (49)	66	50 / 50	2.0 (50)	49	50 / 50
26	4.0 (50)	50 / 50	3.1 (50)	78	50 / 50	2.6 (50)	65	50 / 50	2.0 (50)	50	50 / 50
30	4.0 (50)	50 / 50	3.2 (50)	80	50 / 50	2.7 (50)	68	50 / 50	2.2 (50)	55	50 / 50
34	4.0 (50)	50 / 50	3.2 (50)	80	50 / 50	2.8 (50)	70	50 / 50	2.2 (50)	55	50 / 50
38	3.9 (49)	50 / 50	3.2 (50)	82	50 / 50	2.6 (50)	67	50 / 50	2.2 (50)	56	50 / 50
42	3.9 (50)	50 / 50	3.2 (49)	82	50 / 50	2.6 (50)	67	50 / 50	2.2 (50)	56	50 / 50
46	4.0 (49)	49 / 50	3.1 (50)	78	50 / 50	2.7 (50)	68	50 / 50	2.3 (50)	58	50 / 50
50	4.0 (49)	49 / 50	3.2 (49)	80	49 / 50	2.7 (50)	68	50 / 50	2.3 (49)	58	49 / 50
54	3.9 (48)	48 / 50	3.0 (49)	77	49 / 50	2.7 (50)	69	50 / 50	2.2 (49)	56	49 / 50
58	3.8 (48)	48 / 50	3.1 (49)	82	49 / 50	2.6 (49)	68	49 / 50	2.2 (49)	58	49 / 50
62	3.9 (48)	48 / 50	3.0 (49)	77	49 / 50	2.6 (49)	67	49 / 50	2.3 (49)	59	49 / 50
66	3.9 (48)	48 / 50	3.0 (48)	77	48 / 50	2.8 (48)	72	49 / 50	2.4 (48)	62	48 / 50
70	3.7 (48)	48 / 50	3.0 (48)	81	48 / 50	2.7 (49)	73	49 / 50	2.3 (48)	62	48 / 50
74	3.9 (48)	48 / 50	3.0 (46)	77	46 / 50	2.7 (48)	69	48 / 50	2.5 (47)	64	47 / 50
78	3.9 (47)	47 / 50	3.1 (45)	79	45 / 50	2.8 (46)	72	46 / 50	2.8 (46)	72	46 / 50
82	4.1 (41)	41 / 50	3.2 (43)	78	43 / 50	3.0 (45)	73	45 / 50	3.1 (45)	76	45 / 50
86	3.9 (39)	39 / 50	3.1 (42)	79	42 / 50	2.9 (42)	74	42 / 50	3.3 (44)	85	44 / 50
90	4.0 (39)	39 / 50	3.1 (40)	78	40 / 50	3.1 (39)	78	39 / 50	3.4 (43)	85	43 / 50
94	4.1 (36)	36 / 50	3.5 (36)	85	36 / 50	3.5 (34)	85	34 / 50	4.0 (40)	98	40 / 50
98	4.2 (35)	35 / 50	3.3 (33)	79	33 / 50	3.7 (33)	88	33 / 50	3.7 (40)	88	40 / 50
102	4.1 (28)	30 / 50	3.5 (30)	85	30 / 50	3.8 (28)	93	28 / 50	3.8 (35)	93	35 / 50
104	4.3 (21)	24 / 50	3.3 (29)	77	29 / 50	4.1 (28)	95	28 / 50	3.8 (33)	88	34 / 50

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

TABLE 5 FOOD CONSUMPTION CHANGES OF MALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Week on Study	Control		500 ppm			1000 ppm			2000 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	3.7 (50)	50 / 50	3.6 (50)	97	50 / 50	3.6 (50)	97	50 / 50	3.4 (50)	92	50 / 50
2	3.9 (50)	50 / 50	3.8 (50)	97	50 / 50	3.8 (50)	97	50 / 50	3.7 (50)	95	50 / 50
3	3.9 (50)	50 / 50	3.9 (50)	100	50 / 50	3.9 (50)	100	50 / 50	3.7 (50)	95	50 / 50
4	4.0 (50)	50 / 50	4.0 (50)	100	50 / 50	3.9 (50)	98	50 / 50	3.8 (50)	95	50 / 50
5	4.0 (50)	50 / 50	3.9 (50)	97	50 / 50	3.9 (50)	98	50 / 50	3.8 (50)	95	50 / 50
6	4.1 (50)	50 / 50	4.1 (50)	100	50 / 50	3.9 (50)	95	50 / 50	4.0 (50)	98	50 / 50
7	4.1 (50)	50 / 50	4.0 (50)	98	50 / 50	3.9 (50)	95	50 / 50	4.1 (50)	100	50 / 50
8	4.2 (50)	50 / 50	4.1 (50)	98	50 / 50	4.1 (50)	98	50 / 50	4.0 (50)	95	50 / 50
9	4.2 (50)	50 / 50	4.0 (50)	95	50 / 50	3.9 (50)	93	50 / 50	4.0 (50)	95	50 / 50
10	4.3 (50)	50 / 50	4.1 (50)	95	50 / 50	4.1 (50)	95	50 / 50	4.1 (50)	95	50 / 50
11	4.2 (50)	50 / 50	4.3 (49)	102	50 / 50	4.1 (50)	98	50 / 50	4.1 (50)	98	50 / 50
12	4.3 (50)	50 / 50	4.3 (50)	100	50 / 50	4.2 (50)	98	50 / 50	4.1 (50)	95	50 / 50
13	4.2 (50)	50 / 50	4.1 (50)	98	50 / 50	4.0 (50)	95	50 / 50	4.0 (50)	95	50 / 50
14	4.4 (50)	50 / 50	4.3 (50)	98	50 / 50	4.2 (50)	95	50 / 50	4.1 (50)	93	50 / 50
18	4.4 (50)	50 / 50	4.3 (50)	98	50 / 50	4.2 (50)	95	50 / 50	4.1 (50)	93	50 / 50
22	4.5 (50)	50 / 50	4.2 (50)	93	50 / 50	4.2 (50)	93	50 / 50	4.1 (50)	91	50 / 50
26	4.5 (50)	50 / 50	4.3 (50)	96	50 / 50	4.2 (50)	93	50 / 50	4.0 (50)	89	50 / 50
30	4.7 (50)	50 / 50	4.3 (50)	91	50 / 50	4.2 (50)	89	50 / 50	3.9 (50)	83	50 / 50
34	4.9 (50)	50 / 50	4.6 (50)	94	50 / 50	4.5 (50)	92	50 / 50	4.3 (50)	88	50 / 50
38	5.0 (50)	50 / 50	4.7 (50)	94	50 / 50	4.6 (50)	92	50 / 50	4.3 (50)	86	50 / 50
42	4.9 (50)	50 / 50	4.6 (49)	94	49 / 50	4.6 (50)	94	50 / 50	4.4 (49)	90	49 / 50
46	5.0 (50)	50 / 50	4.6 (49)	92	49 / 50	4.5 (50)	90	50 / 50	4.3 (49)	86	49 / 50
50	4.9 (50)	50 / 50	4.8 (49)	98	49 / 50	4.5 (50)	92	50 / 50	4.5 (49)	92	49 / 50
54	5.0 (50)	50 / 50	4.6 (49)	92	49 / 50	4.5 (50)	90	50 / 50	4.3 (49)	86	49 / 50
58	4.7 (50)	50 / 50	4.3 (48)	91	48 / 50	4.2 (50)	89	50 / 50	4.0 (49)	85	49 / 50
62	4.6 (50)	50 / 50	3.9 (48)	85	48 / 50	4.2 (48)	91	49 / 50	4.0 (49)	87	49 / 50
66	5.0 (50)	50 / 50	4.6 (47)	92	47 / 50	4.5 (49)	90	49 / 50	4.4 (49)	88	49 / 50
70	5.1 (50)	50 / 50	4.8 (46)	94	46 / 50	4.7 (49)	92	49 / 50	4.5 (49)	88	49 / 50
74	5.1 (50)	50 / 50	4.7 (46)	92	46 / 50	4.8 (49)	94	49 / 50	4.6 (49)	90	49 / 50
78	5.1 (50)	50 / 50	4.6 (45)	90	45 / 50	4.6 (49)	90	49 / 50	4.3 (48)	84	48 / 50
82	5.2 (48)	48 / 50	4.8 (44)	92	44 / 50	4.7 (49)	90	49 / 50	4.5 (48)	87	48 / 50
86	4.9 (48)	48 / 50	4.7 (43)	96	43 / 50	4.5 (49)	92	49 / 50	4.2 (47)	86	47 / 50
90	5.1 (47)	47 / 50	4.8 (43)	94	43 / 50	4.6 (48)	90	48 / 50	4.3 (43)	84	43 / 50
94	5.0 (45)	45 / 50	4.6 (41)	92	41 / 50	4.5 (47)	90	47 / 50	3.9 (41)	78	41 / 50
98	4.9 (41)	41 / 50	4.5 (40)	92	40 / 50	4.4 (46)	90	46 / 50	4.2 (40)	86	40 / 50
102	5.0 (39)	39 / 50	4.4 (39)	88	39 / 50	4.4 (42)	88	42 / 50	4.1 (40)	82	40 / 50
104	4.8 (37)	38 / 50	4.4 (38)	92	38 / 50	4.4 (42)	92	42 / 50	4.1 (39)	85	39 / 50

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

TABLE 6 FOOD CONSUMPTION CHANGES OF FEMALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Week on Study	Control		1000 ppm			2000 ppm			4000 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	3.0 (50)	50 / 50	3.1 (50)	103	50 / 50	2.8 (50)	93	50 / 50	2.2 (50)	73	50 / 50
2	3.4 (50)	50 / 50	3.3 (50)	97	50 / 50	3.1 (50)	91	50 / 50	3.1 (50)	91	50 / 50
3	3.4 (50)	50 / 50	3.4 (49)	100	50 / 50	3.2 (50)	94	50 / 50	3.1 (50)	91	50 / 50
4	3.5 (50)	50 / 50	3.4 (50)	97	50 / 50	3.2 (50)	91	50 / 50	2.9 (50)	83	50 / 50
5	3.4 (50)	50 / 50	3.3 (50)	97	50 / 50	3.2 (50)	94	50 / 50	3.0 (50)	88	50 / 50
6	3.5 (50)	50 / 50	3.5 (50)	100	50 / 50	3.4 (50)	97	50 / 50	3.1 (50)	89	50 / 50
7	3.7 (50)	50 / 50	3.5 (50)	95	50 / 50	3.4 (50)	92	50 / 50	3.2 (50)	86	50 / 50
8	3.7 (49)	50 / 50	3.6 (50)	97	50 / 50	3.3 (50)	89	50 / 50	3.2 (50)	86	50 / 50
9	3.7 (50)	50 / 50	3.7 (50)	100	50 / 50	3.5 (50)	95	50 / 50	3.3 (50)	89	50 / 50
10	3.7 (50)	50 / 50	3.7 (50)	100	50 / 50	3.5 (50)	95	50 / 50	3.2 (50)	86	50 / 50
11	3.7 (50)	50 / 50	3.7 (50)	100	50 / 50	3.6 (50)	97	50 / 50	3.3 (50)	89	50 / 50
12	3.7 (50)	50 / 50	3.7 (49)	100	50 / 50	3.6 (50)	97	50 / 50	3.3 (50)	89	50 / 50
13	3.8 (50)	50 / 50	3.8 (50)	100	50 / 50	3.7 (50)	97	50 / 50	3.4 (50)	89	50 / 50
14	3.9 (50)	50 / 50	3.8 (50)	97	50 / 50	3.8 (50)	97	50 / 50	3.5 (50)	90	50 / 50
18	4.0 (50)	50 / 50	3.9 (50)	98	50 / 50	3.8 (50)	95	50 / 50	3.5 (50)	88	50 / 50
22	4.0 (50)	50 / 50	3.8 (50)	95	50 / 50	3.7 (50)	93	50 / 50	3.4 (50)	85	50 / 50
26	4.1 (50)	50 / 50	4.0 (50)	98	50 / 50	3.8 (50)	93	50 / 50	3.6 (50)	88	50 / 50
30	4.0 (50)	50 / 50	3.8 (50)	95	50 / 50	3.6 (50)	90	50 / 50	3.4 (50)	85	50 / 50
34	4.1 (50)	50 / 50	3.9 (50)	95	50 / 50	3.8 (50)	93	50 / 50	3.6 (50)	88	50 / 50
38	4.2 (50)	50 / 50	4.0 (50)	95	50 / 50	3.9 (50)	93	50 / 50	3.6 (50)	86	50 / 50
42	4.0 (50)	50 / 50	3.9 (50)	98	50 / 50	3.7 (50)	93	50 / 50	3.5 (50)	88	50 / 50
46	4.0 (49)	49 / 50	4.0 (50)	100	50 / 50	3.7 (50)	93	50 / 50	3.5 (50)	88	50 / 50
50	4.1 (49)	49 / 50	3.8 (49)	93	49 / 50	3.6 (50)	88	50 / 50	3.4 (49)	83	49 / 50
54	4.1 (48)	48 / 50	3.7 (49)	90	49 / 50	3.5 (50)	85	50 / 50	3.3 (49)	80	49 / 50
58	3.8 (48)	48 / 50	3.4 (49)	89	49 / 50	3.2 (49)	84	49 / 50	3.1 (49)	82	49 / 50
62	3.8 (48)	48 / 50	3.5 (49)	92	49 / 50	3.3 (49)	87	49 / 50	3.1 (49)	82	49 / 50
66	4.1 (48)	48 / 50	3.7 (48)	90	48 / 50	3.5 (49)	85	49 / 50	3.4 (48)	83	48 / 50
70	4.2 (48)	48 / 50	3.9 (48)	93	48 / 50	3.7 (49)	88	49 / 50	3.4 (48)	81	48 / 50
74	4.1 (48)	48 / 50	3.9 (46)	95	46 / 50	3.6 (48)	88	48 / 50	3.4 (47)	83	47 / 50
78	4.0 (47)	47 / 50	3.8 (45)	95	45 / 50	3.5 (46)	88	46 / 50	3.3 (46)	83	46 / 50
82	4.0 (41)	41 / 50	3.8 (43)	95	43 / 50	3.6 (45)	90	45 / 50	3.3 (45)	83	45 / 50
86	4.2 (39)	39 / 50	3.7 (42)	88	42 / 50	3.6 (42)	86	42 / 50	3.3 (44)	79	44 / 50
90	4.1 (39)	39 / 50	3.7 (40)	90	40 / 50	4.0 (39)	98	39 / 50	3.7 (43)	90	43 / 50
94	4.1 (36)	36 / 50	3.8 (36)	93	36 / 50	3.7 (34)	90	34 / 50	3.3 (40)	80	40 / 50
98	4.0 (35)	35 / 50	3.7 (33)	92	33 / 50	3.6 (33)	90	33 / 50	3.2 (40)	80	40 / 50
102	4.0 (30)	30 / 50	3.7 (30)	92	30 / 50	3.7 (28)	92	28 / 50	3.2 (35)	80	35 / 50
104	4.4 (24)	24 / 50	3.6 (29)	82	29 / 50	3.7 (28)	84	28 / 50	3.1 (34)	70	34 / 50

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

TABLE 7 INCIDENCES OF EXTERNAL AND INTERNAL MASSES IN CLINICAL OBSERVATION OF MALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

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	0/50	0/49	2/47	2/50(1/12)
500 ppm	0/50	0/50	0/50	0/49	0/49	1/47	3/45	1/43	4/50(3/12)
1000 ppm	0/50	0/50	0/50	0/50	1/50	1/49	2/49	2/48	3/50(1/ 8)
2000 ppm	0/50	0/50	0/50	0/49	0/49	1/49	0/48	2/42	3/50(1/11)
Internal mass									
Control	0/50	1/50	1/50	1/50	1/50	6/50	10/49	10/47	15/50(5/12)
500 ppm	0/50	1/50	1/50	1/49	2/49	2/47	1/45	8/43	11/50(5/12)
1000 ppm	0/50	1/50	3/50	3/50	4/50	5/49	5/49	13/48	16/50(2/ 8)
2000 ppm	0/50	1/50	1/50	0/49	0/49	0/49	4/48	6/42	10/50(6/11)

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

TABLE 8 INCIDENCES OF EXTERNAL AND INTERNAL MASSES IN CLINICAL OBSERVATION OF FEMALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

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	1/50	2/48	2/48	3/46	3/37	3/50(1/26)
1000 ppm	0/50	0/50	0/50	0/50	0/49	0/48	0/45	0/37	0/50(0/21)
2000 ppm	0/50	0/50	0/50	0/50	0/50	0/49	0/46	2/38	2/50(1/22)
4000 ppm	0/50	0/50	0/50	0/50	1/49	2/48	2/46	2/41	4/50(4/16)
Internal mass									
Control	0/50	0/50	0/50	0/50	0/48	4/48	6/46	6/37	11/50(8/26)
1000 ppm	0/50	1/50	1/50	3/50	5/49	11/48	14/45	14/37	26/50(19/21)
2000 ppm	0/50	0/50	0/50	0/50	3/50	10/49	17/46	20/38	31/50(18/22)
4000 ppm	0/50	0/50	1/50	2/50	2/49	6/48	11/46	21/41	27/50(13/16)

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

TABLE 9 HEMATOLOGY OF MALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Group name	Control	500 ppm	1000 ppm	2000 ppm	
No. of examined animals	36	38	42	39	
Red blood cell ($10^6/\mu$ L)	9.60 ± 0.91	9.59 ± 1.28	9.43 ± 1.92	9.10 ± 0.84	**
Hemoglobin (g/dL)	13.8 ± 1.1	13.6 ± 1.5	13.5 ± 2.3	13.3 ± 1.2	**
MCV (fL)	45.8 ± 1.9	45.3 ± 2.9	46.9 ± 6.3	46.9 ± 1.2	**
MCHC (g/dL)	31.5 ± 0.7	31.4 ± 1.0	31.1 ± 1.3	31.1 ± 0.6	**
Platelet ($10^3/\mu$ L)	1911 ± 411	1985 ± 418	2084 ± 470	2279 ± 303	**
WBC ($10^3/\mu$ L)	4.47 ± 8.86	2.96 ± 1.61	3.05 ± 2.90	2.00 ± 1.31	**
Differential WBC (%)					
N-Seg	30 ± 17	29 ± 13	36 ± 14	42 ± 17	**
Eosino	2 ± 1	2 ± 3	1 ± 1	1 ± 3	**
Mono	5 ± 3	5 ± 2	4 ± 2	3 ± 1	**

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

TABLE 10 HEMATOLOGY OF FEMALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Group name	Control	1000 ppm	2000 ppm		4000 ppm	
No. of examined animals	22	27	25		28	
Hemoglobin (g/dL)	14.1 ± 2.3	13.6 ± 1.7	12.9 ± 2.7		13.4 ± 1.2	**
MCV (fL)	45.7 ± 2.3	46.7 ± 2.8	47.4 ± 5.1		46.7 ± 2.1	**
MCHC (g/dL)	31.6 ± 1.1	31.5 ± 1.1	30.9 ± 1.6	*	30.7 ± 0.6	**
Platelet ($10^3/\mu$L)	1210 ± 273	1329 ± 374	1399 ± 453		1641 ± 454	**
Differential WBC (%)						
Eosino	3 ± 4	1 ± 1	1 ± 1	**	1 ± 1	**

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

TABLE 11 BIOCHEMISTRY OF MALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Group name	Control	500 ppm		1000 ppm		2000 ppm	
No. of examined animals	37	38		42		39	
Total protein (g/dL)	5.1 ± 0.6	5.3 ± 0.7		5.3 ± 0.9		5.6 ± 0.8	**
Albumin (g/dL)	2.9 ± 0.4	3.0 ± 0.4		3.0 ± 0.5	*	3.2 ± 0.3	**
Triglyceride (mg/dL)	47 ± 27	41 ± 24		41 ± 16		31 ± 11	**
GPT (IU/L)	49 ± 77	135 ± 355		178 ± 514	**	119 ± 300	**
ALP (IU/L)	124 ± 28	220 ± 244	**	337 ± 396	**	279 ± 182	**
CPK (IU/L)	42 ± 13	47 ± 20		120 ± 337	*	84 ± 129	**
Urea nitrogen (mg/dL)	23.4 ± 9.4	22.8 ± 3.3		28.4 ± 12.1	**	30.9 ± 11.3	**
Sodium (mEq/L)	152 ± 1	152 ± 1		152 ± 3		153 ± 2	**
Potassium (mEq/L)	4.3 ± 0.4	4.1 ± 0.3		4.2 ± 0.4		4.0 ± 0.4	**

Mean ± S.D.

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

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

TABLE 12 BIOCHEMISTRY OF FEMALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Group name	Control	1000 ppm		2000 ppm		4000 ppm	
No. of examined animals	23	27		26		31	
Total protein (g/dL)	5.5 ± 1.1	5.3 ± 0.5		5.4 ± 0.9		5.7 ± 0.7	*
Albumin (g/dL)	2.9 ± 0.3	3.1 ± 0.3		3.2 ± 0.5	**	3.5 ± 0.4	**
A/G Ratio	1.3 ± 0.3	1.4 ± 0.2		1.5 ± 0.2		1.6 ± 0.2	**
T-cholesterol (mg/dL)	85 ± 37	93 ± 37		133 ± 81	**	169 ± 74	**
Phospholipid (mg/dL)	163 ± 61	177 ± 53		244 ± 116	**	286 ± 104	**
GPT (IU/L)	40 ± 23	74 ± 123		120 ± 189		207 ± 316	**
ALP (IU/L)	171 ± 53	254 ± 88	*	443 ± 468	**	598 ± 559	**
CPK (IU/L)	73 ± 65	64 ± 43		117 ± 184		112 ± 88	**
Urea nitrogen (mg/dL)	23.6 ± 24.3	24.5 ± 10.6		27.5 ± 11.9	**	30.7 ± 13.9	**
Sodium (mEq/L)	151 ± 2	151 ± 2		152 ± 3		155 ± 4	**
Chloride (mEq/L)	123 ± 3	123 ± 4		124 ± 5		126 ± 4	**

Mean ± S.D.

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

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

TABLE 13 URINALYSIS OF MALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Group	Grade	Control	500 ppm	1000 ppm	2000 ppm
Number of examined animals		38	38	42	39
pH	6.0	1	1	7	6
	6.5	6	19	28	25
	7.0	16	12	7	8
	7.5	14	6	0	0
	8.0	1	0	0	0
	8.5	0	0	0	0
	Chi square test		*	**	**

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

TABLE 14 URINALYSIS OF FEMALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Group	Grade	Control	1000 ppm	2000 ppm	4000 ppm
Number of examined animals		27	29	28	34
pH	6.0	1	2	9	25
	6.5	3	14	16	9
	7.0	6	8	3	0
	7.5	5	4	0	0
	8.0	10	1	0	0
	8.5	2	0	0	0
	Chi square test		**	**	**
Protein	±	0	2	6	13
	+	9	9	10	16
	2+	16	16	11	4
	3+	2	2	1	1
	4+	0	0	0	0
	Chi square test				**
Ketone body	−	3	2	3	13
	±	20	18	22	20
	+	4	7	1	1
	2+	0	2	2	0
	Chi square test				**

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

TABLE 15 ORGAN WEIGHTS OF MALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Group name	Control	500 ppm	1000 ppm	2000 ppm
No. of examined animals	37	38	42	39
Body weight (g)	48.1 ± 7.0	40.3 ± 5.3 **	35.1 ± 4.1 **	31.0 ± 2.6 **
Adrenals (g)	0.011 ± 0.003	0.010 ± 0.003	0.010 ± 0.003	0.009 ± 0.003
Adrenals (%)	0.023 ± 0.009	0.025 ± 0.008	0.030 ± 0.009 **	0.031 ± 0.011 **
Testes (g)	0.219 ± 0.044	0.221 ± 0.051	0.204 ± 0.033	0.209 ± 0.030
Testes (%)	0.461 ± 0.094	0.557 ± 0.156 *	0.586 ± 0.102 **	0.679 ± 0.109 **
Heart (g)	0.232 ± 0.026	0.216 ± 0.018 **	0.210 ± 0.019 **	0.197 ± 0.021 **
Heart (%)	0.495 ± 0.111	0.547 ± 0.094 *	0.602 ± 0.065 **	0.639 ± 0.068 **
Lungs (g)	0.238 ± 0.091	0.222 ± 0.046	0.208 ± 0.037 *	0.195 ± 0.027 **
Lungs (%)	0.504 ± 0.196	0.556 ± 0.114 *	0.600 ± 0.127 **	0.637 ± 0.128 **
Kidneys (g)	0.964 ± 1.964	0.757 ± 0.893	0.675 ± 0.447 *	0.765 ± 0.918
Kidneys (%)	2.119 ± 4.555	1.925 ± 2.408 *	1.932 ± 1.234 **	2.473 ± 2.930 **
Spleen (g)	0.092 ± 0.080	0.143 ± 0.210	0.169 ± 0.271	0.092 ± 0.112
Spleen (%)	0.199 ± 0.171	0.373 ± 0.584 *	0.506 ± 0.867 **	0.298 ± 0.365 *
Liver (g)	1.792 ± 0.601	1.902 ± 0.633	2.092 ± 0.964	1.872 ± 0.504
Liver (%)	3.919 ± 2.259	4.945 ± 2.625 **	6.162 ± 3.224 **	6.115 ± 1.984 **
Brain (g)	0.453 ± 0.017	0.456 ± 0.017	0.453 ± 0.020	0.453 ± 0.018
Brain (%)	0.967 ± 0.173	1.151 ± 0.157 **	1.308 ± 0.156 **	1.470 ± 0.127 **

Mean ± S.D.

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

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

TABLE 16 ORGAN WEIGHTS OF FEMALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Group name	Control	1000 ppm	2000 ppm	4000 ppm
No. of examined animals	24	29	28	34
Body weight (g)	31.1 ± 3.4	28.4 ± 4.4	26.4 ± 5.6 **	21.5 ± 2.1 **
Adrenals (g)	0.013 ± 0.003	0.013 ± 0.004	0.012 ± 0.002	0.010 ± 0.002 **
Adrenals (%)	0.040 ± 0.009	0.045 ± 0.018	0.046 ± 0.009	0.049 ± 0.015 *
Ovaries (g)	0.097 ± 0.113	0.268 ± 0.866	0.052 ± 0.077	0.041 ± 0.037
Ovaries (%)	0.310 ± 0.383	0.893 ± 2.780	0.201 ± 0.268	0.193 ± 0.198
Heart (g)	0.183 ± 0.026	0.169 ± 0.031	0.158 ± 0.032 **	0.139 ± 0.025 **
Heart (%)	0.600 ± 0.129	0.597 ± 0.086	0.612 ± 0.136	0.649 ± 0.122
Lungs (g)	0.249 ± 0.243	0.197 ± 0.053	0.191 ± 0.028	0.173 ± 0.024 **
Lungs (%)	0.831 ± 0.904	0.700 ± 0.187	0.745 ± 0.157 *	0.810 ± 0.100 **
Kidneys (g)	0.501 ± 0.273	1.056 ± 2.744	2.107 ± 6.004	0.442 ± 0.338 **
Kidneys (%)	1.655 ± 0.992	3.077 ± 6.025 *	5.634 ± 12.482 **	2.070 ± 1.574 **
Spleen (g)	0.169 ± 0.159	0.298 ± 0.672	0.168 ± 0.128	0.115 ± 0.178 **
Spleen (%)	0.576 ± 0.598	1.048 ± 2.384	0.642 ± 0.487	0.510 ± 0.734
Liver (g)	1.490 ± 0.300	1.580 ± 0.437	1.993 ± 1.208	2.024 ± 0.976
Liver (%)	4.891 ± 1.346	5.611 ± 1.529	7.834 ± 5.039 **	9.451 ± 4.632 **
Brain (g)	0.471 ± 0.021	0.466 ± 0.017	0.455 ± 0.025 *	0.443 ± 0.021 **
Brain (%)	1.533 ± 0.186	1.674 ± 0.231	1.775 ± 0.254 **	2.081 ± 0.225 **

Mean ± S.D.

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

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

TABLE 17 INCIDENCES OF SELECTED LESIONS OF MALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Group		Control	500 ppm	1000 ppm	2000 ppm	Peto	Cochran-
Number of examined animals		50	50	50	50	test	Armitage test
Organ / Findings	Grade of nonneoplastic finding						
Nasal cavity							
Eosinophilic change: respiratory epithelium	1+	31	25	25	36		
	2+	0	1	3	6		
	3+	1	1	0	3		
	Chi square test				**		
Respiratoty metaplasia: olfactory epithelium	1+	18	7	12	11		
	2+	0	0	1	0		
	Chi square test		*				
Lung							
Bronchiolar-alveolar adenoma		5	4	5	2		
Bronchiolar-alveolar carcinoma		9	4	5	5		
Stomach							
Hyperplasia:glandular stomach	1+	6	1	0	1		
	3+	1	0	0	0		
	Chi square test			*			
Liver							
Acidophilic cell focus	1+	1	9	5	5		
	2+	1	1	4	0		
	Chi square test		*				
Basophilic cell focus	1+	2	5	6	7		
	2+	0	0	3	1		
	3+	0	0	0	1		
	Chi square test						
Hemangioma		6	4	1	0 *		↓ ↓
Hepatocellular adenoma 1)		12	25 **	34 **	35 **	↑ ↑	↑ ↑
Hepatocellular carcinoma 2)		6	9	12	10		
1)+2)		18	29 *	39 **	38 **	↑ ↑	↑ ↑
Gall bladder							
Hyperplasia	1+	0 a)	13	8 b)	8 c)		
	Chi square test		**	*	*		
Papillary adenoma		0	2	4	5 *	↑	↑
Kidney							
Hydronephrosis	1+	0	0	0	0		
	2+	2	0	0	0		
	3+	1	1	2	3		
	4+	0	1	0	1		
	Chi square test						
Brain							
Mineralization	1+	21	8	20	16		
	Chi square test		**				
All site							
Hemangioma		7	5	3	1 *		↓

Grade 1+: Slight 2+: Moderate 3+: Marked 4+: Severe

Significant difference * : $p<0.05$ ** : $p<0.01$ Chi square test for non-neoplastic lesion

Fisher's exact test for neoplastic lesion

↑(↓) : $p<0$ ↑↑(↓↓) : $p<0.01$ Peto or Cochran-Armitage test for neoplastic lesion

a): No. of examined animal is 46. b): No. of examined animal is 49. c): No. of examined animal is 47.

The combined incidences indicate the tumor-bearing animals but not the tumors.

TABLE 18 INCIDENCES OF SELECTED LESIONS OF FEMALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

Group		Control	1000 ppm	2000 ppm	4000 ppm	Peto	Cochran-Armitage
Number of examined animals		50	50	50	50	test	test
Organ / Findings	Grade of nonneoplastic finding						
Nasal cavity							
Eosinophilic change: olfactory epithelium	1+	7	1	11	18		
	Chi square test				*		
Eosinophilic change: respiratory epithelium	1+	30	26	15	9		
	2+	5	18	32	28		
	3+	2	1	1	11		
	Chi square test		**	**	**		
Respiratory metaplasia : gland	1+	14	19	22	31		
	2+	0	0	5	3		
	Chi square test			**	**		
Nasopharynx							
Eosinophilic change	1+	2	5	3	13		
	2+	1	0	0	0		
	Chi square test				**		
Lung							
Metastasis : liver tumor		2	3	5	2		
Lymph node							
Malignant lymphoma		22	16	6 **	3 **		↓ ↓
Spleen							
Extramedullary hematopoiesis	1+	14	5	10	9		
	2+	5	1	7	2		
	3+	1	1	2	4		
	Chi square test		*				
Liver							
Clear cell focus	1+	0	4	3	6		
	2+	0	0	0	0		
	3+	0	0	0	2		
	Chi square test				*		
Acidophilic cell focus	1+	1	3	3	12		
	2+	0	1	0	5		
	3+	0	0	0	1		
	4+	1	0	0	0		
	Chi square test				**		
Basophilic cell focus	1+	0	5	4	9		
	2+	1	2	0	1		
	Chi square test				**		
Hepatocellular adenoma 1)		6	22 **	23 **	34 **	↑ ↑	↑ ↑
Hepatocellular carcinoma 2)		1	4	11 **	17 **	↑ ↑	↑ ↑
1)+2)		6	23 **	31 **	41 **	↑ ↑	↑ ↑
Gall bladder							
Hyperplasia	1+	0	2	14	10		
	Chi square test			**	**		
Papillary adenoma		0	1	5 *	3		

Grade 1+: Slight 2+: Moderate 3+: Marked 4+: Severe

Significant difference * : $p<0.05$ ** : $p<0.01$ Chi square test for non-neoplastic lesion

Fisher's exact test for neoplastic lesion

↑(↓) : $p<0.05$ ↑↑(↓↓) : $p<0.01$ Peto or Cochran-Armitage test for neoplastic lesion

The combined incidences indicate the tumor-bearing animals but not the tumors.

TABLE 18 INCIDENCES OF SELECTED LESIONS OF FEMALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE (Continued)

Group		Control	1000 ppm	2000 ppm	4000 ppm	Peto	Cochran-
Number of examined animals		50	50	50	50	test	Armitage test
Organ / Findings	Grade of nonneoplastic finding						
Kidney							
Inflammatory polyp	1+	0	2	0	1		
	2+	2	2	2	2		
	3+	0	5	8	3		
	Chi square test			*			
Hydronephrosis	1+	1	4	0	4		
	2+	0	1	2	0		
	3+	1	7	11	7		
	Chi square test		*	**	*		
Pituitary							
Hyperplasia	1+	5	8	3	4		
	2+	6	2	1	0		
	3+	1	0	0	0		
	Chi square test				*		
Adenoma		6	3	1	1		↓
Adrenal							
Spindle-cell hyperplasia	1+	23	18	17	36		
	2+	24	29	32	11		
	3+	0	0	1	0		
	Chi square test				*		
Uterus							
Endometrial stromal polyp		3	0	0	0		↓
Histiocytic sarcoma		9	18	* 10	10		
All site							
Malignant lymphoma		23	17	7 **	4 **		↓ ↓

Grade 1+: Slight 2+: Moderate 3+: Marked 4+: Severe

Significant difference * : $p<0.05$ ** : $p<0.01$ Chi square test for non-neoplastic lesion

Fisher's exact test for neoplastic lesion

↑(↓) : $p<0.05$ ↑↑(↓↓) : $p<0.01$ Peto or Cochran-Armitage test for neoplastic lesion

The combined incidences indicate the tumor-bearing animals but not the tumors.

TABLE 19 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 with bearing tumors	Incidence (%)	Min. - Max. (%)
Liver		<1296>			
	Hepatocellular adenoma 1)		231	17.8	4 - 34
	Hepatocellular carcinoma 2)		265	20.4	2 - 42
	Hepatoblastoma 3)		7	0.5	0 - 6
	2)+3)		267	20.6	2 - 46
	1)+2)+3)		456	35.2	8 - 72

27 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

TABLE 20 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 with bearing tumors	Incidence (%)	Min. - Max. (%)
Liver		<1298>			
	Hepatocellular adenoma 1)		66	5.1	0 - 10
	Hepatocellular carcinoma 2)		32	2.5	0 - 8
	Hepatoblastoma 3)		0	0.0	0 - 0
	2)+3)		33	2.4	0 - 8
	1)+2)+3)		95	7.3	4 - 14

27 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

TABLE 21 CAUSE OF DEATH OF MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

	Male				Female			
Group	Control	500 ppm	1000 ppm	2000 ppm	Control	1000 ppm	2000 ppm	4000 ppm
Number of dead or moribund animals	12	12	8	11	26	21	22	16
No microscopical confirmation	0	1	1	0	0	0	1	0
Integumentary system lesion	0	0	0	1	0	0	0	0
Renal lesion	0	0	0	0	1	0	0	1
Urinary retention	1	1	0	1	0	0	0	0
Hydronephrosis	1	1	1	2	0	2	6	4
Tumor death : leukemia	3	4	1	3	14	8	1	1
subcutis	0	0	1	0	0	0	0	0
lung	0	0	0	0	0	0	1	0
spleen	1	1	0	0	1	1	0	0
salivary gland	0	0	1	0	0	0	0	0
small intestine	0	0	0	1	0	0	0	0
large intestine	0	0	0	0	0	1	0	0
liver	4	3	2	2	2	1	3	2
pancreas	1	0	0	0	0	0	0	0
pituitary	0	0	0	0	1	0	0	1
ovary	—	—	—	—	0	0	0	1
uterus	—	—	—	—	6	8	9	6
spinal cord	0	0	0	1	0	0	0	0
peripheral nerves	0	1	1	0	1	0	0	0
bone	1	0	0	0	0	0	0	0
peritoneum	0	0	0	0	0	0	1	0

FIGURES

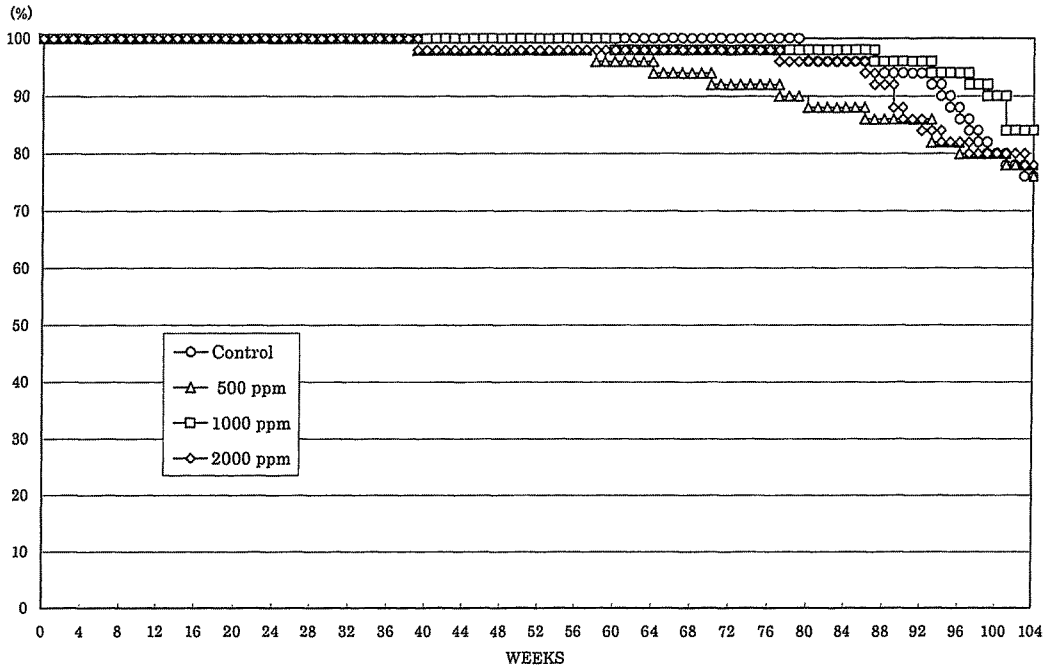


FIGURE 1 SURVIVAL ANIMAL RATE OF MALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

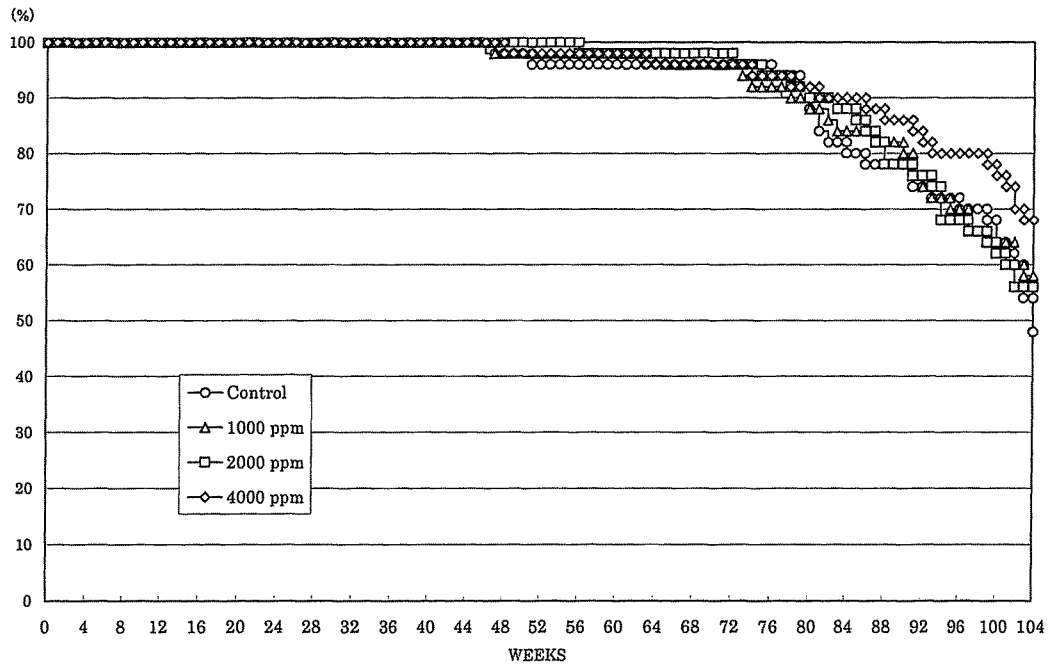


FIGURE 2 SURVIVAL ANIMAL RATE OF FEMALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

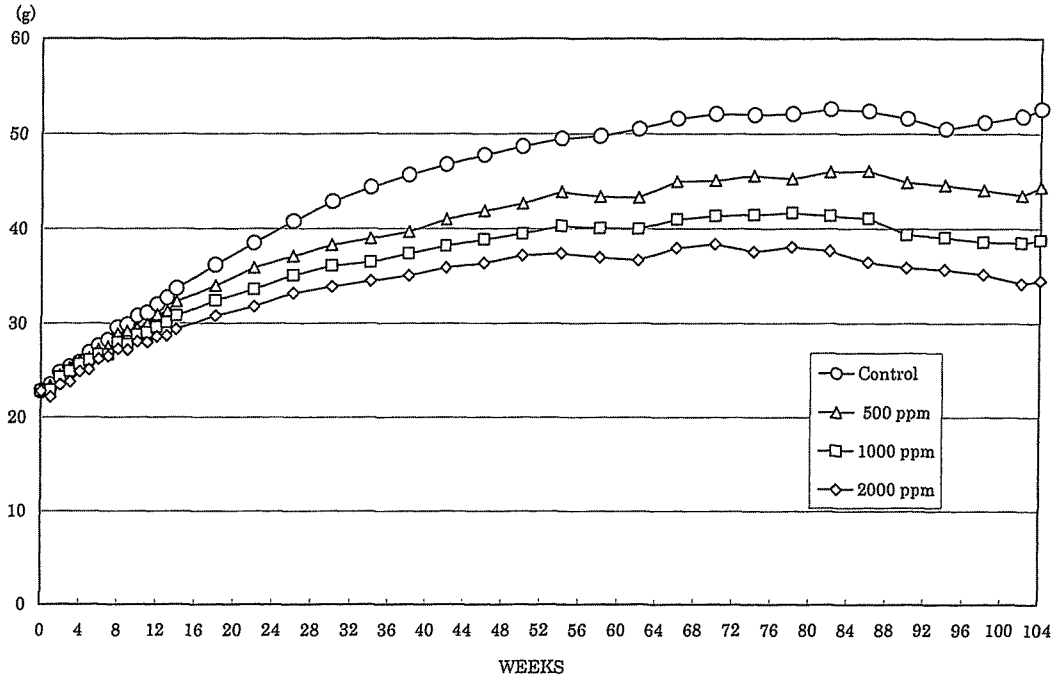


FIGURE 3 BODY WEIGHT CHANGES OF MALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

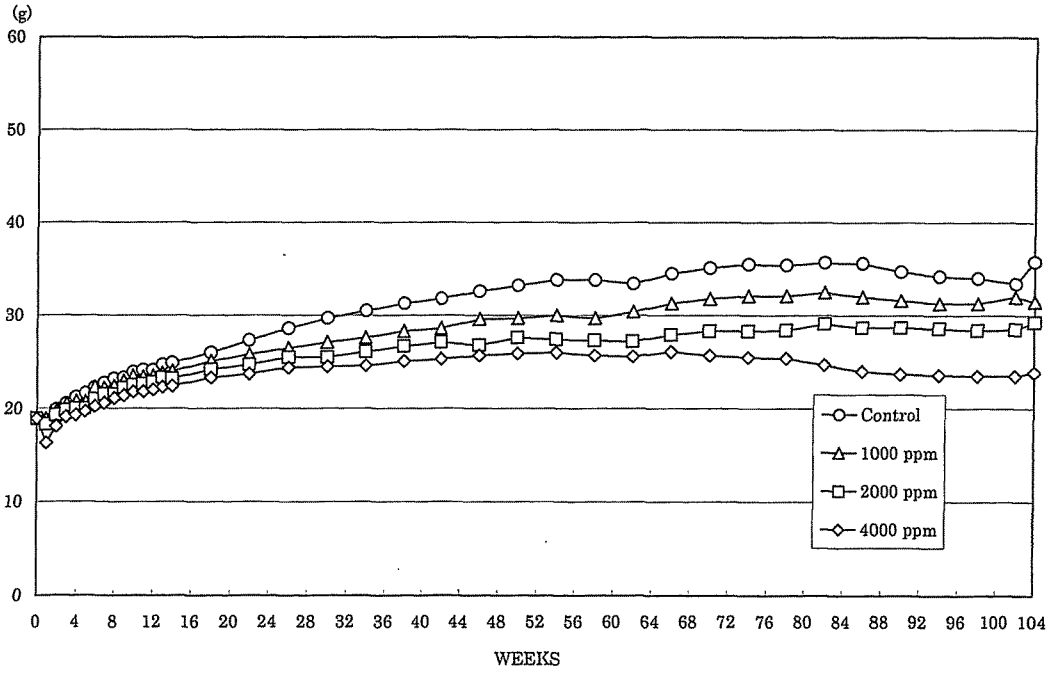


FIGURE 4 BODY WEIGHT CHANGES OF FEMALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

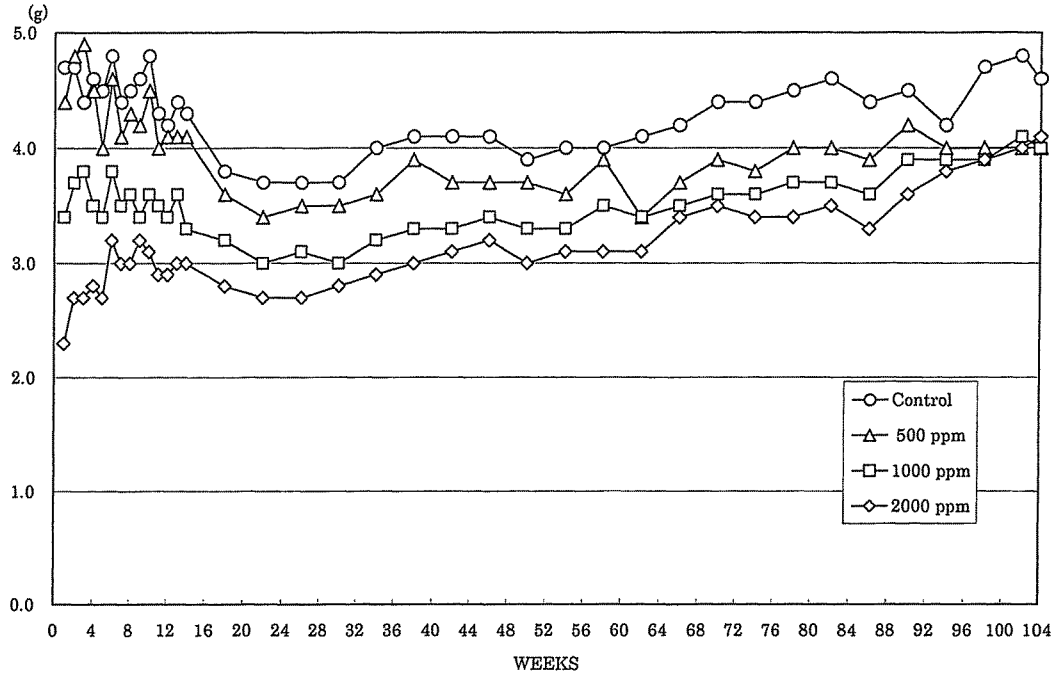


FIGURE 5 WATER CONSUMPTION CHANGES OF MALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

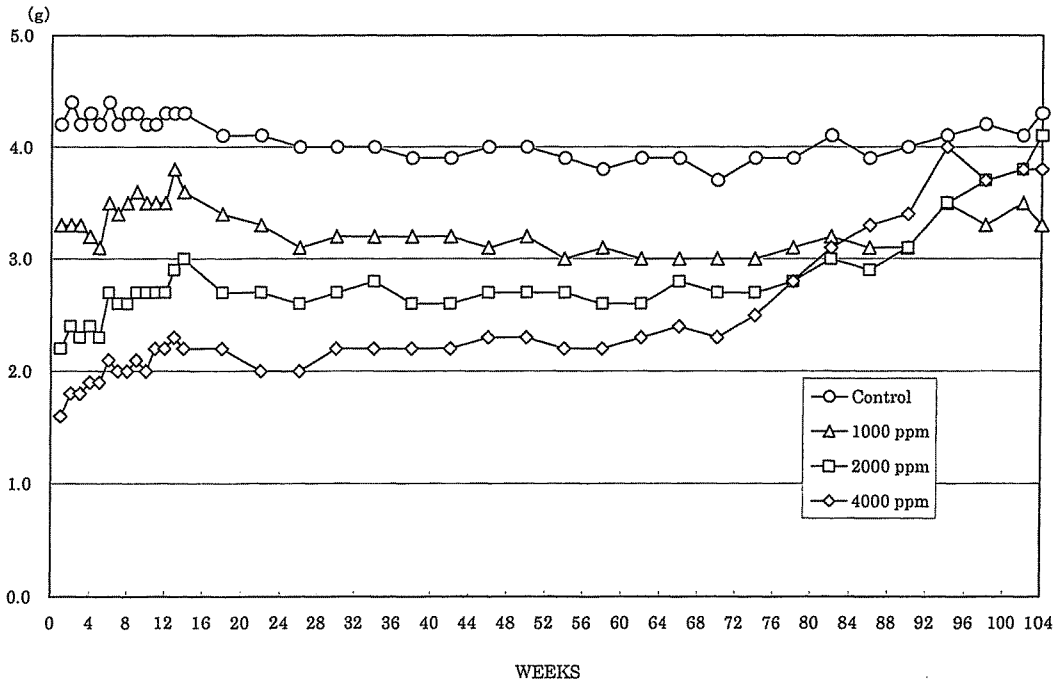


FIGURE 6 WATER CONSUMPTION CHANGES OF FEMALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

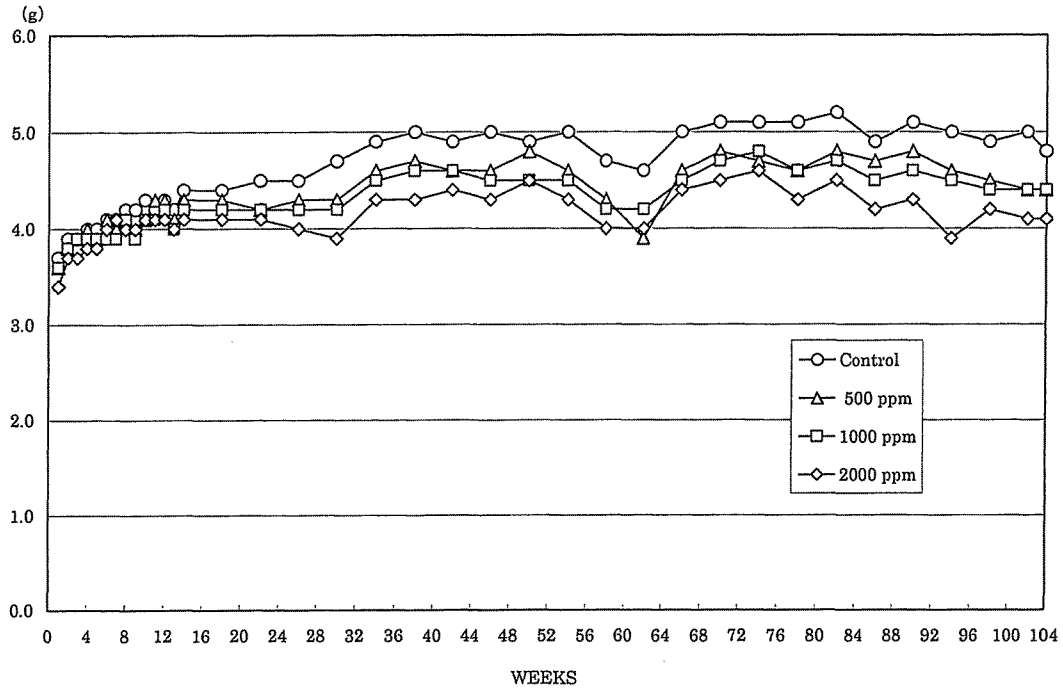


FIGURE 7 FOOD CONSUMPTION CHANGES OF MALE MICE
IN THE 2-YEAR DRINKING WATER STUDY
OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

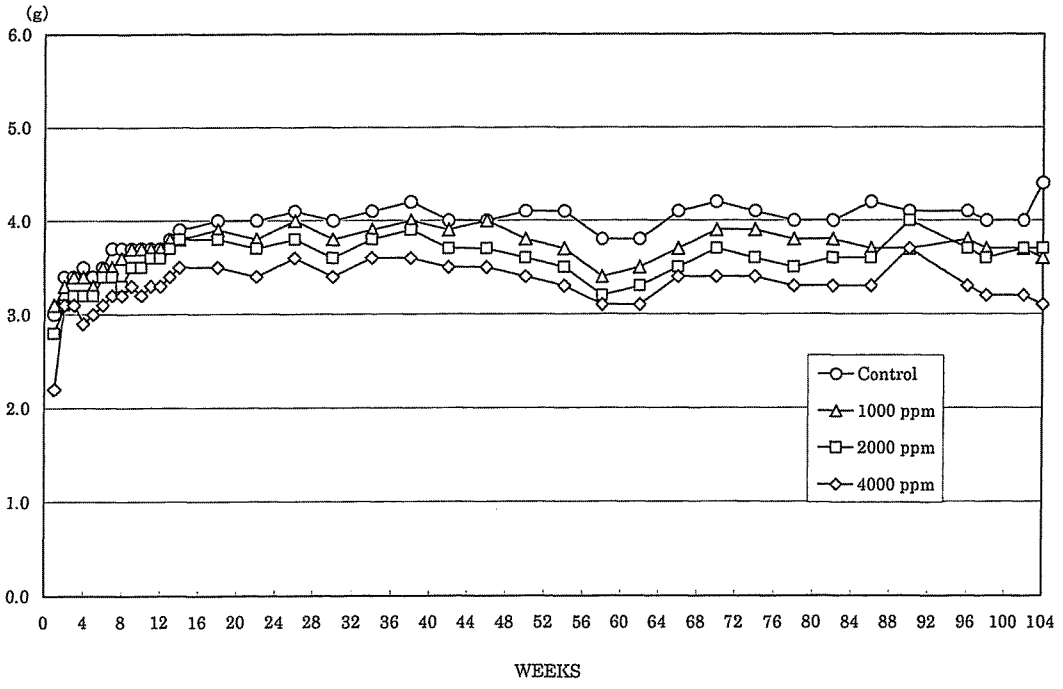
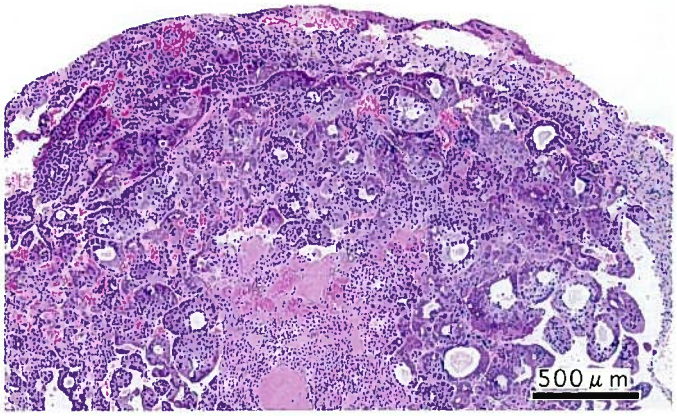
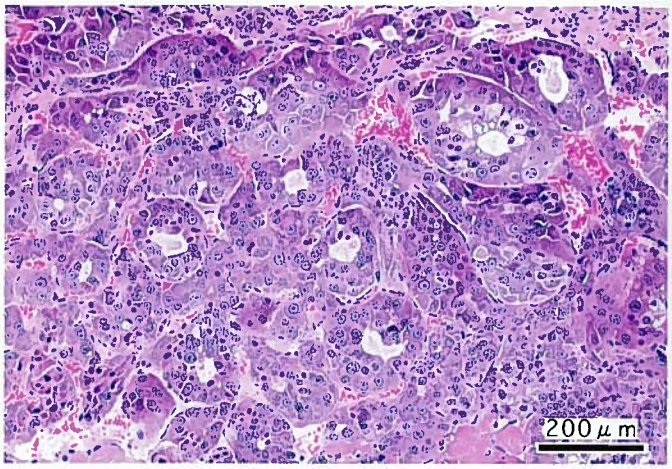


FIGURE 8 FOOD CONSUMPTION CHANGES OF FEMALE MICE
IN THE 2-YEAR DRINKING WATER STUDY
OF *o*-PHENYLENEDIAMINE DIHYDROCHLORIDE

PHOTOGRAPHS



PHOTOGRAPH 1



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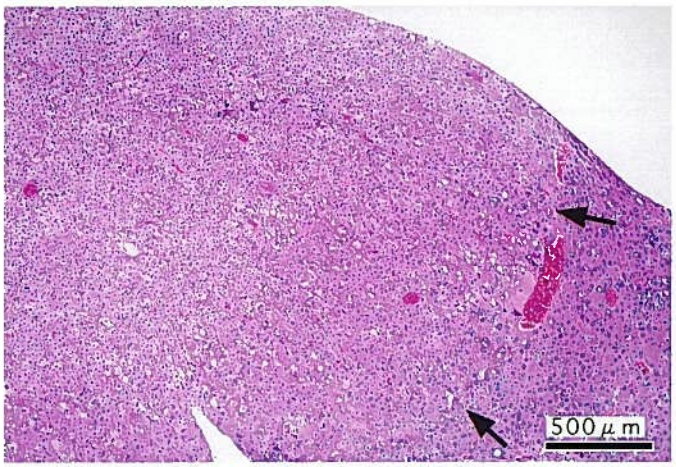

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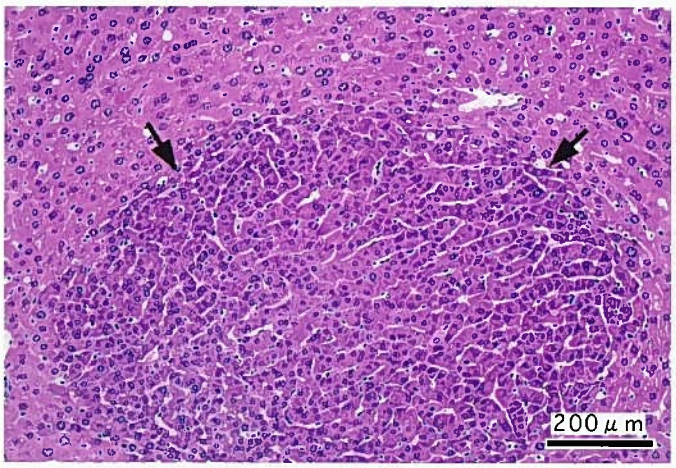

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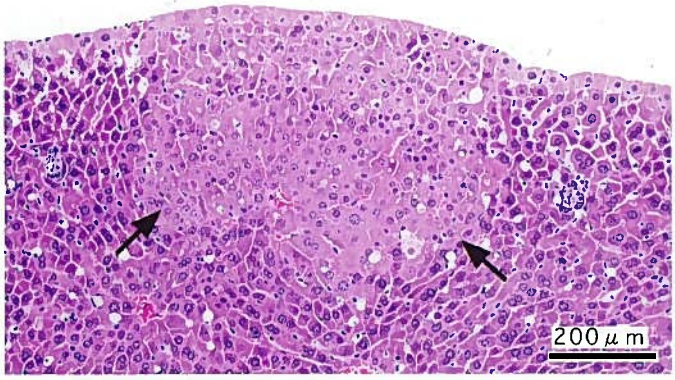

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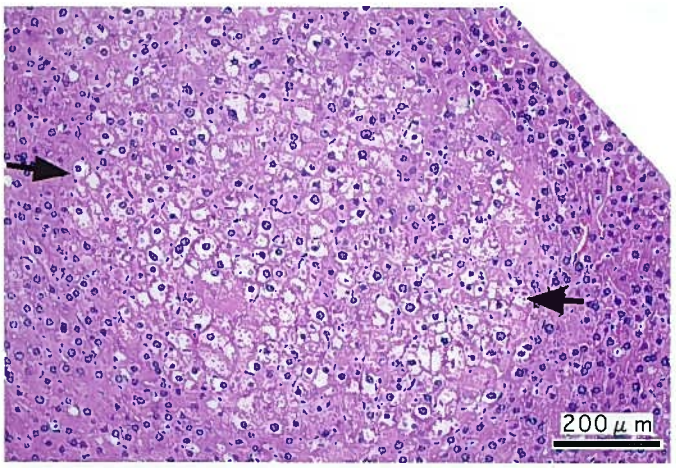
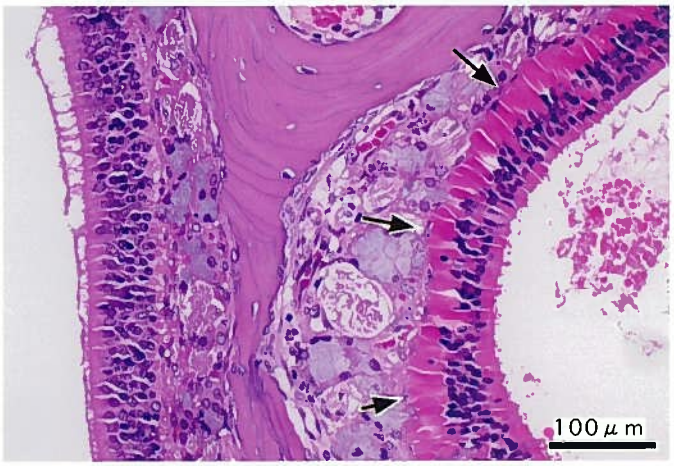


PHOTOGRAPH 6

PHOTOGRAPH 7

PHOTOGRAPH 8



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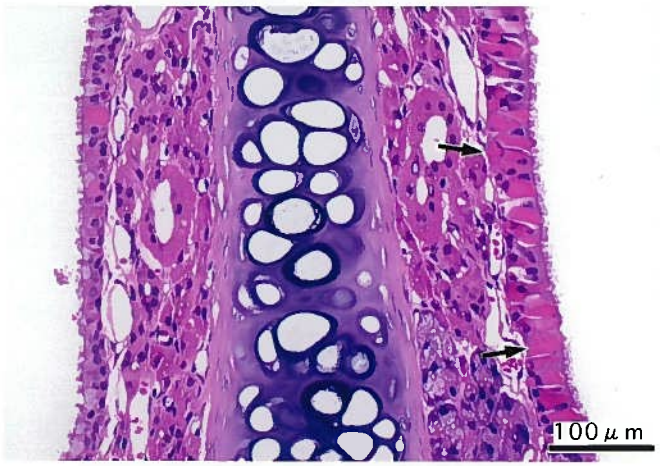
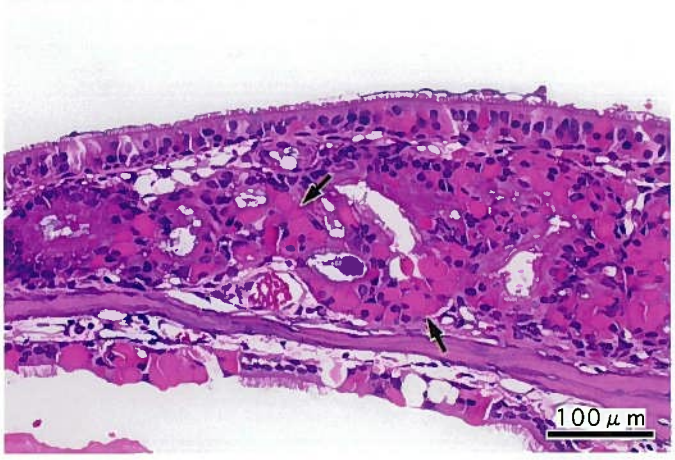


PHOTOGRAPH 10



PHOTOGRAPH 11

PHOTOGRAPH 12