Summary of Feed Carcinogenicity Study of *p*-Nitroanisole in F344 Rats

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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.

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Summary of Feed Carcinogenicity Study of *p*-Nitroanisole in F344 Rats

Purpose, materials and methods

p-Nitroanisole (*p*-NA, 1-methoxy-4-nitrobenzene, CAS No. 100-17-4) is 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*-nitroanisole were examined by feeding groups of 50 F344/DuCrj(Fischer) rats of both sexes *p*-NA-containing diets for 2 years (104 weeks). The dietary concentration of *p*-NA was 0, 2000, 4000 or 8000 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 8000 ppm-fed groups of both sexes were decreased as compared with the respective controls, and the decreased survival rates were attributed to the increased number of deaths due to chronic progressive nephropathy (chronic nephropathy, CPN) in the males (45 cases out of 50). The 8000 ppm-fed female group also showed an increased number of deaths due to CPN, in addition to a greater number of deaths due to uterine tumors (adenocarcinomas) in all the *p*-NA-fed female groups compared to in the control. Yellow urine and yellow coloration of the fur were observed in all the *p*-NA-fed groups of both sexes. Body weight was decreased in the 8000 ppm-fed males throughout the administration period, and in the 4000 ppm-fed males during the later half of the 2-year administration period. Food consumption of the 8000 ppm-fed males was decreased during the later half of the 2-year administration period. Food consumption of all the *p*-NA-fed female groups was decreased throughout the 2-year administration period. The compound-related anemia was observed in both males and females, as evidenced by decreases in red blood cell counts and hemoglobin concentrations. The renal lesion was suggested by increased urea nitrogen and/or altered electrolyte parameters in both the males and females.

The incidence of hepatocellular adenomas was significantly increased in the males fed 4000 and 8000 ppm and in the 8000 ppm-fed females as compared with the respective controls. The incidences of pre-neoplastic lesions such as basophilic cell foci and spongiosis hepatis in the liver were increased in the males fed 4000 ppm and above. The incidence of uterine adenocarcinomas was significantly increased in the females fed 4000 ppm and above. The uterine adenocarcinoma metastasized to other organs. Severity of CPN was increased in all the *p*-NA-fed male groups, while incidence and severity of CPN were increased in the females fed 4000 and 8000 ppm.

Conclusions

In rats, there was some evidence of carcinogenic activity of *p*-NA in males, based on the increased incidences of hepatocellular adenomas, and there was clear evidence of carcinogenic activity of *p*-NA in females, based on the increased incidences of uterine adenocarcinomas.

TABLES

TABLES (Continued)

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

Week on Study	Control		2000ppm			4000ppm			8000ppm		
	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	121 (50)	50 / 50	121 (50)	100	50 / 50	121 (50)	100	50 / 50	121 (50)	100	50 / 50
1	147 (50)	50 / 50	147 (50)	100	50 / 50	141 (50)	96	50 / 50	129 (50)	88	50 / 50
2	178 (50)	50 / 50	180 (50)	101	50 / 50	175 (50)	98	50 / 50	157 (50)	88	50 / 50
3	203 (50)	50 / 50	203 (50)	100	50 / 50	200 (50)	99	50 / 50	181 (50)	89	50 / 50
4	221 (50)	50 / 50	221 (50)	100	50 / 50	219 (50)	99	50 / 50	199 (50)	90	50 / 50
5	237 (50)	50 / 50	236 (50)	100	50 / 50	235 (50)	99	50 / 50	213 (50)	90	50 / 50
6	251 (50)	50 / 50	250 (50)	100	50 / 50	249 (50)	99	50 / 50	224 (50)	89	50 / 50
7	265 (50)	50 / 50	264 (50)	100	50 / 50	263 (50)	99	50 / 50	237 (50)	89	50 / 50
8	276 (50)	50 / 50	276 (50)	100	50 / 50	274 (50)	99	50 / 50	248 (50)	90	50 / 50
9	286 (50)	50 / 50	288 (50)	101	50 / 50	287 (50)	100	50 / 50	256 (50)	90	50 / 50
10	296 (50)	50 / 50	298 (50)	101	50 / 50	297 (50)	100	50 / 50	266 (50)	90	50 / 50
11	304 (50)	50 / 50	306 (50)	101	50 / 50	306 (50)	101	50 / 50	274 (50)	90	50 / 50
12	310 (50)	50 / 50	312 (50)	101	50 / 50	311 (50)	100	50 / 50	278 (50)	90	50 / 50
13	318 (50)	50 / 50	320 (50)	101	50 / 50	321 (50)	101	50 / 50	288 (50)	91	50 / 50
14	324 (50)	50 / 50	328 (50)	101	50 / 50	327 (50)	101	50 / 50	294 (50)	91	50 / 50
18	341 (50)	50 / 50	347 (50)	102	50 / 50	347 (50)	102	50 / 50	313 (50)	92	50 / 50
22	357 (50)	50 / 50	366 (50)	103	50 / 50	365 (50)	102	50 / 50	329 (50)	92	50 / 50
26	366 (50)	50 / 50	377 (50)	103	50 / 50	376 (50)	103	50 / 50	339 (50)	93	50 / 50
30	373 (50)	50 / 50	386 (50)	103	50 / 50	386 (49)	103	49 / 50	346 (50)	93	50 / 50
34	382 (50)	50 / 50	395 (50)	103	50 / 50	396 (49)	104	49 / 50	354 (50)	93	50 / 50
38	391 (50)	50 / 50	405 (50)	104	50 / 50	407 (49)	104	49 / 50	361 (50)	92	50 / 50
42	400 (50)	50 / 50	414 (50)	104	50 / 50	414 (49)	104	49 / 50	367 (50)	92	50 / 50
46	405 (50)	50 / 50	419 (49)	103	49 / 50	420 (49)	104	49 / 50	372 (49)	92	49 / 50
50	407 (50)	50 / 50	423 (49)	104	49 / 50	422 (49)	104	49 / 50	373 (49)	92	49 / 50
54	411 (50)	50 / 50	427 (49)	104	49 / 50	426 (49)	104	49 / 50	372 (48)	91	48 / 50
58	414 (50)	50 / 50	431 (49)	104	49 / 50	425 (48)	103	48 / 50	365 (48)	88	48 / 50
62	417 (49)	49 / 50	433 (49)	104	49 / 50	424 (48)	102	48 / 50	357 (48)	86	48 / 50
66	420 (49)	49 / 50	435 (49)	104	49 / 50	421 (48)	100	48 / 50	349 (48)	83	48 / 50
70	422 (49)	49 / 50	435 (49)	103	49 / 50	416 (48)	99	48 / 50	341 (47)	81	47 / 50
74	419 (49)	49 / 50	434 (49)	104	49 / 50	411 (47)	98	47 / 50	332 (45)	79	45 / 50
78	419 (49)	49 / 50	434 (49)	104	49 / 50	407 (47)	97	47 / 50	319 (41)	76	41 / 50
82	418 (49)	49 / 50	430 (49)	103	49 / 50	401 (47)	96	47 / 50	298 (34)	71	34 / 50
86	416 (49)	49 / 50	427 (47)	103	47 / 50	392 (45)	94	45 / 50	296 (23)	71	23 / 50
90	416 (46)	46 / 50	423 (45)	102	45 / 50	385 (45)	93	45 / 50	305 (16)	73	16 / 50
94	410 (44)	44 / 50	417 (43)	102	43 / 50	375 (44)	91	44 / 50	276 (14)	67	14 / 50
98	403 (42)	42 / 50	408 (39)	101	39 / 50	361 (38)	90	38 / 50	292 (4)	72	4 / 50
102	395 (38)	38 / 50	395 (39)	100	39 / 50	345 (35)	87	35 / 50	250 (4)	63	4 / 50
104	389 (37)	37 / 50	386 (39)	99	39 / 50	333 (32)	86	32 / 50	232 (2)	60	2 / 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 RATS IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Week on Study	Control		2000ppm			4000ppm			8000ppm		
	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 >			< 49 >		
0	97 (50)	50 / 50	97 (50)	100	50 / 50	97 (50)	100	50 / 50	97 (49)	100	49 / 49
1	111 (50)	50 / 50	108 (50)	97	50 / 50	103 (50)	93	50 / 50	97 (49)	87	49 / 49
2	122 (50)	50 / 50	117 (50)	96	50 / 50	112 (50)	92	50 / 50	108 (49)	89	49 / 49
3	132 (50)	50 / 50	124 (50)	94	50 / 50	119 (50)	90	50 / 50	117 (49)	89	49 / 49
4	139 (50)	50 / 50	129 (50)	93	50 / 50	125 (50)	90	50 / 50	124 (49)	89	49 / 49
5	145 (50)	50 / 50	135 (50)	93	50 / 50	130 (50)	90	50 / 50	128 (49)	88	49 / 49
6	150 (50)	50 / 50	138 (50)	92	50 / 50	134 (50)	89	50 / 50	132 (49)	88	49 / 49
7	155 (50)	50 / 50	142 (50)	92	50 / 50	137 (50)	88	50 / 50	135 (49)	87	49 / 49
8	158 (50)	50 / 50	145 (50)	92	50 / 50	140 (50)	89	50 / 50	138 (49)	87	49 / 49
9	161 (50)	50 / 50	148 (50)	92	50 / 50	144 (50)	89	50 / 50	141 (49)	88	49 / 49
10	163 (50)	50 / 50	152 (50)	93	50 / 50	147 (50)	90	50 / 50	144 (49)	88	49 / 49
11	167 (50)	50 / 50	155 (50)	93	50 / 50	150 (50)	90	50 / 50	148 (49)	89	49 / 49
12	169 (50)	50 / 50	157 (50)	93	50 / 50	152 (50)	90	50 / 50	150 (49)	89	49 / 49
13	169 (50)	50 / 50	158 (50)	93	50 / 50	154 (50)	91	50 / 50	154 (49)	91	49 / 49
14	170 (50)	50 / 50	157 (50)	92	50 / 50	156 (50)	92	50 / 50	155 (49)	91	49 / 49
18	176 (50)	50 / 50	161 (50)	91	50 / 50	157 (50)	89	50 / 50	156 (49)	89	49 / 49
22	182 (50)	50 / 50	166 (50)	91	50 / 50	162 (50)	89	50 / 50	160 (49)	88	49 / 49
26	188 (50)	50 / 50	171 (50)	91	50 / 50	166 (50)	88	50 / 50	163 (49)	87	49 / 49
30	192 (50)	50 / 50	175 (50)	91	50 / 50	168 (50)	88	50 / 50	167 (49)	87	49 / 49
34	197 (50)	50 / 50	178 (50)	90	50 / 50	171 (49)	87	49 / 50	169 (49)	86	49 / 49
38	198 (50)	50 / 50	178 (50)	90	50 / 50	172 (49)	87	49 / 50	171 (49)	86	49 / 49
42	203 (50)	50 / 50	182 (50)	90	50 / 50	174 (49)	86	49 / 50	172 (49)	85	49 / 49
46	207 (50)	50 / 50	185 (50)	89	50 / 50	176 (49)	85	49 / 50	175 (49)	85	49 / 49
50	211 (50)	50 / 50	188 (50)	89	50 / 50	179 (49)	85	49 / 50	177 (49)	84	49 / 49
54	217 (50)	50 / 50	191 (50)	88	50 / 50	181 (49)	83	49 / 50	180 (49)	83	49 / 49
58	223 (50)	50 / 50	195 (49)	87	49 / 50	185 (48)	83	48 / 50	180 (49)	81	49 / 49
62	226 (50)	50 / 50	198 (49)	88	49 / 50	188 (48)	83	48 / 50	182 (49)	81	49 / 49
66	231 (50)	50 / 50	201 (49)	87	49 / 50	190 (48)	82	48 / 50	183 (49)	79	49 / 49
70	237 (50)	50 / 50	204 (48)	86	48 / 50	193 (47)	81	47 / 50	184 (49)	78	49 / 49
74	244 (50)	50 / 50	210 (48)	86	48 / 50	198 (47)	81	47 / 50	186 (48)	76	48 / 49
78	246 (50)	50 / 50	214 (48)	87	48 / 50	201 (47)	82	47 / 50	185 (48)	75	48 / 49
82	252 (49)	49 / 50	218 (47)	87	47 / 50	203 (46)	81	46 / 50	186 (48)	74	48 / 49
86	256 (49)	49 / 50	220 (45)	86	45 / 50	206 (45)	80	45 / 50	185 (48)	72	48 / 49
90	260 (48)	48 / 50	224 (45)	86	45 / 50	207 (43)	80	43 / 50	187 (44)	72	44 / 49
94	264 (47)	47 / 50	226 (42)	86	42 / 50	210 (42)	80	42 / 50	184 (43)	70	43 / 49
98	265 (46)	46 / 50	228 (42)	86	42 / 50	215 (39)	81	39 / 50	183 (38)	69	38 / 49
102	261 (46)	46 / 50	228 (40)	87	40 / 50	212 (37)	81	37 / 50	183 (32)	70	32 / 49
104	259 (45)	45 / 50	226 (38)	87	38 / 50	209 (35)	81	35 / 50	180 (31)	69	31 / 49

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

TABLE 3 FOOD CONSUMPTION CHANGES OF MALE RATS IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Week on Study	Control Av. Fc.	Control No. of Surviv. < 50 >	2000ppm Av. Fc.	2000ppm % of cont.	2000ppm No. of Surviv. < 50 >	4000ppm Av. Fc.	4000ppm % of cont.	4000ppm No. of Surviv. < 50 >	8000ppm Av. Fc.	8000ppm % of cont.	8000ppm No. of Surviv. < 50 >
1	11.9 (50)	50 / 50	11.8 (50)	99	50 / 50	10.9 (50)	92	50 / 50	8.8 (50)	74	50 / 50
2	13.4 (50)	50 / 50	13.6 (50)	101	50 / 50	13.1 (50)	98	50 / 50	11.5 (50)	86	50 / 50
3	13.6 (50)	50 / 50	14.0 (50)	103	50 / 50	14.0 (50)	103	50 / 50	12.8 (50)	94	50 / 50
4	14.0 (50)	50 / 50	14.2 (50)	101	50 / 50	14.4 (50)	103	50 / 50	13.7 (49)	98	50 / 50
5	14.2 (50)	50 / 50	14.3 (50)	101	50 / 50	14.5 (50)	102	50 / 50	13.6 (49)	96	50 / 50
6	14.3 (50)	50 / 50	14.4 (50)	101	50 / 50	14.6 (50)	102	50 / 50	13.8 (50)	97	50 / 50
7	14.5 (50)	50 / 50	14.7 (50)	101	50 / 50	14.7 (50)	101	50 / 50	13.8 (50)	95	50 / 50
8	14.4 (50)	50 / 50	14.7 (50)	102	50 / 50	14.7 (50)	102	50 / 50	14.1 (50)	98	50 / 50
9	14.4 (50)	50 / 50	15.0 (50)	104	50 / 50	14.9 (50)	103	50 / 50	14.1 (50)	98	50 / 50
10	14.8 (50)	50 / 50	15.2 (50)	103	50 / 50	15.1 (50)	102	50 / 50	14.4 (50)	97	50 / 50
11	14.3 (50)	50 / 50	15.0 (50)	105	50 / 50	15.1 (50)	106	50 / 50	14.4 (50)	101	50 / 50
12	14.1 (50)	50 / 50	14.5 (50)	103	50 / 50	14.4 (50)	102	50 / 50	13.8 (50)	98	50 / 50
13	14.5 (50)	50 / 50	14.7 (50)	101	50 / 50	14.7 (50)	101	50 / 50	14.2 (50)	98	50 / 50
14	14.3 (50)	50 / 50	14.6 (50)	102	50 / 50	14.6 (50)	102	50 / 50	14.2 (50)	99	50 / 50
18	14.7 (50)	50 / 50	14.9 (50)	101	50 / 50	14.9 (50)	101	50 / 50	14.3 (50)	97	50 / 50
22	15.8 (50)	50 / 50	16.1 (50)	102	50 / 50	16.2 (50)	103	50 / 50	15.5 (50)	98	50 / 50
26	15.9 (50)	50 / 50	16.2 (50)	102	50 / 50	16.5 (50)	104	50 / 50	15.4 (50)	97	50 / 50
30	15.4 (50)	50 / 50	16.2 (50)	105	50 / 50	16.3 (49)	106	49 / 50	15.4 (50)	100	50 / 50
34	15.7 (50)	50 / 50	16.2 (50)	103	50 / 50	16.7 (48)	106	49 / 50	16.0 (50)	102	50 / 50
38	15.9 (50)	50 / 50	16.1 (49)	101	50 / 50	17.0 (48)	107	49 / 50	16.0 (50)	101	50 / 50
42	16.0 (50)	50 / 50	16.1 (50)	101	50 / 50	16.8 (49)	105	49 / 50	15.9 (50)	99	50 / 50
46	16.1 (50)	50 / 50	16.0 (48)	99	49 / 50	16.7 (48)	104	49 / 50	15.9 (49)	99	49 / 50
50	16.0 (50)	50 / 50	16.1 (48)	101	49 / 50	16.6 (47)	104	49 / 50	15.6 (49)	98	49 / 50
54	15.9 (50)	50 / 50	16.1 (48)	101	49 / 50	16.9 (48)	106	49 / 50	15.7 (48)	99	48 / 50
58	15.8 (50)	50 / 50	15.9 (48)	101	49 / 50	16.4 (47)	104	48 / 50	15.1 (48)	96	48 / 50
62	16.0 (49)	49 / 50	16.2 (48)	101	49 / 50	16.6 (47)	104	48 / 50	15.1 (48)	94	48 / 50
66	16.0 (49)	49 / 50	16.2 (49)	101	49 / 50	16.4 (48)	103	48 / 50	14.9 (48)	93	48 / 50
70	15.9 (49)	49 / 50	15.9 (47)	100	49 / 50	16.3 (46)	103	48 / 50	15.2 (46)	96	47 / 50
74	16.1 (48)	49 / 50	16.3 (47)	101	49 / 50	16.5 (46)	102	47 / 50	15.1 (44)	94	45 / 50
78	16.3 (49)	49 / 50	16.3 (48)	100	49 / 50	16.3 (46)	100	47 / 50	14.7 (40)	90	41 / 50
82	15.9 (49)	49 / 50	16.2 (49)	102	49 / 50	16.4 (44)	103	47 / 50	14.9 (34)	94	34 / 50
86	16.1 (49)	49 / 50	16.1 (47)	100	47 / 50	16.4 (42)	102	45 / 50	15.0 (23)	93	23 / 50
90	15.9 (45)	46 / 50	16.0 (44)	101	45 / 50	16.4 (42)	103	45 / 50	16.7 (15)	105	16 / 50
94	15.5 (44)	44 / 50	15.5 (43)	100	43 / 50	15.8 (39)	102	44 / 50	15.1 (13)	97	14 / 50
98	15.3 (41)	42 / 50	15.6 (39)	102	39 / 50	15.6 (35)	102	38 / 50	17.7 (4)	116	4 / 50
102	15.3 (38)	38 / 50	15.6 (39)	102	39 / 50	16.2 (35)	106	35 / 50	13.8 (4)	90	4 / 50
104	15.1 (37)	37 / 50	15.3 (39)	101	39 / 50	16.1 (32)	107	32 / 50	12.2 (2)	81	2 / 50

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

TABLE 4 FOOD CONSUMPTION CHANGES OF FEMALE RATS IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Week on Study	Control		2000ppm			4000ppm			8000ppm		
	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 >			< 49 >		
1	9.7 (50)	50 / 50	9.1 (50)	94	50 / 50	8.4 (50)	87	50 / 50	8.5 (49)	88	49 / 49
2	10.0 (50)	50 / 50	9.1 (50)	91	50 / 50	8.4 (50)	84	50 / 50	8.1 (49)	81	49 / 49
3	10.2 (50)	50 / 50	9.1 (50)	89	50 / 50	8.2 (50)	80	50 / 50	8.2 (49)	80	49 / 49
4	10.2 (50)	50 / 50	9.1 (50)	89	50 / 50	8.5 (50)	83	50 / 50	8.5 (49)	83	49 / 49
5	10.3 (50)	50 / 50	9.1 (50)	88	50 / 50	8.5 (50)	83	50 / 50	8.5 (49)	83	49 / 49
6	10.3 (50)	50 / 50	9.3 (50)	90	50 / 50	8.6 (50)	83	50 / 50	8.4 (48)	82	49 / 49
7	10.0 (50)	50 / 50	9.3 (50)	93	50 / 50	8.5 (50)	85	50 / 50	8.3 (48)	83	49 / 49
8	9.8 (50)	50 / 50	9.2 (50)	94	50 / 50	8.5 (50)	87	50 / 50	8.5 (49)	87	49 / 49
9	9.9 (50)	50 / 50	9.3 (50)	94	50 / 50	8.6 (50)	87	50 / 50	8.4 (49)	85	49 / 49
10	9.7 (50)	50 / 50	9.3 (50)	96	50 / 50	8.6 (50)	89	50 / 50	8.3 (45)	86	49 / 49
11	9.8 (50)	50 / 50	9.5 (50)	97	50 / 50	8.8 (50)	90	50 / 50	8.6 (49)	88	49 / 49
12	9.6 (50)	50 / 50	9.2 (50)	96	50 / 50	8.6 (50)	90	50 / 50	8.5 (49)	89	49 / 49
13	9.7 (50)	50 / 50	9.4 (50)	97	50 / 50	8.8 (49)	91	50 / 50	8.8 (49)	91	49 / 49
14	9.6 (50)	50 / 50	9.0 (50)	94	50 / 50	8.7 (50)	91	50 / 50	8.4 (49)	88	49 / 49
18	10.2 (50)	50 / 50	9.3 (50)	91	50 / 50	8.8 (50)	86	50 / 50	8.6 (49)	84	49 / 49
22	10.8 (50)	50 / 50	9.8 (50)	91	50 / 50	8.9 (50)	82	50 / 50	8.9 (49)	82	49 / 49
26	10.7 (50)	50 / 50	9.7 (50)	91	50 / 50	8.8 (50)	82	50 / 50	8.6 (49)	80	49 / 49
30	10.9 (50)	50 / 50	10.0 (50)	92	50 / 50	8.9 (50)	82	50 / 50	9.1 (49)	83	49 / 49
34	11.0 (50)	50 / 50	10.0 (50)	91	50 / 50	8.9 (49)	81	49 / 50	9.0 (49)	82	49 / 49
38	11.1 (50)	50 / 50	10.1 (50)	91	50 / 50	9.0 (49)	81	49 / 50	9.4 (49)	85	49 / 49
42	11.5 (50)	50 / 50	10.3 (50)	90	50 / 50	9.2 (49)	80	49 / 50	9.4 (49)	82	49 / 49
46	11.9 (50)	50 / 50	10.5 (50)	88	50 / 50	9.2 (49)	77	49 / 50	9.4 (49)	79	49 / 49
50	11.5 (50)	50 / 50	10.6 (50)	92	50 / 50	9.3 (49)	81	49 / 50	9.5 (49)	83	49 / 49
54	11.7 (50)	50 / 50	10.6 (50)	91	50 / 50	9.4 (49)	80	49 / 50	9.6 (49)	82	49 / 49
58	12.1 (50)	50 / 50	10.9 (49)	90	49 / 50	9.9 (48)	82	48 / 50	9.7 (49)	80	49 / 49
62	12.0 (50)	50 / 50	11.1 (49)	93	49 / 50	10.0 (48)	83	48 / 50	9.9 (49)	83	49 / 49
66	12.3 (50)	50 / 50	11.3 (49)	92	49 / 50	10.2 (48)	83	48 / 50	10.2 (49)	83	49 / 49
70	12.2 (50)	50 / 50	11.0 (48)	90	48 / 50	10.4 (47)	85	47 / 50	10.5 (49)	86	49 / 49
74	12.9 (50)	50 / 50	11.6 (48)	90	48 / 50	10.6 (47)	82	47 / 50	10.5 (48)	81	48 / 49
78	12.8 (50)	50 / 50	11.4 (48)	89	48 / 50	10.5 (47)	82	47 / 50	10.5 (48)	82	48 / 49
82	12.9 (49)	49 / 50	11.7 (47)	91	47 / 50	10.9 (46)	84	46 / 50	11.1 (48)	86	48 / 49
86	13.3 (49)	49 / 50	12.2 (45)	92	45 / 50	10.9 (45)	82	45 / 50	11.1 (48)	83	48 / 49
90	13.4 (48)	48 / 50	12.6 (45)	94	45 / 50	11.0 (43)	82	43 / 50	11.2 (43)	84	44 / 49
94	13.0 (47)	47 / 50	12.2 (42)	94	42 / 50	11.0 (42)	85	42 / 50	11.1 (43)	85	43 / 49
98	13.2 (46)	46 / 50	12.1 (42)	92	42 / 50	11.5 (39)	87	39 / 50	11.0 (38)	83	38 / 49
102	12.8 (46)	46 / 50	12.5 (40)	98	40 / 50	11.7 (37)	91	37 / 50	11.3 (32)	88	32 / 49
104	12.4 (45)	45 / 50	12.1 (38)	98	38 / 50	11.7 (35)	94	35 / 50	11.3 (31)	91	31 / 49

< > : 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 O
MALE RATS 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	1/50	2/50	1/50	5/49	7/49	8/45	13/50(2/13)
2000 ppm	0/50	1/50	0/50	0/50	0/49	2/49	3/49	6/45	9/50(1/11)
4000 ppm	0/50	1/50	2/50	1/49	5/49	6/48	11/47	16/44	22/50(5/18)
8000 ppm	0/50	1/50	2/50	5/50	9/48	7/48	2/41	0/16	15/50(15/48)
Internal mass									
Control	0/50	0/50	0/50	0/50	0/50	0/49	0/49	2/45	2/50(2/13)
2000 ppm	0/50	0/50	0/50	0/50	0/49	0/49	0/49	1/45	1/50(1/11)
4000 ppm	0/50	0/50	0/50	0/49	0/49	0/48	0/47	0/44	0/50(0/18)
8000 ppm	0/50	0/50	0/50	0/50	0/48	0/48	0/41	0/16	0/50(0/48)

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 O
FEMALE RATS 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	1/50	0/50	5/50	4/48	8/50(0/ 5)
2000 ppm	0/50	0/50	0/50	2/50	3/50	3/49	7/48	4/45	10/50(5/12)
4000 ppm	0/50	0/50	2/50	3/49	5/49	4/48	4/47	7/43	13/50(6/15)
8000 ppm	0/49	1/49	0/49	3/49	5/49	1/49	4/48	4/43	11/49(4/18)
Internal mass									
Control	0/50	0/50	0/50	0/50	0/50	0/50	1/50	0/48	1/50(1/ 5)
2000 ppm	0/50	0/50	0/50	0/50	1/50	1/49	2/48	3/45	6/50(6/12)
4000 ppm	0/50	0/50	0/50	0/49	1/49	0/48	1/47	2/43	4/50(2/15)
8000 ppm	0/49	0/49	0/49	0/49	0/49	0/49	0/48	0/43	0/49(0/18)

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 RATS IN THE 2-YEAR FEED STUDYOF *p*-NITROANISOLE

Group name	Control	2000 ppm	4000 ppm	8000 ppm
No. of examined animals	37	38	31	2
Red blood cell ($10^6/\mu$ L)	8.66 ± 0.82	8.18 ± 1.59	6.51 ± 1.75 **	4.55 ± 1.43 ?
Hemoglobin (g/dL)	14.7 ± 1.5	13.4 ± 2.5 *	10.4 ± 2.9 **	8.2 ± 3.1 ?
Hematocrit (%)	44.0 ± 3.7	40.9 ± 6.7 *	32.9 ± 7.7 **	26.8 ± 7.8 ?
MCH (pg)	16.9 ± 1.0	16.5 ± 1.3	16.0 ± 1.1 **	17.9 ± 1.2 ?
MCHC (g/dL)	33.3 ± 1.1	32.6 ± 1.6 *	31.2 ± 2.4 **	30.3 ± 2.8 ?
Platelet ($10^3/\mu$ L)	886 ± 250	921 ± 312	1197 ± 243 **	1691 ± 74 ?
Differential WBC (%)				
N-seg	37 ± 10	39 ± 9	45 ± 10 **	66 ± 11 ?
Eosino	2 ± 2	2 ± 1	1 ± 1 **	0 ± 0 ?
Lympho	53 ± 9	51 ± 10	44 ± 10 **	28 ± 5 ?
Other	3 ± 5	3 ± 5	5 ± 3 **	5 ± 5 ?

Mean ± S.D.
*) Significant difference, $p<0.05$ (Test of Dunnett)
**) Significant difference, $p<0.01$ (Test of Dunnett)
?)The statistical test was not applied, because number of data in this group was less than three.

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

Group name	Control	2000 ppm	4000 ppm	8000 ppm
No. of examined animals	43	37	33	29
Red blood cell ($10^6/\mu$ L)	8.08 ± 1.04	8.04 ± 0.74	7.30 ± 0.46 **	6.73 ± 0.89 **
Hemoglobin (g/dL)	14.9 ± 1.9	14.5 ± 1.3	13.2 ± 0.9 **	11.8 ± 1.3 **
Hematocrit (%)	43.1 ± 4.8	42.6 ± 3.6	39.7 ± 2.0 **	36.5 ± 3.5 **
MCV (fL)	53.7 ± 2.8	53.1 ± 1.8	54.4 ± 1.9 **	54.6 ± 3.4 *
MCH (pg)	18.4 ± 0.7	18.1 ± 0.7	18.1 ± 0.7	17.6 ± 0.8 **
MCHC (g/dL)	34.4 ± 1.5	34.0 ± 0.7 *	33.2 ± 0.7 **	32.2 ± 0.9 **
Platelet ($10^3/\mu$ L)	653 ± 122	643 ± 78	759 ± 120 **	1059 ± 210 **
WBC ($10^3/\mu$ L)	3.22 ± 2.56	6.03 ± 9.44	4.51 ± 2.13 **	6.12 ± 3.79 **
Differential WBC (%)				
N-band	1 ± 1	1 ± 1	2 ± 1 **	2 ± 1
N-seg	33 ± 8	38 ± 13	39 ± 10 *	49 ± 18 **
Eosino	2 ± 1	1 ± 1	1 ± 2	1 ± 1 **
Mono	4 ± 2	3 ± 2	3 ± 1 **	3 ± 2
Lympho	58 ± 9	54 ± 12	52 ± 10	42 ± 17 **

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

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

Group name	Control	2000 ppm		4000 ppm		8000 ppm	
No. of examined animals	37	38		31		2	
Albumin (g/dL)	3.4 ± 0.2	3.3 ± 0.3	*	3.0 ± 0.3	**	2.3 ± 0.2	?
A/G ratio	1.1 ± 0.1	1.0 ± 0.1	*	0.9 ± 0.1	**	0.8 ± 0.1	?
Glucose (mg/dL)	173 ± 31	172 ± 28		156 ± 29	*	151 ± 6	?
T-cholesterol (mg/dL)	148 ± 40	172 ± 55		262 ± 66	**	281 ± 65	?
Triglyceride (mg/dL)	57 ± 40	79 ± 77		111 ± 60	**	29 ± 6	?
Phospholipid (mg/dL)	213 ± 54	249 ± 100		372 ± 88	**	387 ± 29	?
GPT (IU/L)	38 ± 8	39 ± 38	*	69 ± 84		123 ± 74	?
γ-GTP (IU/L)	7 ± 3	14 ± 6	**	49 ± 45	**	85 ± 2	?
Urea nitrogen (mg/dL)	18.6 ± 3.4	22.9 ± 9.8	*	56.7 ± 40.5	**	189.9 ± 58.5	?
Creatinine (mg/dL)	0.6 ± 0.1	0.6 ± 0.1		1.1 ± 0.7	**	2.0 ± 0.4	?
Potassium (mEq/L)	3.4 ± 0.3	3.5 ± 0.4		3.8 ± 0.5	**	4.9 ± 1.1	?
Chloride (mEq/L)	106 ± 2	107 ± 2		104 ± 3	**	102 ± 4	?
Calcium (mg/dL)	10.4 ± 0.5	10.4 ± 0.4		11.2 ± 1.1	**	12.0 ± 1.2	?
Inorganic phosphorus (mg/dL)	4.0 ± 0.5	4.1 ± 0.8		7.1 ± 4.1	**	19.0 ± 5.9	?

Mean ± S.D.
*) Significant difference, $p<0.05$ (Test of Dunnett)
**) Significant difference, $p<0.01$ (Test of Dunnett)
?)The statistical test was not applied, because number of data in this group was less than three.

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

Group name	Control	2000 ppm		4000 ppm		8000 ppm	
No. of examined animals	43	37		34		30	
Total protein (g/dL)	6.9 ± 0.5	6.8 ± 0.5		7.4 ± 0.4	**	6.8 ± 0.9	
Albumin (g/dL)	3.9 ± 0.4	3.8 ± 0.3		3.9 ± 0.3		3.3 ± 0.6	**
A/G ratio	1.3 ± 0.2	1.3 ± 0.1		1.2 ± 0.1	**	1.0 ± 0.2	**
T-cholesterol (mg/dL)	127 ± 37	122 ± 27		214 ± 104	**	330 ± 101	**
Phospholipid (mg/dL)	224 ± 59	212 ± 43		332 ± 146	**	467 ± 142	**
GOT (IU/L)	115 ± 33	155 ± 192		74 ± 42	**	117 ± 113	**
GPT (IU/L)	50 ± 14	74 ± 97		37 ± 17	**	61 ± 58	
LDH (IU/L)	272 ± 83	279 ± 165		197 ± 60	**	240 ± 143	*
ALP (IU/L)	120 ± 73	186 ± 290		93 ± 32	*	127 ± 84	
γ-GTP (IU/L)	3 ± 2	4 ± 3		4 ± 3		20 ± 24	**
Urea nitrogen (mg/dL)	17.0 ± 3.0	20.4 ± 4.8	**	21.7 ± 3.5	**	53.8 ± 63.5	**
Creatinine (mg/dL)	0.5 ± 0.1	0.6 ± 0.1		0.6 ± 0.1		0.7 ± 0.3	**
Sodium (mEq/L)	141 ± 1	141 ± 2		140 ± 2		139 ± 2	**
Potassium (mEq/L)	3.4 ± 0.3	3.4 ± 0.3		3.4 ± 0.3		4.0 ± 0.7	**
Chloride (mEq/L)	105 ± 3	105 ± 3		104 ± 3	*	102 ± 5	**
Calcium (mg/dL)	10.3 ± 0.5	10.2 ± 0.5		10.7 ± 0.6	**	11.1 ± 0.9	**
Inorganic phosphorus (mg/dL)	3.5 ± 0.7	3.9 ± 0.6		4.0 ± 0.8	*	7.1 ± 6.6	**

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

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

Group name	Grade	Control	2000 ppm	4000 ppm	8000 ppm
Number of examined animals		37	39	34	3
pH	6.0	1	1	1	0
	6.5	4	2	8	2
	7.0	7	3	6	1
	7.5	14	20	16	0
	8.0	11	10	3	0
	8.5	0	3	0	0
	Chi square test				**
Glucose	−	37	39	31	2
	±	0	0	1	1
	+	0	0	2	0
	2+	0	0	0	0
	3+	0	0	0	0
	Chi square test				**

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

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

Group name	Grade	Control	2000 ppm	4000 ppm	8000 ppm
Number of examined animals		45	39	35	31
Protein	-	0	0	0	0
	±	3	1	0	0
	+	16	18	0	0
	2+	15	12	15	3
	3+	10	8	19	26
	4+	1	0	1	2
	Chi square test			**	**
Occult blood	−	43	29	28	23
	±	0	1	1	5
	+	1	1	2	0
	2+	0	3	0	0
	3+	1	5	4	3
	Chi square test				*

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

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

Group name	Control	2000 ppm	4000 ppm	8000 ppm
No. of examined animals	37	39	32	2
Body weight (g)	371 ± 34	366 ± 39	313 ± 34 **	216 ± 6 ?
Adrenals (g)	0.072 ± 0.014	0.139 ± 0.368	0.089 ± 0.066	0.077 ± 0.030 ?
Adrenals (%)	0.020 ± 0.006	0.038 ± 0.096	0.030 ± 0.025 **	0.036 ± 0.013 ?
Testes (g)	3.484 ± 1.254	4.411 ± 3.496	4.515 ± 2.197	1.735 ± 0.738 ?
Testes (%)	0.939 ± 0.338	1.182 ± 0.861	1.442 ± 0.668 **	0.799 ± 0.321 ?
Heart (g)	1.198 ± 0.091	1.270 ± 0.182	1.236 ± 0.169	1.270 ± 0.301 ?
Heart (%)	0.325 ± 0.034	0.351 ± 0.065	0.403 ± 0.090 **	0.587 ± 0.124 ?
Lungs (g)	1.362 ± 0.087	1.562 ± 0.394 **	1.560 ± 0.284 **	1.247 ± 0.021 ?
Lungs (%)	0.369 ± 0.031	0.437 ± 0.156 **	0.506 ± 0.126 **	0.578 ± 0.025 ?
Kidneys (g)	2.577 ± 0.287	2.839 ± 0.419 **	3.358 ± 0.541 **	3.559 ± 0.054 ?
Kidneys (%)	0.703 ± 0.128	0.791 ± 0.200 *	1.094 ± 0.264 **	1.649 ± 0.069 ?
Spleen (g)	0.962 ± 0.448	1.452 ± 2.882	1.098 ± 0.262 **	0.531 ± 0.081 ?
Spleen (%)	0.259 ± 0.112	0.417 ± 0.915	0.349 ± 0.072 **	0.246 ± 0.030 ?
Liver (g)	9.809 ± 1.142	11.564 ± 2.085 **	14.804 ± 1.525 **	16.305 ± 4.564 ?
Liver (%)	2.655 ± 0.299	3.194 ± 0.715 **	4.768 ± 0.551 **	7.523 ± 1.916 ?
Brain (g)	2.069 ± 0.092	2.084 ± 0.061	2.118 ± 0.054 **	2.042 ± 0.131 ?
Brain (%)	0.562 ± 0.050	0.576 ± 0.067	0.685 ± 0.079 **	0.945 ± 0.036 ?

Mean ± S.D.

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

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

?)The statistical test was not applied, because number of data in this group was less than three.

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

Group name	Control	2000 ppm		4000 ppm		8000 ppm	
No. of examined animals	45	38		35		31	
Body weight (g)	244 ± 29	211 ± 31	**	194 ± 25	**	165 ± 20	**
Adrenals (g)	0.072 ± 0.010	0.067 ± 0.011	*	0.065 ± 0.010	**	0.064 ± 0.010	**
Adrenals (%)	0.030 ± 0.005	0.032 ± 0.007		0.034 ± 0.007	*	0.039 ± 0.008	**
Ovaries (g)	0.169 ± 0.226	0.155 ± 0.168		0.265 ± 0.763		0.124 ± 0.089	
Overies (%)	0.069 ± 0.092	0.079 ± 0.109	**	0.140 ± 0.410	**	0.075 ± 0.052	**
Heart (g)	0.877 ± 0.069	0.827 ± 0.092	*	0.819 ± 0.070	**	0.842 ± 0.088	
Heart (%)	0.363 ± 0.041	0.396 ± 0.050	*	0.425 ± 0.042	**	0.518 ± 0.082	**
Lungs (g)	1.025 ± 0.095	1.026 ± 0.075		1.006 ± 0.073		1.013 ± 0.096	
Lungs (%)	0.427 ± 0.074	0.497 ± 0.087	**	0.524 ± 0.064	**	0.624 ± 0.108	**
Kidneys (g)	1.699 ± 0.152	1.649 ± 0.178		1.870 ± 0.283	*	2.318 ± 0.501	**
Kidneys (%)	0.703 ± 0.077	0.793 ± 0.111	*	0.975 ± 0.179	**	1.423 ± 0.344	**
Spleen (g)	0.691 ± 0.594	0.524 ± 0.201		0.618 ± 0.150	*	0.689 ± 0.230	*
Spleen (%)	0.286 ± 0.247	0.249 ± 0.084		0.318 ± 0.063	**	0.411 ± 0.110	**
Liver (g)	6.287 ± 0.966	5.997 ± 1.233		7.447 ± 1.405	**	10.078 ± 2.085	**
Liver (%)	2.590 ± 0.331	2.854 ± 0.456		3.841 ± 0.596	**	6.098 ± 0.944	**
Brain (g)	1.895 ± 0.047	1.922 ± 0.042	*	1.933 ± 0.046	**	1.932 ± 0.056	**
Brain (%)	0.789 ± 0.104	0.931 ± 0.148	**	1.010 ± 0.125	**	1.188 ± 0.131	**

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 RATS IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Group name No. of examined animals	Control <50>	2000 ppm <50>	4000 ppm <50>	8000 ppm <50>		
Liver						
Hepatocellular adenoma	0 (0%) [a)]	1 (2%)	13(26%) **	11 (22%) **	⬆⬆	⇧⇧
Testis						
Interstitial cell tumor	34 (64%)	45 (90%) **	48 (96%) **	48 (96%) **	⬆⬆	⇧⇧
Spleen						
Mononuclear cell leukemia	8 (16%)	5 (10%)	3 (6%)	0 (0%) **		⇩⇩

[a)] : No. of animals bearing tumor (incidence ; %)
[**)] : 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)

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

Group name No. of examined animals	Control <50>	2000 ppm <50>	4000 ppm <50>	8000 ppm <49>		
Uterus						
Adenocarcinoma	1 (2%) [a)]	4 (8%)	8 (16%) *	8 (16%) *	⬆⬆	⇧
Liver						
Hepatocellular adenoma	0 (0%)	0 (0%)	0 (0%)	5 (10%) *	⬆⬆	⇧⇧
Spleen						
Mononuclear cell leukemia	8 (16%)	7 (14%)	1 (2%) *	1 (2%) *		⇩⇩

[a)] : No. of animals bearing tumor (incidence ; %)
[*)] : Significant difference, $p<0.05$ (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 17
INCIDENCES OF SELECTED NON-NEOPLASTIC LESIONS OF MALE RATS IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Group name	Control				2000 ppm				4000 ppm				8000 ppm			
No. 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	12	14	7	0	13	9	7	0	13	14	12	0	8	2	0	0 **
Lung																
Bronchiolar-alveolar cell hyperplasia	8	1	0	0	0	1	0	0 *	0	0	0	0 **	0	0	0	0 **
Uremic pneumonitis	0	0	0	0	0	0	0	0	5	0	1	0 *	18	3	0	0 **
Spleen																
Deposit of hemosiderin	35	5	0	0	24	15	1	0 *	18	22	6	0 **	34	12	0	0
Heart																
Mineralization	0	0	0	0	0	0	0	0	4	1	0	0	18	0	0	0 **
Artery/aort																
Mineralization	0	0	0	0	0	0	0	0	3	0	0	0	16	0	0	0 **
Tongue																
Edema	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0 **
Mineralization	0	0	0	0	0	0	0	0	5	0	0	0	19	0	0	0 **
Stmach																
Mineralization	0	0	0	0	1	0	0	0	4	5	0	0 **	16	15	4	0 **
Liver																
Granulation	11	1	0	0	8	2	2	0	11	8	1	0	2	0	0	0 *
Basophilic cell focus	4	0	0	0	7	1	0	0	23	4	0	0 **	19	2	0	0 **
Spongiosis hepatis	0	0	0	0	5	0	0	0	8	1	1	0 *	11	1	0	0 **
Pancreas																
Atrophy	6	0	0	0	7	0	0	0	3	0	0	0	0	0	0	0 *
Kidney																
Cyst	0	0	0	0	1	0	0	0	2	0	0	0	8	0	0	0 **
Chronic nephropathy	22	15	8	1	10	14	24	1 **	0	1	25	23 **	1	0	1	48 **
Mineralization : papilla	8	0	0	0	7	0	0	0	8	26	14	0 **	37	9	0	0 **
Urothelial hyperplasia : pelvis	12	0	0	0	6	0	0	0	34	1	0	0 **	35	0	0	0 **
Parathyroid																
Hyperplasia	0	0	0	0	0	0	0	0	6	0	0	0 *	23	0	0	0 **
Adrenal																
Hemorrhage	0	0	0	0	0	0	0	0	2	0	0	0	13	0	0	0 **
Necrosis : cortex	0	0	0	0	0	0	0	0	2	0	0	0	10	0	0	0 **
Prostate																
Hyperplasia	8	0	0	0	9	0	0	0	6	1	0	0	0	0	0	0 **
Muscle																
Mineralization	0	0	0	0	0	0	0	0	4	0	0	0	7	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 RATS IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

Group name	Control				2000 ppm				4000 ppm				8000 ppm			
No. of examined animals	50				50				50				49			
Grade	<1>	<2>	<3>	<4>	<1>	<2>	<3>	<4>	<1>	<2>	<3>	<4>	<1>	<2>	<3>	<4>
Nasal cavity																
Mineralization	25	0	0	0	13	0	0	0 *	16	0	0	0	8	0	0	0 **
Eosinophilic change : olfactory epithelium	3	15	31	0	4	15	25	0	11	15	22	0	7	23	12	0 **
Lung																
Uremic pneumonitis	0	0	0	0	0	0	0	0	0	0	0	0	5	3	0	0 *
Spleen																
Deposit of hemosiderin	7	30	7	0	6	25	12	0	5	17	25	0 **	12	18	14	0
Heart																
Mineralization	0	0	0	0	0	0	0	0	1	0	0	0	5	0	0	0
Myocardial fibrosis	16	0	0	0	21	0	0	0	24	0	0	0	27	0	0	0 *
Tongue																
Edema	0	0	0	0	0	0	0	0	1	0	0	0	6	0	0	0 *
Mineralization	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
Stmach																
Mineralization	0	0	0	0	0	0	0	0	0	0	0	0	10	1	0	0 **
Liver																
Granulation	26	2	5	0	21	5	4	0	26	1	5	0	11	5	3	0 **
Kidney																
Chronic nephropathy	12	2	3	0	8	2	1	0	9	16	9	1 **	4	5	22	17 **
Mineralization : cortico-medullary junction	6	0	0	0	5	0	0	0	2	0	0	0	0	0	0	0 *
Mineralization : papilla	5	0	0	0	14	0	0	0 *	19	15	7	0 **	17	15	11	0 **
Urothelial hyperplasia : pelvis	1	0	0	0	2	0	0	0	4	1	0	0	11	0	0	0 **
Adrenal																
Hemorrhage	0	0	0	0	1	0	0	0	1	0	0	0	2	4	0	0 *
Peliosis-like lesion	31	0	0	0	23	0	0	0	18	0	0	0 *	6	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 19
CAUSE OF DEATH OF RATS IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

		Male				Female			
Group name		Control	2000ppm	4000ppm	8000ppm	Control	2000ppm	4000ppm	8000ppm
Number of dead or moribund animals		13	11	18	48	5	12	15	18
No microscopical confirmation		0	0	1	0	0	0	1	0
Cadiovascular lesion		0	1	0	0	0	0	1	0
Digestive system lesion		1	0	0	0	0	0	0	0
Urinary retention		0	0	1	0	0	0	0	0
Chronic nephropathy		0	0	4	45	0	0	0	12
Arteritis		0	0	0	1	0	0	0	0
Tumor death	leukemia	6	4	3	0	2	6	0	1
	skin/app	0	0	0	1	0	0	0	0
	subcutis	0	0	1	0	0	1	0	0
	tongue	0	0	0	0	0	0	0	1
	spleen	1	0	1	0	0	0	0	0
	oral cavity	1	0	0	0	0	0	0	0
	kidney	0	0	1	0	0	0	0	0
	pituitary	2	1	3	0	2	0	1	1
	thyroid	0	0	1	0	0	0	0	0
	uterus	—	—	—	—	1	5	7	3
	mammary gland	0	0	0	0	0	0	1	0
	brain	0	1	1	0	0	0	1	0
	spinal cord	0	1	0	0	0	0	0	0
	Zymbal gland	0	1	0	0	0	0	1	0
	bone	0	1	1	1	0	0	1	0
	retroperit	0	0	0	0	0	0	1	0
	peritoneum	1	0	1	0	0	0	0	0
	abdominal cavity	1	0	0	0	0	0	0	0

TABLE 20 HISTORICAL CONTROL DATA OF SELECTED NEOPLASTIC LESIONS
IN JAPAN BIOASSAY RESEARCH CENTER : F344/DuCrj MALE RATS

Organs	Tumors	No. of animals examined	No. of animals bearing tumor	Incidence (%)	Min. - Max. (%)
Spleen		<1599>			
	Mononuclear cell leukemia		190	11.9	4 - 22
Liver		<1599>			
	Hepatocellular adenoma		26	1.6	0 - 6
Testis		<1598>			
	Interstitial cell tumor		1368	85.6	56 - 98

32 carcinogenicity studies examined in Japan Bioassay Research Center were used.
Study No. : 0043, 0059, 0061, 0063, 0065, 0067, 0095, 0104, 0115, 0130, 0141, 0158, 0162, 0189, 0205, 0210, 0224, 0242, 0267, 0269, 0278, 0284, 0288, 0294, 0296, 0318, 0328, 0342, 0347, 0365, 0371, 0399

TABLE 21 HISTORICAL CONTROL DATA OF SELECTED NEOPLASTIC LESIONS
IN JAPAN BIOASSAY RESEARCH CENTER : F344/DuCrj FEMALE RATS

Organs	Tumors	No. of animals examined	No. of animals bearing tumor	Incidence (%)	Min. - Max. (%)
Spleen		<1547>			
	Mononuclear cell leukemia		201	13.0	2 - 26
Liver		<1547>			
	Hepatocellular adenoma		20	1.3	0 - 6
Uterus		<1547>			
	Adenocarcinoma		6	0.4	0 - 4
	Endometrial stromal polyp		221	14.3	2 - 28

31 carcinogenicity studies examined in Japan Bioassay Research Center were used.
Study No. : 0043, 0059, 0061, 0063, 0065, 0067, 0095, 0104, 0115, 0130, 0141, 0158, 0162, 0189, 0205, 0210, 0224, 0242, 0267, 0269, 0278, 0284, 0296, 0303, 0318, 0328, 0342, 0347, 0365, 0371, 0399

FIGURES

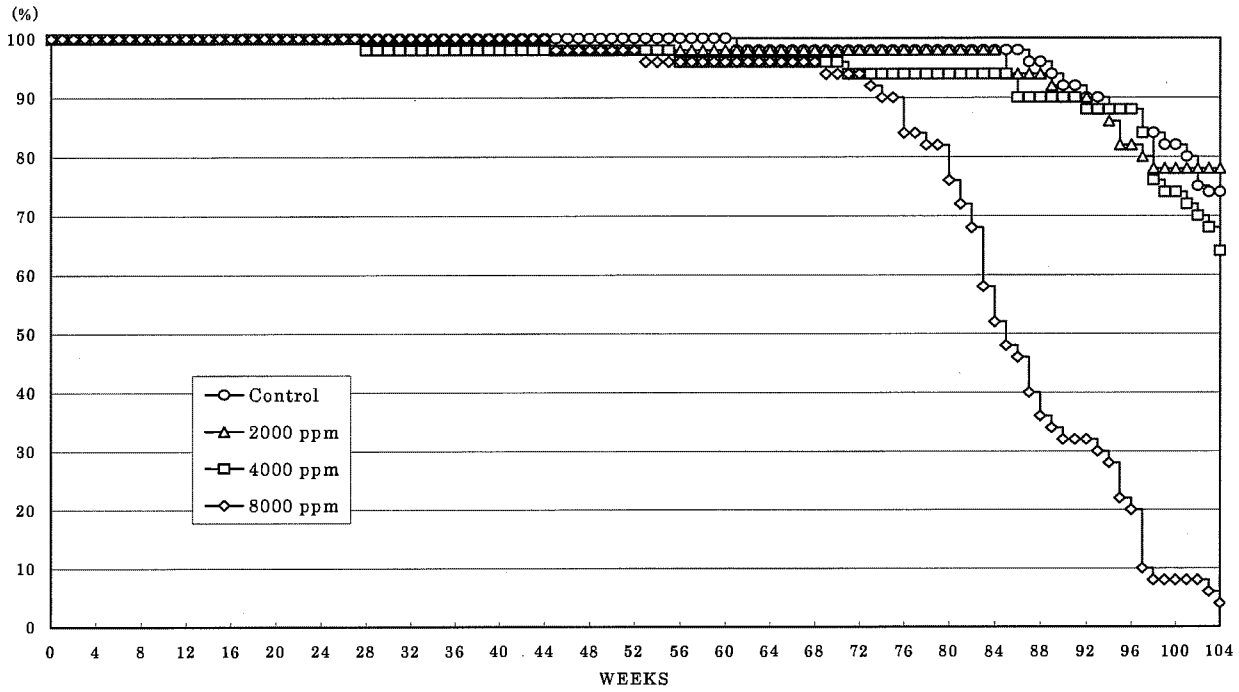


FIGURE 1 SURVIVAL ANIMAL RATE OF MALE RATS IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

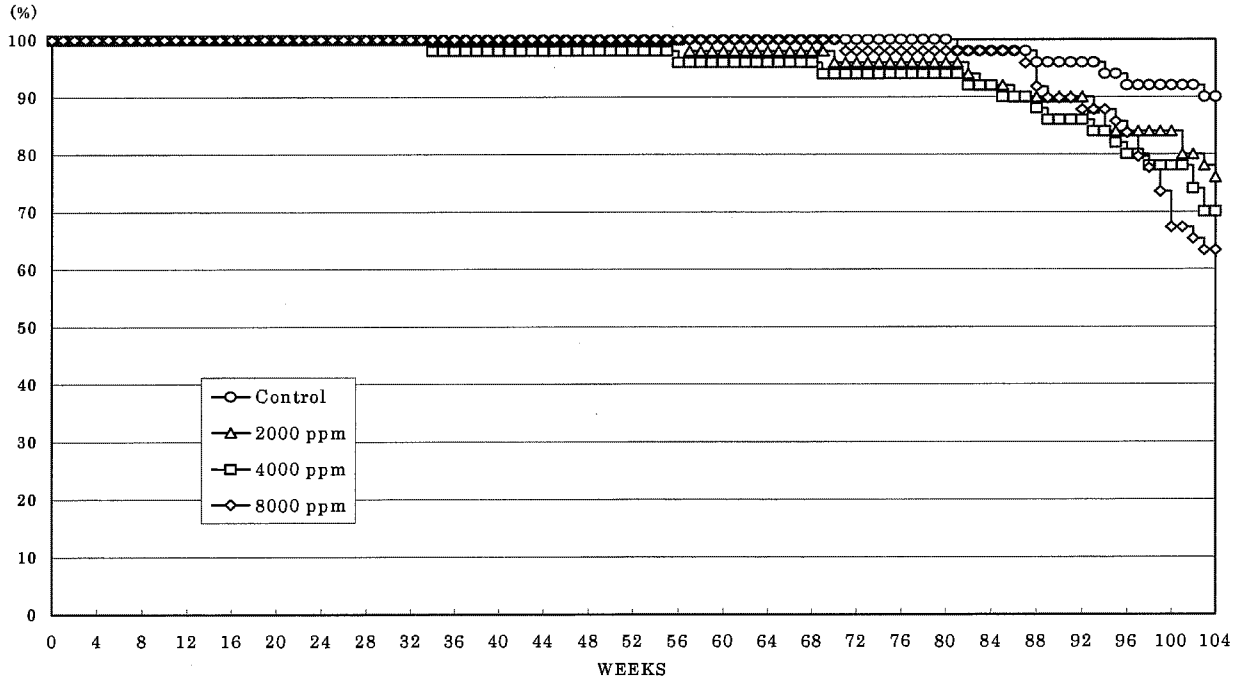


FIGURE 2 SURVIVAL ANIMAL RATE OF FEMALE RATS THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

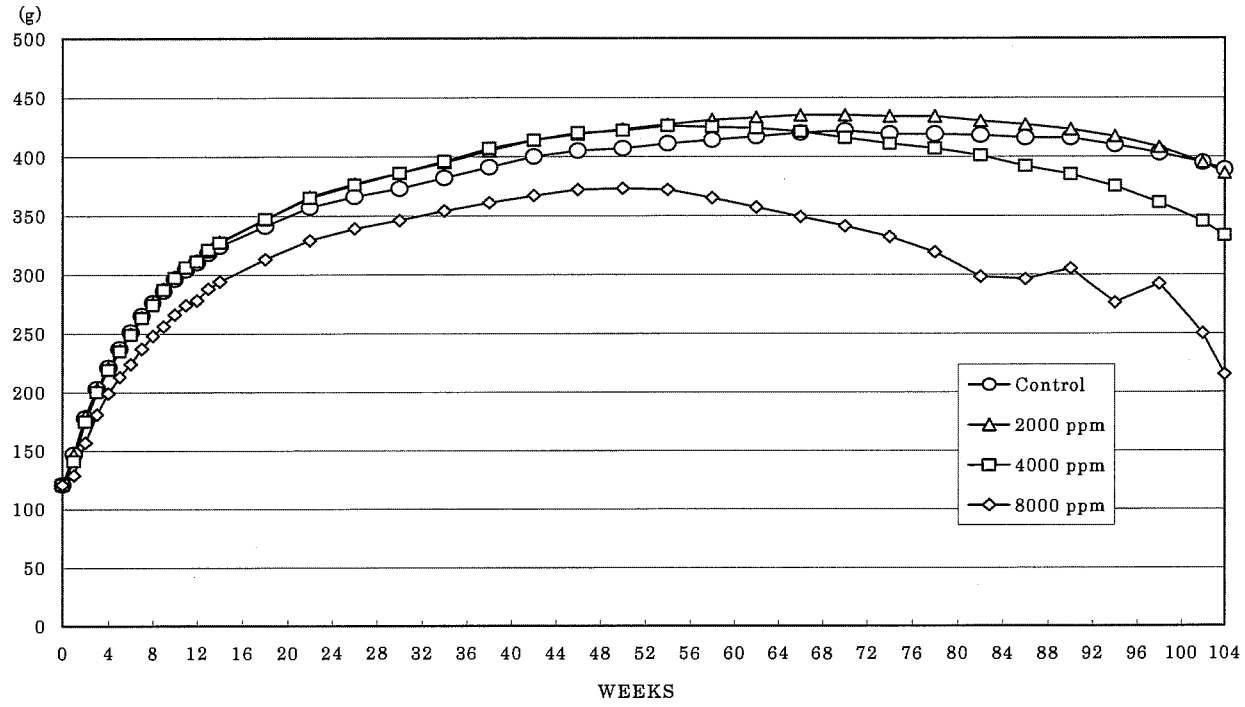


FIGURE 3 BODY WEIGHT CHANGES OF MALE RATS IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

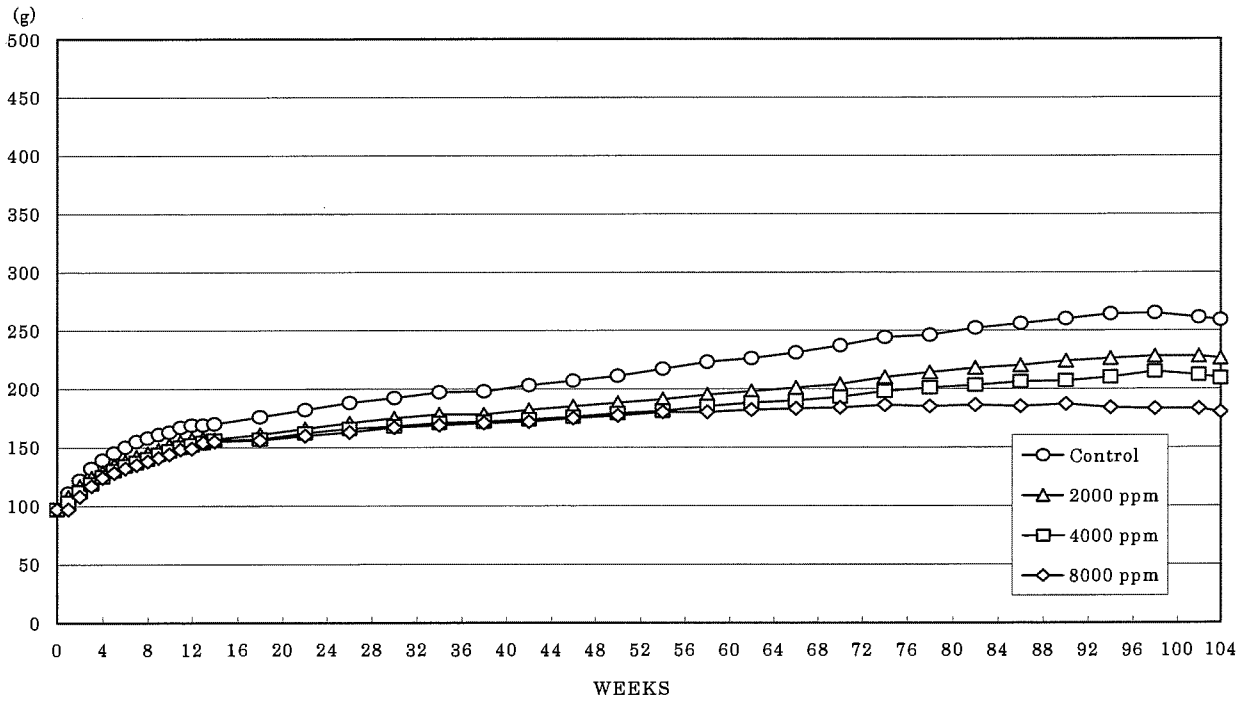


FIGURE 4 BODY WEIGHT CHANGES OF FEMALE RATS IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

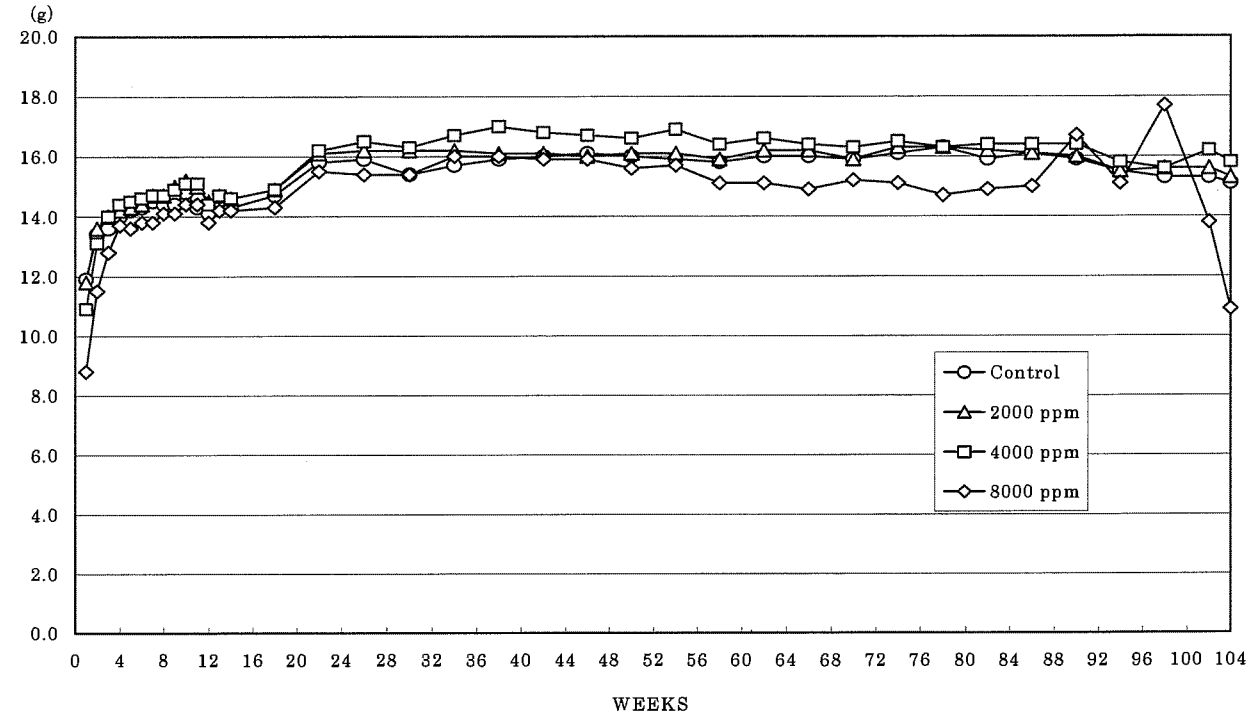


FIGURE 5 FOOD CONSUMPTION CHANGES OF MALE RATS IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE

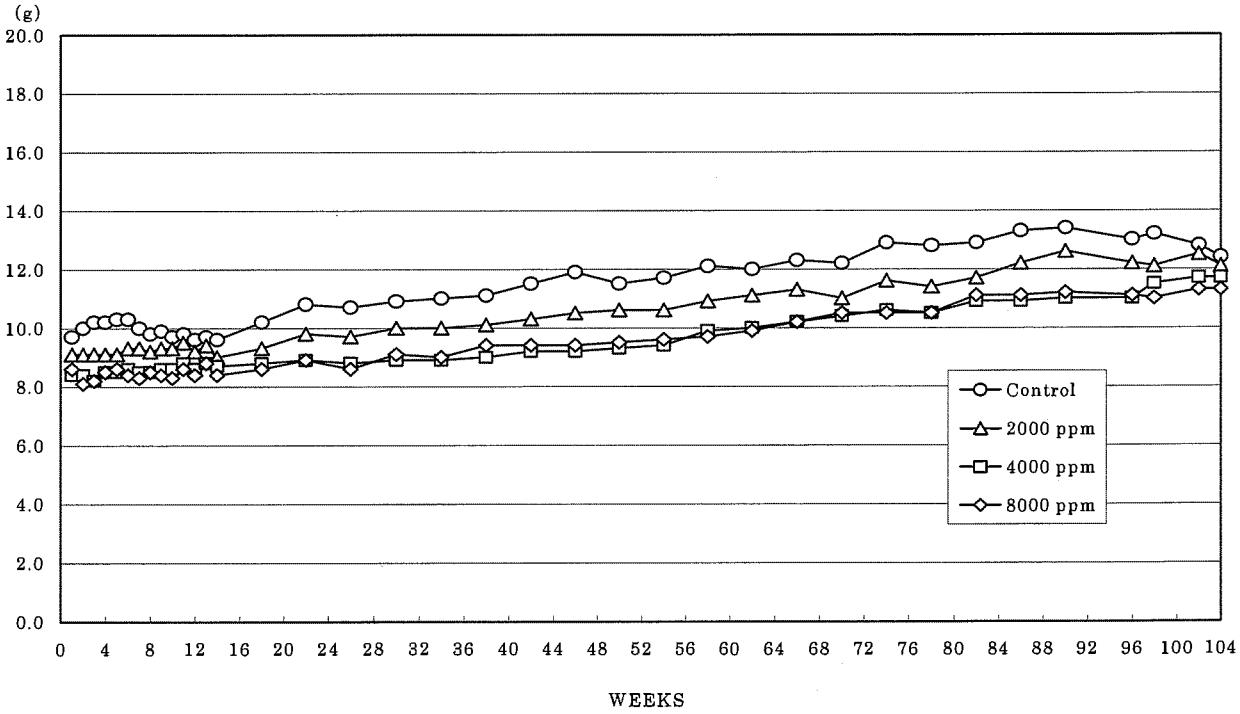
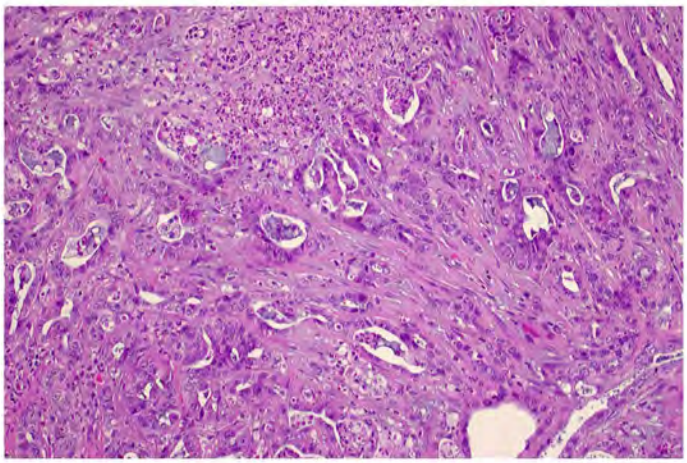
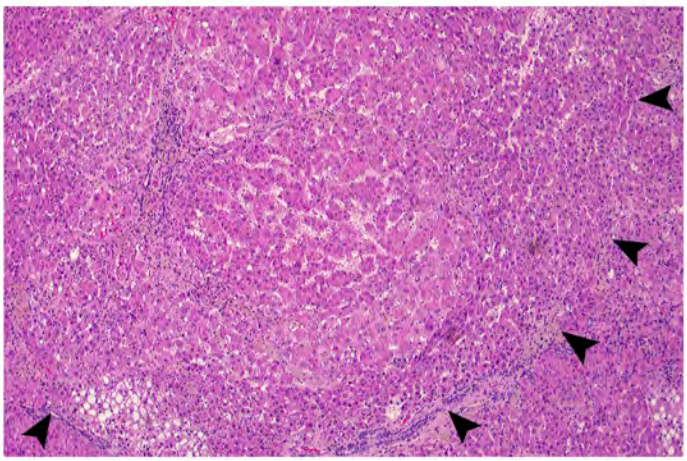


FIGURE 6 FOOD CONSUMPTION CHANGES OF FEMALE RATS IN THE 2-YEAR FEED STUDY OF *p*-NITROANISOLE



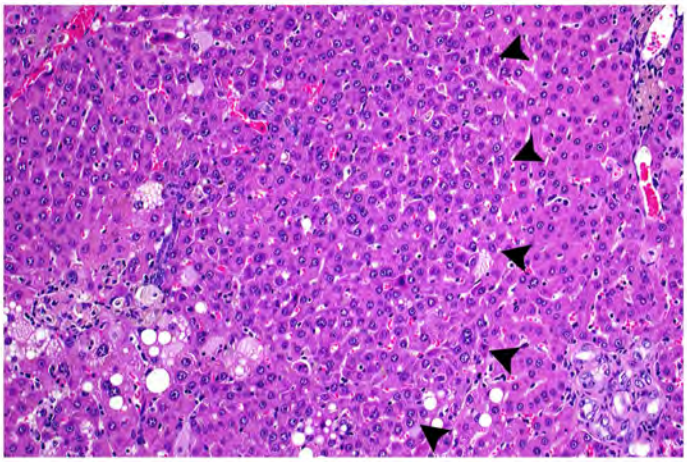
200 μm

Photograph 1
Uterus: Adenocarcima.
Rat, Female, 8000 ppm, Animal No. 0401-2311 (H&E)



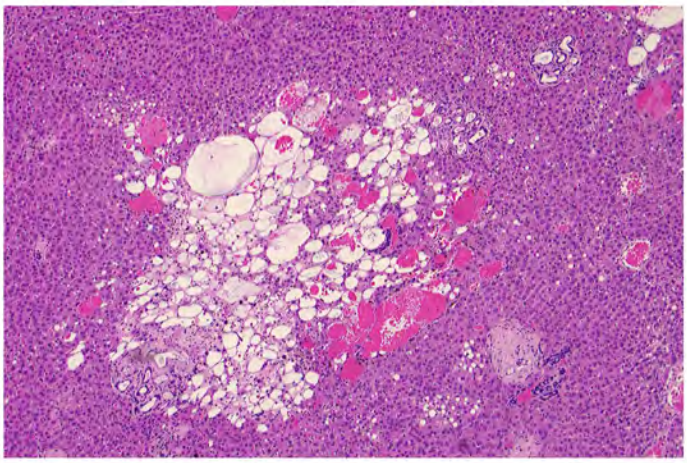
500 μm

Photograph 2
Liver: Hepatocellular adenoma (arrow heads).
Rat, Male, 8000 ppm, Animal No. 0401-1313 (H&E)



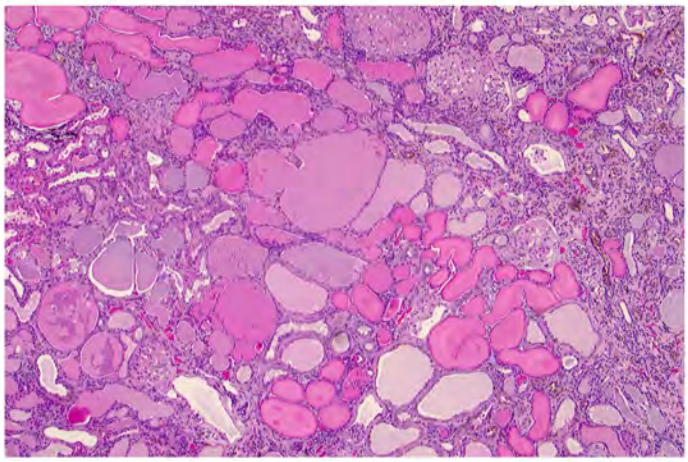
200 μm

Photograph 3
Liver: Basophilic cell focus (arrow heads).
Rat, Male, 8000 ppm, Animal No. 0401-1309 (H&E)



500 μm

Photograph 4
Liver: Spongiosis hepatis.
Rat, Male, 8000 ppm, Animal No. 0399-1349 (H&E)



500 μm

Photograph 5
Kidney: Chronic nephropathy.
Rat, Male, 8000 ppm, Animal No. 0401-1309 (H&E)