Summary of Feed Carcinogenicity Study of 1-Chloro-2-Nitrobenzene in F344 Rats

April 2006

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 April 13, 2006.

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

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Purpose, materials and methods

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

The carcinogenicity and chronic toxicity of *o*-CNB were examined by feeding groups of 50 F344/DuCrlCrlj rats of both sexes *o*-CNB-containing diets for 2 years (104 weeks). The dietary concentration of *o*-CNB was 0, 80, 400 or 2000 ppm (w/w). The highest dose level was chosen so as not to exceed the maximum tolerated dose (MTD), based on both growth rate and toxicity in the previous 13-week toxicity study. *o*-CNB was analyzed for purity and stability by both infrared spectrometry and high performance liquid chromatography before and after its use. The *o*-CNB concentrations in the diet were determined by high performance liquid chromatography at the time of preparation, and on the 8th day after preparation stored at room temperature. The animals were observed daily for clinical signs and mortality. Body weight and food consumption were measured once a week for the first 14 weeks and every 4 weeks thereafter. Animals found dead, in a moribund state, or surviving to the end of the 2-year administration period underwent complete necropsy. Urinalysis was performed near the end of the administration period. For hematology and blood biochemistry, the surviving animals were bled under ether anesthesia, after they were fasted overnight, at the terminal necropsy. Organs and tissues were removed, weighed and examined for macroscopic lesions at necropsy. The organs and tissues were fixed and embedded in paraffin. Tissue sections of 5 μm thick were prepared and stained with hematoxylin and eosin and examined for histopathology. Incidences of neoplastic lesions were statistically analyzed by Fisher's exact test. A positive trend of the dose-response relation for the neoplastic incidence was analyzed by Peto's test. Incidences of non-neoplastic lesions and urinalysis were analyzed by Chi-square test. Changes in body weight, food consumption, hematological and blood biochemical parameters, and organ weights were analyzed by Dunnett's test. The present study was conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) Good Laboratory Practice and with reference to the OECD Guideline for Testing of Chemicals 451 "Carcinogenicity Studies".

Results

Some of the 2000 ppm-fed males died after the 53rd week, and all of them died before the

103rd week of the 2-year administration period. The markedly decreased survival rate of the 2000 ppm-fed male group was attributed to the increased number of deaths due to chronic progressive nephropathy (chronic nephropathy, CPN). The high dose level of 2000 ppm for males was thought to exceed the MTD. However, no significant difference in survival rate was found between any of the other *o*-CNB-fed male and female groups and the respective controls. Body weights of the 2000 ppm-fed males and females were significantly decreased throughout the 2-year administration period. The body weight of 2000 ppm-fed males was decreased to 89% at the 34th week of the 2-year administration period as compared with the male control. The terminal body weight of the 2000 ppm-fed females was suppressed to 82%, as compared with the control. The body weights of the 400 ppm-fed males were also suppressed in the late period of 2-year administration. The yellow urine, which may have been colored by a metabolite of *o*-CNB, was observed in the 2000 ppm-fed males and females throughout the 2-year administration period. There was no significant difference in food consumption between any *o*-CNB-fed group of either sex and the respective control except for the 2000 ppm-fed males during both the early and late periods of 2-year administration.

The increased incidences of tumor were observed in the liver (hepatocellular carcinomas and hepatocellular adenomas) of the males and females and in the kidney (renal cell adenomas) of the females. The incidences of hepatocellular adenomas and carcinomas were increased dose-dependently in males and females. The significantly increased combined incidence of these two hepatocellular tumors was noted in the 400 ppm-fed males and in the 2000 ppm-fed females. The incidence of renal cell adenomas occurred in the 2000 ppm-fed females. As pre-neoplastic lesions in the liver, the incidences of acidophilic cell foci in both the 400ppm-fed males and females, basophilic cell foci and spongiosis hepatis in the 400 ppm-fed males and clear cell foci in the 2000 ppm-fed females were significantly increased. As a pre-neoplastic lesion in the kidney, the increased incidence of atypical tubule hyperplasia in the proximal tubules was noted in the 2000 ppm-fed females. Renal cell carcinomas occurred in the 2000 ppm-fed males.

Severities of CPN were increased dose-dependently in both the *o*-CNB-fed males and females. The incidences of brown pigment deposit in the proximal tubules and urothelial hyperplasia were increased. In the spleen, hemosiderin deposition, engorgement of erythrocytes, angiectasis and capsule hyperplasia were observed. Hydropic degeneration and brown pigment deposit in centrilobular hepatocytes were observed in the *o*-CNB-fed females.

Conclusions

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

TABLES

TABLES (CONTINUED)

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

Week on Study	Control		80 ppm			400 ppm			2000 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	126 (50)	50 / 50	126 (50)	100	50 / 50	126 (50)	100	50 / 50	126 (50)	100	50 / 50
1	153 (50)	50 / 50	154 (50)	101	50 / 50	152 (50)	99	50 / 50	141 (50)	92	50 / 50
2	186 (50)	50 / 50	186 (50)	100	50 / 50	183 (50)	98	50 / 50	167 (50)	90	50 / 50
3	206 (50)	50 / 50	207 (50)	100	50 / 50	203 (50)	99	50 / 50	184 (50)	89	50 / 50
4	228 (50)	50 / 50	231 (50)	101	50 / 50	226 (50)	99	50 / 50	204 (50)	89	50 / 50
5	243 (50)	50 / 50	244 (50)	100	50 / 50	240 (50)	99	50 / 50	218 (50)	90	50 / 50
6	255 (50)	50 / 50	257 (50)	101	50 / 50	253 (50)	99	50 / 50	231 (50)	91	50 / 50
7	268 (50)	50 / 50	271 (50)	101	50 / 50	266 (50)	99	50 / 50	244 (50)	91	50 / 50
8	278 (50)	50 / 50	281 (50)	101	50 / 50	277 (50)	100	50 / 50	254 (50)	91	50 / 50
9	289 (50)	50 / 50	290 (50)	100	50 / 50	286 (50)	99	50 / 50	264 (50)	91	50 / 50
10	298 (50)	50 / 50	299 (50)	100	50 / 50	295 (50)	99	50 / 50	270 (50)	91	50 / 50
11	308 (50)	50 / 50	308 (50)	100	50 / 50	303 (50)	98	50 / 50	277 (50)	90	50 / 50
12	313 (50)	50 / 50	313 (50)	100	50 / 50	307 (50)	98	50 / 50	282 (50)	90	50 / 50
13	319 (50)	50 / 50	320 (50)	100	50 / 50	314 (50)	98	50 / 50	288 (50)	90	50 / 50
14	324 (50)	50 / 50	325 (50)	100	50 / 50	319 (50)	98	50 / 50	294 (50)	91	50 / 50
18	343 (50)	50 / 50	345 (50)	101	50 / 50	340 (50)	99	50 / 50	312 (50)	91	50 / 50
22	356 (50)	50 / 50	360 (50)	101	50 / 50	356 (50)	100	50 / 50	324 (50)	91	50 / 50
26	367 (50)	50 / 50	370 (50)	101	50 / 50	367 (50)	100	50 / 50	333 (50)	91	50 / 50
30	382 (50)	50 / 50	387 (50)	101	50 / 50	382 (50)	100	50 / 50	342 (50)	90	50 / 50
34	392 (50)	50 / 50	395 (50)	101	50 / 50	391 (50)	100	50 / 50	349 (50)	89	50 / 50
38	397 (50)	50 / 50	401 (50)	101	50 / 50	396 (50)	100	50 / 50	352 (50)	89	50 / 50
42	406 (50)	50 / 50	409 (50)	101	50 / 50	404 (50)	100	50 / 50	356 (50)	88	50 / 50
46	411 (50)	50 / 50	415 (50)	101	50 / 50	410 (49)	100	49 / 50	359 (50)	87	50 / 50
50	415 (50)	50 / 50	420 (50)	101	50 / 50	412 (49)	99	49 / 50	357 (50)	86	50 / 50
54	419 (50)	50 / 50	424 (50)	101	50 / 50	416 (49)	99	49 / 50	359 (49)	86	49 / 50
58	422 (50)	50 / 50	426 (50)	101	50 / 50	417 (49)	99	49 / 50	358 (49)	85	49 / 50
62	429 (49)	49 / 50	431 (50)	100	50 / 50	421 (49)	98	49 / 50	354 (49)	83	49 / 50
66	431 (49)	49 / 50	433 (50)	100	50 / 50	421 (49)	98	49 / 50	348 (49)	81	49 / 50
70	432 (49)	49 / 50	433 (50)	100	50 / 50	419 (49)	97	49 / 50	339 (48)	78	48 / 50
74	436 (49)	49 / 50	435 (50)	100	50 / 50	420 (49)	96	49 / 50	331 (45)	76	45 / 50
78	438 (49)	49 / 50	437 (49)	100	49 / 50	419 (48)	96	48 / 50	318 (37)	73	37 / 50
82	435 (49)	49 / 50	432 (48)	99	48 / 50	410 (48)	94	48 / 50	306 (25)	70	25 / 50
86	428 (49)	49 / 50	427 (48)	100	48 / 50	403 (48)	94	48 / 50	297 (18)	69	18 / 50
90	429 (45)	45 / 50	421 (46)	98	46 / 50	395 (45)	92	45 / 50	301 (10)	70	10 / 50
94	421 (43)	43 / 50	414 (45)	98	45 / 50	381 (45)	90	45 / 50	274 (7)	65	7 / 50
98	415 (42)	42 / 50	408 (44)	98	44 / 50	375 (44)	90	44 / 50	281 (2)	68	2 / 50
102	408 (40)	40 / 50	400 (40)	98	40 / 50	360 (41)	88	41 / 50	213 (1)	52	1 / 50
104	395 (40)	40 / 50	394 (40)	100	40 / 50	355 (39)	90	39 / 50	－ (－)	－	0 / 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 1-CHLORO-2-NITROBENZENE

Week on Study	Control		80 ppm			400 ppm			2000 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	99 (50)	50 / 50	99 (50)	100	50 / 50	99 (50)	100	50 / 50	99 (50)	100	50 / 50
1	111 (50)	50 / 50	110 (50)	99	50 / 50	110 (50)	99	50 / 50	104 (50)	94	50 / 50
2	124 (50)	50 / 50	123 (50)	99	50 / 50	123 (50)	99	50 / 50	119 (50)	96	50 / 50
3	130 (50)	50 / 50	129 (50)	99	50 / 50	129 (50)	99	50 / 50	125 (50)	96	50 / 50
4	140 (50)	50 / 50	138 (50)	99	50 / 50	139 (50)	99	50 / 50	134 (50)	96	50 / 50
5	146 (50)	50 / 50	144 (50)	99	50 / 50	145 (50)	99	50 / 50	141 (50)	97	50 / 50
6	150 (50)	50 / 50	148 (50)	99	50 / 50	149 (50)	99	50 / 50	144 (50)	96	50 / 50
7	154 (50)	50 / 50	152 (50)	99	50 / 50	151 (50)	98	50 / 50	147 (50)	95	50 / 50
8	157 (50)	50 / 50	154 (50)	98	50 / 50	155 (50)	99	50 / 50	150 (50)	96	50 / 50
9	160 (50)	50 / 50	157 (50)	98	50 / 50	158 (50)	99	50 / 50	152 (50)	95	50 / 50
10	164 (50)	50 / 50	160 (50)	98	50 / 50	161 (50)	98	50 / 50	155 (50)	95	50 / 50
11	167 (50)	50 / 50	164 (50)	98	50 / 50	164 (50)	98	50 / 50	160 (50)	96	50 / 50
12	168 (50)	50 / 50	165 (50)	98	50 / 50	165 (50)	98	50 / 50	161 (50)	96	50 / 50
13	170 (50)	50 / 50	166 (50)	98	50 / 50	167 (50)	98	50 / 50	163 (50)	96	50 / 50
14	171 (50)	50 / 50	167 (50)	98	50 / 50	168 (50)	98	50 / 50	163 (50)	95	50 / 50
18	178 (50)	50 / 50	174 (50)	98	50 / 50	175 (50)	98	50 / 50	169 (50)	95	50 / 50
22	182 (50)	50 / 50	178 (50)	98	50 / 50	179 (50)	98	50 / 50	172 (50)	95	50 / 50
26	188 (50)	50 / 50	183 (50)	97	50 / 50	184 (50)	98	50 / 50	176 (50)	94	50 / 50
30	192 (50)	50 / 50	188 (50)	98	50 / 50	189 (50)	98	50 / 50	180 (50)	94	50 / 50
34	196 (50)	50 / 50	191 (50)	97	50 / 50	192 (50)	98	50 / 50	183 (50)	93	50 / 50
38	199 (50)	50 / 50	194 (50)	97	50 / 50	196 (50)	98	50 / 50	185 (50)	93	50 / 50
42	203 (50)	50 / 50	199 (50)	98	50 / 50	200 (50)	99	50 / 50	189 (50)	93	50 / 50
46	208 (50)	50 / 50	202 (50)	97	50 / 50	204 (50)	98	50 / 50	190 (50)	91	50 / 50
50	211 (50)	50 / 50	206 (50)	98	50 / 50	207 (50)	98	50 / 50	193 (50)	91	50 / 50
54	215 (50)	50 / 50	210 (50)	98	50 / 50	211 (50)	98	50 / 50	196 (50)	91	50 / 50
58	221 (50)	50 / 50	214 (50)	97	50 / 50	216 (50)	98	50 / 50	199 (50)	90	50 / 50
62	227 (50)	50 / 50	221 (50)	97	50 / 50	221 (50)	97	50 / 50	203 (49)	89	49 / 50
66	231 (50)	50 / 50	226 (50)	98	50 / 50	227 (50)	98	50 / 50	207 (49)	90	49 / 50
70	232 (49)	49 / 50	230 (50)	99	50 / 50	230 (50)	99	50 / 50	210 (48)	91	48 / 50
74	244 (49)	49 / 50	240 (50)	98	50 / 50	239 (49)	98	49 / 50	215 (48)	88	48 / 50
78	250 (48)	48 / 50	250 (50)	100	50 / 50	248 (49)	99	49 / 50	220 (48)	88	48 / 50
82	253 (48)	48 / 50	254 (50)	100	50 / 50	249 (49)	98	49 / 50	217 (48)	86	48 / 50
86	257 (47)	47 / 50	259 (50)	101	50 / 50	255 (49)	99	49 / 50	221 (48)	86	48 / 50
90	262 (45)	45 / 50	264 (50)	101	50 / 50	257 (49)	98	49 / 50	221 (48)	84	48 / 50
94	262 (44)	44 / 50	266 (50)	102	50 / 50	258 (49)	98	49 / 50	222 (47)	85	47 / 50
98	264 (43)	43 / 50	269 (48)	102	48 / 50	258 (47)	98	47 / 50	220 (44)	83	44 / 50
102	265 (41)	41 / 50	263 (44)	99	44 / 50	259 (46)	98	46 / 50	217 (40)	82	40 / 50
104	265 (41)	41 / 50	266 (42)	100	42 / 50	256 (45)	97	45 / 50	216 (39)	82	39 / 50

< > : 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 1-CHLORO-2-NITROBENZENE

Week on Study	Control		80 ppm			400 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	12.5 (50)	50 / 50	12.5 (50)	100	50 / 50	12.3 (50)	98	50 / 50	10.5 (50)	84	50 / 50
2	13.6 (50)	50 / 50	13.8 (50)	101	50 / 50	13.4 (50)	99	50 / 50	12.4 (50)	91	50 / 50
3	14.3 (50)	50 / 50	14.5 (50)	101	50 / 50	14.1 (50)	99	50 / 50	13.0 (50)	91	50 / 50
4	14.6 (50)	50 / 50	14.8 (50)	101	50 / 50	14.6 (50)	100	50 / 50	13.5 (50)	92	50 / 50
5	14.6 (50)	50 / 50	14.9 (50)	102	50 / 50	14.8 (50)	101	50 / 50	13.7 (50)	94	50 / 50
6	14.0 (50)	50 / 50	14.4 (50)	103	50 / 50	14.1 (50)	101	50 / 50	13.4 (50)	96	50 / 50
7	14.4 (50)	50 / 50	14.7 (50)	102	50 / 50	14.4 (50)	100	50 / 50	13.7 (50)	95	50 / 50
8	14.2 (50)	50 / 50	14.6 (50)	103	50 / 50	14.5 (50)	102	50 / 50	13.9 (50)	98	50 / 50
9	14.3 (50)	50 / 50	14.7 (50)	103	50 / 50	14.4 (50)	101	50 / 50	13.8 (50)	97	50 / 50
10	14.5 (50)	50 / 50	14.7 (50)	101	50 / 50	14.6 (50)	101	50 / 50	13.8 (49)	95	50 / 50
11	14.7 (50)	50 / 50	14.9 (50)	101	50 / 50	14.7 (50)	100	50 / 50	13.9 (50)	95	50 / 50
12	14.6 (50)	50 / 50	14.7 (50)	101	50 / 50	14.4 (50)	99	50 / 50	14.0 (50)	96	50 / 50
13	14.6 (50)	50 / 50	14.8 (50)	101	50 / 50	14.7 (50)	101	50 / 50	14.0 (50)	96	50 / 50
14	14.4 (50)	50 / 50	14.6 (50)	101	50 / 50	14.5 (50)	101	50 / 50	13.9 (50)	97	50 / 50
18	14.5 (50)	50 / 50	14.8 (50)	102	50 / 50	14.7 (50)	101	50 / 50	14.3 (50)	99	50 / 50
22	14.5 (50)	50 / 50	14.9 (50)	103	50 / 50	15.2 (50)	105	50 / 50	14.6 (50)	101	50 / 50
26	14.8 (50)	50 / 50	15.2 (50)	103	50 / 50	15.3 (50)	103	50 / 50	15.0 (50)	101	50 / 50
30	15.0 (50)	50 / 50	15.3 (50)	102	50 / 50	15.4 (50)	103	50 / 50	15.0 (49)	100	50 / 50
34	15.3 (49)	50 / 50	15.5 (50)	101	50 / 50	15.6 (50)	102	50 / 50	15.6 (50)	102	50 / 50
38	15.1 (50)	50 / 50	15.6 (50)	103	50 / 50	15.6 (50)	103	50 / 50	15.4 (46)	102	50 / 50
42	15.2 (49)	50 / 50	15.5 (50)	102	50 / 50	15.5 (50)	102	50 / 50	15.3 (49)	101	50 / 50
46	15.4 (49)	50 / 50	15.6 (49)	101	50 / 50	15.9 (49)	103	49 / 50	15.6 (48)	101	50 / 50
50	15.5 (50)	50 / 50	15.9 (50)	103	50 / 50	15.8 (49)	102	49 / 50	15.6 (48)	101	50 / 50
54	15.5 (50)	50 / 50	15.9 (50)	103	50 / 50	15.9 (49)	103	49 / 50	15.7 (46)	101	49 / 50
58	15.7 (50)	50 / 50	15.7 (49)	100	50 / 50	16.0 (49)	102	49 / 50	15.6 (45)	99	49 / 50
62	15.9 (49)	49 / 50	16.0 (50)	101	50 / 50	16.3 (49)	103	49 / 50	15.6 (45)	98	49 / 50
66	15.5 (49)	49 / 50	15.6 (49)	101	50 / 50	16.0 (49)	103	49 / 50	15.1 (45)	97	49 / 50
70	15.7 (49)	49 / 50	15.8 (50)	101	50 / 50	16.3 (49)	104	49 / 50	15.2 (45)	97	48 / 50
74	15.9 (49)	49 / 50	16.0 (50)	101	50 / 50	16.4 (48)	103	49 / 50	14.5 (41)	91	45 / 50
78	16.3 (49)	49 / 50	16.1 (48)	99	49 / 50	16.5 (48)	101	48 / 50	14.5 (35)	89	37 / 50
82	15.7 (46)	49 / 50	15.6 (47)	99	48 / 50	15.7 (45)	100	48 / 50	13.3 (21)	85	25 / 50
86	15.4 (49)	49 / 50	15.5 (48)	101	48 / 50	16.0 (47)	104	48 / 50	14.1 (18)	92	18 / 50
90	15.9 (41)	45 / 50	16.1 (45)	101	46 / 50	15.8 (43)	99	45 / 50	13.4 (6)	84	10 / 50
94	15.2 (37)	43 / 50	15.5 (43)	102	45 / 50	15.4 (42)	101	45 / 50	11.7 (7)	77	7 / 50
98	15.4 (37)	42 / 50	15.2 (41)	99	44 / 50	15.4 (36)	100	44 / 50	13.0 (2)	84	2 / 50
102	15.1 (36)	40 / 50	14.7 (37)	97	40 / 50	15.4 (34)	102	41 / 50	7.6 (1)	50	1 / 50
104	14.8 (37)	40 / 50	15.4 (38)	104	40 / 50	16.3 (35)	110	39 / 50	－ (－)	－	0 / 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 1-CHLORO-2-NITROBENZENE

Week on Study	Control		80 ppm			400 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	9.5 (50)	50 / 50	9.3 (50)	98	50 / 50	9.2 (50)	97	50 / 50	7.5 (50)	79	50 / 50
2	9.4 (50)	50 / 50	9.3 (50)	99	50 / 50	9.5 (50)	101	50 / 50	9.3 (50)	99	50 / 50
3	9.6 (50)	50 / 50	9.4 (50)	98	50 / 50	9.6 (50)	100	50 / 50	9.4 (50)	98	50 / 50
4	9.6 (50)	50 / 50	9.4 (50)	98	50 / 50	9.7 (50)	101	50 / 50	9.4 (50)	98	50 / 50
5	9.8 (50)	50 / 50	9.4 (50)	96	50 / 50	9.9 (50)	101	50 / 50	9.7 (50)	99	50 / 50
6	9.2 (50)	50 / 50	8.9 (50)	97	50 / 50	9.1 (50)	99	50 / 50	9.1 (50)	99	50 / 50
7	9.4 (50)	50 / 50	8.9 (50)	95	50 / 50	9.0 (50)	96	50 / 50	9.1 (50)	97	50 / 50
8	9.3 (50)	50 / 50	8.8 (50)	95	50 / 50	9.0 (50)	97	50 / 50	9.0 (50)	97	50 / 50
9	9.4 (50)	50 / 50	8.8 (50)	94	50 / 50	9.0 (50)	96	50 / 50	9.0 (50)	96	50 / 50
10	9.4 (50)	50 / 50	8.8 (50)	94	50 / 50	8.9 (50)	95	50 / 50	8.8 (50)	94	50 / 50
11	9.7 (50)	50 / 50	9.1 (50)	94	50 / 50	9.1 (50)	94	50 / 50	9.1 (50)	94	50 / 50
12	9.5 (50)	50 / 50	9.0 (50)	95	50 / 50	8.9 (50)	94	50 / 50	8.9 (50)	94	50 / 50
13	9.5 (50)	50 / 50	8.9 (50)	94	50 / 50	9.1 (50)	96	50 / 50	9.1 (50)	96	50 / 50
14	9.3 (50)	50 / 50	8.8 (50)	95	50 / 50	9.1 (50)	98	50 / 50	9.0 (50)	97	50 / 50
18	9.5 (50)	50 / 50	9.1 (50)	96	50 / 50	9.1 (50)	96	50 / 50	9.1 (50)	96	50 / 50
22	9.8 (50)	50 / 50	9.2 (50)	94	50 / 50	9.5 (50)	97	50 / 50	9.4 (50)	96	50 / 50
26	10.1 (50)	50 / 50	9.4 (50)	93	50 / 50	9.6 (50)	95	50 / 50	9.5 (50)	94	50 / 50
30	9.8 (50)	50 / 50	9.5 (50)	97	50 / 50	9.7 (50)	99	50 / 50	9.5 (50)	97	50 / 50
34	10.4 (50)	50 / 50	9.7 (50)	93	50 / 50	10.1 (50)	97	50 / 50	9.8 (50)	94	50 / 50
38	10.3 (50)	50 / 50	9.7 (50)	94	50 / 50	10.4 (50)	101	50 / 50	9.9 (50)	96	50 / 50
42	10.5 (50)	50 / 50	9.9 (50)	94	50 / 50	10.2 (50)	97	50 / 50	10.0 (50)	95	50 / 50
46	10.7 (50)	50 / 50	10.4 (50)	97	50 / 50	10.5 (50)	98	50 / 50	10.2 (50)	95	50 / 50
50	11.0 (50)	50 / 50	10.7 (50)	97	50 / 50	10.8 (50)	98	50 / 50	10.4 (50)	95	50 / 50
54	11.2 (50)	50 / 50	10.9 (50)	97	50 / 50	11.1 (50)	99	50 / 50	10.7 (50)	96	50 / 50
58	11.6 (50)	50 / 50	11.2 (50)	97	50 / 50	11.4 (50)	98	50 / 50	10.9 (50)	94	50 / 50
62	11.9 (50)	50 / 50	11.6 (50)	97	50 / 50	11.8 (50)	99	50 / 50	11.4 (49)	96	49 / 50
66	11.7 (50)	50 / 50	11.5 (50)	98	50 / 50	11.9 (50)	102	50 / 50	11.5 (49)	98	49 / 50
70	11.7 (49)	49 / 50	11.9 (50)	102	50 / 50	11.9 (49)	102	50 / 50	11.6 (48)	99	48 / 50
74	12.9 (49)	49 / 50	12.9 (50)	100	50 / 50	12.5 (49)	97	49 / 50	12.1 (48)	94	48 / 50
78	12.9 (48)	48 / 50	13.2 (50)	102	50 / 50	13.0 (49)	101	49 / 50	12.7 (48)	98	48 / 50
82	12.4 (48)	48 / 50	12.8 (50)	103	50 / 50	12.4 (49)	100	49 / 50	12.3 (48)	99	48 / 50
86	12.8 (47)	47 / 50	12.9 (50)	101	50 / 50	13.0 (49)	102	49 / 50	12.8 (48)	100	48 / 50
90	13.2 (45)	45 / 50	13.3 (50)	101	50 / 50	13.1 (49)	99	49 / 50	13.0 (48)	98	48 / 50
94	12.5 (44)	44 / 50	12.5 (50)	100	50 / 50	12.4 (49)	99	49 / 50	12.8 (47)	102	47 / 50
98	12.5 (43)	43 / 50	12.6 (48)	101	48 / 50	12.7 (47)	102	47 / 50	12.8 (44)	102	44 / 50
102	12.6 (41)	41 / 50	12.1 (44)	96	44 / 50	12.9 (45)	102	46 / 50	13.0 (40)	103	40 / 50
104	12.8 (41)	41 / 50	13.0 (42)	102	42 / 50	12.9 (45)	101	45 / 50	13.3 (39)	104	39 / 50

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

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

Group Name	Control	80 ppm	400 ppm	2000 ppm
No. of examined animals	39	40	39	0
RED BLOOD CELL ($10^6/\mu$ L)	7.44 ± 1.94	8.30 ± 1.24 *	7.42 ± 1.18	—
HEMOGLOBIN (g/dL)	12.9 ± 3.5	14.0 ± 2.1	12.3 ± 1.8 **	—
HEMATOCRIT (%)	37.1 ± 8.6	40.4 ± 4.8	35.5 ± 4.8 **	—
MCV (fL)	51.8 ± 10.0	49.2 ± 5.5 *	48.1 ± 2.5 **	—
MCH (pg)	17.6 ± 2.8	16.9 ± 1.4 *	16.6 ± 1.0 **	—
PLATELET ($10^3/\mu$ L)	786 ± 274	833 ± 277	949 ± 154 **	—
METHEMOGLOBIN (%)	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2 **	—

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

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

Group Name	Control	80 ppm	400 ppm	2000 ppm
No. of examined animals	41	42	45	38
RED BLOOD CELL ($10^6/\mu$ L)	7.82 ± 0.86	7.61 ± 1.48	7.80 ± 0.55	6.71 ± 0.57 **
HEMOGLOBIN (g/dL)	14.7 ± 1.6	14.2 ± 2.8	14.1 ± 1.2 **	12.2 ± 0.9 **
HEMATOCRIT (%)	40.7 ± 3.6	39.7 ± 6.1	39.8 ± 2.9	35.4 ± 2.4 **
MCV (fL)	52.3 ± 2.9	53.8 ± 9.4	51.0 ± 1.7 **	52.9 ± 2.3
MCH (pg)	18.9 ± 1.0	18.8 ± 1.3	18.1 ± 1.0 **	18.2 ± 0.7 **
MCHC (g/dL)	36.1 ± 1.4	35.4 ± 3.2	35.4 ± 1.1 **	34.4 ± 0.6 **
PLATELET ($10^3/\mu$ L)	612 ± 142	644 ± 154	725 ± 123 **	730 ± 115 **
RETICULOCYTE (%)	3.3 ± 2.9	4.6 ± 8.1	3.1 ± 1.4	5.7 ± 1.4 **
METHEMOGLOBIN (%)	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2 **	1.3 ± 0.4 **
WBC ($10^3/\mu$ L)	3.63 ± 2.09	7.90 ± 22.29	3.30 ± 1.33	6.15 ± 15.18 *
Differential WBC (%)				
N-SEG	40 ± 10	38 ± 10	42 ± 9	46 ± 11 *

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

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

Group Name	Control	80 ppm		400 ppm		2000 ppm
No. of examined animals	39	40		39		0
ALBUMIN (g/dL)	2.9 ± 0.2	2.9 ± 0.2		2.7 ± 0.3	**	—
A/G RATIO	0.8 ± 0.1	0.8 ± 0.1		0.7 ± 0.1	**	—
T-CHOLESTEROL (mg/dL)	159 ± 43	191 ± 42	**	277 ± 52	**	—
TRIGLYCERIDE (mg/dL)	89 ± 63	93 ± 51		207 ± 88	**	—
PHOSPHOLIPID (mg/dL)	235 ± 68	261 ± 57		377 ± 76	**	—
LDH (IU/L)	254 ± 296	176 ± 96		166 ± 90	*	—
G-GTP (IU/L)	6 ± 3	13 ± 6	**	35 ± 23	**	—
UREA NITROGEN (mg/dL)	18.0 ± 4.1	19.4 ± 3.2		43.9 ± 31.7	**	—
CREATININE (mg/dL)	0.6 ± 0.1	0.6 ± 0.1		0.9 ± 0.3	**	—
CHLORIDE (mEq/L)	105 ± 2	104 ± 2		103 ± 2	**	—
CALCIUM (mg/dL)	10.4 ± 0.4	10.5 ± 0.3		11.2 ± 0.6	**	—
INORGANIC PHOSPHORUS (mg/dL)	4.2 ± 0.7	4.3 ± 0.5		5.7 ± 2.2	**	—

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

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

Group Name	Control	80 ppm	400 ppm		2000 ppm	
No. of examined animals	41	42	45		38	
ALBUMIN (g/dL)	3.6 ± 0.4	3.6 ± 0.4	3.6 ± 0.3		3.2 ± 0.3	**
A/G RATIO	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1		0.9 ± 0.1	**
T-BILIRUBIN (mg/dL)	0.15 ± 0.05	0.29 ± 0.96	0.14 ± 0.02		0.21 ± 0.03	**
GLUCOSE (mg/dL)	148 ± 20	155 ± 25	160 ± 18	*	158 ± 14	
T-CHOLESTEROL (mg/dL)	126 ± 28	146 ± 40	188 ± 48	**	267 ± 61	**
TRIGLYCERIDE (mg/dL)	66 ± 65	70 ± 54	77 ± 44		211 ± 107	**
PHOSPHOLIPID (mg/dL)	223 ± 49	256 ± 101	292 ± 70	**	414 ± 93	**
ALT (IU/L)	67 ± 70	73 ± 89	67 ± 28		134 ± 117	**
LDH (IU/L)	268 ± 93	285 ± 229	200 ± 70	**	189 ± 73	**
ALP (IU/L)	139 ± 78	167 ± 346	110 ± 64	**	161 ± 35	**
G-GTP (IU/L)	2 ± 2	3 ± 3	6 ± 3	**	77 ± 25	**
UREA NITROGEN (mg/dL)	16.1 ± 2.7	17.7 ± 4.8	18.0 ± 4.2	*	28.8 ± 12.7	**
CREATININE (mg/dL)	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1		0.6 ± 0.2	*
SODIUM (mEq/L)	141 ± 2	140 ± 2	140 ± 2		139 ± 2	*
CHLORIDE (mEq/L)	103 ± 2	103 ± 2	103 ± 2		102 ± 2	**
CALCIUM (mg/dL)	10.7 ± 0.9	10.5 ± 0.3	10.8 ± 0.4		11.2 ± 0.4	**
INORGANIC PHOSPHORUS (mg/dL)	4.1 ± 0.7	4.0 ± 0.7	4.0 ± 0.6		5.0 ± 1.3	**

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

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

Group Name		Control	80 ppm	400 ppm	2000 ppm
No. of examined animals		40	40	39	0
	Grade				
pH	5.0	0	0	0	−
	6.0	3	0	1	−
	6.5	0	2	7	−
	7.0	7	5	10	−
	7.5	21	21	19	−
	8.0	9	12	2	−
	8.5	0	0	0	−
	Chi square test			*	

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

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

Group Name		Control	80 ppm	400 ppm	2000 ppm
No. of examined animals		41	42	46	40
	Grade				
pH	5.0	0	0	0	0
	6.0	1	3	1	1
	6.5	4	4	3	9
	7.0	9	11	7	16
	7.5	7	5	15	9
	8.0	17	18	19	5
	8.5	3	1	1	0
	Chi square test				*
Protein	−	0	0	0	0
	±	5	1	0	0
	+	12	12	2	0
	2+	14	14	9	1
	3+	7	11	24	23
	4+	3	4	11	16
	Chi square test			**	**
Ketone body	−	12	8	14	36
	±	29	34	29	4
	+	0	0	2	0
	2+	0	0	0	0
	3+	0	0	0	0
	4+	0	0	1	0
	Chi square test				**
Bilirubin	−	41	42	41	21
	+	0	0	5	19
	2+	0	0	0	0
	3+	0	0	0	0
	Chi square test			*	**

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

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

Group Name	Control	80 ppm		400 ppm		2000 ppm
No. of examined animals	40	40		39		0
Body weight (g)	376 ± 47	375 ± 26		334 ± 27	**	—
Adrenals (g)	0.068 ± 0.013	0.069 ± 0.035		0.066 ± 0.009		—
Adrenals (%)	0.019 ± 0.007	0.019 ± 0.010		0.020 ± 0.004	**	—
Heart (g)	1.256 ± 0.133	1.239 ± 0.164		1.186 ± 0.090		—
Heart (%)	0.342 ± 0.081	0.332 ± 0.052		0.357 ± 0.040	**	—
Lungs (g)	1.480 ± 0.330	1.430 ± 0.215		1.345 ± 0.118	*	—
Lungs (%)	0.409 ± 0.160	0.383 ± 0.066		0.404 ± 0.041	**	—
Kidneys (g)	2.846 ± 0.488	2.867 ± 0.273		3.175 ± 0.380	**	—
Kidneys (%)	0.782 ± 0.273	0.769 ± 0.102		0.961 ± 0.180	**	—
Spleen (g)	1.875 ± 1.944	1.484 ± 2.403		1.023 ± 0.288		—
Spleen (%)	0.551 ± 0.684	0.393 ± 0.605		0.306 ± 0.085		—
Liver (g)	10.985 ± 1.234	11.916 ± 1.482	**	14.722 ± 1.500	**	—
Liver (%)	2.990 ± 0.672	3.184 ± 0.408	*	4.424 ± 0.471	**	—
Brain (g)	2.111 ± 0.053	2.124 ± 0.043		2.129 ± 0.057		—
Brain (%)	0.573 ± 0.095	0.569 ± 0.042		0.642 ± 0.056	**	—

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

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

Group Name	Control	80 ppm	400 ppm	2000 ppm
No. of examined animals	41	42	45	39
Body weight (g)	251 ± 30	252 ± 33	242 ± 29	201 ± 21 **
Adrenals (g)	0.069 ± 0.009	0.068 ± 0.008	0.070 ± 0.034	0.058 ± 0.010 **
Adrenals (%)	0.028 ± 0.005	0.028 ± 0.005	0.029 ± 0.012	0.029 ± 0.005
Ovaries (g)	0.143 ± 0.063	0.274 ± 0.482	0.126 ± 0.017	0.193 ± 0.321
Ovaries (%)	0.057 ± 0.024	0.106 ± 0.167	0.053 ± 0.011	0.097 ± 0.160 **
Heart (g)	0.868 ± 0.082	0.895 ± 0.094	0.875 ± 0.076	0.866 ± 0.065
Heart (%)	0.349 ± 0.035	0.362 ± 0.070	0.366 ± 0.053	0.435 ± 0.051 **
Lungs (g)	0.993 ± 0.196	0.985 ± 0.116	0.959 ± 0.133	0.937 ± 0.081
Lungs (%)	0.400 ± 0.087	0.400 ± 0.091	0.401 ± 0.077	0.471 ± 0.064 **
Kidneys (g)	1.753 ± 0.151	1.776 ± 0.153	1.889 ± 0.216 *	2.503 ± 0.292 **
Kidneys (%)	0.705 ± 0.071	0.717 ± 0.116	0.785 ± 0.088 **	1.257 ± 0.187 **
Spleen (g)	0.820 ± 0.962	0.993 ± 1.613	0.614 ± 0.183	1.040 ± 0.308 **
Spleen (%)	0.324 ± 0.350	0.445 ± 0.925	0.255 ± 0.079	0.522 ± 0.159 **
Liver (g)	6.621 ± 1.325	7.355 ± 1.056 *	8.662 ± 1.468 **	14.596 ± 1.258 **
Liver (%)	2.641 ± 0.416	2.972 ± 0.664	3.571 ± 0.376 **	7.314 ± 0.845 **
Brain (g)	1.910 ± 0.052	1.911 ± 0.053	1.918 ± 0.041	1.909 ± 0.050
Brain (%)	0.772 ± 0.089	0.773 ± 0.111	0.804 ± 0.113	0.959 ± 0.095 **

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

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

Group Name	Control	80 ppm	400 ppm	2000 ppm	Peto	Cochran-
Number of examined animals	50	50	50	50	test	Armitage test
liver	<50>	<50>	<50>	<50>		
hepatocellular adenoma	2 (4 %)	3 (6 %)	7 (14 %)	1 (2 %)	↑	
hepatocellular carcinoma	0 (0 %)	0 (0 %)	3 (6 %)	1 (2 %)	↑ ↑	↑
kidney	<50>	<50>	<50>	<50>		
renal cell adenoma	0 (0 %)	1 (2 %)	0 (0 %)	1 (2 %)		
renal cell carcinoma	0 (0 %)	0 (0 %)	0 (0 %)	4 (8 %)		
testis	<50>	<50>	<50>	<50>		
interstitial cell tumor	38 (76 %)	42 (84 %)	45 (90 %)	33 (66 %)	↑ ↑	
spleen	<50>	<50>	<50>	<50>		
mononuclear cell leukemia	10 (20 %)	4 (8 %)	2 (4 %) *	0 (0 %)		↓
pituitary gland	<50>	<50>	<50>	<50>		
adenoma	18 (36 %)	9 (18 %) *	11 (22 %)	1 (2 %)		
thyroid	<50>	<50>	<50>	<49>		
C-cell adenoma	7 (14 %)	4 (8 %)	1 (2 %) *	1 (2 %)		↓

Significant difference * : p≦0.05 ** : p≦0.01 Fisher's exact test for neoplastic lesion
↑(↓) : p≦0.05 ↑ ↑(↓ ↓) : p≦0.01 Peto or Cochran-Armitage test for neoplastic lesion
< > : Number of animals examined at the site
Statistical analysis is not applied to the data of 2000 ppm group, since they exceeded the MTD.

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

Group Name	Control	80 ppm	400 ppm	2000 ppm	Peto	Cochran-
Number of examined animals	50	50	50	50	test	Armitage test
liver	<50>	<50>	<50>	<50>		
hepatocellular adenoma	0 (0 %)	0 (0 %)	2 (4 %)	20 (40 %) **	↑ ↑	↑ ↑
hepatocellular carcinoma	0 (0 %)	0 (0 %)	0 (0 %)	4 (8 %)	↑ ↑	↑ ↑
kidney	<50>	<50>	<50>	<50>		
renal cell adenoma	0 (0 %)	0 (0 %)	0 (0 %)	2 (4 %)	↑	
clitoral gland	<50>	<50>	<50>	<50>		
adenoma	2 (4 %)	1 (2 %)	1 (2 %)	3 (6 %)	↑	
uterus	<50>	<50>	<50>	<50>		
endometrial stromal polyp	11 (22 %)	10 (20 %)	12 (24 %)	4 (8 %) *		

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

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

Group Name	Control				80 ppm				400 ppm				2000 ppm			
Number of examined animals	50				50				50				50			
Grade of non-neoplastic lesion	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
nasal cavity	<50>				<50>				<50>				<50>			
eosinophilic change:olfactory epithelium	13	17	4	0	18	15	2	0	14	11	0	0	0	0	0	0
inflammation:foreign body	14	6	0	0	13	10	0	0	13	5	0	0	6	1	0	0
inflammation:respiratory epithelium	0	0	0	0	0	0	0	0	1	2	0	0	16	22	2	0
respiratory metaplasia:gland	30	7	0	0	35	1	0	0	30	0	0	0 *	31	3	0	0
lung	<50>				<50>				<50>				<50>			
uremic pneumonitis	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0
spleen	<50>				<50>				<50>				<50>			
angiectasis	0	0	0	0	0	0	0	0	0	0	0	0	9	7	0	0
deposit of hemosiderin	23	1	0	0	27	2	0	0	32	7	0	0 **	15	28	0	0
engorgement of erythrocyte	0	0	0	0	3	0	0	0	11	0	0	0 **	2	1	0	0
capsule hyperplasia	0	0	0	0	0	0	0	0	0	0	0	0	49	0	0	0
heart	<50>				<50>				<50>				<50>			
myocardial fibrosis	32	4	0	0	34	1	0	0	32	5	0	0	12	35	0	0
artery/aorta	<50>				<50>				<50>				<50>			
mineralization	0	0	0	0	0	0	0	0	1	0	0	0	32	0	0	0
stomach	<50>				<50>				<50>				<50>			
mineralization:glandular stomach	0	0	0	0	0	0	0	0	3	0	0	0	27	0	0	0
liver	<50>				<50>				<50>				<50>			
necrosis:single cell	0	0	0	0	0	0	0	0	0	0	0	0	17	1	0	0
fatty change	0	0	0	0	0	1	0	0	0	0	0	0	16	0	0	0
hydropic change:central	0	0	0	0	0	0	0	0	0	0	0	0	45	3	0	0
acidophilic cell focus	0	2	0	0	1	2	0	0	4	18	2	0 **	3	4	0	0
basophilic cell focus	5	1	0	0	3	4	0	0	1	16	3	0 **	1	0	0	0
spongiosis hepatis	3	1	0	0	7	1	0	0	6	27	2	0 **	0	0	0	0
bile duct hyperplasia	0	49	0	0	0	47	0	0	19	24	0	0 **	0	0	0	0
deposit of brown pigment	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0	0
pancreas	<50>				<50>				<50>				<50>			
atrophy	9	4	2	0	9	6	2	0	9	2	0	0	0	1	0	0

Grade 1: Slight 2 : Moderate 3 : Marked 4 :Severe
< > : Number of animals examined at the site
Significant difference ; * : $p \leqq 0.05$ ** : $p \leqq 0.01$ Test of Chi Square
Statistical analysis is not applied to the data of 2000 ppm group, since they exceeded the MTD.

TABLE 15 INCIDENCES OF SELECTED NON-NEOPLASTIC LESIONS OF MALE RATS IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE (Continued)

Group Name	Control				80 ppm				400 ppm				2000 ppm			
Number of examined animals	50				50				50				50			
Grade of non-neoplastic lesion	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
kidney	<50>				<50>				<50>				<50>			
cyst	1	0	0	0	0	1	0	0	4	4	0	0 *	1	19	0	0
chronic nephropathy	20	20	3	0	6	37	4	1 **	0	6	28	15 **	0	1	0	49
mineralization:cortex	0	0	0	0	0	0	0	0	0	2	0	0	2	42	0	0
urothelial hyperplasia:pelvis	0	0	0	0	1	0	0	0	7	25	0	0 **	5	43	0	0
atypical tubule hyperplasia	0	0	0	0	1	0	0	0	1	0	0	0	2	4	0	0
deposit of brown pigment:proximal tubule	0	0	0	0	0	0	0	0	41	1	0	0 **	0	44	5	0
thyroid	<50>				<50>				<50>				<50>			
C-cell hyperplasia	2	1	0	0	7	5	0	0 *	5	1	0	0	0	0	0	0
parathyroid	<50>				<50>				<50>				<50>			
hyperplasia	0	0	0	0	0	0	0	0	3	0	0	0	27	0	0	0
testis	<50>				<50>				<50>				<50>			
hyperplasia	16	0	0	0	21	0	0	0	27	0	0	0 *	24	0	0	0
prostate	<50>				<50>				<50>				<50>			
hyperplasia	3	4	0	0	5	4	0	0	2	2	0	0	0	0	0	0
eye	<50>				<50>				<50>				<50>			
retinal atrophy	19	16	1	0	14	26	3	0	17	23	2	0	8	8	1	0
keratitis	0	0	0	0	0	0	0	0	1	0	0	0	12	16	5	0

Grade 1: Slight 2 : Moderate 3 : Marked 4 :Severe
< > : Number of animals examined at the site
Significant difference ; * : $p \leqq 0.05$ ** : $p \leqq 0.01$ Test of Chi Square
Statistical analysis is not applied to the data of 2000 ppm group, since they exceeded the MTD.

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

Group Name	Control				80 ppm				400 ppm				2000 ppm			
Number of examined animals	50				50				50				50			
Grade of non-neoplastic lesion	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
nasal cavity	<50>				<50>				<50>				<50>			
eosinophilic change:olfactory epithelium	1	31	18	0	3	25	21	0	6	33	10	0	6	0	0	0 **
eosinophilic change:respiratory epithelium	25	0	0	0	32	0	0	0	27	0	0	0	3	0	0	0 **
spleen	<50>				<50>				<50>				<50>			
angiectasis	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
deposit of hemosiderin	24	9	0	0	27	14	0	0	23	23	0	0 **	33	12	0	0 *
increased extramedullary hematopoiesis	10	5	0	0	6	3	0	0	14	2	0	0	22	6	0	0 *
engorgement of erythrocyte	0	0	0	0	1	0	0	0	5	0	0	0	26	1	0	0 **
capsule hyperplasia	0	0	0	0	0	0	0	0	0	0	0	0	46	0	0	0 **
liver	<50>				<50>				<50>				<50>			
necrosis:single cell	0	0	0	0	0	0	0	0	1	0	0	0	5	1	0	0 *
hydropic change:central	0	0	0	0	0	0	0	0	0	0	0	0	38	3	0	0 **
granulation	12	5	4	0	16	4	2	0	7	3	0	0	4	1	0	0 **
clear cell focus	1	1	0	0	1	0	0	0	2	0	0	0	1	7	3	0 *
acidophilic cell focus	0	0	0	0	0	0	0	0	4	4	0	0 *	1	29	6	0 **
basophilic cell focus	24	5	0	0	22	0	0	0 *	9	0	0	0 **	5	0	0	0 **
bile duct hyperplasia	18	9	0	0	19	8	0	0	36	4	0	0 **	1	0	0	0 **
deposit of brown pigment	0	0	0	0	0	0	0	0	0	0	0	0	43	1	0	0 **
kidney	<50>				<50>				<50>				<50>			
chronic nephropathy	15	5	0	0	22	11	0	0 *	27	16	2	0 **	2	14	31	2 **
urothelial hyperplasia:pelvis	0	0	0	0	0	0	0	0	0	0	0	0	3	4	0	0 *
atypical tubule hyperplasia	0	0	0	0	0	0	0	0	0	0	0	0	4	1	0	0
deposit of brown pigment:proximal tubule	0	0	0	0	0	0	0	0	48	0	0	0 **	3	46	0	0 **
thyroid	<50>				<50>				<50>				<50>			
C-cell hyperplasia	6	2	0	0	4	1	0	0	7	1	0	0	1	0	0	0 *
adrenal gland	<50>				<50>				<50>				<50>			
focal fatty change:cortex	6	0	0	0	3	1	0	0	6	0	0	0	0	0	0	0 *

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

TABLE 17 HISTORICAL CONTROL DATA OF SELECTED NEOPLASTIC LESIONS IN JAPAN BIOASSAY RESEARCH CENTER : F344/DuCrlCrlj MALE RATS

Organs Tumors	No. of animals examined	No. of animals bearing tumor	Incidence (%)	Min. - Max. (%)
Liver	1949			
Hepatocellular adenoma		34	1.7	0 - 8
Hepatocellular carcinoma		6	0.3	0 - 2
Kidney	1949			
Renal cell adenoma		2	0.1	0 - 2
Renal cell carcinoma		4	0.2	0 - 4
Testis	1948			
Interstitial cell tumor		1659	85.2	56 - 98

39 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, 0396, 0399, 0401, 0407, 0417, 0421, 0437, 0448

TABLE 18 HISTORICAL CONTROL DATA OF SELECTED NEOPLASTIC LESIONS IN JAPAN BIOASSAY RESEARCH CENTER : F344/DuCrlCrlj FEMALE RATS

Organs Tumors	No. of animals examined	No. of animals bearing tumor	Incidence (%)	Min. - Max. (%)
Liver	1797			
Hepatocellular adenoma		21	1.2	0 - 6
Hepatocellular carcinoma		2	0.1	0 - 2
Kidney	1797			
Renal cell adenoma		2	0.1	0 - 2
Clitoral gland	1797			
Adenoma		51	2.8	0 - 8

36 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, 0401, 0417, 0421, 0437, 0448

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

		Male				Female			
Group name		Control	80 ppm	400 ppm	2000 ppm	Control	80 ppm	400 ppm	2000 ppm
Number of dead or moribund animals		10	10	11	50	9	8	5	11
No microscopical confirmation		0	1	1	0	1	0	0	0
Chronic nephropathy		0	0	3	47	0	0	0	0
Arteritis		0	0	0	1	0	0	0	0
Tumor death ：	leukemia	6	1	1	0	2	1	1	1
	subcutis	1	0	0	0	1	0	1	0
	bone marrow	0	1	0	0	0	0	0	0
	thymus	0	0	1	0	0	0	0	0
	oral cavity	0	0	0	0	0	0	0	1
	tongue	0	0	1	0	0	0	0	0
	pancreas	0	0	1	0	0	0	0	0
	kidney	1	0	0	0	0	1	0	0
	urinary bladder	0	0	0	0	0	0	0	1
	pituitary gland	2	3	3	0	2	2	1	0
	adrenal gland	0	0	0	0	0	0	0	1
	ovary	—	—	—	—	0	0	0	1
	uterus	—	—	—	—	3	2	2	2
	mammary gland	0	0	0	0	0	2	0	0
	preputial gland	0	3	0	0	—	—	—	—
	clitoral gland	—	—	—	—	0	0	0	2
	brain	0	0	0	0	0	0	0	1
	peripheral nerves	0	1	0	0	0	0	0	0
	Zymbal gland	0	0	0	0	0	0	0	1
	bone	0	0	0	1	0	0	0	0
	peritoneum	0	0	0	1	0	0	0	0

FIGURES

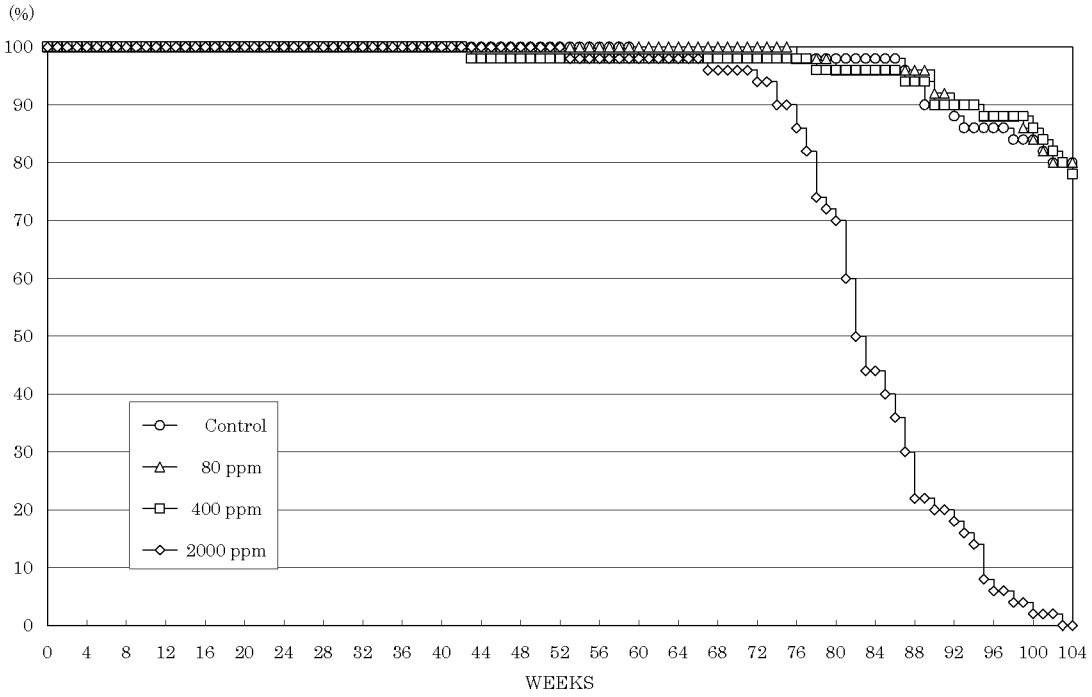


FIGURE 1 SURVIVAL ANIMAL RATE OF MALE RATS IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

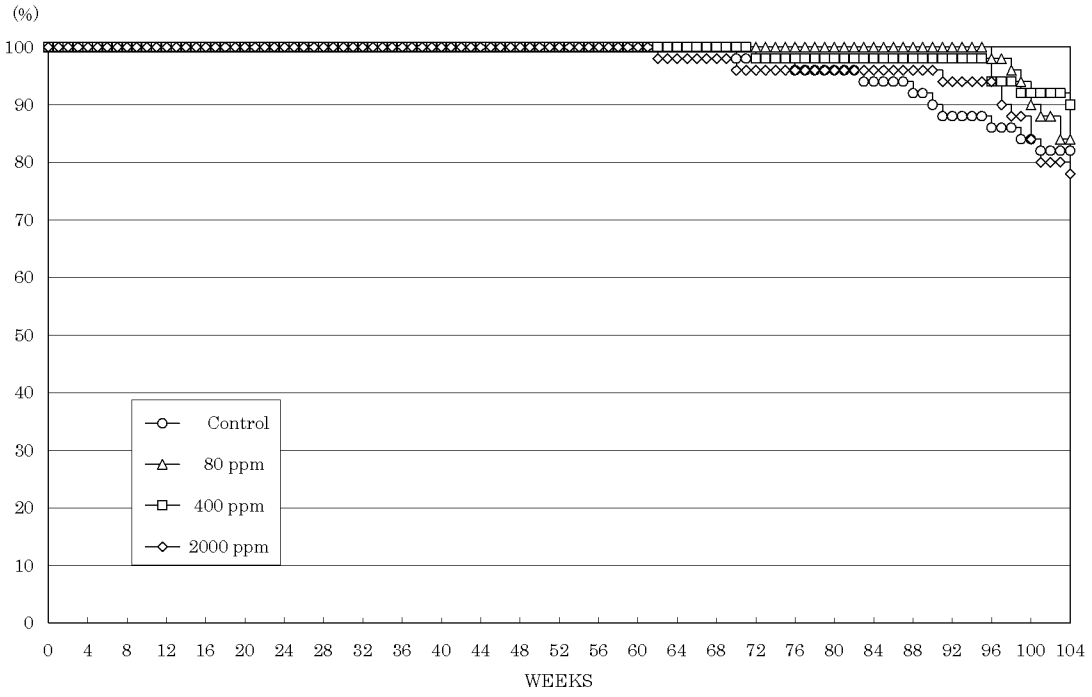


FIGURE 2 SURVIVAL ANIMAL RATE OF FEMALE RATS THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

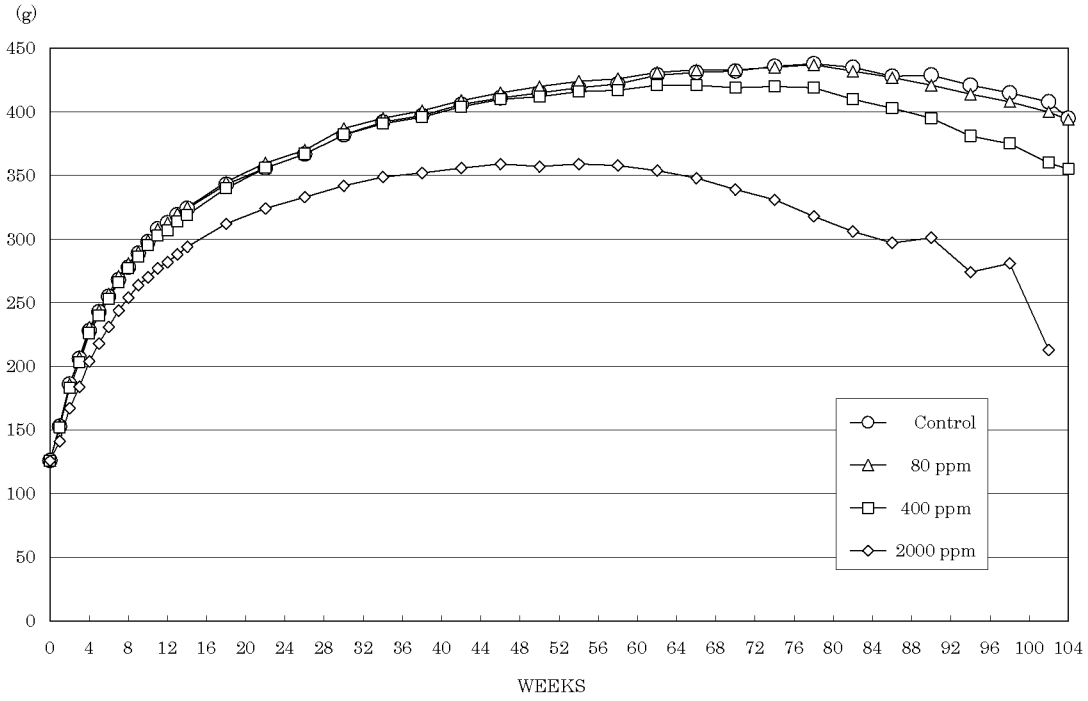


FIGURE 3 BODY WEIGHT CHANGES OF MALE RATS IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

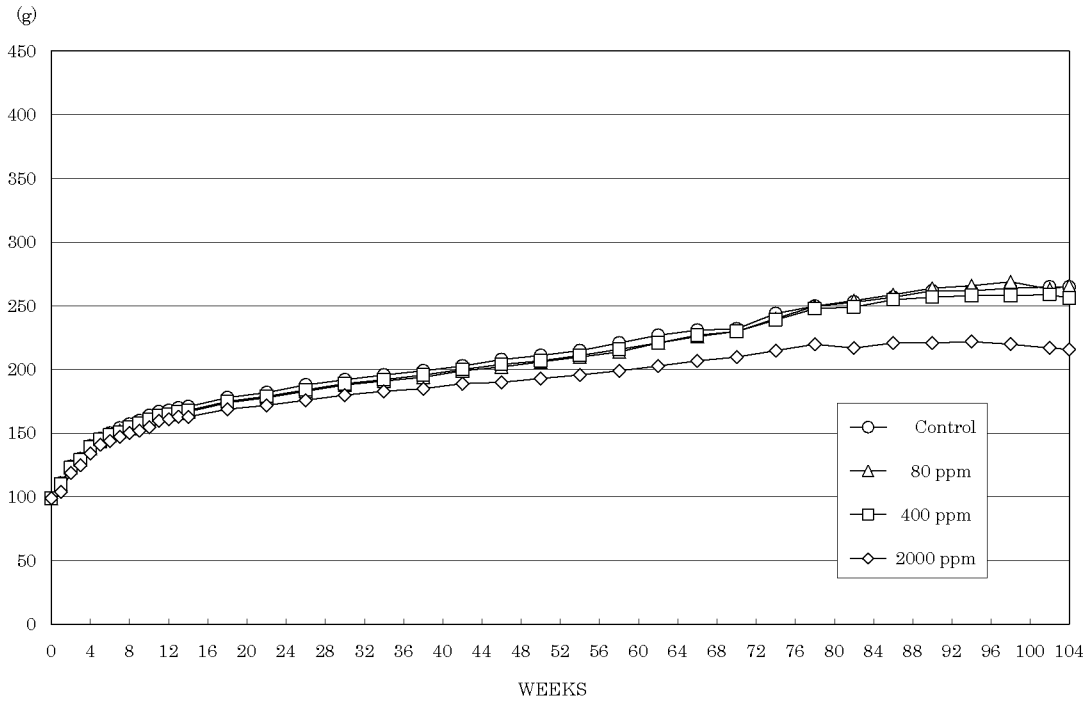


FIGURE 4 BODY WEIGHT CHANGES OF FEMALE RATS IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

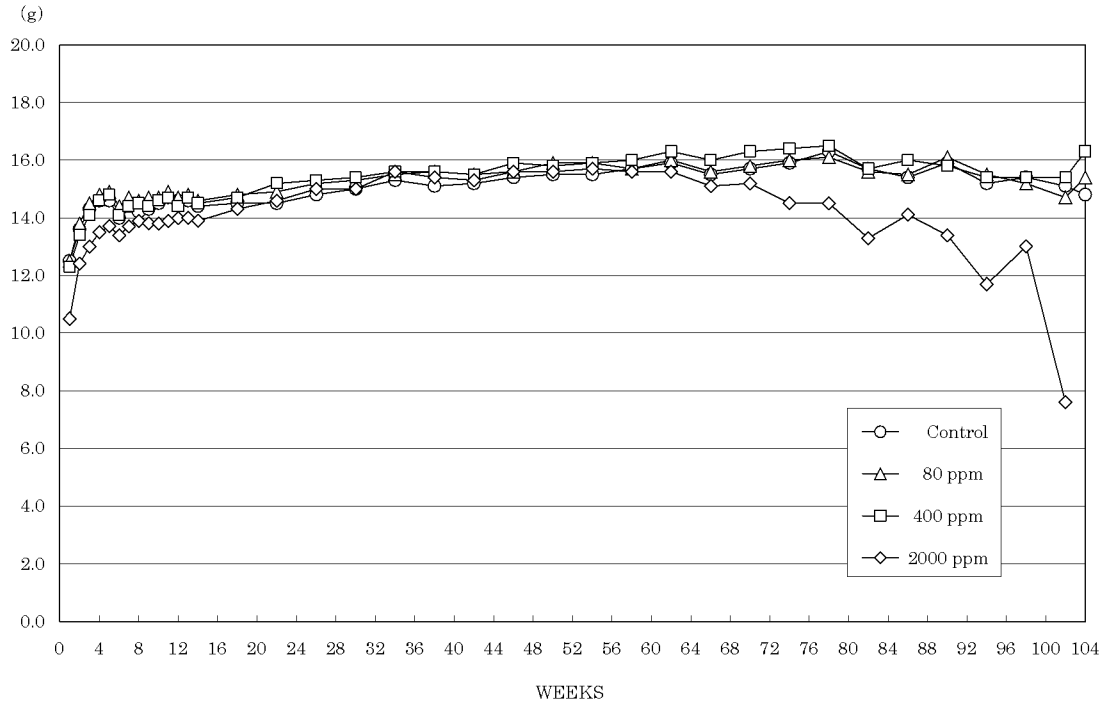


FIGURE 5 FOOD CONSUMPTION CHANGES OF MALE RATS IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE

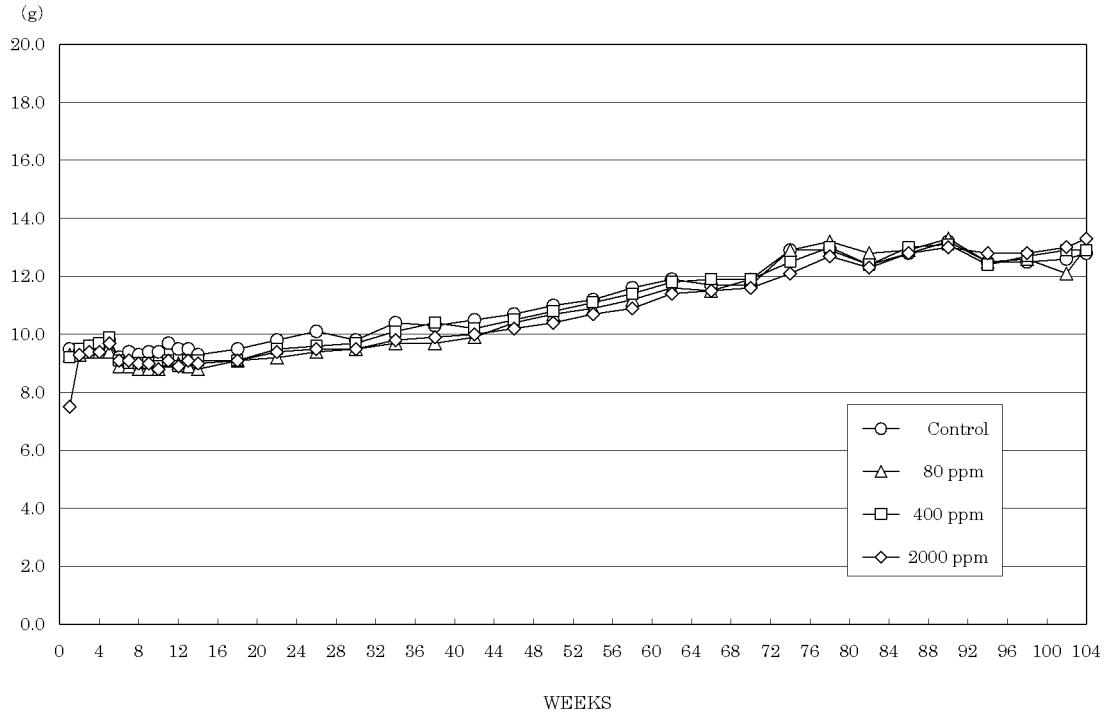
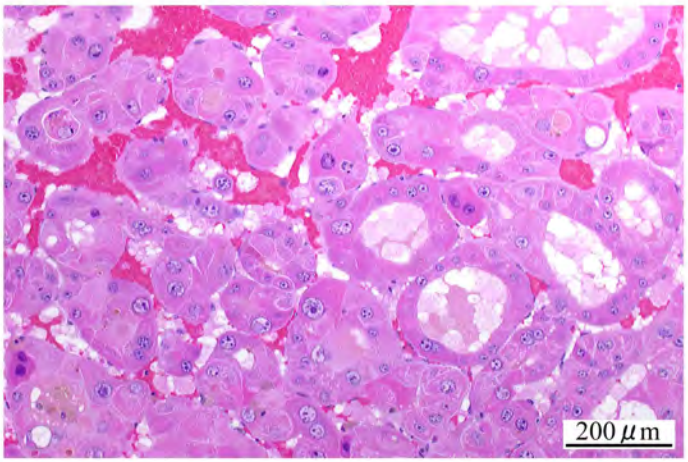
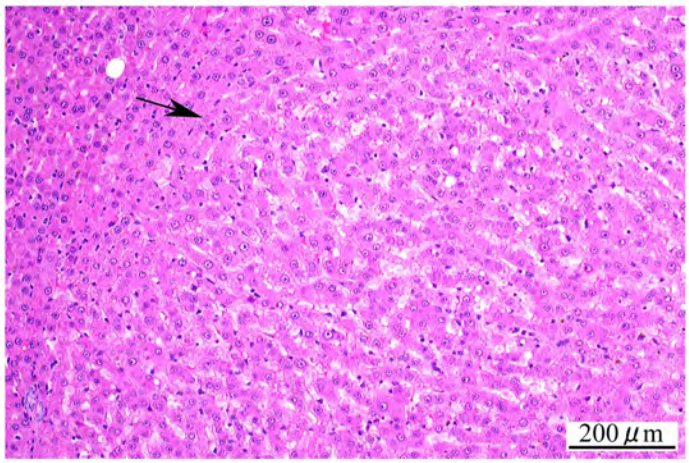


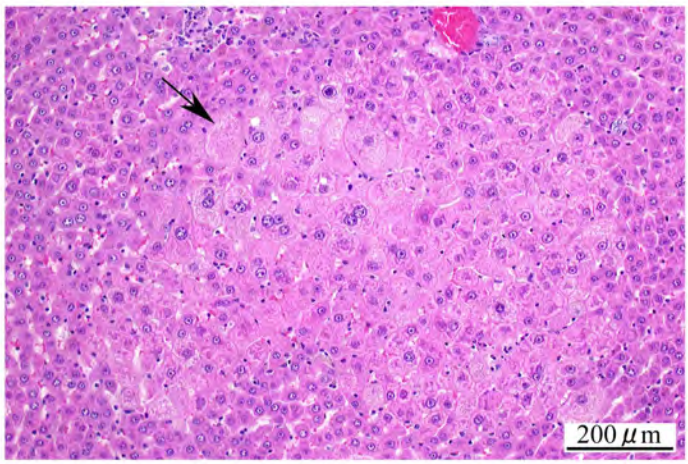
FIGURE 6 FOOD CONSUMPTION CHANGES OF FEMALE RATS IN THE 2-YEAR FEED STUDY OF 1-CHLORO-2-NITROBENZENE



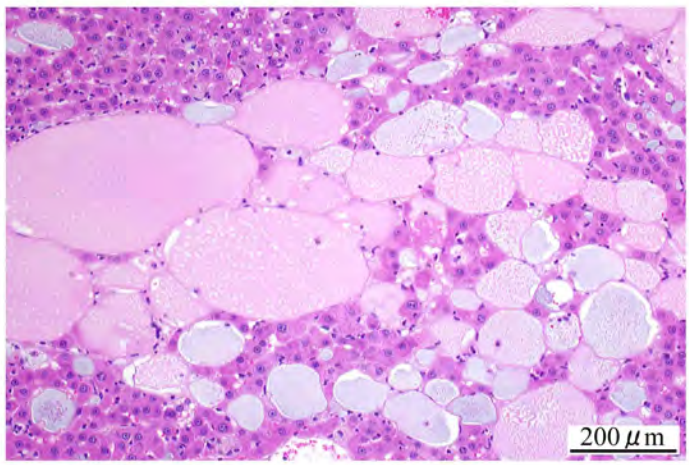
Photograph 1
Liver: Hepatocellular carcinoma
Rat, Female, 2000 ppm, Animal No. 0461-2345 (H&E)



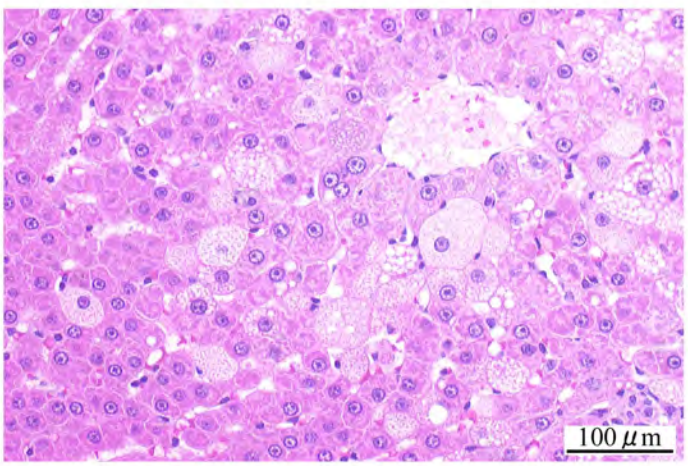
Photograph 2
Liver: Hepatocellular adenoma (Arrow)
Rat, Male, 400 ppm, Animal No. 0461-1216 (H&E)



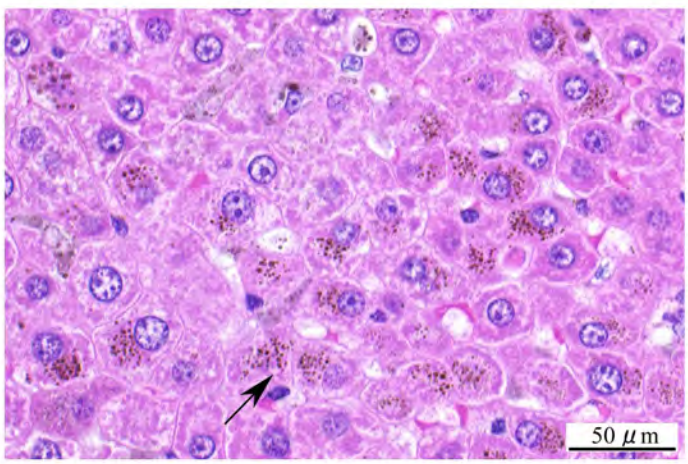
Photograph 3
Liver: Acidophilic cell focus (Arrow)
Rat, Female, 2000 ppm, Animal No. 0461-2311 (H&E)



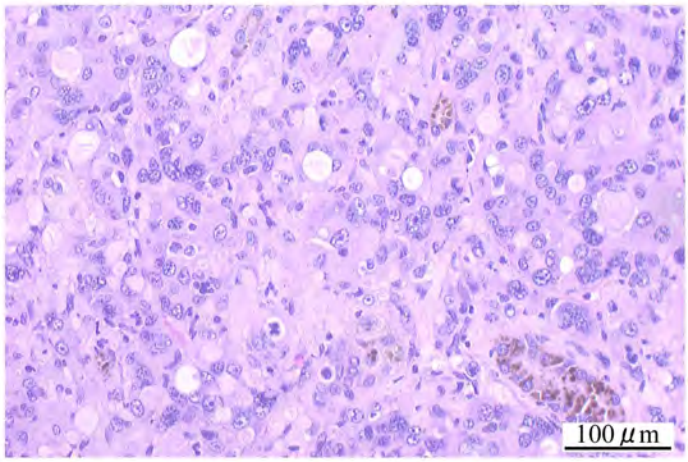
Photograph 4
Liver: Spongiosis hepatis
Rat, Male, 400 ppm, Animal No. 0461-1231 (H&E)



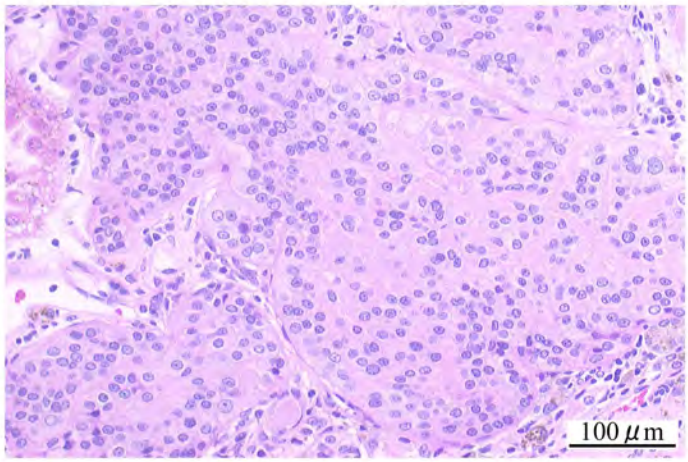
Photograph 5
Liver: Hydropic change: central
Rat, Female, 2000 ppm, Animal No. 0461-2304 (H&E)



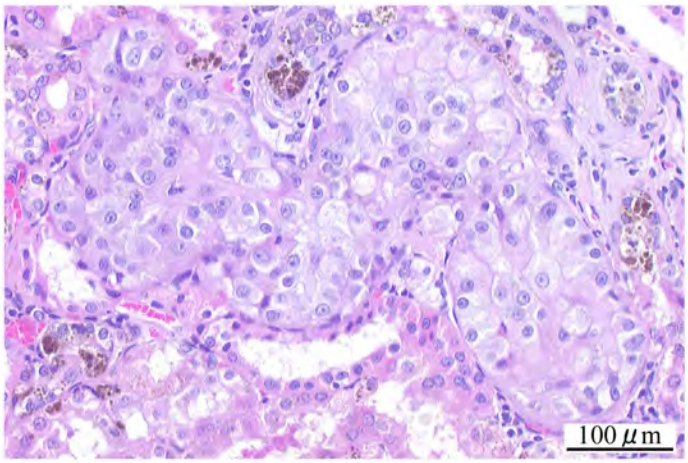
Photograph 6
Liver: Deposit of brown pigment (Arrow)
Rat, Female, 2000 ppm, Animal No. 0461-2323 (H&E)



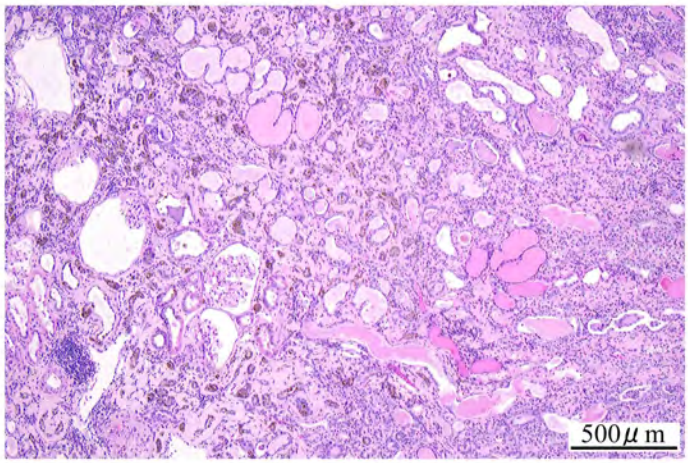
Photograph 7
Kidney: Renal cell carcinoma
Rat, Male, 2000 ppm, Animal No. 0461-1319 (H&E)



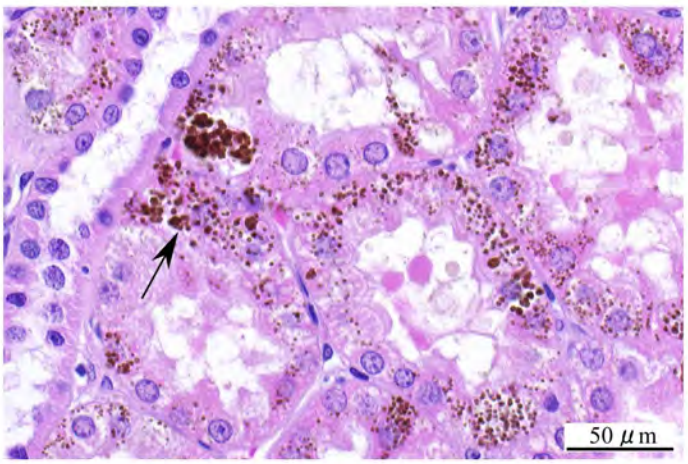
Photograph 8
Kidney: Renal cell adenoma
Rat, Female, 2000 ppm, Animal No. 0461-2341 (H&E)



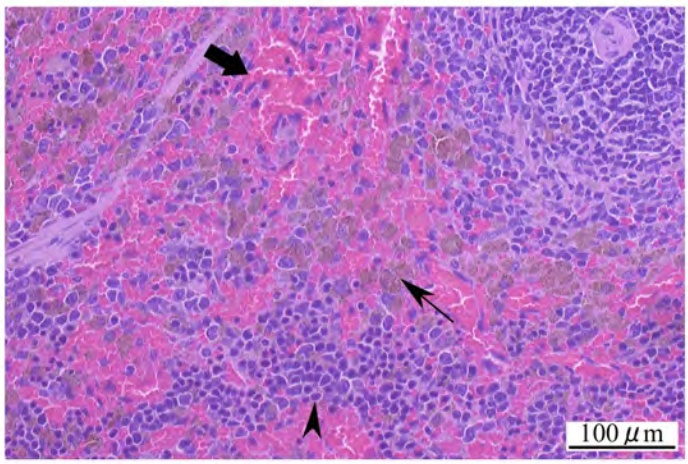
Photograph 9
Kidney: Atypical tubule hyperplasia
Rat, Female, 2000 ppm, Animal No. 0461-2336 (H&E)



Photograph 10
Kidney: Chronic nephropathy
Rat, Male, 2000 ppm, Animal No. 0461-1312 (H&E)



Photograph 11
Kidney: Deposit of brown pigment: proximal tubule (Arrow)
Rat, Female, 2000 ppm, Animal No. 0461-2336 (H&E)



Photograph 12
Spleen: Deposit of hemosiderin (Arrow), increased extramedullary hematopoiesis (Arrowhead) and engorgement of erythrocyte (Widearrow)
Rat, Female, 2000 ppm, Animal No. 0461-2345 (H&E)