Summary of Inhalation Carcinogenicity Study of Propiononitrile in B6D2F1 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 27, 2007.

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

Summary of Inhalation Carcinogenicity Study of Propiononitrile in B6D2F1 Mice

Purpose, materials and methods

Propiononitrile (CAS No. 107-12-0) is a colorless liquid with a boiling point of 97.2°C. It is soluble in water and miscible with alcohol and ether.

The carcinogenicity and chronic toxicity of propiononitrile were examined by inhalation exposure of groups of 50 B6D2F1/Crlj mice of both sexes to propiononitrile vapor at a target concentration of 0 (clean air), 12.5, 25 or 50 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. Propiononitrile 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. Propiononitrile vapor-air mixture was generated by bubbling clean air through the propiononitrile liquid and supplied to the inhalation exposure chambers. Air concentrations of propiononitrile 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 studies were conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) Good Laboratory Practice and with reference to the OECD Guideline for Testing of Chemicals 451 "Carcinogenicity Studies".

Results

No significant difference in survival rate, clinical sign, body weight or food consumption was found between any propiononitrile-exposed group of either sex and the respective control. Reticulocyte counts were increased in the 50 ppm-exposed females.

No significant increase in the incidence of neoplastic or non-neoplastic lesions was found in any propiononitrile-exposed group of either sex as compared with the respective control.

Conclusions

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

TABLES

TABLE 1 CONCENTRATIONS OF PROPIONONITRILE IN THE INHALATION CHAMBER OF THE 2-YEAR INHALATION STUDY

Group Name	Concentration(ppm) Mean ± S.D.
Control	0.0 ± 0.0
12.5 ppm	12.5 ± 0.2
25 ppm	25.1 ± 0.2
50 ppm	49.9 ± 1.1

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

Week on Study	Control		12.5 ppm			25 ppm			50 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	23.2 (50)	50 / 50	23.2 (50)	100	50 / 50	23.2 (50)	100	50 / 50	23.2 (50)	100	50 / 50
1	24.8 (50)	50 / 50	24.6 (50)	99	50 / 50	24.8 (50)	100	50 / 50	24.6 (50)	99	50 / 50
2	25.8 (50)	50 / 50	25.5 (50)	99	50 / 50	25.9 (50)	100	50 / 50	25.7 (50)	100	50 / 50
3	26.6 (50)	50 / 50	26.3 (49)	99	49 / 50	26.7 (50)	100	50 / 50	26.5 (50)	100	50 / 50
4	27.4 (50)	50 / 50	27.2 (49)	99	49 / 50	27.1 (50)	99	50 / 50	26.8 (50)	98	50 / 50
5	28.0 (50)	50 / 50	27.6 (49)	99	49 / 50	27.8 (50)	99	50 / 50	27.5 (50)	98	50 / 50
6	28.5 (50)	50 / 50	27.8 (49)	98	49 / 50	28.3 (50)	99	50 / 50	27.7 (50)	97	50 / 50
7	28.9 (50)	50 / 50	28.3 (49)	98	49 / 50	28.6 (50)	99	50 / 50	28.0 (50)	97	50 / 50
8	29.5 (50)	50 / 50	28.9 (49)	98	49 / 50	29.4 (50)	100	50 / 50	28.6 (49)	97	49 / 50
9	30.1 (50)	50 / 50	29.7 (49)	99	49 / 50	30.1 (50)	100	50 / 50	29.5 (49)	98	49 / 50
10	30.8 (50)	50 / 50	30.3 (49)	98	49 / 50	30.7 (50)	100	50 / 50	30.0 (49)	97	49 / 50
11	31.2 (50)	50 / 50	30.5 (49)	98	49 / 50	31.1 (50)	100	50 / 50	29.9 (49)	96	49 / 50
12	32.0 (50)	50 / 50	31.5 (49)	98	49 / 50	31.8 (50)	99	50 / 50	30.9 (49)	97	49 / 50
13	32.5 (50)	50 / 50	32.1 (49)	99	49 / 50	32.3 (50)	99	50 / 50	31.4 (49)	97	49 / 50
17	34.7 (50)	50 / 50	34.4 (49)	99	49 / 50	34.8 (50)	100	50 / 50	33.7 (49)	97	49 / 50
21	36.6 (50)	50 / 50	36.2 (49)	99	49 / 50	36.5 (50)	100	50 / 50	36.1 (48)	99	48 / 50
25	38.5 (50)	50 / 50	38.0 (49)	99	49 / 50	38.3 (50)	99	50 / 50	37.6 (48)	98	48 / 50
29	40.0 (50)	50 / 50	40.0 (49)	100	49 / 50	39.9 (49)	100	49 / 50	39.1 (48)	98	48 / 50
33	41.5 (50)	50 / 50	41.0 (49)	99	49 / 50	41.3 (49)	100	49 / 50	40.6 (48)	98	48 / 50
37	42.8 (50)	50 / 50	42.2 (49)	99	49 / 50	42.7 (49)	100	49 / 50	41.8 (48)	98	48 / 50
41	43.4 (50)	50 / 50	43.4 (49)	100	49 / 50	44.1 (49)	102	49 / 50	43.2 (48)	100	48 / 50
45	44.2 (50)	50 / 50	44.5 (49)	101	49 / 50	45.1 (49)	102	49 / 50	44.0 (48)	100	48 / 50
49	45.4 (48)	48 / 50	45.5 (49)	100	49 / 50	46.0 (49)	101	49 / 50	45.4 (47)	100	47 / 50
53	45.8 (48)	48 / 50	46.3 (48)	101	48 / 50	46.8 (48)	102	48 / 50	45.7 (45)	100	45 / 50
57	46.4 (48)	48 / 50	46.7 (48)	101	48 / 50	46.9 (48)	101	48 / 50	45.8 (45)	99	45 / 50
58	46.6 (48)	48 / 50	46.8 (48)	100	48 / 50	47.0 (48)	101	48 / 50	45.9 (45)	98	45 / 50
62	47.0 (48)	48 / 50	47.4 (48)	101	48 / 50	48.0 (48)	102	48 / 50	46.5 (45)	99	45 / 50
66	47.6 (48)	48 / 50	48.3 (48)	101	48 / 50	48.4 (48)	102	48 / 50	47.2 (45)	99	45 / 50
70	48.0 (48)	48 / 50	48.8 (47)	102	47 / 50	48.3 (48)	101	48 / 50	47.5 (45)	99	45 / 50
74	48.8 (48)	48 / 50	49.9 (46)	102	46 / 50	48.7 (48)	100	48 / 50	48.2 (45)	99	45 / 50
78	49.6 (47)	47 / 50	50.6 (44)	102	44 / 50	49.7 (46)	100	46 / 50	49.2 (44)	99	44 / 50
82	49.6 (46)	46 / 50	50.6 (42)	102	42 / 50	50.1 (42)	101	42 / 50	49.1 (44)	99	44 / 50
86	49.6 (44)	44 / 50	50.7 (42)	102	42 / 50	50.1 (40)	101	40 / 50	49.0 (42)	99	42 / 50
90	49.5 (43)	43 / 50	51.5 (40)	104	40 / 50	49.9 (40)	101	40 / 50	48.3 (40)	98	40 / 50
94	50.0 (40)	40 / 50	51.4 (40)	103	40 / 50	49.6 (40)	99	40 / 50	48.1 (38)	96	38 / 50
98	48.9 (39)	39 / 50	49.8 (40)	102	40 / 50	49.7 (38)	102	38 / 50	48.4 (34)	99	34 / 50
102	47.7 (37)	37 / 50	50.4 (36)	106	36 / 50	49.0 (34)	103	34 / 50	48.2 (32)	101	32 / 50
104	47.3 (35)	35 / 50	49.9 (35)	105	35 / 50	48.6 (34)	103	34 / 50	48.0 (31)	101	31 / 50

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

TABLE 3 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF PROPIONONITRILE

Week on Study	Control		12.5 ppm			25 ppm			50 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	19.1 (50)	50 / 50	19.1 (50)	100	50 / 50	19.1 (50)	100	50 / 50	19.1 (50)	100	50 / 50
1	20.2 (50)	50 / 50	20.1 (50)	100	50 / 50	20.1 (50)	100	50 / 50	20.1 (50)	100	50 / 50
2	21.2 (50)	50 / 50	20.9 (50)	99	50 / 50	20.9 (50)	99	50 / 50	20.9 (50)	99	50 / 50
3	21.6 (50)	50 / 50	21.1 (50)	98	50 / 50	21.4 (50)	99	50 / 50	21.5 (50)	100	50 / 50
4	22.4 (50)	50 / 50	21.9 (50)	98	50 / 50	21.9 (50)	98	50 / 50	21.9 (50)	98	50 / 50
5	22.7 (50)	50 / 50	22.3 (50)	98	50 / 50	22.5 (50)	99	50 / 50	22.6 (50)	100	50 / 50
6	23.2 (50)	50 / 50	22.8 (50)	98	50 / 50	22.8 (50)	98	50 / 50	22.9 (50)	99	50 / 50
7	23.7 (50)	50 / 50	23.2 (50)	98	50 / 50	23.4 (50)	99	50 / 50	23.0 (50)	97	50 / 50
8	24.0 (50)	50 / 50	23.4 (50)	98	50 / 50	23.9 (50)	100	50 / 50	23.5 (50)	98	50 / 50
9	24.0 (50)	50 / 50	23.8 (50)	99	50 / 50	24.1 (50)	100	50 / 50	24.0 (50)	100	50 / 50
10	24.0 (50)	50 / 50	23.9 (50)	100	50 / 50	24.2 (50)	101	50 / 50	24.1 (50)	100	50 / 50
11	24.5 (50)	50 / 50	24.1 (50)	98	50 / 50	24.4 (50)	100	50 / 50	23.8 (50)	97	50 / 50
12	25.1 (50)	50 / 50	24.5 (50)	98	50 / 50	25.0 (50)	100	50 / 50	24.6 (50)	98	50 / 50
13	25.1 (50)	50 / 50	25.0 (50)	100	50 / 50	25.2 (50)	100	50 / 50	24.8 (50)	99	50 / 50
17	26.2 (50)	50 / 50	26.4 (50)	101	50 / 50	26.1 (50)	100	50 / 50	25.9 (50)	99	50 / 50
21	27.1 (50)	50 / 50	27.1 (50)	100	50 / 50	27.6 (50)	102	50 / 50	27.0 (50)	100	50 / 50
25	28.1 (50)	50 / 50	27.8 (50)	99	50 / 50	28.4 (50)	101	50 / 50	27.5 (50)	98	50 / 50
29	29.0 (50)	50 / 50	28.9 (50)	100	50 / 50	29.3 (50)	101	50 / 50	28.6 (50)	99	50 / 50
33	29.2 (50)	50 / 50	29.1 (50)	100	50 / 50	29.5 (50)	101	50 / 50	28.9 (50)	99	50 / 50
37	29.8 (50)	50 / 50	29.9 (50)	100	50 / 50	30.0 (50)	101	50 / 50	29.3 (50)	98	50 / 50
41	30.1 (49)	49 / 50	30.1 (50)	100	50 / 50	30.8 (50)	102	50 / 50	30.2 (50)	100	50 / 50
45	31.2 (49)	49 / 50	31.0 (50)	99	50 / 50	31.2 (50)	100	50 / 50	30.6 (50)	98	50 / 50
49	31.5 (49)	49 / 50	31.6 (50)	100	50 / 50	32.1 (50)	102	50 / 50	31.5 (50)	100	50 / 50
53	31.2 (49)	49 / 50	31.4 (50)	101	50 / 50	31.9 (50)	102	50 / 50	31.2 (50)	100	50 / 50
57	32.0 (49)	49 / 50	31.7 (49)	99	49 / 50	32.0 (50)	100	50 / 50	31.2 (50)	98	50 / 50
58	31.8 (49)	49 / 50	31.4 (49)	99	49 / 50	31.7 (50)	100	50 / 50	31.3 (50)	98	50 / 50
62	32.5 (49)	49 / 50	31.9 (49)	98	49 / 50	32.7 (48)	101	48 / 50	31.8 (50)	98	50 / 50
66	33.0 (47)	47 / 50	32.7 (48)	99	48 / 50	33.1 (47)	100	47 / 50	31.8 (50)	96	50 / 50
70	33.0 (47)	47 / 50	32.5 (47)	98	47 / 50	34.0 (47)	103	47 / 50	32.0 (50)	97	50 / 50
74	33.5 (46)	46 / 50	33.1 (46)	99	46 / 50	33.7 (45)	101	45 / 50	32.8 (49)	98	49 / 50
78	34.4 (45)	45 / 50	33.7 (42)	98	42 / 50	35.0 (45)	102	45 / 50	33.6 (47)	98	47 / 50
82	34.3 (45)	45 / 50	33.3 (40)	97	40 / 50	34.8 (42)	101	42 / 50	33.4 (45)	97	45 / 50
86	34.6 (44)	44 / 50	33.7 (39)	97	39 / 50	34.8 (38)	101	38 / 50	33.8 (44)	98	44 / 50
90	33.9 (41)	41 / 50	33.3 (37)	98	37 / 50	34.8 (36)	103	36 / 50	33.7 (41)	99	41 / 50
94	34.3 (38)	38 / 50	33.6 (35)	98	35 / 50	35.1 (36)	102	36 / 50	33.8 (38)	99	38 / 50
98	34.0 (32)	32 / 50	33.5 (31)	99	31 / 50	34.9 (32)	103	32 / 50	33.8 (34)	99	34 / 50
102	35.3 (29)	29 / 50	35.1 (29)	99	29 / 50	34.8 (28)	99	28 / 50	34.3 (29)	97	29 / 50
104	35.4 (28)	28 / 50	34.4 (28)	97	28 / 50	34.0 (26)	96	26 / 50	35.0 (26)	99	26 / 50

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

TABLE 4 FOOD CONSUMPTION CHANGES OF MALE MICE IN THE 2-YEAR INHALATION STUDY OF PROPIONONITRILE

Week on Study	Control		12.5 ppm			25 ppm			50 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	4.1 (50)	50 / 50	4.1 (50)	100	50 / 50	4.2 (50)	102	50 / 50	4.2 (50)	102	50 / 50
2	4.1 (50)	50 / 50	4.0 (50)	98	50 / 50	4.2 (50)	102	50 / 50	4.1 (50)	100	50 / 50
3	4.1 (50)	50 / 50	4.1 (49)	100	49 / 50	4.3 (50)	105	50 / 50	4.1 (50)	100	50 / 50
4	4.2 (50)	50 / 50	4.3 (49)	102	49 / 50	4.2 (50)	100	50 / 50	4.2 (50)	100	50 / 50
5	4.2 (50)	50 / 50	4.2 (49)	100	49 / 50	4.2 (50)	100	50 / 50	4.2 (50)	100	50 / 50
6	4.3 (50)	50 / 50	4.3 (49)	100	49 / 50	4.3 (50)	100	50 / 50	4.2 (50)	98	50 / 50
7	4.3 (50)	50 / 50	4.4 (49)	102	49 / 50	4.3 (50)	100	50 / 50	4.3 (50)	100	50 / 50
8	4.4 (50)	50 / 50	4.5 (49)	102	49 / 50	4.4 (50)	100	50 / 50	4.4 (49)	100	49 / 50
9	4.4 (50)	50 / 50	4.5 (49)	102	49 / 50	4.4 (50)	100	50 / 50	4.5 (49)	102	49 / 50
10	4.4 (50)	50 / 50	4.6 (49)	105	49 / 50	4.5 (50)	102	50 / 50	4.4 (49)	100	49 / 50
11	4.5 (50)	50 / 50	4.5 (49)	100	49 / 50	4.5 (50)	100	50 / 50	4.5 (49)	100	49 / 50
12	4.5 (50)	50 / 50	4.6 (49)	102	49 / 50	4.5 (50)	100	50 / 50	4.5 (49)	100	49 / 50
13	4.5 (50)	50 / 50	4.5 (49)	100	49 / 50	4.5 (50)	100	50 / 50	4.5 (49)	100	49 / 50
17	4.5 (50)	50 / 50	4.6 (49)	102	49 / 50	4.5 (50)	100	50 / 50	4.5 (49)	100	49 / 50
21	4.5 (50)	50 / 50	4.6 (49)	102	49 / 50	4.5 (50)	100	50 / 50	4.6 (48)	102	48 / 50
25	4.5 (50)	50 / 50	4.6 (49)	102	49 / 50	4.6 (50)	102	50 / 50	4.6 (48)	102	48 / 50
29	4.6 (50)	50 / 50	4.8 (49)	104	49 / 50	4.7 (49)	102	49 / 50	4.7 (48)	102	48 / 50
33	4.7 (50)	50 / 50	4.8 (49)	102	49 / 50	4.8 (49)	102	49 / 50	4.8 (48)	102	48 / 50
37	4.7 (50)	50 / 50	4.9 (49)	104	49 / 50	4.8 (49)	102	49 / 50	4.9 (48)	104	48 / 50
41	4.7 (50)	50 / 50	4.8 (49)	102	49 / 50	4.8 (49)	102	49 / 50	4.8 (48)	102	48 / 50
45	4.7 (50)	50 / 50	4.8 (49)	102	49 / 50	4.8 (49)	102	49 / 50	4.8 (48)	102	48 / 50
49	4.8 (48)	48 / 50	4.9 (49)	102	49 / 50	4.8 (49)	100	49 / 50	4.8 (47)	100	47 / 50
53	4.8 (48)	48 / 50	4.9 (48)	102	48 / 50	4.9 (48)	102	48 / 50	4.8 (45)	100	45 / 50
57	4.8 (48)	48 / 50	4.9 (48)	102	48 / 50	4.9 (48)	102	48 / 50	4.9 (45)	102	45 / 50
58	4.9 (48)	48 / 50	4.9 (48)	100	48 / 50	4.9 (48)	100	48 / 50	4.9 (45)	100	45 / 50
62	5.0 (48)	48 / 50	5.0 (48)	100	48 / 50	5.0 (48)	100	48 / 50	4.9 (45)	98	45 / 50
66	5.0 (48)	48 / 50	5.0 (48)	100	48 / 50	5.0 (48)	100	48 / 50	5.0 (45)	100	45 / 50
70	5.1 (48)	48 / 50	5.1 (47)	100	47 / 50	5.1 (48)	100	48 / 50	5.1 (45)	100	45 / 50
74	5.2 (48)	48 / 50	5.2 (46)	100	46 / 50	5.3 (48)	102	48 / 50	5.2 (45)	100	45 / 50
78	5.1 (47)	47 / 50	5.1 (44)	100	44 / 50	5.1 (46)	100	46 / 50	5.1 (44)	100	44 / 50
82	5.2 (46)	46 / 50	5.2 (42)	100	42 / 50	5.2 (42)	100	42 / 50	5.2 (44)	100	44 / 50
86	5.1 (44)	44 / 50	5.1 (42)	100	42 / 50	5.3 (40)	104	40 / 50	5.2 (42)	102	42 / 50
90	5.1 (43)	43 / 50	5.4 (40)	106	40 / 50	5.3 (40)	104	40 / 50	5.2 (40)	102	40 / 50
94	5.0 (40)	40 / 50	5.1 (40)	102	40 / 50	5.2 (40)	104	40 / 50	5.2 (38)	104	38 / 50
98	4.9 (39)	39 / 50	4.9 (40)	100	40 / 50	5.1 (38)	104	38 / 50	5.1 (34)	104	34 / 50
102	5.0 (37)	37 / 50	5.2 (36)	104	36 / 50	5.1 (34)	102	34 / 50	5.1 (32)	102	32 / 50
104	4.9 (35)	35 / 50	5.2 (35)	106	35 / 50	5.1 (34)	104	34 / 50	5.2 (31)	106	31 / 50

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

TABLE 5 FOOD CONSUMPTION CHANGES OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF PROPIONONITRILE

Week on Study	Control		12.5 ppm			25 ppm			50 ppm		
	Av. FC.	No. of Surviv. <50>	Av. FC.	% of cont.	No. of Surviv. <50>	Av. FC.	% of cont.	No. of Surviv. <50>	Av. FC.	% of cont.	No. of Surviv. <50>
1	3.5 (50)	50 / 50	3.6 (50)	103	50 / 50	3.5 (50)	100	50 / 50	3.6 (50)	103	50 / 50
2	3.5 (50)	50 / 50	3.5 (50)	100	50 / 50	3.5 (50)	100	50 / 50	3.5 (50)	100	50 / 50
3	3.6 (50)	50 / 50	3.6 (50)	100	50 / 50	3.6 (50)	100	50 / 50	3.6 (50)	100	50 / 50
4	3.8 (50)	50 / 50	3.9 (50)	103	50 / 50	3.8 (50)	100	50 / 50	3.8 (50)	100	50 / 50
5	3.8 (50)	50 / 50	3.9 (50)	103	50 / 50	3.8 (50)	100	50 / 50	3.8 (50)	100	50 / 50
6	3.9 (50)	50 / 50	4.0 (50)	103	50 / 50	4.1 (50)	105	50 / 50	3.9 (50)	100	50 / 50
7	4.1 (50)	50 / 50	4.2 (50)	102	50 / 50	4.1 (50)	100	50 / 50	4.0 (50)	98	50 / 50
8	4.2 (50)	50 / 50	4.3 (50)	102	50 / 50	4.1 (50)	98	50 / 50	4.2 (50)	100	50 / 50
9	4.2 (50)	50 / 50	4.2 (50)	100	50 / 50	4.1 (50)	98	50 / 50	4.2 (50)	100	50 / 50
10	4.2 (49)	50 / 50	4.3 (50)	102	50 / 50	4.2 (50)	100	50 / 50	4.1 (50)	98	50 / 50
11	4.2 (49)	50 / 50	4.3 (50)	102	50 / 50	4.2 (50)	100	50 / 50	4.2 (50)	100	50 / 50
12	4.3 (50)	50 / 50	4.3 (50)	100	50 / 50	4.2 (44)	98	50 / 50	4.2 (50)	98	50 / 50
13	4.2 (50)	50 / 50	4.2 (50)	100	50 / 50	4.1 (50)	98	50 / 50	4.2 (50)	100	50 / 50
17	4.2 (50)	50 / 50	4.4 (50)	105	50 / 50	4.3 (50)	102	50 / 50	4.2 (50)	100	50 / 50
21	4.3 (50)	50 / 50	4.4 (50)	102	50 / 50	4.3 (50)	100	50 / 50	4.3 (50)	100	50 / 50
25	4.4 (50)	50 / 50	4.4 (49)	100	50 / 50	4.3 (50)	98	50 / 50	4.3 (50)	98	50 / 50
29	4.6 (50)	50 / 50	4.6 (50)	100	50 / 50	4.5 (50)	98	50 / 50	4.5 (50)	98	50 / 50
33	4.5 (50)	50 / 50	4.6 (50)	102	50 / 50	4.6 (50)	102	50 / 50	4.6 (50)	102	50 / 50
37	4.5 (50)	50 / 50	4.7 (50)	104	50 / 50	4.6 (50)	102	50 / 50	4.6 (50)	102	50 / 50
41	4.4 (49)	49 / 50	4.5 (50)	102	50 / 50	4.5 (50)	102	50 / 50	4.5 (50)	102	50 / 50
45	4.6 (49)	49 / 50	4.7 (50)	102	50 / 50	4.6 (50)	100	50 / 50	4.4 (50)	96	50 / 50
49	4.5 (49)	49 / 50	4.5 (50)	100	50 / 50	4.5 (50)	100	50 / 50	4.5 (50)	100	50 / 50
53	4.4 (49)	49 / 50	4.5 (50)	102	50 / 50	4.4 (50)	100	50 / 50	4.4 (50)	100	50 / 50
57	4.5 (49)	49 / 50	4.4 (49)	98	49 / 50	4.4 (50)	98	50 / 50	4.4 (50)	98	50 / 50
58	4.4 (49)	49 / 50	4.4 (49)	100	49 / 50	4.3 (50)	98	50 / 50	4.4 (50)	100	50 / 50
62	4.5 (49)	49 / 50	4.5 (49)	100	49 / 50	4.6 (48)	102	48 / 50	4.5 (50)	100	50 / 50
66	4.6 (47)	47 / 50	4.5 (48)	98	48 / 50	4.5 (47)	98	47 / 50	4.6 (50)	100	50 / 50
70	4.6 (47)	47 / 50	4.6 (47)	100	47 / 50	4.7 (47)	102	47 / 50	4.5 (50)	98	50 / 50
74	4.7 (46)	46 / 50	4.6 (46)	98	46 / 50	4.8 (45)	102	45 / 50	4.7 (49)	100	49 / 50
78	4.6 (45)	45 / 50	4.4 (42)	96	42 / 50	4.6 (45)	100	45 / 50	4.6 (47)	100	47 / 50
82	4.7 (45)	45 / 50	4.5 (40)	96	40 / 50	4.8 (42)	102	42 / 50	4.6 (45)	98	45 / 50
86	4.7 (44)	44 / 50	4.7 (39)	100	39 / 50	4.6 (38)	98	38 / 50	4.7 (44)	100	44 / 50
90	4.6 (41)	41 / 50	4.8 (37)	104	37 / 50	4.7 (36)	102	36 / 50	4.8 (41)	104	41 / 50
94	4.6 (38)	38 / 50	4.8 (35)	104	35 / 50	4.8 (36)	104	36 / 50	4.8 (38)	104	38 / 50
98	4.6 (32)	32 / 50	4.6 (31)	100	31 / 50	4.5 (32)	98	32 / 50	4.7 (34)	102	34 / 50
102	4.8 (29)	29 / 50	5.0 (29)	104	29 / 50	5.0 (28)	104	28 / 50	4.8 (29)	100	29 / 50
104	4.9 (28)	28 / 50	4.9 (28)	100	28 / 50	4.7 (26)	96	26 / 50	4.9 (26)	100	26 / 50

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

TABLE 6 HEMATOLOGY OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF PROPIONONITRILE

Group Name	Control	12.5 ppm	25 ppm	50 ppm	
No. of examined animals	26	27	24	25	
RETICULOCYTE (%)	4.6 ± 5.5	3.9 ± 3.6	2.5 ± 0.7	5.0 ± 6.6	**

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

TABLE 7 URINALYSIS OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF PROPIONONITRILE

Group Name		Control	12.5 ppm	25 ppm	50 ppm
No. of examined animals		28	29	26	27
	Grade				
pH	5.0	0	0	0	0
	6.0	1	2	1	2
	6.5	1	1	1	4
	7.0	2	6	1	2
	7.5	14	8	4	1
	8.0	8	10	19	16
	8.5	2	2	0	2
	Chi square test			*	**

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

TABLE 8 INCIDENCES OF SELECTED NEOPLASTIC LESIONS OF MALE MICE IN THE 2-YEAR INHALATION STUDY OF PROPIONONITRILE

Group Name	Control	12.5 ppm	25 ppm	50 ppm	Peto test	Cochran-Armitage test
Number of examined animals	50	50	50	50		
liver	<50>	<50>	<50>	<50>		
hemangioma	1 (2 %)	0 (0 %)	2 (4 %)	3 (6 %)		
hemangiosarcoma	2 (4 %)	5 (10 %)	5 (10 %)	5 (10 %)		
hemangioma + hemangiosarcoma	3 (6 %)	5 (10 %)	7 (14 %)	8 (16 %)	↑	
Harderian gland	<50>	<50>	<50>	<50>		
adenoma	2 (4 %)	1 (2 %)	1 (2 %)	5 (10 %)	↑	

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 9 HISTORICAL CONTROL DATA OF SELECTED NEOPLASTIC LESIONS IN JAPAN BIOASSAY RESEARCH CENTER： B6D2F1/Crlj MALE MICE

Organs Tumors	No. of animals examined	No. of animals bearing tumor	Incidence (%)	Min. - Max. (%)
Liver	1796			
Hemangioma 1)		57	3.2	0 - 14
Hemangiosarcoma 2)		73	4.1	0 - 14
1) + 2)		130	7.2	0 - 16
Harderian gland	1795			
Adenoma		88	4.9	0 - 10

36 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, 0372, 0402, 0406, 0418, 0422, 0438, 0449, 0458, 0462

TABLE 10 CAUSE OF DEATH OF MICE IN THE 2-YEAR INHALATION STUDY OF PROPIONONITRILE

		Male				Female			
Group name		Control	12.5 ppm	25 ppm	50 ppm	Control	12.5 ppm	25 ppm	50 ppm
Number of dead or moribund animals		15	15	16	19	22	22	24	24
no microscopical confirmation		0	0	0	0	0	0	0	1
integumentary system lesion		0	0	0	1	0	0	0	0
renal lesion		0	0	0	0	1	0	0	0
reproductive system lesion		0	0	0	0	0	1	0	0
central nervous system lesion		0	0	0	0	1	0	0	0
urinary retention		1	2	2	0	0	0	0	0
amyloidosis		0	0	0	0	0	0	1	0
arteritis		1	0	0	0	0	0	0	0
hydronephrosis		2	3	1	5	0	0	0	0
tumor death ：	leukemia	0	3	3	3	9	11	12	9
	subcutis	0	2	1	1	0	0	0	2
	lung	3	1	1	1	0	0	0	0
	spleen	0	0	1	1	1	1	0	0
	salivary gland	2	0	0	0	0	0	0	0
	liver	4	4	5	3	3	1	2	2
	urinaly bladder	0	0	1	0	0	0	0	0
	pituitary gland	0	0	0	0	2	2	0	2
	thyroid	0	0	0	0	1	0	0	0
	adrenal gland	1	0	0	0	0	0	0	0
	epididymis	1	0	1	1	—	—	—	—
	uterus	—	—	—	—	4	6	9	8
	brain	0	0	0	1	0	0	0	0
	peripheral nerves	0	0	0	2	0	0	0	0

FIGURES

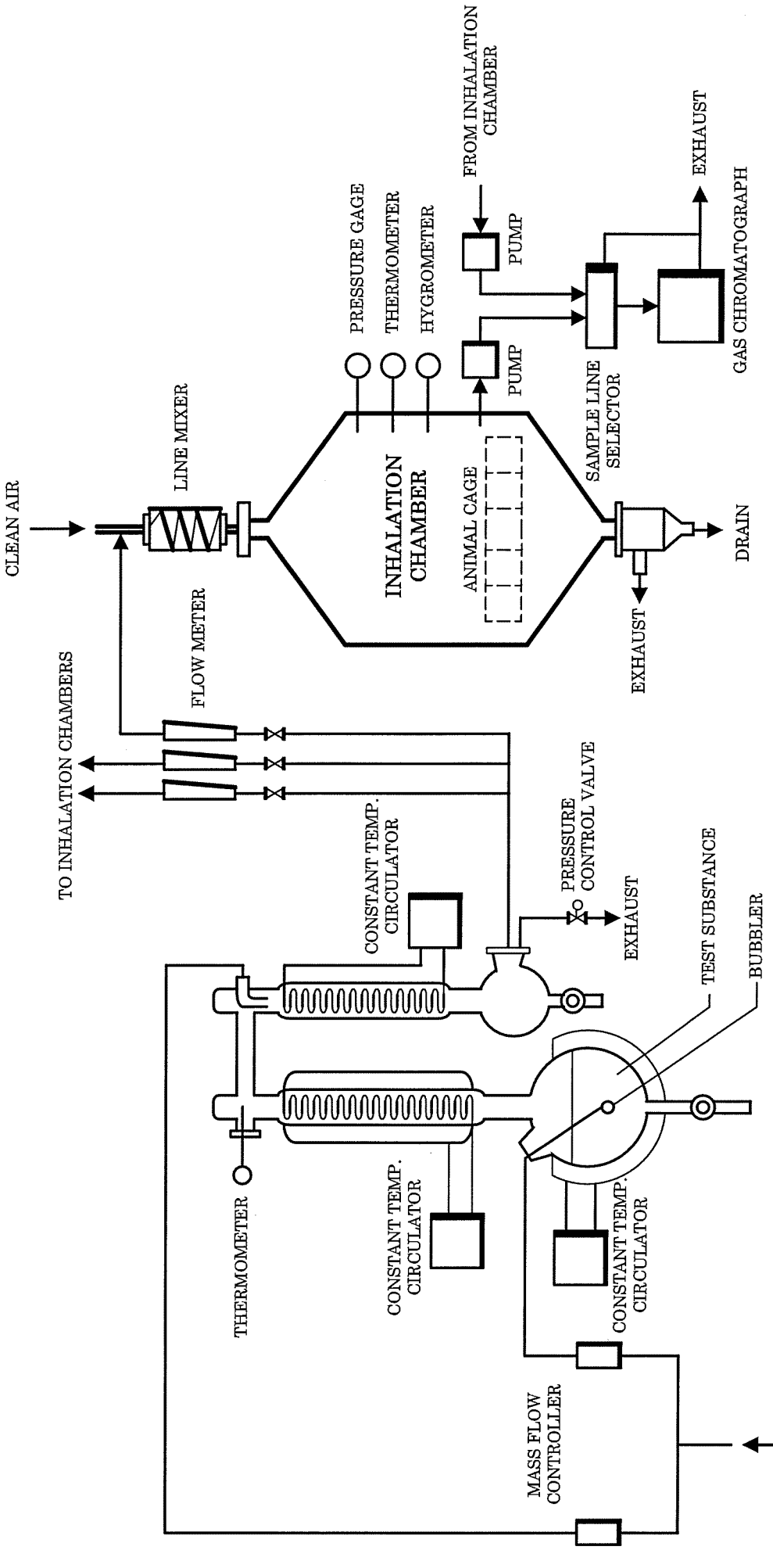

FIGURE 1 PROPIONONITRILE VAPOR GENERATION SYSTEM AND INHALATION SYSTEM

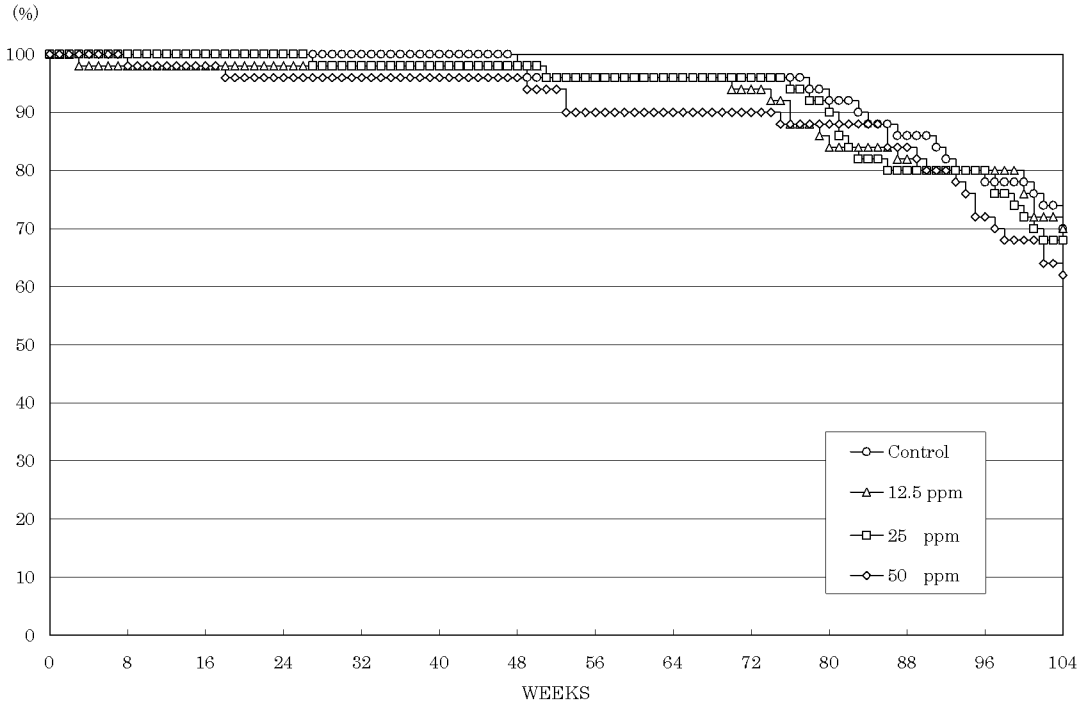

FIGURE 2 SURVIVAL ANIMAL RATE OF MALE MICE IN THE 2-YEAR INHALATION STUDY OF PROPIONONITRILE

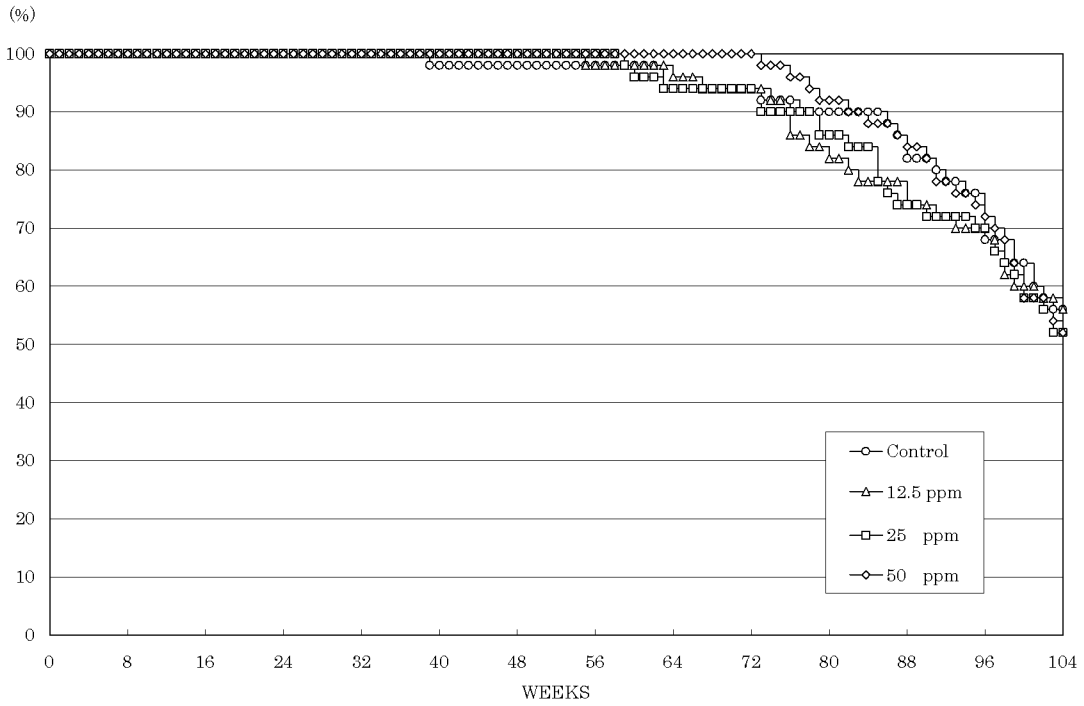

FIGURE 3 SURVIVAL ANIMAL RATE OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF PROPIONONITRILE

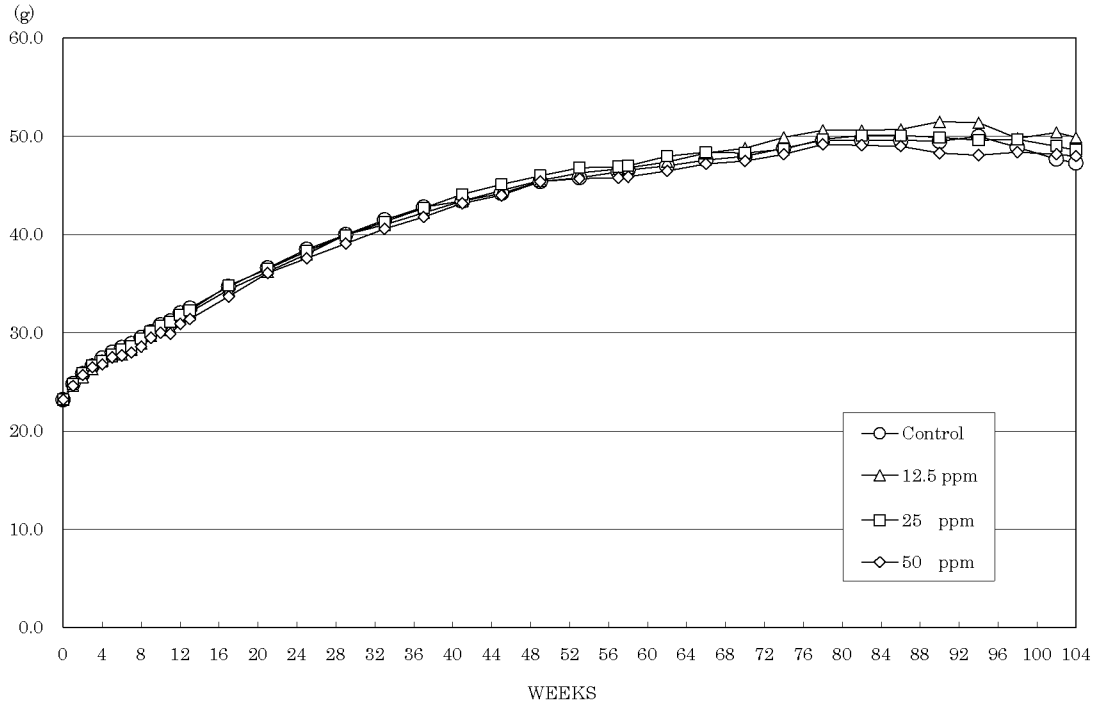

FIGURE 4 BODY WEIGHT CHANGES OF MALE MICE IN THE 2-YEAR INHALATION STUDY OF PROPIONONITRILE

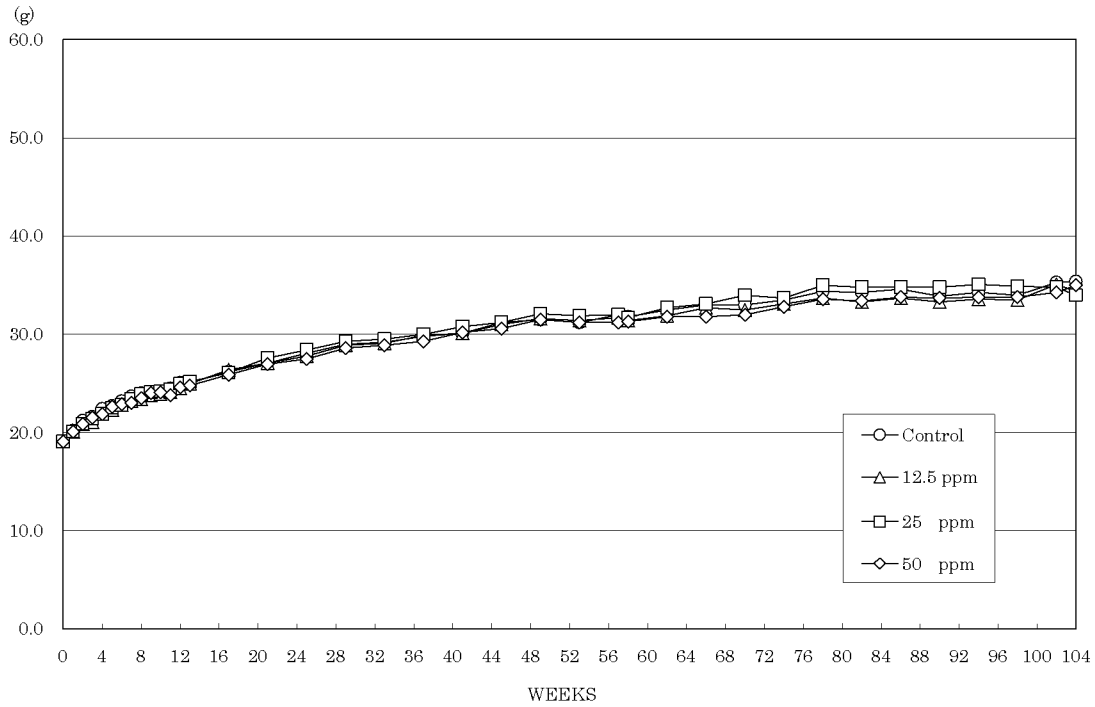

FIGURE 5 BODY WEIGHT CHANGES OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF PROPIONONITRILE

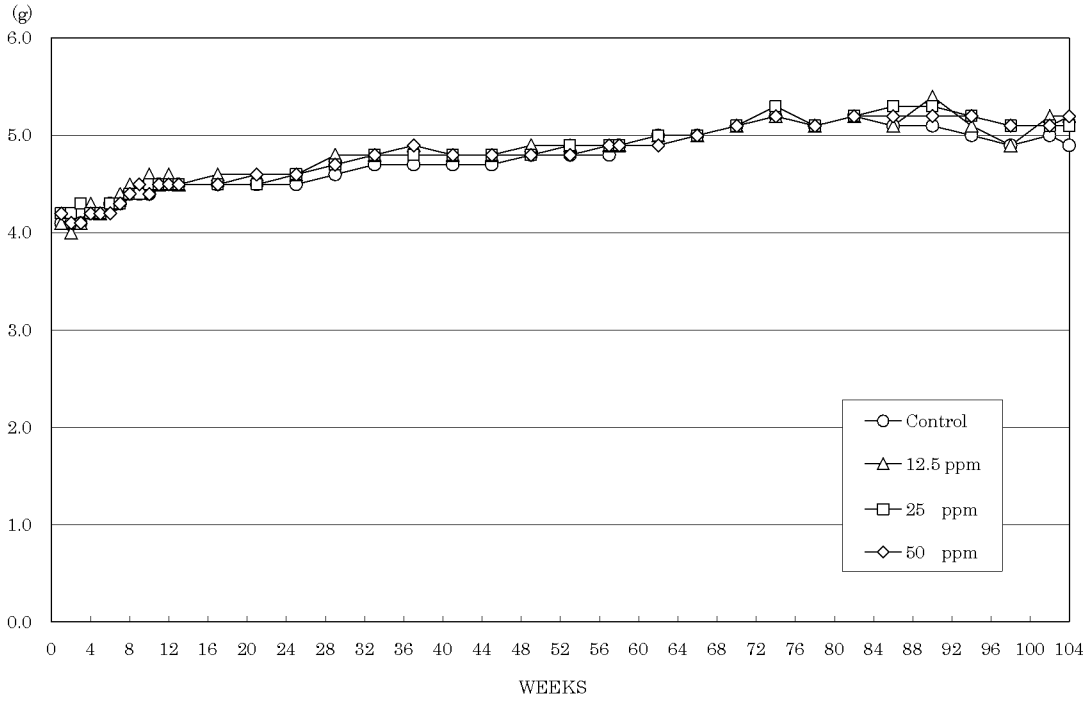

FIGURE 6 FOOD CONSUMPTION CHANGES OF MALE MICE IN THE 2-YEAR INHALATION STUDY OF PROPIONONITRILE

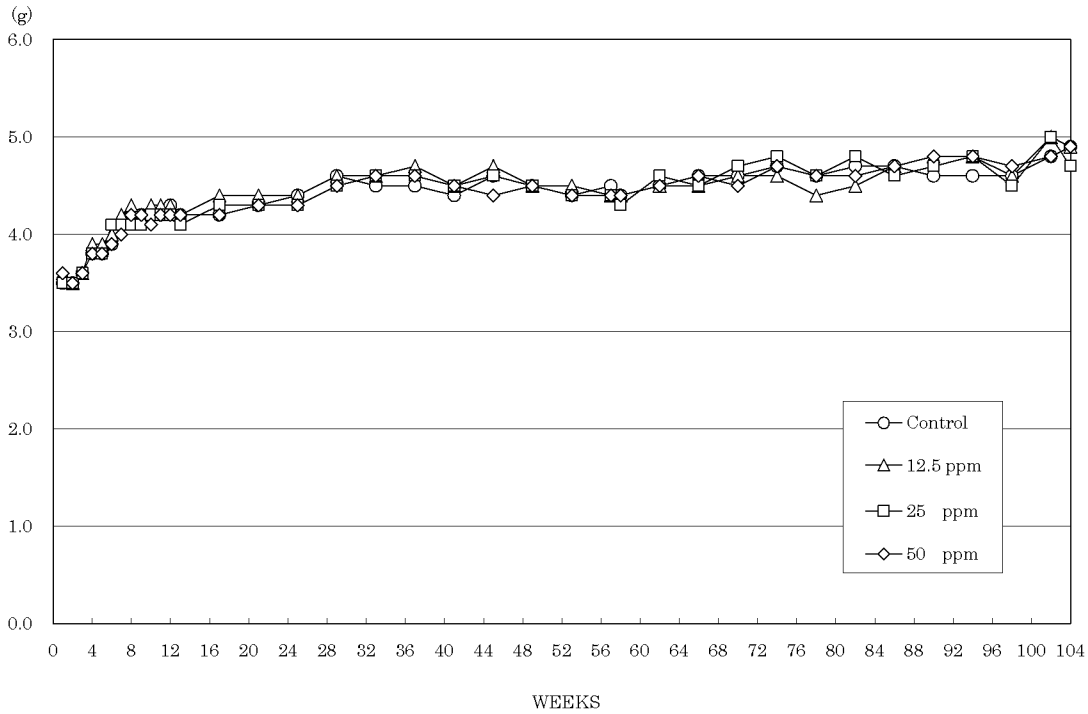

FIGURE 7 FOOD CONSUMPTION CHANGES OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF PROPIONONITRILE