Summary of Inhalation Carcinogenicity Study of Cyclohexene 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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Purpose, materials and methods

Cyclohexene (CAS No. 110-83-8) is a colorless liquid with a boiling point of 83.3°C and a vapor pressure of 89 mm Hg at 25°C, and is insoluble in water.

The carcinogenicity and chronic toxicity of cyclohexene were examined by inhalation exposure of groups of 50 F344/DuCrj (Fischer) rats of both sexes to cyclohexene vapor at a target concentration of 0 (clean air), 600, 1200 or 2400 ppm (v/v) for 6 hours/day, 5 days/week for 2 years (104 weeks). 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. Cyclohexene was analyzed for purity and stability by both infrared spectrometry and gas chromatography before and after its use. Stainless-steel inhalation exposure chambers (volume: 7600 L) were used throughout the 2-year exposure period. Cyclohexene vapor-air mixture was generated by bubbling clean air through the cyclohexene liquid, and supplied to the inhalation exposure chambers. Air concentrations of cyclohexene vapor in the inhalation exposure chambers were monitored at 15 min intervals by gas chromatography. 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 exposure period underwent complete necropsy. Urinalysis was performed near the end of the exposure 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

No significant difference in survival rate, clinical sign or food consumption was found between any cyclohexene-exposed group of either sex and the respective control. Body weights of the 2400 ppm-exposed males and females were significantly decreased throughout the 2-year exposure period as compared with the respective controls.

The combined incidences of hepatocellular adenomas and carcinomas in the males were slightly increased in a dose-related manner. As pre-neoplastic lesions, the incidences of acidophilic cell foci and spongiosis hepatis in the liver were increased in the cyclohexene-exposed males. No significant difference in the incidence of neoplastic lesions was found between any cyclohexene-exposed female group and the female control. As non-neoplastic lesions, chronic progressive nephropathy (chronic nephropathy) in the male kidneys, focal follicular cell hyperplasia in the male thyroid, and degeneration of granular cells in the cerebellum of males and females were observed. A no-observed-adverse-effect-level (NOAEL) for cyclohexene is thought to be 600 ppm for the hepatic endpoint of spongiosis hepatis and increased liver weight in the rat.

Conclusions

In rats, there was no evidence of carcinogenic activity of cyclohexene in males, based on no increase in the incidence of hepatocellular adenomas or carcinomas in males, although the combined incidences of these two hepatocellular tumors were increased slightly but dose-dependently. No increase in the incidence of neoplastic lesions in other organs was noted in the cyclohexene-exposed males. There was no evidence of carcinogenic activity of cyclohexene in females, based on no increase in the incidence of neoplastic lesions.

TABLES

TABLES (CONTINUED)

TABLE 1 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Weeks on Study	Control Av.Wt.	Control No.of Surviv. <50>	600ppm Av.Wt.	600ppm % of cont.	600ppm No.of Surviv. <50>	1200ppm Av.Wt.	1200ppm % of cont.	1200ppm No.of Surviv. <50>	2400ppm Av.Wt.	2400ppm % of cont.	2400ppm 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	159 (50)	50/50	156 (50)	98	50/50	156 (50)	98	50/50	153 (50)	96	50/50
2	192 (50)	50/50	186 (50)	97	50/50	185 (50)	96	50/50	177 (50)	92	50/50
3	217 (50)	50/50	211 (50)	97	50/50	209 (50)	96	50/50	199 (50)	92	50/50
4	237 (50)	50/50	230 (50)	97	50/50	228 (50)	96	50/50	218 (50)	92	50/50
5	250 (50)	50/50	244 (50)	98	50/50	242 (50)	97	50/50	231 (50)	92	50/50
6	260 (50)	50/50	254 (50)	98	50/50	254 (50)	98	50/50	241 (50)	93	50/50
7	273 (50)	50/50	268 (50)	98	50/50	267 (50)	98	50/50	255 (50)	93	50/50
8	285 (50)	50/50	281 (50)	99	50/50	280 (50)	98	50/50	266 (50)	93	50/50
9	297 (50)	50/50	292 (50)	98	50/50	291 (50)	98	50/50	274 (50)	92	50/50
10	302 (50)	50/50	299 (50)	99	50/50	299 (50)	99	50/50	283 (50)	94	50/50
11	310 (50)	50/50	308 (50)	99	50/50	306 (50)	99	50/50	290 (50)	94	50/50
12	315 (50)	50/50	314 (50)	100	50/50	314 (50)	100	50/50	296 (50)	94	50/50
13	322 (50)	50/50	320 (50)	99	50/50	321 (50)	100	50/50	303 (50)	94	50/50
14	327 (50)	50/50	326 (50)	100	50/50	326 (50)	100	50/50	310 (50)	95	50/50
18	345 (50)	50/50	344 (50)	100	50/50	342 (50)	99	50/50	323 (50)	94	50/50
22	357 (50)	50/50	357 (50)	100	50/50	358 (50)	100	50/50	336 (50)	94	50/50
26	370 (50)	50/50	371 (50)	100	50/50	371 (50)	100	50/50	345 (50)	93	50/50
30	378 (50)	50/50	380 (50)	101	50/50	380 (50)	101	50/50	353 (50)	93	50/50
34	388 (50)	50/50	392 (50)	101	50/50	391 (50)	101	50/50	363 (49)	94	49/50
38	396 (50)	50/50	399 (50)	101	50/50	399 (50)	101	50/50	371 (49)	94	49/50
42	404 (50)	50/50	408 (50)	101	50/50	410 (50)	101	50/50	383 (49)	95	49/50
46	411 (50)	50/50	413 (50)	100	50/50	414 (50)	101	50/50	389 (49)	95	49/50
50	415 (50)	50/50	416 (50)	100	50/50	417 (49)	100	49/50	390 (49)	94	49/50
54	419 (50)	50/50	419 (50)	100	50/50	420 (49)	100	49/50	392 (49)	94	49/50
58	422 (50)	50/50	423 (50)	100	50/50	424 (49)	100	49/50	395 (49)	94	49/50
62	428 (49)	49/50	427 (50)	100	50/50	431 (49)	101	49/50	401 (49)	94	49/50
66	433 (49)	49/50	430 (50)	99	50/50	434 (49)	100	49/50	400 (49)	92	49/50
70	433 (49)	49/50	431 (50)	100	50/50	433 (49)	100	49/50	401 (48)	93	48/50
74	432 (48)	48/50	430 (50)	100	50/50	432 (48)	100	48/50	401 (48)	93	48/50
78	432 (48)	48/50	426 (50)	99	50/50	429 (48)	99	48/50	399 (48)	92	48/50
82	432 (47)	47/50	429 (49)	99	49/50	428 (48)	99	48/50	399 (47)	92	47/50
86	427 (47)	47/50	425 (49)	100	49/50	425 (48)	100	48/50	395 (45)	93	45/50
90	426 (44)	44/50	423 (49)	99	49/50	422 (48)	99	48/50	389 (43)	91	43/50
94	426 (43)	43/50	422 (46)	99	46/50	424 (47)	100	47/50	389 (42)	91	42/50
98	420 (43)	43/50	414 (45)	99	45/50	419 (45)	100	45/50	385 (41)	92	41/50
102	413 (40)	40/50	410 (42)	99	42/50	409 (44)	99	44/50	379 (40)	92	40/50
104	402 (40)	40/50	407 (40)	101	40/50	398 (44)	99	44/50	372 (38)	93	38/50

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

TABLE 2 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Weeks on Study	Control Av.Wt.	Control No.of Surviv. <50>	600ppm Av.Wt.	600ppm % of cont.	600ppm No.of Surviv. <50>	1200ppm Av.Wt.	1200ppm % of cont.	1200ppm No.of Surviv. <50>	2400ppm Av.Wt.	2400ppm % of cont.	2400ppm No.of Surviv. <50>
0	98 (50)	50/50	98 (50)	100	50/50	98 (50)	100	50/50	98 (50)	100	50/50
1	114 (50)	50/50	112 (50)	98	50/50	113 (50)	99	50/50	111 (50)	97	50/50
2	128 (50)	50/50	125 (50)	98	50/50	126 (50)	98	50/50	123 (50)	96	50/50
3	138 (50)	50/50	136 (50)	99	50/50	136 (50)	99	50/50	133 (50)	96	50/50
4	148 (50)	50/50	144 (50)	97	50/50	144 (50)	97	50/50	141 (50)	95	50/50
5	155 (50)	50/50	151 (50)	97	50/50	152 (50)	98	50/50	147 (50)	95	50/50
6	159 (50)	50/50	156 (50)	98	50/50	156 (50)	98	50/50	151 (50)	95	50/50
7	165 (50)	50/50	161 (50)	98	50/50	162 (50)	98	50/50	157 (50)	95	50/50
8	170 (50)	50/50	167 (50)	98	50/50	167 (50)	98	50/50	162 (50)	95	50/50
9	175 (50)	50/50	172 (50)	98	50/50	171 (50)	98	50/50	166 (50)	95	50/50
10	178 (50)	50/50	175 (50)	98	50/50	174 (50)	98	50/50	169 (50)	95	50/50
11	183 (50)	50/50	179 (50)	98	50/50	178 (50)	97	50/50	172 (50)	94	50/50
12	185 (50)	50/50	182 (50)	98	50/50	181 (50)	98	50/50	175 (50)	95	50/50
13	187 (50)	50/50	183 (50)	98	50/50	184 (50)	98	50/50	176 (50)	94	50/50
14	189 (50)	50/50	186 (50)	98	50/50	186 (50)	98	50/50	180 (50)	95	50/50
18	197 (50)	50/50	190 (50)	96	50/50	192 (50)	97	50/50	184 (50)	93	50/50
22	201 (50)	50/50	197 (50)	98	50/50	199 (50)	99	50/50	190 (50)	95	50/50
26	209 (50)	50/50	204 (50)	98	50/50	205 (50)	98	50/50	195 (50)	93	50/50
30	211 (50)	50/50	208 (50)	99	50/50	211 (50)	100	50/50	199 (50)	94	50/50
34	218 (50)	50/50	216 (50)	99	50/50	219 (50)	100	50/50	206 (50)	94	50/50
38	223 (50)	50/50	222 (50)	100	50/50	224 (50)	100	50/50	210 (50)	94	50/50
42	227 (50)	50/50	228 (50)	100	50/50	230 (50)	101	50/50	218 (50)	96	50/50
46	231 (50)	50/50	232 (50)	100	50/50	233 (50)	101	50/50	222 (50)	96	50/50
50	234 (50)	50/50	235 (50)	100	50/50	236 (50)	101	50/50	222 (50)	95	50/50
54	240 (50)	50/50	240 (50)	100	50/50	240 (50)	100	50/50	226 (50)	94	50/50
58	246 (50)	50/50	246 (50)	100	50/50	246 (50)	100	50/50	229 (50)	93	50/50
62	252 (50)	50/50	256 (49)	102	49/50	256 (50)	102	50/50	237 (50)	94	50/50
66	259 (50)	50/50	260 (49)	100	49/50	261 (50)	101	50/50	238 (49)	92	49/50
70	260 (50)	50/50	264 (49)	102	49/50	263 (50)	101	50/50	240 (48)	92	48/50
74	264 (49)	49/50	268 (49)	102	49/50	268 (50)	102	50/50	245 (48)	93	48/50
78	268 (49)	49/50	270 (49)	101	49/50	272 (50)	101	50/50	247 (48)	92	48/50
82	275 (49)	49/50	276 (49)	100	49/50	276 (50)	100	50/50	254 (47)	92	47/50
86	278 (49)	49/50	280 (48)	101	48/50	280 (50)	101	50/50	257 (46)	92	46/50
90	283 (49)	49/50	282 (48)	100	48/50	282 (48)	100	48/50	257 (46)	91	46/50
94	283 (45)	45/50	284 (47)	100	47/50	286 (48)	101	48/50	259 (43)	92	43/50
98	282 (45)	45/50	285 (47)	101	47/50	288 (48)	102	48/50	263 (41)	93	41/50
102	284 (43)	43/50	285 (44)	100	44/50	292 (47)	103	47/50	264 (38)	93	38/50
104	280 (42)	42/50	282 (44)	101	44/50	291 (47)	104	47/50	262 (36)	94	36/50

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

TABLE 3 INCIDENCES OF EXTERNAL AND INTERNAL MASSES IN CLINICAL OBSERVATION OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

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	2/49	4/48	13/44	14/50 (2/10)
600ppm	0/50	0/50	0/50	0/50	1/50	4/50	7/49	14/49	14/50 (2/10)
1200ppm	0/50	0/50	0/50	0/50	0/49	0/49	2/48	12/47	12/50 (1/6)
2400ppm	0/50	1/50	1/50	0/49	0/49	3/49	6/48	9/43	11/50 (2/12)
Internal mass									
Control	0/50	0/50	0/50	0/50	0/50	0/49	0/48	1/44	1/50 (0/10)
600ppm	0/50	0/50	0/50	0/50	0/50	0/50	0/49	1/49	1/50 (1/10)
1200ppm	0/50	0/50	0/50	0/50	0/49	0/49	0/48	1/47	1/50 (0/6)
2400ppm	0/50	0/50	0/50	0/49	0/49	0/49	1/48	1/43	2/50 (1/12)

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

TABLE 4 INCIDENCES OF EXTERNAL AND INTERNAL MASSES IN CLINICAL OBSERVATION OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

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	5/50	8/49	11/48	12/50 (4/8)
600ppm	0/50	0/50	0/50	0/50	0/50	1/49	6/49	12/47	13/50 (2/6)
1200ppm	0/50	0/50	0/50	0/50	2/50	2/50	7/50	14/48	15/50 (0/3)
2400ppm	0/50	0/50	0/50	0/50	1/50	2/49	4/48	7/46	7/50 (2/14)
Internal mass									
Control	0/50	0/50	0/50	0/50	0/50	1/50	1/49	1/48	2/50 (2/8)
600ppm	0/50	0/50	0/50	0/50	0/50	1/49	1/49	2/47	3/50 (1/6)
1200ppm	0/50	0/50	0/50	0/50	0/50	0/50	0/50	1/48	1/50 (0/3)
2400ppm	0/50	0/50	0/50	0/50	0/50	0/49	0/48	3/46	3/50 (3/14)

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

TABLE 5 FOOD CONSUMPTION CHANGES OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Weeks on Study	Control Av.Fc. <50>	600ppm Av.Fc. <50>	600ppm % of cont.	1200ppm Av.Fc. <50>	1200ppm % of cont.	2400ppm Av.Fc. <50>	2400ppm % of cont.
1	16.0 (50)	15.7 (50)	98	15.3 (50)	96	15.0 (50)	94
2	17.5 (50)	17.5 (50)	100	17.0 (50)	97	16.7 (50)	95
3	18.3 (50)	18.1 (50)	99	17.8 (50)	97	17.5 (50)	96
4	18.6 (50)	18.4 (50)	99	18.2 (50)	98	17.9 (50)	96
5	18.1 (50)	18.1 (50)	100	18.2 (50)	101	18.2 (50)	101
6	18.0 (50)	17.9 (50)	99	18.1 (50)	101	17.9 (50)	99
7	17.5 (50)	17.6 (50)	101	17.6 (50)	101	17.8 (50)	102
8	17.5 (50)	17.7 (50)	101	17.5 (50)	100	17.7 (50)	101
9	17.6 (50)	17.9 (50)	102	17.8 (50)	101	18.0 (50)	102
10	17.5 (50)	17.8 (50)	102	17.8 (50)	102	17.9 (50)	102
11	17.4 (50)	17.8 (50)	102	17.7 (50)	102	18.0 (50)	103
12	17.5 (50)	17.7 (50)	101	17.6 (50)	101	17.6 (50)	101
13	17.1 (50)	17.5 (50)	102	17.4 (50)	102	17.6 (50)	103
14	17.1 (50)	17.3 (50)	101	17.4 (50)	102	17.4 (50)	102
18	16.9 (50)	17.2 (50)	102	17.0 (50)	101	17.0 (50)	101
22	17.2 (50)	17.5 (50)	102	17.6 (50)	102	17.3 (50)	101
26	17.2 (50)	17.5 (50)	102	17.5 (50)	102	17.3 (50)	101
30	17.0 (50)	17.4 (50)	102	17.5 (50)	103	17.2 (50)	101
34	17.0 (50)	17.6 (50)	104	17.8 (50)	105	17.6 (49)	104
38	16.4 (50)	16.7 (50)	102	16.9 (50)	103	16.9 (49)	103
42	16.6 (50)	17.2 (50)	104	17.4 (50)	105	17.9 (49)	108
46	16.7 (50)	17.2 (50)	103	17.3 (50)	104	17.5 (49)	105
50	16.6 (50)	16.9 (50)	102	16.9 (49)	102	17.1 (49)	103
54	17.0 (50)	17.3 (50)	102	17.4 (49)	102	17.2 (49)	101
58	16.6 (50)	17.3 (50)	104	17.4 (49)	105	17.3 (49)	104
62	17.0 (49)	17.5 (50)	103	17.7 (49)	104	17.6 (49)	104
66	17.1 (49)	17.2 (50)	101	17.3 (49)	101	17.3 (49)	101
70	17.1 (49)	17.4 (50)	102	17.3 (49)	101	17.3 (48)	101
74	17.3 (48)	17.5 (50)	101	17.8 (48)	103	18.0 (48)	104
78	16.9 (48)	17.1 (50)	101	17.2 (48)	102	17.4 (48)	103
82	16.9 (47)	17.1 (49)	101	17.2 (48)	102	17.2 (47)	102
86	16.4 (47)	16.6 (49)	101	17.3 (48)	105	17.0 (45)	104
90	16.8 (44)	17.2 (49)	102	17.4 (48)	104	17.1 (43)	102
94	16.8 (43)	17.3 (46)	103	17.6 (47)	105	17.4 (42)	104
98	16.5 (43)	16.5 (45)	100	17.6 (45)	107	17.0 (41)	103
102	16.1 (40)	16.7 (42)	104	17.3 (44)	107	16.8 (40)	104
104	14.9 (40)	16.6 (40)	111	16.5 (44)	111	16.8 (38)	113

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

TABLE 6 FOOD CONSUMPTION CHANGES OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Weeks on Study	Control Av.Fc. <50>	600ppm Av.Fc. <50>	600ppm % of cont.	1200ppm Av.Fc. <50>	1200ppm % of cont.	2400ppm Av.Fc. <50>	2400ppm % of cont.
1	11.9 (50)	11.5 (50)	97	11.4 (50)	96	11.1 (50)	93
2	12.3 (50)	12.4 (50)	101	12.1 (50)	98	11.8 (50)	96
3	12.6 (50)	12.6 (50)	100	12.7 (50)	101	12.3 (50)	98
4	12.8 (50)	12.6 (50)	98	12.7 (50)	99	12.7 (49)	99
5	12.9 (50)	12.8 (50)	99	12.8 (50)	99	12.5 (50)	97
6	11.9 (50)	12.1 (50)	102	12.2 (50)	103	12.2 (50)	103
7	12.1 (50)	12.1 (50)	100	12.6 (50)	104	12.5 (50)	103
8	12.1 (50)	12.1 (50)	100	12.0 (50)	99	12.0 (50)	99
9	12.4 (50)	12.1 (50)	98	12.2 (50)	98	12.1 (50)	98
10	12.3 (50)	12.4 (50)	101	11.9 (50)	97	12.1 (50)	98
11	12.5 (50)	12.1 (50)	97	12.2 (50)	98	12.1 (50)	97
12	12.2 (50)	12.6 (50)	103	12.1 (50)	99	12.2 (50)	100
13	12.1 (50)	12.6 (50)	104	12.2 (50)	101	12.0 (50)	99
14	12.1 (50)	12.1 (50)	100	12.1 (50)	100	12.1 (50)	100
18	12.0 (50)	11.7 (50)	98	11.7 (50)	98	11.7 (50)	98
22	12.0 (50)	12.4 (50)	103	12.4 (50)	103	12.2 (50)	102
26	11.8 (50)	12.0 (50)	102	11.9 (50)	101	11.6 (50)	98
30	11.9 (50)	11.9 (50)	100	12.4 (50)	104	12.0 (50)	101
34	11.9 (50)	12.2 (50)	103	12.2 (50)	103	12.6 (50)	106
38	11.6 (50)	12.0 (50)	103	11.8 (50)	102	11.7 (50)	101
42	11.6 (50)	12.2 (50)	105	12.1 (50)	104	12.4 (50)	107
46	11.9 (50)	12.2 (50)	103	12.3 (50)	103	12.4 (50)	104
50	11.8 (50)	11.9 (50)	101	12.1 (50)	103	12.1 (50)	103
54	12.1 (50)	12.7 (50)	105	12.6 (50)	104	12.1 (50)	100
58	12.0 (50)	12.6 (50)	105	12.4 (50)	103	12.1 (50)	101
62	12.2 (50)	12.9 (49)	106	12.7 (50)	104	12.4 (50)	102
66	12.3 (50)	12.6 (49)	102	12.6 (50)	102	12.5 (49)	102
70	12.3 (50)	12.9 (49)	105	12.6 (50)	102	12.2 (48)	99
74	12.8 (49)	13.1 (49)	102	13.4 (50)	105	13.3 (48)	104
78	12.7 (49)	12.7 (49)	100	13.2 (50)	104	12.8 (48)	101
82	12.6 (49)	13.0 (49)	103	12.9 (50)	102	12.8 (47)	102
86	12.3 (49)	12.9 (48)	105	12.9 (50)	105	12.9 (46)	105
90	12.9 (49)	12.9 (48)	100	13.3 (48)	103	12.7 (46)	98
94	12.8 (45)	13.4 (47)	105	13.4 (48)	105	12.8 (43)	100
98	12.4 (45)	13.0 (47)	105	13.7 (48)	110	13.0 (41)	105
102	12.7 (43)	12.6 (44)	99	13.5 (47)	106	12.9 (38)	102
104	12.6 (42)	12.6 (44)	100	13.4 (47)	106	12.7 (36)	101

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

TABLE 7 BIOCHEMISTRY OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Group Name	Control	600 ppm		1200 ppm		2400 ppm	
No. of examined animals	40	38		43		38	
T-cholesterol (mg/dL)	168 ± 61	190 ± 66		230 ± 92	**	205 ± 70	*
Triglyceride (mg/dL)	91 ± 68	128 ± 82		199 ± 162	**	154 ± 127	*
Phospholipid (mg/dL)	242 ± 86	269 ± 79		330 ± 135	**	291 ± 88	*
γ-GTP (IU/L)	10 ± 9	12 ± 7		16 ± 10	**	23 ± 18	**
Urea nitrogen (mg/dL)	19.9 ± 6.2	21.8 ± 4.9	*	28.2 ± 19.0	**	28.7 ± 24.7	**
Creatinine (mg/dL)	0.6 ± 0.1	0.6 ± 0.1		0.8 ± 0.4	**	0.8 ± 0.8	**
Carcium (mg/dL)	10.4 ± 0.3	10.6 ± 0.5		11.0 ± 0.6	**	10.8 ± 0.4	**
Inorganic phosphorus (mg/dL)	4.1 ± 0.7	4.3 ± 0.7		4.8 ± 1.2	**	4.9 ± 3.4	

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

TABLE 8 URINALYSIS OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Group Name	Grade	Control	600 ppm	1200 ppm	2400 ppm
No. of examined animals		39	40	44	39
Protein	-	0	0	0	0
	±	0	0	0	0
	1+	0	0	0	0
	2+	5	4	1	2
	3+	25	20	20	18
	4+	9	16	23	19
	Chi square test			*	*

Significant difference: * : $p<0.05$ ** : $p<0.01$ Chi square test

TABLE 9 URINALYSIS OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Group Name	Grade	Control	600 ppm	1200 ppm	2400 ppm
No. of examined animals		42	44	47	37
pH	5.0	0	0	0	0
	6.0	0	0	1	1
	6.5	7	4	4	3
	7.0	6	9	3	1
	7.5	9	11	10	5
	8.0	19	17	23	18
	8.5	1	3	6	9
	Chi square test				*

Significant difference: * : $p<0.05$ ** : $p<0.01$ Chi square test

TABLE 10 ORGAN WEIGHTS OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Group Name	Control	600 ppm	1200 ppm	2400 ppm
No. of examined animals	40	40	44	38
Body weight (g)	379 ± 44	383 ± 37	371 ± 32	347 ± 31 **
Testes (g)	3.942 ± 1.405	4.754 ± 1.396	4.284 ± 1.836	4.816 ± 1.517 *
Testes (%)	1.038 ± 0.340	1.243 ± 0.342	1.147 ± 0.487	1.379 ± 0.420 **
Heart (g)	1.246 ± 0.098	1.244 ± 0.128	1.264 ± 0.107	1.222 ± 0.103
Heart (%)	0.332 ± 0.039	0.327 ± 0.036	0.343 ± 0.043	0.354 ± 0.036 *
Lung (g)	1.487 ± 0.133	1.512 ± 0.150	1.565 ± 0.294	1.498 ± 0.188
Lung (%)	0.399 ± 0.075	0.398 ± 0.050	0.425 ± 0.093	0.435 ± 0.075 **
Kidneys (g)	2.619 ± 0.288	2.757 ± 0.244	2.864 ± 0.310 **	2.713 ± 0.288
Kidneys (%)	0.705 ± 0.156	0.725 ± 0.086	0.776 ± 0.097 **	0.784 ± 0.083 **
Liver (g)	10.838 ± 1.751	11.435 ± 1.443	12.353 ± 1.854 **	11.976 ± 2.211 *
Liver (%)	2.882 ± 0.523	2.984 ± 0.226	3.341 ± 0.528 **	3.452 ± 0.618 **
Brain (g)	2.025 ± 0.048	2.025 ± 0.052	2.026 ± 0.080	1.974 ± 0.057 **
Brain (%)	0.543 ± 0.078	0.534 ± 0.059	0.550 ± 0.050	0.573 ± 0.055 **

Mean ± S.D.

Significant difference: * : $p<0.05$ ** : $p<0.01$ Test of Dunnett

TABLE 11 ORGAN WEIGHTS OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Group Name	Control	600 ppm	1200 ppm	2400 ppm
No. of examined animals	42	44	47	36
Body weight (g)	263 ± 44	263 ± 34	273 ± 30	244 ± 25 *
Adrenals (g)	0.082 ± 0.015	0.089 ± 0.063	0.083 ± 0.011	0.081 ± 0.008
Adrenals (%)	0.032 ± 0.007	0.034 ± 0.022	0.031 ± 0.005	0.033 ± 0.004 *
Ovaries(g)	0.218 ± 0.396	0.158 ± 0.113	0.137 ± 0.053	0.135 ± 0.027
Ovaries(%)	0.079 ± 0.124	0.060 ± 0.042	0.051 ± 0.022	0.055 ± 0.010 **
Lung (g)	1.066 ± 0.144	1.087 ± 0.209	1.083 ± 0.110	1.077 ± 0.082
Lung (%)	0.414 ± 0.069	0.423 ± 0.120	0.400 ± 0.047	0.446 ± 0.064 **
Spleen (g)	0.582 ± 0.230	0.816 ± 1.065	0.827 ± 1.316	0.566 ± 0.178
Spleen (%)	0.225 ± 0.090	0.340 ± 0.532	0.307 ± 0.491	0.232 ± 0.066 *
Liver (g)	6.969 ± 1.802	7.024 ± 1.149	7.348 ± 1.247	6.654 ± 0.769
Liver (%)	2.670 ± 0.609	2.697 ± 0.503	2.697 ± 0.352	2.729 ± 0.220 **
Brain(g)	1.851 ± 0.052	1.859 ± 0.062	1.855 ± 0.066	1.789 ± 0.052 **
Brain(%)	0.723 ± 0.119	0.720 ± 0.112	0.687 ± 0.070	0.741 ± 0.090

Mean ± S.D.

Significant difference: * : $p<0.05$ ** : $p<0.01$ Test of Dunnett

TABLE 12 INCIDENCES OF SELECTED LESIONS OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Group		Control	600 ppm	1200 ppm	2400 ppm	Peto	Cochran-Armitage
Number of examined animals		50	50	50	50	test	test
Organ / Findings	Grade of non-neoplastic finding						
Nasal cavity							
Mineralization	1+	32	25	37	21		
	Chi square test				*		
Liver							
Acidophilic cell focus	1+	3	7	8	8		
	2+	0	0	1	3		
	Chi square test				*		
Clear cell focus	1+	7	8	9	8		
	2+	3	0	3	1		
	Chi square test						
Basophilic cell focus	1+	9	6	8	9		
	2+	2	3	4	4		
	Chi square test						
Vacuolated cell focus	1+	2	0	1	1		
	Chi square test						
Spongiosis hepatis	1+	2	3	9	13		
	2+	0	2	1	5		
	Chi square test			*	**		
Hepatocellular adenoma 1)		2	1	4	4		
Hepatocellular carcinoma 2)		0	0	0	1		
1)+2)		2	1	4	5	↑	
Kidney							
Chronic nephropathy	1+	9	6	4	4		
	2+	23	20	15	15		
	3+	15	20	24	24		
	4+	1	2	6	5		
	Chi square test			*			
Thyroid							
Focal follicular cell	1+	0	1	2	2		
hyperplasia	2+	0	0	1	5		
	Chi square test				*		
Brain							
Degeneration: granular cell	1+	0	0	0	1		
	2+	0	0	0	1		
	3+	0	0	0	4		
	Chi square test						

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 13 INCIDENCES OF SELECTED LESIONS OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Group		Control	600 ppm	1200 ppm	2400 ppm	Peto test	Cochran-Armitage test
Number of examined animals		50	50	50	50		
Organ Findings	Grade of non-neoplastic finding						
Subcutis							
Fibroma		4	2	0	0		↓
Nasal cavity							
Mineralization	1+	31	18	16	17		
	Chi square test		*	**	**		
Brain							
Degeneration: granular cell	1+	0	0	0	0		
	2+	0	0	0	1		
	3+	0	0	0	5		
	Chi square test				*		

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

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 14 CAUSE OF DEATH OF MALE AND FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

	Male				Female			
Group	Control	600 ppm	1200 ppm	2400 ppm	Control	600 ppm	1200 ppm	2400 ppm
Number of dead or moribund animals	10	10	6	12	8	6	3	14
No microscopical confirmation	0	0	1	0	0	0	0	0
Respiratory system lesion	0	0	0	1	0	0	0	0
Central nervous system lesion	0	0	0	2	0	0	0	3
Tumor death : leukemia	3	4	1	1	4	1	1	5
subcutis	0	0	0	1	1	1	0	0
bone marrow	1	0	0	0	0	0	0	0
liver	0	0	0	1	0	0	0	0
pancreas	0	1	0	0	0	0	0	0
pituitary	3	2	2	2	2	3	2	3
thyroid	0	0	1	0	0	0	0	0
adrenal	2	0	0	1	0	0	0	0
uterus	—	—	—	—	0	0	0	1
mammary gland	0	0	0	0	1	0	0	1
clitoral gland	—	—	—	—	0	0	0	1
brain	0	1	0	0	0	1	0	0
Zymbal gland	1	0	0	0	0	0	0	0
bone	0	1	0	2	0	0	0	0
vertebra	0	0	0	1	0	0	0	0
peritoneum	0	1	1	0	0	0	0	0

TABLE 15 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 tumors	Incidence (%)	Min. - Max. (%)
Liver	<1499>			
Hepatocellular adenoma 1)		21	1.4	0 - 6
Hepatocellular carcinoma 2)		4	0.3	0 - 2
1)+2)		25	1.7	0 - 6

30 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

TABLE 16 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 tumors	Incidence (%)	Min. - Max. (%)
Subcutis	<1447>			
Fibroma		16	2.4	0 - 4

29 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

FIGURES

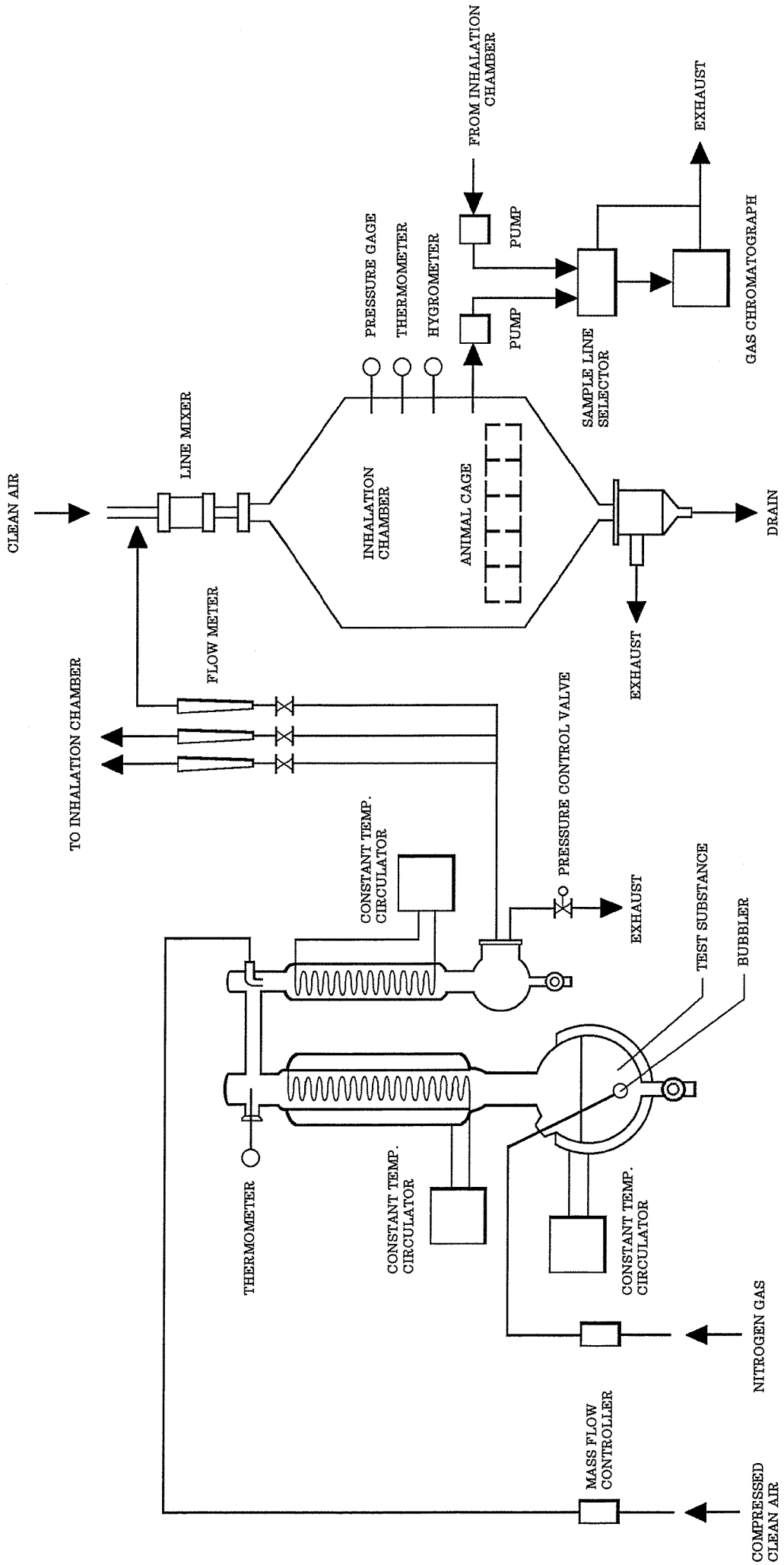


FIGURE 1 CYCLOHEXENE VAPOR GENERATION SYSTEM AND INHALATION SYSTEM

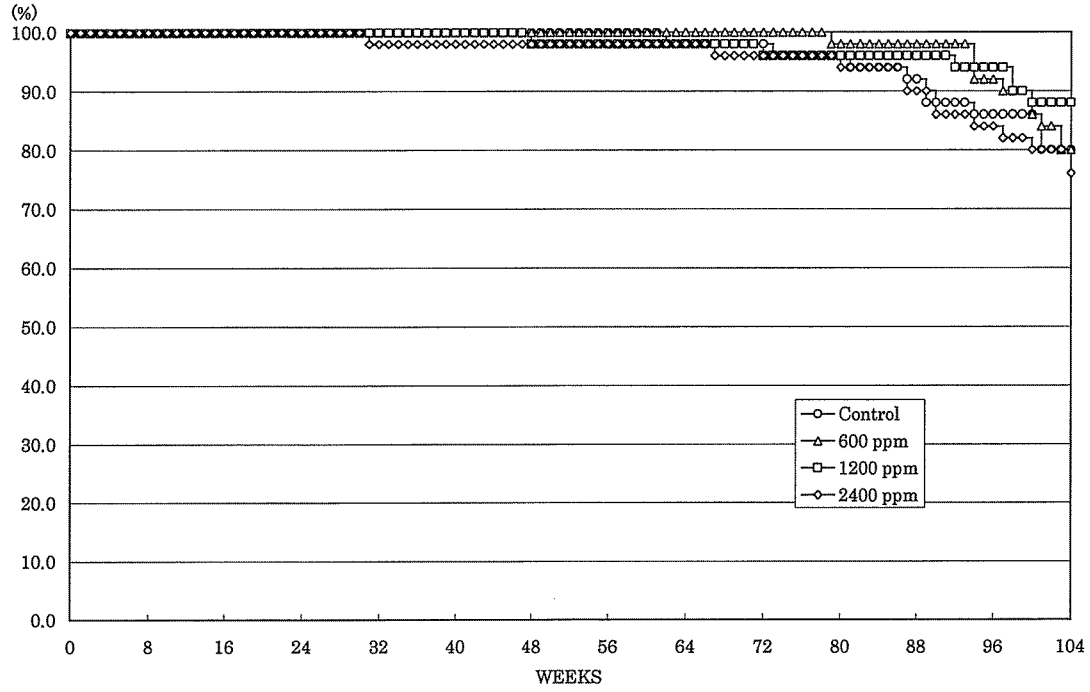


FIGURE 2 SURVIVAL ANIMAL RATE OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

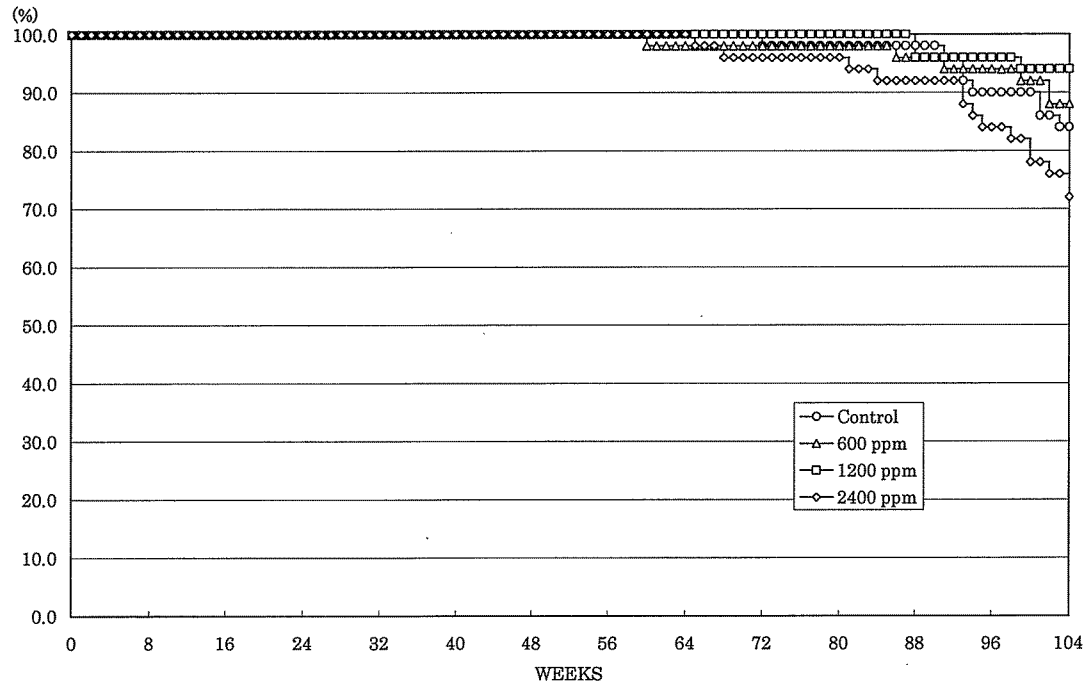


FIGURE 3 SURVIVAL ANIMAL RATE OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

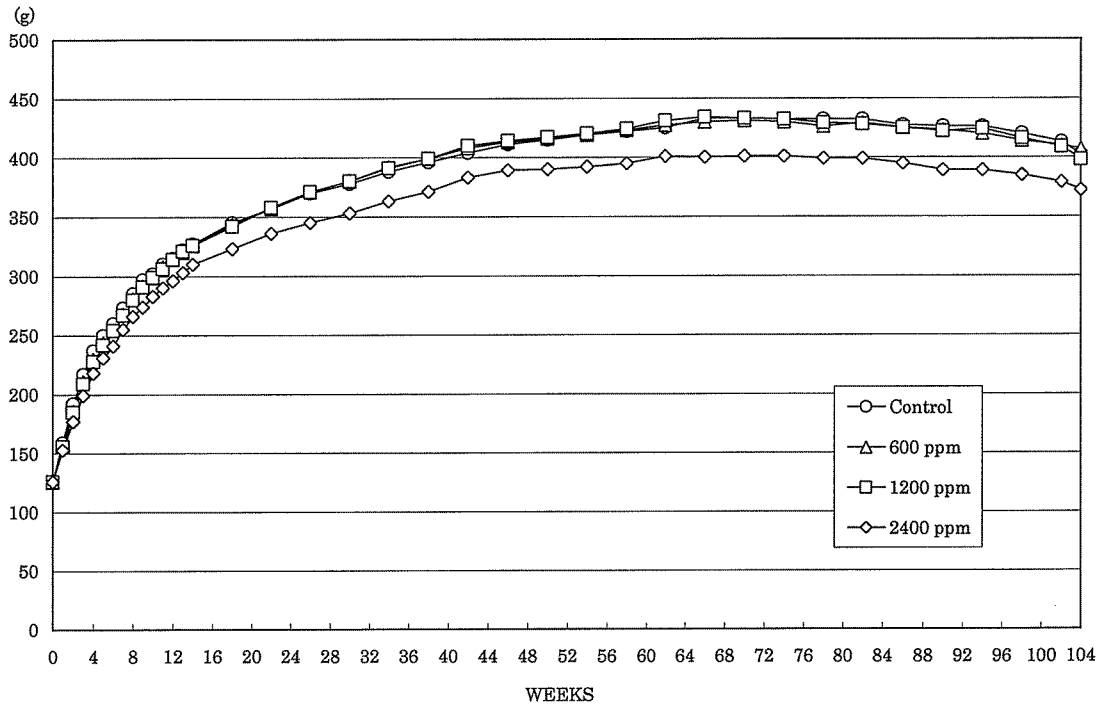


FIGURE 4 BODY WEIGHT CHANGES OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

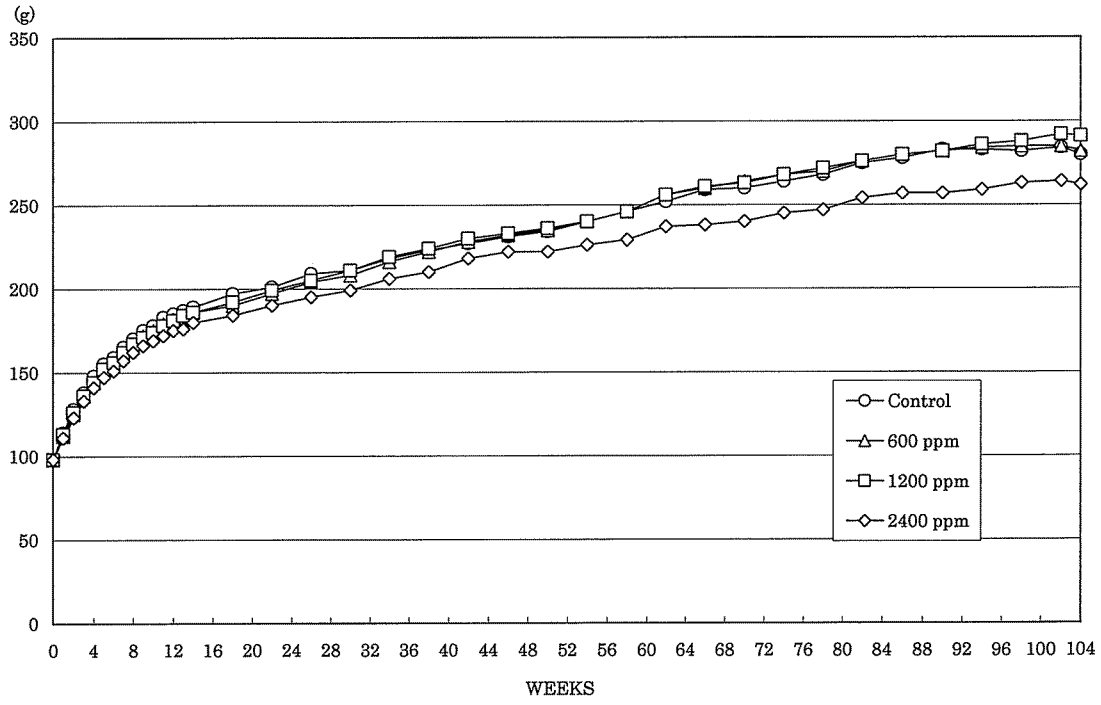


FIGURE 5 BODY WEIGHT CHANGES OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

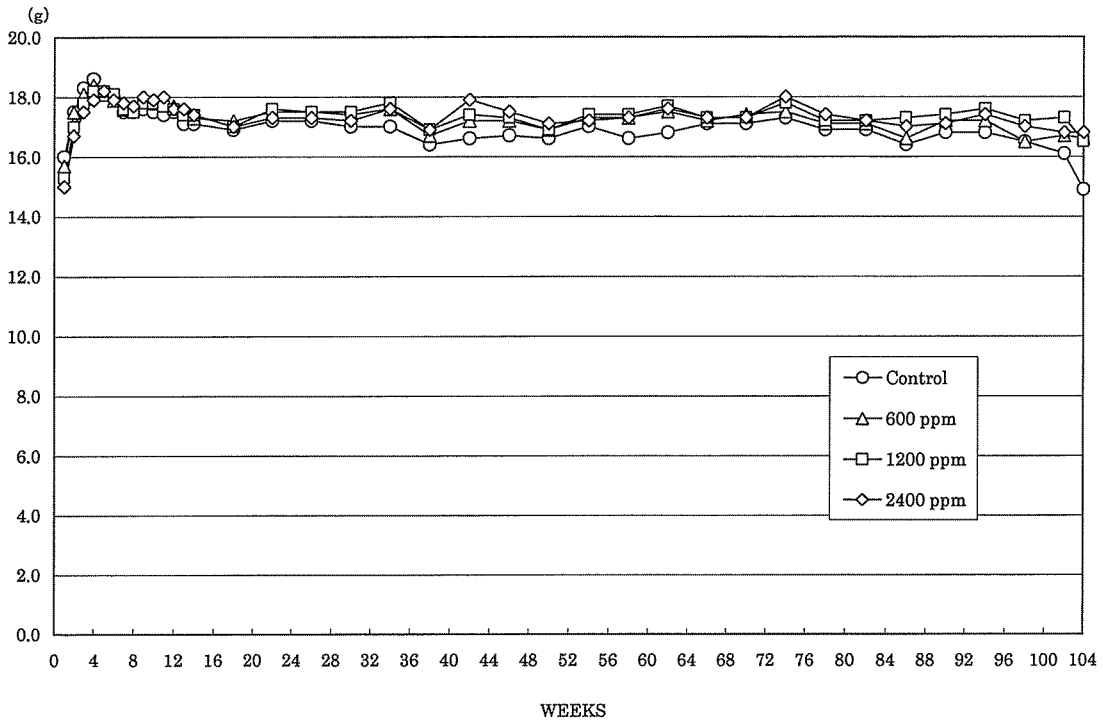


FIGURE 6 FOOD CONSUMPTION CHANGES OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

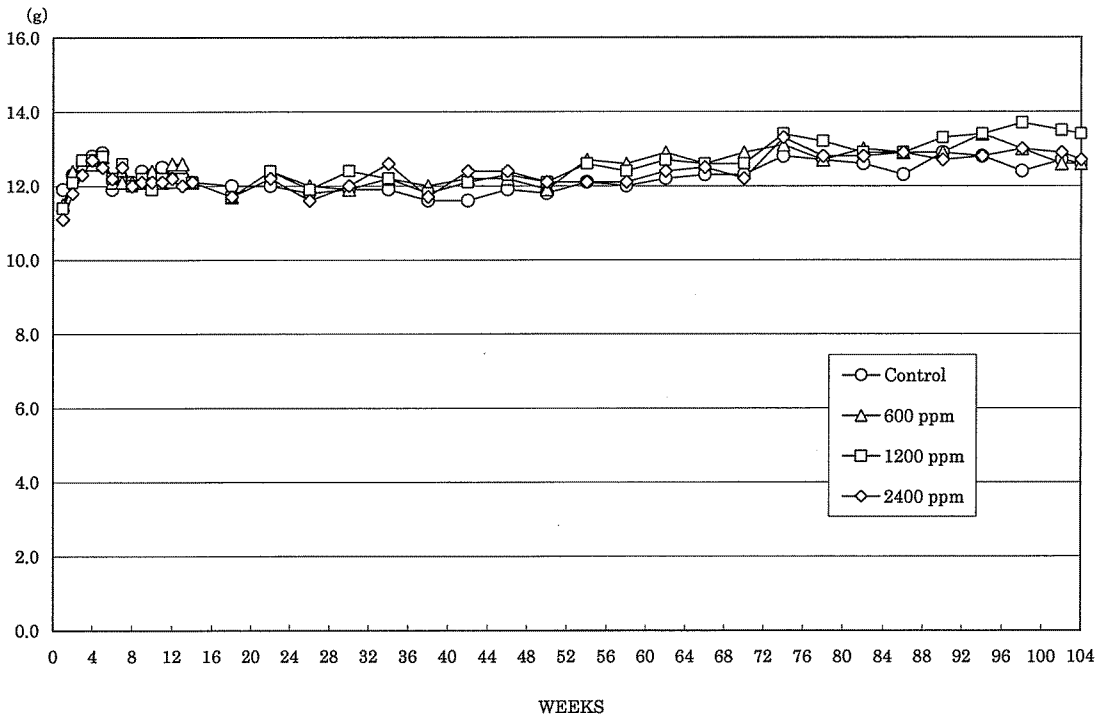
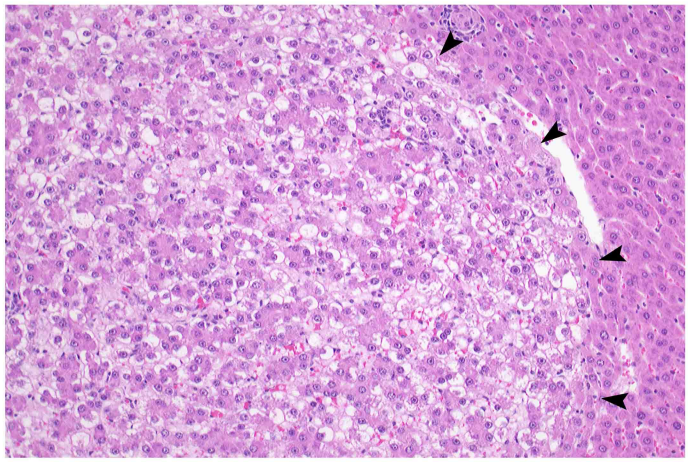
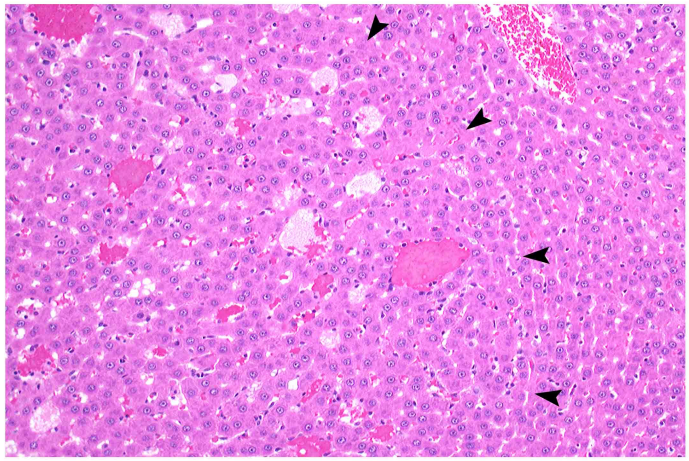


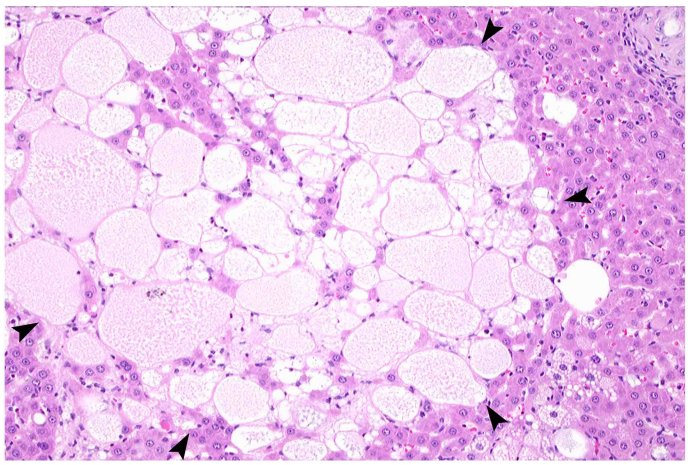
FIGURE 7 FOOD CONSUMPTION CHANGES OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE



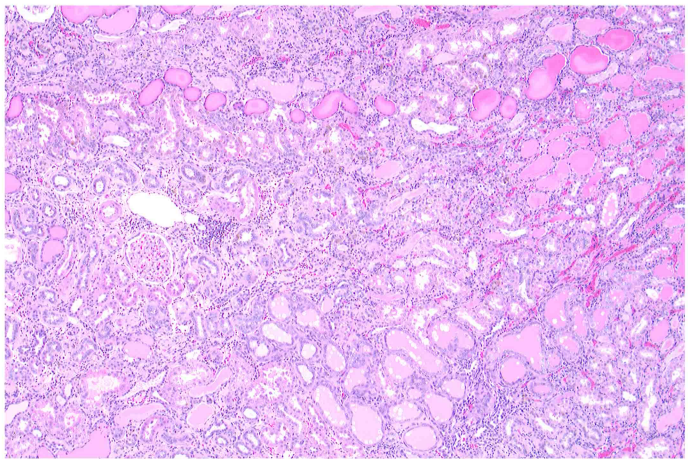
Photograph 1 200 μm
Liver: Hepatocellular adenoma (arrow heads).
Rat, Male, 2400 ppm, Animal No. 0399-1318 (H&E)



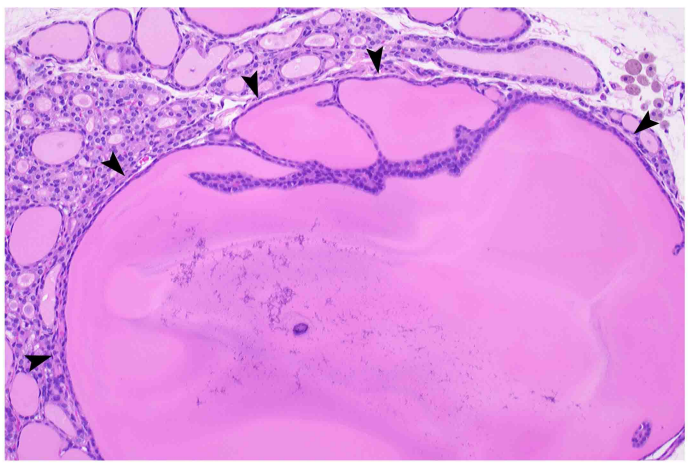
Photograph 2 200 μm
Liver: Acidophilic cell focus (arrow heads).
Rat, Male, 2400 ppm, Animal No. 0399-1315 (H&E)



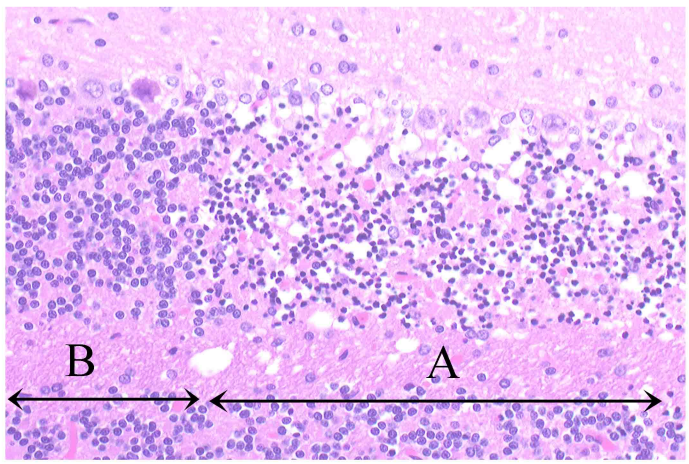
Photograph 3 200 μm
Liver: Spongiosis hepatis (arrow heads).
Rat, Male, 2400 ppm, Animal No. 0399-1317 (H&E)



Photograph 4 500 μm
Kidney: Chronic nephropathy (severe).
Rat, Male, 2400 ppm, Animal No. 0399-1348 (H&E)



Photograph 5 200 μm
Thyroid: Focal follicular cell hyperplasia (arrow heads).
Rat, Male, 2400 ppm, Animal No. 0399-1336 (H&E)



Photograph 6 100 μm
Brain: Degeneration of granular cells in the granular layer of the cerebellum (A). Normal granular layer (B).
Rat, Female, 2400 ppm, Animal No. 0399-2339 (H&E)