Summary of Inhalation Carcinogenicity Study of Cyclohexene in BDF1 Mice

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Japan Bioassay Research Center

Japan Industrial Safety and Health Association

PREFACE

The tests were contracted and supported by the Ministry of Health, Labour and Welfare of Japan. The tests were conducted by Japan Bioassay Research Center (JBRC) and the report was prepared by JBRC and peer reviewed by outside expert pathologist. Complete report was submitted to Ministry of Health, Labour and Welfare of Japan on March 31 2004.

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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 Crj:BDF1 mice of both sexes to cyclohexene vapor at a target concentration of 0 (clean air), 75, 150 or 300 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: 3700 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 300 ppm-exposed males and females were decreased sporadically, but their terminal body weights were recovered to those of the respective controls.

Neither exposure-related increase in the incidence of neoplastic lesions nor in the incidence of non-neoplastic lesions was recognized for any cyclohexene-exposed group of either sex, as compared with the respective control.

Death occurred in the male mice exposed to 600 ppm cyclohexene in the previous 2-week inhalation study, and the decreased body weight and increased relative weights of the liver and kidney were noted in the male mice exposed to 300 ppm in the previous 13-week inhalation study. On the other hand, the 2-year inhalation exposure of mice of both sexes to 300 ppm cyclohexene did not cause any hazardous effect, suggesting that the no-observed-adverse-effect-level (NOAEL) for cyclohexene was 300 ppm in the mouse.

Conclusions

In mice, there was no evidence of carcinogenic activity of cyclohexene in males or females.

TABLES

TABLES(CONTINUED)

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

Weeks on Study	Control Av.Wt.	Control No.of Surviv. <50>	75ppm Av.Wt.	75ppm % of cont.	75ppm No.of Surviv. <50>	150ppm Av.Wt.	150ppm % of cont.	150ppm No.of Surviv. <50>	300ppm Av.Wt.	300ppm % of cont.	300ppm No.of Surviv. <50>
0	22.9 (50)	50/50	22.9 (50)	100	50/50	22.9 (50)	100	50/50	22.9 (50)	100	50/50
1	24.0 (50)	50/50	24.0 (50)	100	50/50	24.2 (50)	101	50/50	23.6 (50)	98	50/50
2	24.9 (50)	50/50	24.8 (50)	100	50/50	24.9 (50)	100	50/50	24.6 (50)	99	50/50
3	25.6 (50)	50/50	25.4 (49)	99	49/50	25.5 (49)	100	49/50	24.9 (50)	97	50/50
4	26.0 (50)	50/50	25.6 (49)	98	49/50	26.0 (49)	100	49/50	25.4 (50)	98	50/50
5	26.4 (50)	50/50	25.8 (49)	98	49/50	26.4 (49)	100	49/50	25.6 (50)	97	50/50
6	27.1 (50)	50/50	26.3 (49)	97	49/50	27.1 (49)	100	49/50	26.2 (50)	97	50/50
7	27.6 (50)	50/50	26.6 (49)	96	49/50	27.4 (49)	99	49/50	26.6 (50)	96	50/50
8	28.2 (50)	50/50	27.4 (49)	97	49/50	28.1 (49)	100	49/50	27.3 (50)	97	50/50
9	28.6 (50)	50/50	27.8 (49)	97	49/50	28.7 (49)	100	49/50	27.5 (50)	96	50/50
10	29.1 (50)	50/50	28.2 (49)	97	49/50	29.3 (49)	101	49/50	28.2 (50)	97	50/50
11	29.7 (50)	50/50	28.8 (49)	97	49/50	30.0 (49)	101	49/50	28.4 (50)	96	50/50
12	30.7 (50)	50/50	29.7 (49)	97	49/50	31.3 (49)	102	49/50	29.3 (50)	95	50/50
13	31.4 (50)	50/50	30.4 (49)	97	49/50	31.6 (49)	101	49/50	29.7 (50)	95	50/50
14	32.0 (50)	50/50	31.0 (49)	97	49/50	32.2 (49)	101	49/50	30.2 (50)	94	50/50
18	34.6 (50)	50/50	34.0 (49)	98	49/50	35.3 (49)	102	49/50	33.2 (50)	96	50/50
22	36.8 (50)	50/50	35.9 (49)	98	49/50	37.5 (49)	102	49/50	35.3 (50)	96	50/50
26	38.8 (49)	49/50	38.4 (49)	99	49/50	39.3 (49)	101	49/50	37.4 (50)	96	50/50
30	40.7 (49)	49/50	40.5 (49)	100	49/50	40.9 (49)	100	49/50	39.1 (50)	96	50/50
34	42.1 (49)	49/50	41.5 (49)	99	49/50	42.3 (49)	100	49/50	40.4 (50)	96	50/50
38	43.1 (49)	49/50	42.9 (49)	100	49/50	43.2 (49)	100	49/50	41.1 (50)	95	50/50
42	44.0 (49)	49/50	44.0 (49)	100	49/50	44.5 (49)	101	49/50	42.3 (50)	96	50/50
46	45.0 (49)	49/50	45.1 (49)	100	49/50	45.1 (49)	100	49/50	43.1 (50)	96	50/50
50	46.0 (49)	49/50	45.9 (49)	100	49/50	46.0 (49)	100	49/50	44.4 (50)	97	50/50
54	46.8 (49)	49/50	46.7 (49)	100	49/50	47.3 (48)	101	48/50	45.1 (50)	96	50/50
58	47.4 (49)	49/50	47.2 (49)	100	49/50	47.9 (48)	101	48/50	45.8 (50)	97	50/50
62	48.1 (49)	49/50	48.0 (49)	100	49/50	48.4 (48)	101	48/50	46.1 (50)	96	50/50
66	49.3 (49)	49/50	49.6 (49)	101	49/50	49.0 (48)	99	48/50	47.6 (49)	97	49/50
70	50.2 (49)	49/50	50.3 (49)	100	49/50	50.1 (48)	100	48/50	48.4 (49)	96	49/50
74	50.5 (49)	49/50	50.8 (49)	101	49/50	50.8 (48)	101	48/50	49.5 (49)	98	49/50
78	50.0 (48)	48/50	51.0 (49)	102	49/50	50.9 (46)	102	46/50	49.2 (49)	98	49/50
82	49.8 (47)	47/50	51.0 (49)	102	49/50	51.0 (46)	102	46/50	49.6 (48)	100	48/50
86	49.5 (47)	47/50	51.8 (47)	105	47/50	51.8 (45)	105	45/50	50.7 (47)	102	47/50
90	49.4 (44)	44/50	51.0 (47)	103	47/50	51.7 (43)	105	43/50	51.1 (46)	103	46/50
94	49.1 (41)	41/50	49.7 (45)	101	45/50	50.8 (41)	103	41/50	50.8 (45)	103	45/50
98	48.8 (39)	39/50	49.3 (44)	101	44/50	49.9 (41)	102	41/50	50.7 (44)	104	44/50
102	48.0 (37)	37/50	49.0 (39)	102	39/50	48.3 (38)	101	38/50	49.6 (43)	103	43/50
104	47.4 (37)	37/50	48.2 (38)	102	38/50	48.5 (35)	102	35/50	48.9 (42)	103	42/50

< > : 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 MICE IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Weeks on Study	Control Av.Wt.	Control No.of Surviv.	75ppm Av.Wt.	75ppm % of cont.	75ppm No.of Surviv.	150ppm Av.Wt.	150ppm % of cont.	150ppm No.of Surviv.	300ppm Av.Wt.	300ppm % of cont.	300ppm No.of Surviv.
	<50>		<50>			<50>			<49>		
0	19.1 (50)	50/50	19.1 (50)	100	50/50	19.1 (50)	100	50/50	19.1 (49)	100	49/49
1	19.7 (50)	50/50	19.7 (50)	100	50/50	19.6 (50)	99	50/50	19.3 (49)	98	49/49
2	20.6 (50)	50/50	20.5 (50)	100	50/50	20.7 (50)	100	50/50	20.2 (49)	98	49/49
3	21.0 (50)	50/50	20.9 (50)	100	50/50	20.9 (50)	100	50/50	20.5 (49)	98	49/49
4	21.6 (50)	50/50	21.7 (50)	100	50/50	21.6 (50)	100	50/50	21.4 (49)	99	49/49
5	22.1 (50)	50/50	21.8 (50)	99	50/50	22.0 (50)	100	50/50	21.5 (49)	97	49/49
6	22.7 (50)	50/50	22.5 (50)	99	50/50	22.6 (50)	100	50/50	22.1 (49)	97	49/49
7	23.1 (50)	50/50	22.8 (50)	99	50/50	22.8 (50)	99	50/50	22.4 (49)	97	49/49
8	23.4 (50)	50/50	23.3 (50)	100	50/50	23.1 (50)	99	50/50	22.9 (49)	98	49/49
9	23.4 (50)	50/50	23.3 (50)	100	50/50	23.3 (50)	100	50/50	23.0 (49)	98	49/49
10	23.9 (50)	50/50	23.4 (50)	98	50/50	23.7 (50)	99	50/50	23.4 (49)	98	49/49
11	24.1 (50)	50/50	24.0 (50)	100	50/50	23.9 (50)	99	50/50	23.7 (49)	98	49/49
12	24.6 (50)	50/50	24.3 (50)	99	50/50	24.4 (50)	99	50/50	23.8 (49)	97	49/49
13	24.5 (50)	50/50	24.3 (50)	99	50/50	24.5 (50)	100	50/50	24.0 (49)	98	49/49
14	24.7 (50)	50/50	24.8 (50)	100	50/50	24.8 (50)	100	50/50	24.2 (49)	98	49/49
18	26.4 (50)	50/50	25.9 (50)	98	50/50	26.1 (50)	99	50/50	25.8 (49)	98	49/49
22	26.8 (50)	50/50	26.4 (50)	99	50/50	26.5 (50)	99	50/50	26.0 (49)	97	49/49
26	27.3 (50)	50/50	27.5 (50)	101	50/50	27.1 (50)	99	50/50	26.3 (49)	96	49/49
30	28.1 (50)	50/50	27.9 (50)	99	50/50	27.8 (50)	99	50/50	27.4 (48)	98	48/49
34	28.7 (50)	50/50	28.2 (50)	98	50/50	28.6 (50)	100	50/50	27.4 (48)	95	48/49
38	28.7 (50)	50/50	28.8 (50)	100	50/50	28.9 (50)	101	50/50	27.5 (48)	96	48/49
42	29.1 (50)	50/50	29.6 (50)	102	50/50	29.6 (50)	102	50/50	28.4 (48)	98	48/49
46	29.5 (50)	50/50	29.9 (50)	101	50/50	30.1 (49)	102	49/50	28.5 (48)	97	48/49
50	29.9 (50)	50/50	30.4 (50)	102	50/50	31.0 (49)	104	49/50	28.9 (48)	97	48/49
54	30.6 (50)	50/50	31.0 (50)	101	50/50	30.8 (48)	101	48/50	29.6 (48)	97	48/49
58	30.5 (50)	50/50	31.6 (50)	104	50/50	31.2 (47)	102	47/50	30.0 (48)	98	48/49
62	31.5 (49)	49/50	32.2 (49)	102	49/50	32.2 (46)	102	46/50	29.6 (46)	94	46/49
66	32.3 (48)	48/50	32.8 (49)	102	49/50	32.6 (46)	101	46/50	30.2 (46)	93	46/49
70	32.7 (48)	48/50	33.1 (47)	101	47/50	33.3 (46)	102	46/50	30.7 (45)	94	45/49
74	33.7 (46)	46/50	34.5 (45)	102	45/50	34.0 (45)	101	45/50	31.8 (45)	94	45/49
78	33.3 (44)	44/50	34.8 (44)	105	44/50	33.2 (44)	100	44/50	32.2 (44)	97	44/49
82	33.7 (43)	43/50	34.5 (42)	102	42/50	33.8 (42)	100	42/50	32.0 (43)	95	43/49
86	33.9 (43)	43/50	35.4 (40)	104	40/50	34.5 (38)	102	38/50	32.8 (39)	97	39/49
90	33.6 (42)	42/50	34.7 (37)	103	37/50	34.5 (36)	103	36/50	33.0 (37)	98	37/49
94	33.1 (39)	39/50	34.3 (35)	104	35/50	34.9 (34)	105	34/50	32.5 (35)	98	35/49
98	33.8 (38)	38/50	35.2 (31)	104	31/50	35.3 (30)	104	30/50	32.9 (32)	97	32/49
102	33.8 (32)	32/50	36.3 (28)	107	28/50	34.9 (25)	103	25/50	33.6 (32)	99	32/49
104	33.4 (27)	27/50	36.2 (25)	108	25/50	34.1 (24)	102	24/50	33.0 (29)	99	29/49

< > : 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 MICE 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	0/49	0/49	0/49	0/49	2/48	3/43	5/50 (2/13)
	75ppm	0/50	0/49	0/49	0/49	1/49	1/49	2/49	1/46	2/50 (1/12)
	150ppm	0/50	0/49	0/49	0/49	0/48	0/48	1/46	2/43	2/50 (1/15)
	300ppm	0/50	0/50	0/50	0/50	0/50	2/49	1/49	3/45	4/50 (1/8)
Internal mass										
	Control	1/50	1/50	0/49	0/49	0/49	2/49	9/48	14/43	19/50 (7/13)
	75ppm	0/50	0/49	0/49	0/49	0/49	1/49	6/49	17/46	18/50 (7/12)
	150ppm	0/50	0/49	0/49	0/49	0/48	0/48	1/46	9/43	10/50 (5/15)
	300ppm	0/50	0/50	1/50	1/50	1/50	3/49	5/49	12/45	14/50 (3/8)

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 MICE 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	0/48	1/43	5/40	5/50 (4/23)
	75ppm	0/50	0/50	0/50	0/50	0/50	1/49	2/43	2/35	3/50 (2/25)
	150ppm	0/50	0/50	0/50	0/50	0/48	1/46	2/43	2/35	5/50 (4/26)
	300ppm	0/49	0/49	0/49	0/48	1/48	0/46	2/44	1/37	3/49 (2/20)
Internal mass										
	Control	0/50	0/50	0/50	0/50	1/50	5/48	4/43	6/40	13/50 (12/23)
	75ppm	0/50	0/50	0/50	1/50	1/50	5/49	4/43	8/35	17/50 (12/25)
	150ppm	0/50	0/50	0/50	1/50	1/48	4/46	5/43	9/35	17/50 (10/26)
	300ppm	1/49	1/49	1/49	2/48	5/48	7/46	8/44	11/37	18/49 (13/20)

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 MICE IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Weeks on Study	Control Av.Fc. <50>	75ppm Av.Fc. <50>	75ppm % of cont.	150ppm Av.Fc. <50>	150ppm % of cont.	300ppm Av.Fc. <50>	300ppm % of cont.
1	4.2 (50)	4.2 (50)	100	4.2 (50)	100	4.1 (50)	98
2	4.1 (50)	4.1 (50)	100	3.9 (50)	95	4.0 (50)	98
3	4.0 (50)	4.0 (49)	100	4.0 (49)	100	4.0 (50)	100
4	4.0 (50)	4.1 (49)	103	4.1 (49)	103	4.1 (50)	103
5	4.1 (50)	4.1 (49)	100	4.2 (49)	102	4.1 (50)	100
6	4.2 (50)	4.3 (49)	102	4.3 (49)	102	4.3 (50)	102
7	4.3 (50)	4.4 (49)	102	4.4 (49)	102	4.3 (50)	100
8	4.4 (50)	4.5 (49)	102	4.4 (49)	100	4.4 (50)	100
9	4.5 (50)	4.6 (49)	102	4.6 (49)	102	4.6 (50)	102
10	4.5 (50)	4.5 (49)	100	4.6 (49)	102	4.7 (50)	104
11	4.6 (50)	4.6 (49)	100	4.6 (49)	100	4.5 (50)	98
12	4.6 (50)	4.6 (49)	100	4.6 (49)	100	4.6 (50)	100
13	4.6 (50)	4.6 (49)	100	4.5 (49)	98	4.6 (50)	100
14	4.6 (50)	4.7 (49)	102	4.6 (49)	100	4.6 (50)	100
18	4.8 (50)	4.6 (49)	96	4.7 (49)	98	4.8 (50)	100
22	4.7 (50)	4.7 (49)	100	4.8 (49)	102	4.8 (50)	102
26	4.6 (49)	4.7 (49)	102	4.6 (49)	100	4.7 (50)	102
30	4.7 (49)	4.8 (49)	102	4.6 (49)	98	4.7 (50)	100
34	4.8 (49)	4.9 (49)	102	4.8 (49)	100	4.8 (50)	100
38	4.8 (49)	4.9 (49)	102	4.8 (49)	100	4.7 (50)	98
42	4.8 (49)	4.9 (49)	102	4.8 (49)	100	4.8 (50)	100
46	4.9 (49)	5.0 (49)	102	4.8 (49)	98	4.9 (50)	100
50	4.9 (49)	4.9 (49)	100	4.7 (49)	96	4.8 (50)	98
54	4.9 (49)	4.9 (49)	100	4.9 (48)	100	4.8 (50)	98
58	5.1 (49)	5.1 (49)	100	5.1 (48)	100	5.0 (50)	98
62	5.1 (49)	5.1 (49)	100	5.1 (48)	100	5.0 (50)	98
66	5.1 (49)	5.2 (49)	102	5.1 (48)	100	5.0 (49)	98
70	5.1 (49)	5.1 (49)	100	5.1 (48)	100	5.1 (49)	100
74	5.3 (49)	5.3 (49)	100	5.2 (48)	98	5.3 (49)	100
78	5.2 (48)	5.2 (49)	100	5.2 (46)	100	5.1 (49)	98
82	5.2 (47)	5.2 (49)	100	5.1 (46)	98	5.2 (48)	100
86	5.2 (47)	5.3 (47)	102	5.1 (45)	98	5.1 (47)	98
90	5.1 (44)	5.2 (47)	102	5.1 (43)	100	5.2 (46)	102
94	5.0 (41)	4.9 (45)	98	5.0 (41)	100	5.1 (45)	102
98	5.1 (39)	5.0 (44)	98	5.0 (41)	98	5.1 (44)	100
102	5.1 (37)	4.9 (39)	96	5.0 (38)	98	5.0 (43)	98
104	4.9 (37)	5.0 (38)	102	4.9 (35)	100	4.9 (42)	100

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

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

Weeks on Study	Control Av.Fc. <50>	75ppm Av.Fc. <50>	75ppm % of cont.	150ppm Av.Fc. <50>	150ppm % of cont.	300ppm Av.Fc. <49>	300ppm % of cont.
1	3.6 (50)	3.6 (50)	100	3.5 (50)	97	3.4 (49)	94
2	3.5 (50)	3.5 (50)	100	3.5 (50)	100	3.5 (49)	100
3	3.6 (50)	3.6 (50)	100	3.6 (50)	100	3.5 (49)	97
4	3.8 (50)	3.8 (50)	100	3.8 (50)	100	3.8 (49)	100
5	4.0 (50)	3.9 (50)	98	4.0 (50)	100	3.8 (49)	95
6	4.1 (50)	4.1 (50)	100	4.1 (50)	100	4.0 (49)	98
7	4.2 (50)	4.2 (50)	100	4.3 (50)	102	4.2 (49)	100
8	4.3 (50)	4.3 (50)	100	4.3 (50)	100	4.4 (49)	102
9	4.5 (50)	4.5 (50)	100	4.5 (50)	100	4.5 (49)	100
10	4.4 (50)	4.4 (50)	100	4.4 (50)	100	4.5 (49)	102
11	4.4 (50)	4.4 (50)	100	4.4 (50)	100	4.4 (49)	100
12	4.2 (50)	4.2 (50)	100	4.2 (50)	100	4.2 (49)	100
13	4.3 (50)	4.3 (50)	100	4.3 (50)	100	4.3 (49)	100
14	4.4 (50)	4.4 (50)	100	4.4 (50)	100	4.4 (49)	100
18	4.4 (50)	4.4 (50)	100	4.4 (50)	100	4.5 (49)	102
22	4.4 (50)	4.3 (50)	98	4.3 (48)	98	4.4 (49)	100
26	4.2 (50)	4.3 (50)	102	4.2 (50)	100	4.3 (49)	102
30	4.4 (50)	4.4 (50)	100	4.3 (50)	98	4.4 (47)	100
34	4.5 (50)	4.5 (50)	100	4.4 (50)	98	4.5 (48)	100
38	4.4 (50)	4.5 (50)	102	4.4 (50)	100	4.4 (48)	100
42	4.4 (50)	4.5 (50)	102	4.4 (50)	100	4.4 (48)	100
46	4.5 (50)	4.5 (50)	100	4.5 (49)	100	4.5 (48)	100
50	4.4 (49)	4.4 (50)	100	4.4 (49)	100	4.3 (48)	98
54	4.5 (50)	4.5 (50)	100	4.3 (48)	96	4.4 (48)	98
58	4.5 (50)	4.5 (50)	100	4.6 (47)	102	4.5 (48)	100
62	4.6 (49)	4.6 (49)	100	4.5 (46)	98	4.4 (46)	96
66	4.7 (48)	4.6 (49)	98	4.6 (46)	98	4.6 (46)	98
70	4.6 (48)	4.6 (47)	100	4.6 (46)	100	4.4 (45)	96
74	4.8 (46)	5.0 (45)	104	4.8 (45)	100	4.8 (45)	100
78	4.7 (44)	4.7 (44)	100	4.5 (44)	96	4.7 (44)	100
82	4.7 (43)	4.8 (42)	102	4.7 (42)	100	4.6 (43)	98
86	4.9 (43)	4.8 (40)	98	4.5 (38)	92	4.6 (39)	94
90	4.6 (42)	4.6 (37)	100	4.6 (36)	100	4.7 (37)	102
94	4.4 (39)	4.6 (35)	105	4.6 (34)	105	4.7 (35)	107
98	4.7 (38)	4.7 (31)	100	4.7 (30)	100	4.6 (32)	98
102	4.7 (32)	5.0 (28)	106	4.7 (25)	100	4.9 (32)	104
104	4.6 (27)	4.8 (25)	104	4.6 (24)	100	4.6 (29)	100

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

TABLE 7 HEMATOLOGY OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Group Name	Control	75 ppm	150 ppm	300 ppm	
No. of examined animals	27	23	20	27	
Platelet ($10^3/\mu$ L)	1241 ± 299	1068 ± 385	1053 ± 480	950 ± 332	*

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

TABLE 8 BIOCHEMISTRY OF MALE MICE IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Group Name	Control	75 ppm	150 ppm	300 ppm	
No. of examined animals	36	38	34	41	
Total protein (g/dL)	5.3 ± 0.8	5.1 ± 0.6	5.2 ± 0.6	4.8 ± 0.5	**
Calcium (mg/dL)	9.3 ± 0.6	9.0 ± 0.5	9.1 ± 0.5	8.9 ± 0.3	**

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

TABLE 9 ORGAN WEIGHTS OF MALE MICE IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Group Name	Control	75 ppm	150 ppm	300 ppm
No. of examined animals	37	38	35	42
Body weight (g)	43.1 ± 8.6	44.1 ± 8.1	44.1 ± 8.0	45.0 ± 6.9
Kidneys (g)	0.659 ± 0.089	0.617 ± 0.046 *	0.641 ± 0.067	0.743 ± 0.320
Kidneys (%)	1.579 ± 0.348	1.444 ± 0.292	1.505 ± 0.373	1.752 ± 1.152

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

TABLE 10 ORGAN WEIGHTS OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Group Name	Control	75 ppm	150 ppm	300 ppm
No. of examined animals	27	25	24	29
Body weight (g)	29.2 ± 3.6	32.1 ± 6.4	30.7 ± 4.2	29.0 ± 2.1
Adrenal (g)	0.015 ± 0.003	0.014 ± 0.003	0.013 ± 0.003	0.014 ± 0.003
Adrenal (%)	0.051 ± 0.013	0.045 ± 0.012	0.042 ± 0.012 *	0.049 ± 0.010
Kidneys (g)	0.448 ± 0.059	0.614 ± 0.355 **	0.462 ± 0.068	0.485 ± 0.279
Kidneys (%)	1.542 ± 0.200	1.997 ± 1.347	1.521 ± 0.251	1.688 ± 1.020
Liver (g)	1.453 ± 0.227	2.464 ± 2.253 **	1.934 ± 1.248	1.502 ± 0.361
Liver (%)	4.975 ± 0.536	7.420 ± 5.535	6.188 ± 3.255	5.206 ± 1.368

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

TABLE 11 INCIDENCES OF SELECTED LESIONS OF MALE MICE IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Group	Control	75 ppm	150 ppm	300 ppm	Peto test	Cochran-Armitage test
Number of examined animals	50	50	50	50		
Organ						
Findings						
Liver						
Hepatocellular adenoma 1)	8	6	9	5		
Hepatocellular carcinoma 2)	12	7	5	3	*	↓
1)+2)	20	13	12	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

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

TABLE 12 INCIDENCES OF SELECTED LESIONS OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

Group	Control	75 ppm	150 ppm	300 ppm	Peto test	Cochran-Armitage test
Number of examined animals	50	50	50	49		
Organ						
Findings						
Lymph node						
Malignant lymphoma	18	23	19	10		↓
Mammary gland						
Adenocarcinoma	3	0	0	0		↓

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

TABLE 13 CAUSE OF DEATH OF MALE AND FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

		Male				Female			
Group		Control	75 ppm	150 ppm	300 ppm	Control	75 ppm	150 ppm	300 ppm
Number of dead or moribund animals		13	12	15	8	23	25	26	20
No microscopical confirmation		0	1	0	0	1	0	0	1
Hemorrhage		1	0	0	0	0	0	0	0
Urinary retention		0	1	0	2	0	0	0	0
Arteritis		0	0	0	0	0	0	1	1
Hydronephrosis		1	1	1	2	1	0	0	1
Tumor death :	leukemia	1	4	1	0	10	16	9	6
	subcutis	1	0	0	0	0	0	2	0
	lung	0	0	3	2	0	0	0	0
	lymph node	0	1	0	0	0	0	0	0
	spleen	0	0	1	0	0	0	0	0
	tongue	0	0	1	0	0	0	0	0
	stomach	0	0	1	0	0	0	0	0
	liver	9	4	5	1	0	0	1	0
	urinary bladder	0	0	1	0	0	0	0	0
	pituitary	0	0	0	0	1	0	3	0
	thyroid	0	0	0	1	0	0	0	0
	uterus	—	—	—	—	9	8	9	9
	mammary gland	0	0	0	0	1	0	0	0
	peripheral nerves	0	0	1	0	0	0	0	1
	Harderian gland	0	0	0	0	0	1	0	0
	bone	0	0	0	0	0	0	1	1

TABLE 14 HISTORICAL CONTROL DATA OF SELECTED NEOPLASTIC LESIONS IN JAPAN BIOASSAY RESEARCH CENTER : Crj:BDF_1 MALE MICE

Organs / Tumors	No. of animals examined	No. of animals bearing tumors	Incidence (%)	Min. - Max. (%)
Liver	<1346>			
Hepatocellular adenoma 1)		241	17.9	4 - 34
Hepatocellular carcinoma 2)		273	20.3	2 - 42
1)+2)		470	34.9	8 - 68

27 carcinogenicity studies examined in Japan Bioassay Research Center were used.
Study No. : 0044, 0060, 0062, 0064, 0066, 0068, 0096, 0105, 0116, 0140, 0159, 0163, 0190, 0206, 0211, 0225, 0243, 0268, 0270, 0279, 0285, 0297, 0319, 0329, 0343, 0348, 0366

TABLE 15 HISTORICAL CONTROL DATA OF SELECTED NEOPLASTIC LESIONS IN JAPAN BIOASSAY RESEARCH CENTER : Crj:BDF_1 FEMALE MICE

Organs / Tumors	No. of animals examined	No. of animals bearing tumors	Incidence (%)	Min. - Max. (%)
Lymph node	<1348>			
Malignant lymphoma		368	28.6	12 - 44
Mammary gland	<1348>			
Adenocarcinoma		24	1.8	0 - 8

27 carcinogenicity studies examined in Japan Bioassay Research Center were used.
Study No. : 0044, 0060, 0062, 0064, 0066, 0068, 0096, 0105, 0116, 0140, 0159, 0163, 0190, 0206, 0211, 0225, 0243, 0268, 0270, 0279, 0285, 0297, 0319, 0329, 0343, 0348, 0366

FIGURES

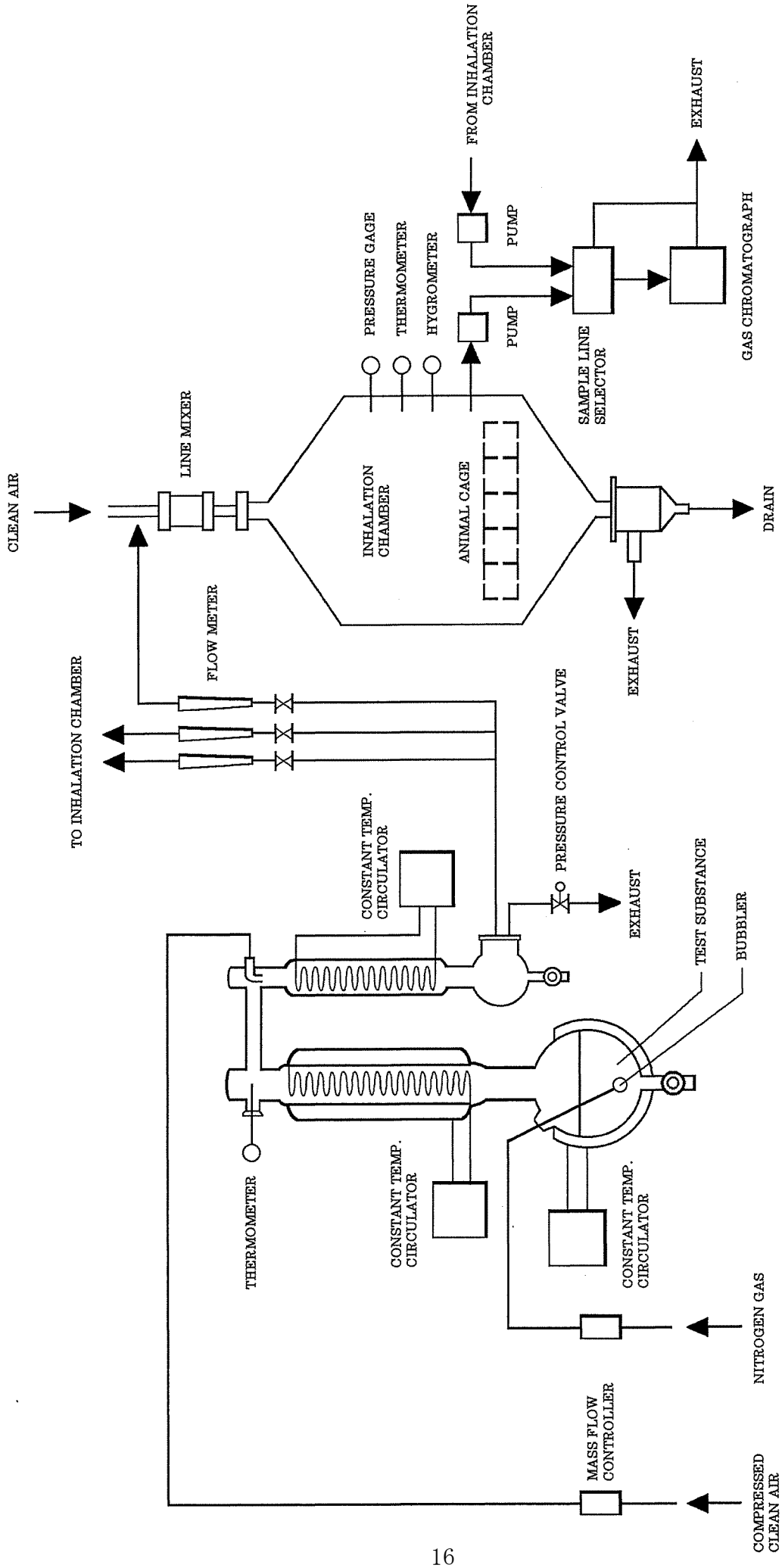

FIGURE 1 CYCLOHEXENE VAPOR GENERATION SYSTEM AND INHALATION SYSTEM

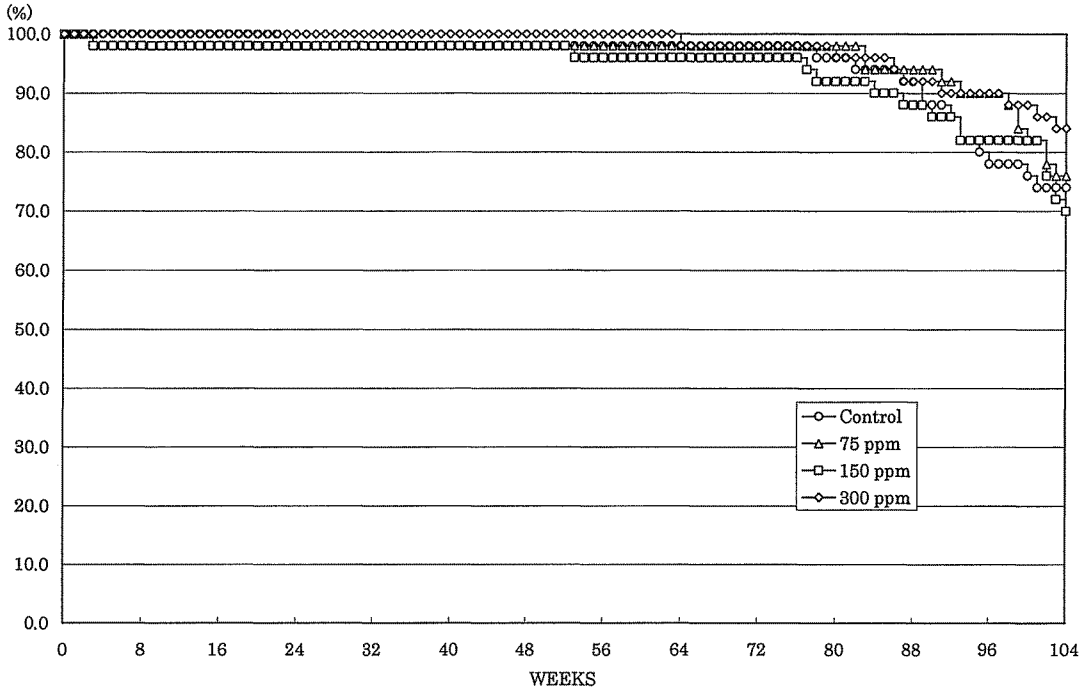

FIGURE 2 SURVIVAL ANIMAL RATE OF MALE MICE IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

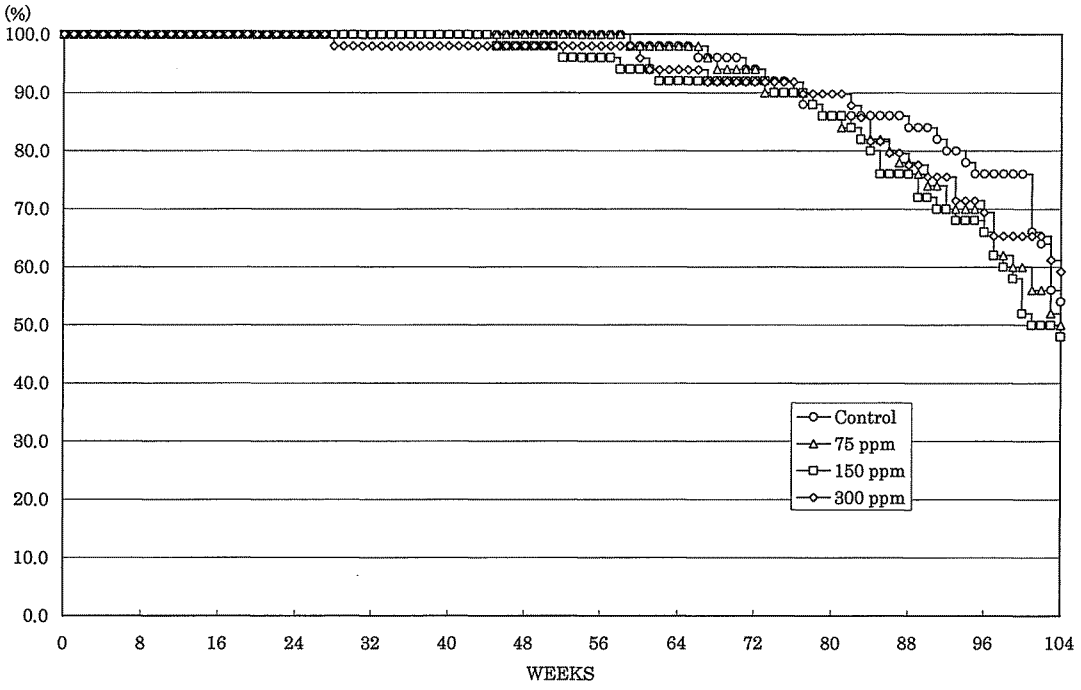

FIGURE 3 SURVIVAL ANIMAL RATE OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

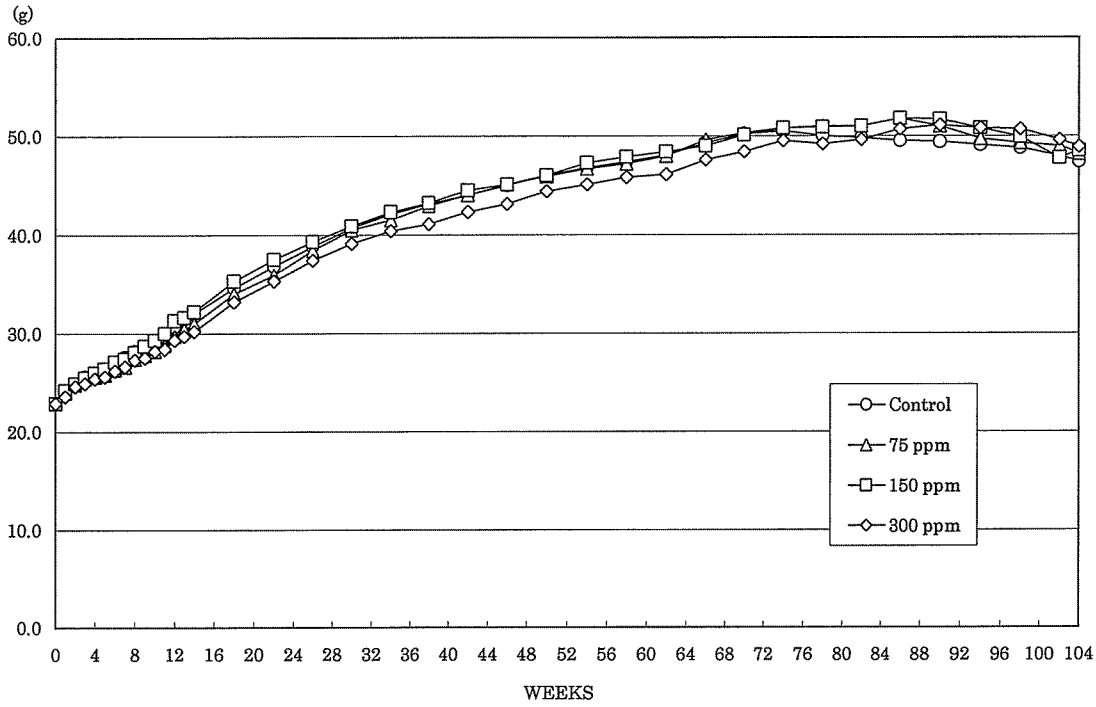

FIGURE 4 BODY WEIGHT CHANGES OF MALE MICE IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

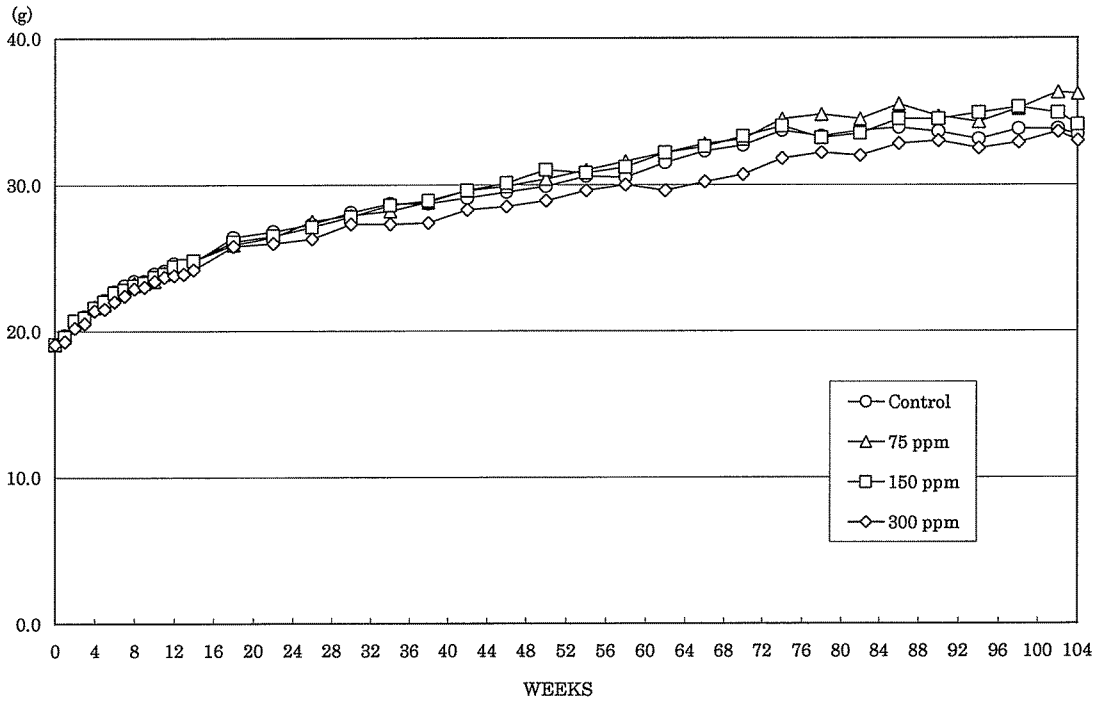

FIGURE 5 BODY WEIGHT CHANGES OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

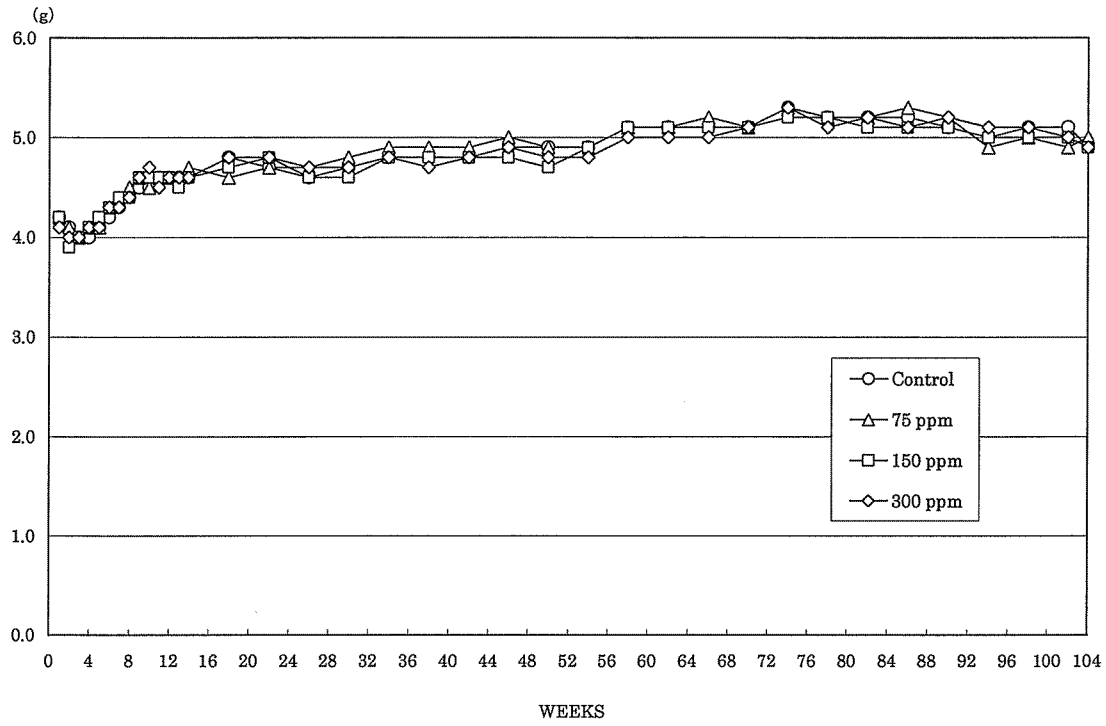

FIGURE 6 FOOD CONSUMPTION CHANGES OF MALE MICE IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE

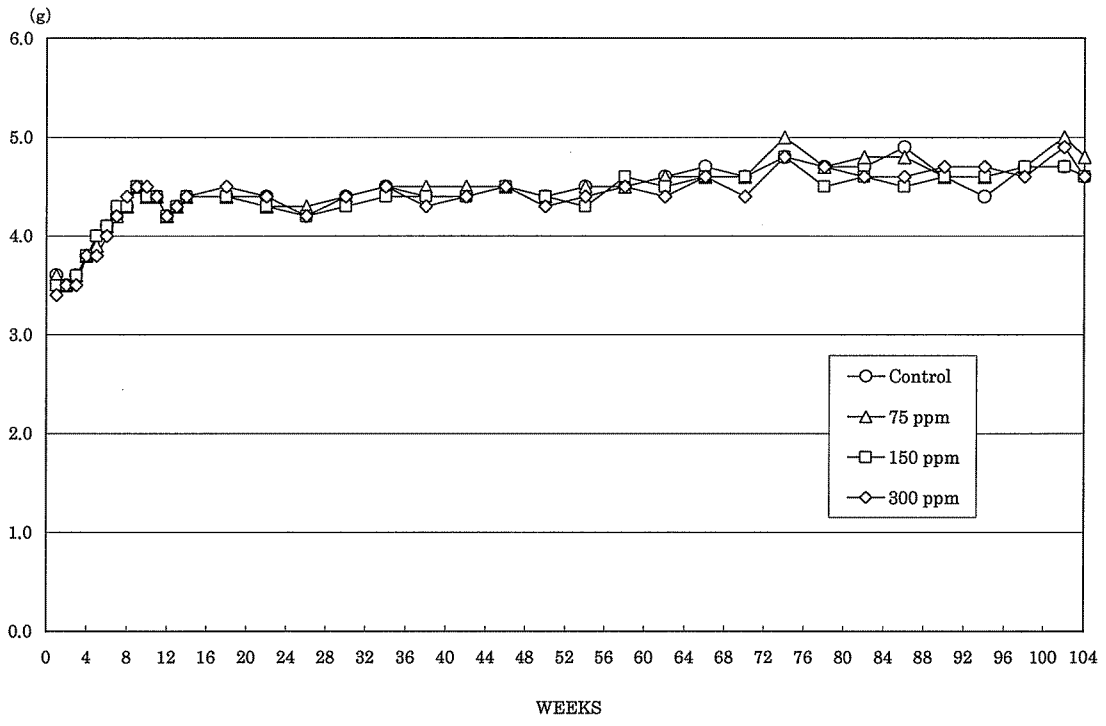

FIGURE 7 FOOD CONSUMPTION CHANGES OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF CYCLOHEXENE