Summary of Inhalation Carcinogenicity Study of 2-Methallyl Chloride 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 Labour 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 Labour of Japan on June 30 1998.

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

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Purpose, materials and methods

2-Methallyl chloride (MAC, 3-chloro-2-methylpropene, CAS No.563-47-3) is a colorless liquid with a boiling point of 71-72°C and a vapor pressure of 101.7 mm Hg at 20°C, and is insoluble in water, but soluble in chloroform, acetone, alcohol, ether and benzene.

The carcinogenicity and chronic toxicity of MAC were examined by inhalation exposure of groups of 50 F344/DuCrj (Fischer) rats of both sexes to MAC vapor at a target concentration of 0 (clean air), 50, 100 or 200 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. MAC 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. MAC vapor-air mixture was generated by bubbling clean air through the MAC liquid, and supplied to the inhalation exposure chambers. Air concentrations of MAC 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 with reference to the Organisation for Economic Co-operation and Development (OECD) Good Laboratory Practice and the OECD Guideline for Testing of Chemicals 451 "Carcinogenicity Studies".

Results

Survival rates of the MAC-exposed males were decreased at the end of the 2-year exposure period in an exposure concentration-related manner as compared with the male control, whereas the survival rates of the MAC-exposed females tended to increase over the female control. However, these changes occurring in both sexes were considered not to be related to the effects of exposure to MAC. Growth rates of the 100 and 200 ppm-exposed males and the 200 ppm-exposed females were slightly suppressed as compared with the respective controls.

The incidence of follicular cell adenomas in the thyroid was slightly increased in the MAC-exposed male groups, and the increased tumor incidence might possibly be related to the effects of exposure to MAC. As a non-neoplastic lesion in the nasal cavity, the incidence and severity of eosinophilic change in the olfactory epithelium were increased in an exposure concentration-related manner in all the MAC-exposed groups of both sexes. Since the eosinophilic change in the olfactory epithelium is known to be age-related, this nasal lesion was considered to be enhanced by the exposure to MAC. As an age-related lesion in the kidney, the incidences of chronic progressive nephropathy (chronic nephropathy) were suppressed in the females exposed to 100 and 200 ppm.

Conclusions

In rats, there was equivocal evidence of carcinogenic activity of MAC in males, based on the slightly increased incidence of follicular cell adenomas in the thyroid, which might possibly be related to the effects of exposure to MAC. There was no evidence of carcinogenic activity of MAC in females, based on no increase in the incidence of neoplastic lesions.

TABLES

TABLE 1 EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE 2-YEAR INHALATION STUDY OF 2-METHALLYL CHLORIDE

2-year study
<Method of Administration> Inhalation
<Number of Groups> Male 4, Female 4
<Size of Groups> 50 males and 50 females of each group
<Animals> Strain and Species F344/DuCrj (Fischer) rat Animal Source Charles River Japan, Inc. Duration Held Before Study 2 wk Age When Placed on Study 6 wk Age When Killed 110～111 wk
<Doses> Male : 0, 50, 100, or 200 ppm Female : 0, 50, 100, or 200 ppm
<Duration of Dosing> 6h/d, 5d/wk, for 104wk
<Animal Maintenance> Feed CRF-1 (Oriental Yeast Co., Ltd.) Sterilized by γ-ray Available *ad libitum* Water Filtrated and sterilized by ultraviolet ray Automatic watering system in duration of quarantine Available *ad libitum* Animal per Cage Single (stainless steel wire) Animal Room Environment Barrier system Temperature : 23±3℃ Fluorescent light 12h/d Chamber Environment Temperature : 22±2℃ Humidity : 55±15% Air changes :12±1/h Pressure :0～-15mmAq
<Type and Frequency of Observation> Clinical Sign Observed 1 per d Body Weight Weighed 1 per wk for 14wk Weighed 1 per 4wks thereafter Food Consumption Weighed 1 per wk for 14wk Weighed 1 per 4wks thereafter

TABLE 1 (continued) EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE 2-YEAR INHALATION STUDY OF 2-METHALLYL CHLORIDE

2-year study
<Hematology> Red blood cell (RBC), Hemoglobin, Hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular hemoglobin (MCH), Mean Corpuscular hemoglobin concentrate (MCHC), Platelet, White blood cell (WBC), Differential WBC. <Biochemistry> Total protein, Albumin, A/G ratio, Total bilirubin, Glucose, Total cholesterol Triglyceride, Phospholipid , Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT), Lactate dehydrogenase (LDH), Alkaline phosphatase (ALP), γ-Glutamyl transpeptidase (γ-GTP) , Creatine phosphokinase (CPK), Urea nitrogen, Creatinine , Sodium, Potassium, Chloride, Calcium, Inorganic phosphorus. <Urinalysis> pH, Protein, Glucose, Ketone body, Bilirubin, Occult blood, Urobilinogen. <Necropsy> Necropsy performed on all animals. <Organ Weight> Organ weight measurement performed on scheduled sacrificed animals. The following organs were weighed; brain, lung, liver, spleen, heart, kidney, adrenal, testis, ovary. <Histopathologic Examination> Histopathologic examination performed on all animals. The following organs were examined; skin, nasal cavity, trachea, lung, bone marrow, lymph node, thymus, spleen, heart, tongue, salivary gland, esophagus, stomach, small intestine, large intestine, liver, pancreas, kidney, urinary bladder, pituitary, thyroid, adrenal, testis, epididymis, seminal vesicle, prostate, ovary, uterus, vagina, mammary gland, brain, spinal cord, peripheral nerve, eye, Harderian gland, muscle, bone, other organs/tissues with gross lesions.

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

Week on Study	Control Av.Wt.	Control No.of Surviv. <50>	50 ppm Av.Wt.	50 ppm % of cont. <50>	50 ppm No.of Surviv.	100 ppm Av.Wt.	100 ppm % of cont. <50>	100 ppm No.of Surviv.	200 ppm Av.Wt.	200 ppm % of cont. <50>	200 ppm No.of Surviv.
0	119 (50)	50/50	118 (50)	99	50/50	119 (50)	100	50/50	119 (50)	100	50/50
1	122 (50)	50/50	122 (50)	100	50/50	123 (50)	101	50/50	123 (50)	101	50/50
1	149 (50)	50/50	149 (50)	100	50/50	151 (50)	101	50/50	146 (50)	98	50/50
2	183 (50)	50/50	184 (50)	101	50/50	185 (50)	101	50/50	177 (50)	97	50/50
3	209 (50)	50/50	211 (50)	101	50/50	211 (50)	101	50/50	200 (50)	96	50/50
4	229 (50)	50/50	234 (50)	102	50/50	231 (50)	101	50/50	216 (50)	94	50/50
5	246 (50)	50/50	252 (50)	102	50/50	246 (50)	100	50/50	230 (50)	93	50/50
6	264 (50)	50/50	267 (50)	101	50/50	261 (50)	99	50/50	243 (50)	92	50/50
7	280 (50)	50/50	282 (50)	101	50/50	275 (50)	98	50/50	257 (50)	92	50/50
8	291 (50)	50/50	295 (50)	101	50/50	287 (50)	99	50/50	267 (50)	92	50/50
9	304 (50)	50/50	306 (50)	101	50/50	299 (50)	98	50/50	278 (50)	91	50/50
10	314 (50)	50/50	313 (50)	100	50/50	305 (50)	97	50/50	287 (50)	91	50/50
11	322 (50)	50/50	321 (50)	100	50/50	313 (50)	97	50/50	295 (50)	92	50/50
12	328 (50)	50/50	328 (50)	100	50/50	320 (50)	98	50/50	301 (50)	92	50/50
13	337 (50)	50/50	338 (50)	100	50/50	329 (50)	98	50/50	310 (50)	92	50/50
14	344 (50)	50/50	345 (50)	100	50/50	336 (50)	98	50/50	317 (50)	92	50/50
18	372 (49)	49/50	372 (50)	100	50/50	362 (50)	97	50/50	337 (50)	91	50/50
22	389 (49)	49/50	388 (50)	100	50/50	379 (50)	97	50/50	348 (50)	89	50/50
26	398 (49)	49/50	397 (50)	100	50/50	385 (50)	97	50/50	357 (50)	90	50/50
30	403 (49)	49/50	403 (50)	100	50/50	391 (50)	97	50/50	365 (50)	91	50/50
34	420 (49)	49/50	419 (50)	100	50/50	404 (50)	96	50/50	381 (50)	91	50/50
38	436 (49)	49/50	438 (50)	100	50/50	420 (50)	96	50/50	394 (50)	90	50/50
42	444 (49)	49/50	446 (50)	100	50/50	429 (50)	97	50/50	402 (50)	91	50/50
46	450 (49)	49/50	449 (50)	100	50/50	433 (50)	96	50/50	403 (50)	90	50/50
50	454 (49)	49/50	458 (50)	101	50/50	440 (50)	97	50/50	412 (50)	91	50/50
54	463 (49)	49/50	466 (50)	101	50/50	445 (50)	96	50/50	418 (50)	90	50/50
58	470 (49)	49/50	471 (50)	100	50/50	451 (50)	96	50/50	420 (50)	89	50/50
62	469 (49)	49/50	474 (49)	101	49/50	452 (50)	96	50/50	423 (50)	90	50/50
66	469 (49)	49/50	476 (49)	101	49/50	452 (50)	96	50/50	422 (50)	90	50/50
70	470 (49)	49/50	476 (49)	101	49/50	454 (50)	97	50/50	425 (50)	90	50/50
74	471 (48)	48/50	477 (49)	101	49/50	455 (50)	97	50/50	425 (50)	90	50/50
78	471 (48)	48/50	474 (48)	101	48/50	452 (50)	96	50/50	424 (50)	90	50/50
82	466 (47)	47/50	474 (48)	102	48/50	452 (50)	97	50/50	424 (49)	91	49/50
86	464 (47)	47/50	472 (47)	102	47/50	458 (49)	99	49/50	428 (46)	92	46/50
90	457 (47)	47/50	471 (45)	103	45/50	460 (49)	101	49/50	427 (44)	93	44/50
94	446 (46)	46/50	459 (44)	103	44/50	444 (44)	100	44/50	421 (41)	94	41/50
98	432 (46)	45/50	450 (42)	104	42/50	436 (43)	101	43/50	408 (38)	94	38/50
102	430 (41)	41/50	441 (38)	103	38/50	420 (37)	98	37/50	405 (32)	94	32/50
104	423 (39)	39/50	430 (36)	102	35/50	417 (33)	99	33/50	402 (30)	95	30/50

< >:No.of effective animals,( ):No.of measured animals Av.Wt.: g

TABLE 3 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF 2-METHALLYL CHLORIDE

Week on Study	Control Av.Wt.	Control No.of Surviv. <50>	50 ppm Av.Wt.	50 ppm % of cont. <50>	50 ppm No.of Surviv.	100 ppm Av.Wt.	100 ppm % of cont. <50>	100 ppm No.of Surviv.	200 ppm Av.Wt.	200 ppm % of cont. <50>	200 ppm No.of Surviv.
0	97 (50)	50/50	97 (50)	100	50/50	97 (50)	100	50/50	97 (50)	100	50/50
1	99 (50)	50/50	99 (50)	100	50/50	100 (50)	101	50/50	100 (50)	101	50/50
1	115 (50)	50/50	113 (50)	98	50/50	115 (50)	100	50/50	112 (50)	97	50/50
2	129 (50)	50/50	129 (50)	100	50/50	130 (50)	101	50/50	126 (50)	98	50/50
3	139 (50)	50/50	140 (50)	101	50/50	141 (50)	101	50/50	136 (50)	98	50/50
4	146 (50)	50/50	148 (50)	101	50/50	149 (50)	102	50/50	142 (50)	97	50/50
5	154 (50)	50/50	156 (50)	101	50/50	156 (50)	101	50/50	148 (50)	96	50/50
6	163 (50)	50/50	162 (50)	99	50/50	162 (50)	99	50/50	154 (50)	94	50/50
7	170 (50)	50/50	170 (50)	100	50/50	169 (50)	99	50/50	162 (50)	95	50/50
8	176 (50)	50/50	175 (50)	99	50/50	173 (50)	98	50/50	167 (50)	95	50/50
9	180 (50)	50/50	179 (50)	99	50/50	177 (50)	98	50/50	170 (50)	94	50/50
10	184 (50)	50/50	183 (50)	99	50/50	180 (50)	98	50/50	174 (50)	95	50/50
11	188 (50)	50/50	188 (50)	100	50/50	184 (50)	98	50/50	178 (50)	95	50/50
12	190 (50)	50/50	190 (50)	100	50/50	187 (50)	98	50/50	181 (50)	95	50/50
13	194 (50)	50/50	194 (50)	100	50/50	191 (50)	98	50/50	184 (50)	95	50/50
14	195 (50)	50/50	195 (50)	100	50/50	193 (50)	99	50/50	184 (50)	94	50/50
18	203 (50)	50/50	204 (50)	100	50/50	200 (50)	99	50/50	193 (50)	95	50/50
22	211 (50)	50/50	210 (50)	100	50/50	207 (50)	98	50/50	200 (50)	95	50/50
26	223 (50)	50/50	223 (50)	100	50/50	219 (50)	98	50/50	212 (50)	95	50/50
30	229 (50)	50/50	231 (50)	101	50/50	225 (50)	98	50/50	217 (50)	95	50/50
34	237 (50)	50/50	238 (50)	100	50/50	232 (50)	98	50/50	225 (49)	95	49/50
38	243 (50)	50/50	248 (50)	102	50/50	238 (50)	98	50/50	230 (49)	95	49/50
42	247 (50)	50/50	251 (50)	102	50/50	242 (50)	98	50/50	233 (49)	94	49/50
46	251 (50)	50/50	257 (50)	102	50/50	247 (50)	98	50/50	238 (49)	95	49/50
50	259 (50)	50/50	266 (50)	103	50/50	253 (50)	98	50/50	242 (49)	93	49/50
54	265 (50)	50/50	272 (50)	103	50/50	259 (50)	98	50/50	249 (49)	94	49/50
58	271 (50)	50/50	280 (50)	103	50/50	265 (50)	98	50/50	253 (48)	93	48/50
62	278 (49)	49/50	285 (50)	103	50/50	270 (50)	97	50/50	259 (48)	93	48/50
66	285 (49)	49/50	292 (50)	102	50/50	276 (50)	97	50/50	265 (48)	93	48/50
70	290 (48)	48/50	298 (49)	103	49/50	282 (50)	97	50/50	270 (48)	93	48/50
74	291 (48)	48/50	306 (47)	105	47/50	287 (50)	99	50/50	275 (48)	95	48/50
78	297 (47)	47/50	306 (47)	103	47/50	290 (50)	98	50/50	279 (48)	94	48/50
82	300 (47)	47/50	312 (47)	104	47/50	294 (50)	98	50/50	286 (48)	95	48/50
86	307 (45)	45/50	316 (47)	103	47/50	303 (49)	99	49/50	292 (48)	95	48/50
90	306 (45)	45/50	322 (45)	105	45/50	308 (49)	101	49/50	290 (48)	95	47/50
94	306 (43)	41/50	323 (45)	106	45/50	310 (48)	101	48/50	296 (46)	97	46/50
98	311 (39)	39/50	322 (45)	104	44/50	312 (48)	100	48/50	295 (46)	95	46/50
102	312 (38)	38/50	324 (41)	104	40/50	311 (47)	100	47/50	299 (44)	96	44/50
104	309 (38)	38/50	322 (40)	104	40/50	311 (45)	101	45/50	297 (44)	96	44/50

< >:No.of effective animals,( ):No.of measured animals Av.Wt.: g

TABLE 4 INCIDENCE OF EXTERNAL AND INTERNAL MASS IN CLINICAL OBSERVATION OF MALE RATS IN THE 2-YEAR STUDY OF 2-METHALLYL CHLORIDE

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	3/49	2/49	2/49	4/48	6/46	12/50(1/11)
	50ppm	0/50	1/50	1/50	2/50	4/50	2/49	3/48	8/44	12/50(7/15)
	100ppm	0/50	0/50	0/50	0/50	1/50	2/50	8/50	18/47	18/50(6/17)
	200ppm	0/50	0/50	0/50	0/50	1/50	2/50	7/50	10/41	11/50(5/20)
Internal mass										
	Control	0/50	0/50	0/49	0/49	0/49	0/49	1/48	3/46	3/50(2/11)
	50ppm	0/50	0/50	0/50	0/50	0/50	0/49	1/48	2/44	3/50(1/15)
	100ppm	0/50	0/50	0/50	0/50	0/50	0/50	0/50	1/47	1/50(1/17)
	200ppm	0/50	0/50	0/50	0/50	0/50	0/50	2/50	3/41	4/50(3/20)

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 INCIDENCE OF EXTERNAL AND INTERNAL MASS IN CLINICAL OBSERVATION OF FEMALE RATS IN THE 2-YEAR STUDY OF 2-METHALLYL CHLORIDE

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	1/49	3/47	9/43	11/50(3/12)
	50ppm	0/50	0/50	0/50	0/50	2/50	2/50	4/47	6/45	8/50(2/10)
	100ppm	0/50	0/50	0/50	0/50	0/50	1/50	3/50	13/48	14/50(2/ 5)
	200ppm	0/50	0/50	0/50	0/49	0/49	1/48	3/48	7/47	8/50(2/ 6)
Internal mass										
	Control	0/50	0/50	0/50	0/50	0/50	0/49	3/47	3/43	5/50(5/12)
	50ppm	0/50	0/50	0/50	0/50	1/50	1/50	1/47	2/45	4/50(2/10)
	100ppm	0/50	0/50	0/50	0/50	0/50	0/50	0/50	1/48	1/50(1/ 5)
	200ppm	0/50	0/50	1/50	0/49	0/49	0/48	1/48	4/47	5/50(3/ 6)

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 6 FOOD CONSUMPTION CHANGES OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF 2-METHALLYL CHLORIDE

Week on Study	Control Av.FC.	Control No.of Surviv. <50>	50 ppm Av.FC.	50 ppm % of cont. <50>	50 ppm No.of Surviv.	100 ppm Av.FC.	100 ppm % of cont. <50>	100 ppm No.of Surviv.	200 ppm Av.FC.	200 ppm % of cont. <50>	200 ppm No.of Surviv.
1	16.0 (50)	50/50	16.2 (50)	101	50/50	15.8 (50)	99	50/50	14.5 (50)	91	50/50
2	17.2 (50)	50/50	17.6 (50)	102	50/50	17.9 (50)	104	50/50	17.2 (50)	100	50/50
3	17.8 (50)	50/50	18.6 (50)	104	50/50	18.4 (50)	103	50/50	17.1 (50)	96	50/50
4	17.5 (50)	50/50	18.5 (50)	106	50/50	18.0 (50)	103	50/50	16.5 (50)	94	50/50
5	18.1 (50)	50/50	18.4 (50)	102	50/50	17.9 (50)	99	50/50	16.8 (50)	93	50/50
6	18.6 (50)	50/50	18.4 (50)	99	50/50	17.7 (50)	95	50/50	17.2 (50)	92	50/50
7	18.8 (50)	50/50	18.7 (50)	99	50/50	18.2 (50)	97	50/50	17.5 (50)	93	50/50
8	18.4 (50)	50/50	18.8 (50)	102	50/50	18.3 (50)	99	50/50	17.1 (50)	93	50/50
9	18.3 (50)	50/50	18.4 (50)	101	50/50	18.2 (50)	99	50/50	17.2 (50)	94	50/50
10	18.8 (50)	50/50	18.2 (50)	97	50/50	18.0 (50)	96	50/50	17.7 (50)	94	50/50
11	18.2 (50)	50/50	18.0 (50)	99	50/50	18.1 (50)	99	50/50	17.7 (50)	97	50/50
12	17.6 (50)	50/50	18.0 (50)	102	50/50	17.8 (50)	101	50/50	17.3 (50)	98	50/50
13	18.5 (50)	50/50	18.9 (50)	102	50/50	18.6 (50)	101	50/50	17.9 (50)	97	50/50
14	17.7 (50)	50/50	18.3 (50)	103	50/50	18.3 (50)	103	50/50	17.2 (50)	97	50/50
18	17.9 (49)	49/50	17.8 (50)	99	50/50	17.7 (46)	99	50/50	16.8 (50)	94	50/50
22	18.0 (49)	49/50	17.8 (50)	99	50/50	17.8 (50)	99	50/50	17.0 (50)	94	50/50
26	18.0 (49)	49/50	18.1 (50)	101	50/50	17.8 (50)	99	50/50	17.3 (50)	96	50/50
30	18.4 (49)	49/50	18.0 (50)	98	50/50	18.8 (50)	102	50/50	18.5 (50)	101	50/50
34	18.2 (49)	49/50	17.9 (50)	98	50/50	17.8 (50)	98	50/50	17.8 (50)	98	50/50
38	18.5 (49)	49/50	18.0 (50)	97	50/50	18.0 (50)	97	50/50	17.4 (50)	94	50/50
42	18.8 (49)	49/50	18.0 (50)	96	50/50	18.3 (50)	97	50/50	18.0 (50)	96	50/50
46	18.5 (49)	49/50	18.0 (50)	97	50/50	18.5 (50)	100	50/50	17.6 (50)	95	50/50
50	18.8 (49)	49/50	18.8 (50)	100	50/50	18.8 (50)	100	50/50	18.2 (50)	97	50/50
54	18.7 (49)	49/50	18.2 (50)	97	50/50	18.0 (50)	96	50/50	17.9 (50)	96	50/50
58	18.4 (49)	49/50	18.2 (50)	99	50/50	18.5 (50)	101	50/50	17.8 (50)	97	50/50
62	18.4 (49)	49/50	18.5 (49)	101	49/50	18.3 (50)	99	50/50	17.9 (50)	97	50/50
66	18.6 (49)	49/50	18.5 (49)	99	49/50	18.2 (50)	98	50/50	17.9 (50)	96	50/50
70	18.7 (49)	49/50	18.8 (49)	101	49/50	18.9 (50)	101	50/50	18.6 (50)	99	50/50
74	18.7 (48)	48/50	18.6 (49)	99	49/50	19.1 (50)	102	50/50	18.4 (50)	98	50/50
78	19.5 (48)	48/50	19.1 (48)	98	48/50	18.9 (50)	97	50/50	18.7 (50)	96	50/50
82	18.9 (47)	47/50	18.6 (48)	98	48/50	18.9 (50)	100	50/50	18.7 (49)	99	49/50
86	19.3 (47)	47/50	18.6 (47)	96	47/50	19.3 (49)	100	49/50	18.9 (46)	98	46/50
90	19.4 (47)	47/50	19.5 (45)	101	45/50	19.5 (49)	101	49/50	19.1 (44)	98	44/50
94	18.7 (46)	46/50	18.4 (44)	98	44/50	18.9 (44)	101	44/50	19.4 (40)	104	41/50
98	18.9 (46)	45/50	19.5 (42)	103	42/50	19.8 (43)	105	43/50	19.0 (38)	101	38/50
102	19.0 (41)	41/50	18.0 (37)	95	38/50	18.6 (37)	98	37/50	18.2 (32)	96	32/50
104	18.5 (39)	39/50	17.7 (36)	96	35/50	18.9 (33)	102	33/50	18.5 (30)	100	30/50

< >:No.of effective animals,( ):No.of measured animals Av.FC.: g

TABLE 7 FOOD CONSUMPTION CHANGES OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF 2-METHALLYL CHLORIDE

Week on Study	Control Av.FC.	Control No.of Surviv. <50>	50 ppm Av.FC.	50 ppm % of cont. <50>	50 ppm No.of Surviv.	100 ppm Av.FC.	100 ppm % of cont. <50>	100 ppm No.of Surviv.	200 ppm Av.FC.	200 ppm % of cont. <50>	200 ppm No.of Surviv.
1	13.0 (50)	50/50	12.5 (50)	96	50/50	12.8 (50)	98	50/50	11.7 (50)	90	50/50
2	12.9 (50)	50/50	13.0 (50)	101	50/50	13.7 (50)	106	50/50	13.3 (50)	103	50/50
3	12.3 (50)	50/50	13.0 (50)	106	50/50	13.3 (50)	108	50/50	12.5 (50)	102	50/50
4	12.2 (50)	50/50	12.3 (50)	101	50/50	12.5 (48)	102	50/50	11.8 (50)	97	50/50
5	12.8 (50)	50/50	12.6 (50)	98	50/50	12.5 (50)	98	50/50	11.8 (50)	92	50/50
6	12.9 (50)	50/50	12.5 (50)	97	50/50	12.2 (50)	95	50/50	12.0 (50)	93	50/50
7	12.8 (50)	50/50	12.8 (50)	100	50/50	12.5 (50)	98	50/50	12.7 (50)	99	50/50
8	12.8 (50)	50/50	12.8 (50)	100	50/50	12.2 (50)	95	50/50	12.6 (50)	98	50/50
9	12.6 (50)	50/50	11.9 (50)	94	50/50	11.8 (50)	94	50/50	12.3 (50)	98	50/50
10	12.4 (50)	50/50	12.2 (50)	98	50/50	12.0 (50)	97	50/50	12.3 (50)	99	50/50
11	12.5 (50)	50/50	12.6 (50)	101	50/50	12.5 (50)	100	50/50	12.7 (50)	102	50/50
12	12.2 (50)	50/50	12.4 (50)	102	50/50	12.2 (50)	100	50/50	12.1 (50)	99	50/50
13	12.7 (50)	50/50	12.4 (50)	98	50/50	12.9 (50)	102	50/50	12.6 (50)	99	50/50
14	11.6 (50)	50/50	11.7 (50)	101	50/50	11.8 (50)	102	50/50	11.7 (50)	101	50/50
18	12.0 (50)	50/50	11.8 (50)	98	50/50	12.1 (50)	101	50/50	12.2 (50)	102	50/50
22	12.2 (50)	50/50	11.8 (50)	97	50/50	12.1 (50)	99	50/50	12.7 (50)	104	50/50
26	13.7 (50)	50/50	13.6 (50)	99	50/50	13.8 (50)	101	50/50	14.0 (50)	102	50/50
30	13.4 (50)	50/50	13.0 (50)	97	50/50	13.1 (50)	98	50/50	13.6 (50)	101	50/50
34	13.1 (50)	50/50	12.7 (50)	97	50/50	13.1 (50)	100	50/50	13.4 (49)	102	49/50
38	12.2 (50)	50/50	12.5 (50)	102	50/50	12.2 (50)	100	50/50	12.4 (49)	102	49/50
42	13.0 (50)	50/50	12.6 (50)	97	50/50	12.5 (50)	96	50/50	13.1 (49)	101	49/50
46	13.1 (50)	50/50	13.0 (50)	99	50/50	13.1 (50)	100	50/50	13.2 (49)	101	49/50
50	14.1 (50)	50/50	13.7 (50)	97	50/50	13.2 (50)	94	50/50	13.1 (49)	93	49/50
54	13.1 (50)	50/50	13.0 (50)	99	50/50	12.9 (50)	98	50/50	12.9 (49)	98	49/50
58	13.2 (50)	50/50	13.3 (50)	101	50/50	13.0 (50)	98	50/50	12.9 (48)	98	48/50
62	13.8 (49)	49/50	13.0 (50)	94	50/50	13.1 (50)	95	50/50	13.4 (48)	97	48/50
66	14.0 (49)	49/50	13.5 (50)	96	50/50	13.5 (50)	96	50/50	13.3 (48)	95	48/50
70	14.4 (48)	48/50	13.8 (49)	96	49/50	13.9 (50)	97	50/50	13.9 (48)	97	48/50
74	13.6 (48)	48/50	14.1 (47)	104	47/50	13.9 (50)	102	50/50	14.0 (48)	103	48/50
78	14.4 (47)	47/50	13.7 (47)	95	47/50	13.9 (50)	97	50/50	14.0 (48)	97	48/50
82	14.2 (47)	47/50	13.9 (47)	98	47/50	13.8 (50)	97	50/50	14.3 (48)	101	48/50
86	14.5 (45)	45/50	14.1 (47)	97	47/50	14.4 (49)	99	49/50	14.4 (48)	99	48/50
90	14.3 (45)	45/50	14.4 (45)	101	45/50	14.4 (49)	101	49/50	13.7 (48)	96	47/50
94	14.4 (42)	41/50	14.1 (45)	98	45/50	13.8 (48)	96	48/50	14.5 (46)	101	46/50
98	14.7 (39)	39/50	14.7 (45)	100	44/50	14.7 (48)	100	48/50	14.4 (46)	98	46/50
102	14.7 (38)	38/50	14.5 (41)	99	40/50	13.7 (47)	93	47/50	14.0 (44)	95	44/50
104	14.5 (38)	38/50	14.1 (40)	97	40/50	13.5 (46)	93	45/50	14.0 (44)	97	44/50

< >:No.of effective animals,( ):No.of measured animals Av.FC.: g

TABLE 8 NEOPLASTIC LESIONS INCIDENCE AND STATISTICAL ANALYSIS IN MALE RATS IN THE 2-YEAR INHALATION STUDY OF 2-METHALLYL CHLORIDE

Group Name	Control	50ppm	100ppm	200ppm
SITE : thyroid				
TUMOR : follicular adenoma(f)				
Tumor rate				
Overall rates(a)	2/50(4.0)	0/50(0.0)	2/50(4.0)	6/50(12.0)
Adjusted rates(b)	5.13	0.0	6.06	19.35
Terminal rates(c)	2/39(5.1)	0/35(0.0)	2/33(6.0)	5/30(16.7)
Statistical analysis				
Peto test				
Standard method(d)	P=------			
Prevalence method(d)	P=0.0052**			
Combined analysis (d)	P=------			
Cochran-Armitage test(e)	P=0.00213*			
Fisher Exact test(e)		P=0.2574	P=0.3088	P=0.1606
SITE : thyroid				
TUMOR : follicular adenoma, follicular adenocarcinoma				
Tumor rate				
Overall rates(a)	4/50(8.0)	4/50(8.0)	3/50(6.0)	10/50(20.0)
Adjusted rates(b)	10.26	11.43	9.09	27.27
Terminal rates(c)	4/39(10.3)	4/35(11.4)	3/33(9.1)	7/30(23.3)
Statistical analysis				
Peto test				
Standard method(d)	P=------			
Prevalence method(d)	P=0.0080**			
Combined analysis (d)	P=------			
Cochran-Armitage test(e)	P=0.0388*			
Fisher Exact test(e)		P=0.3579	P=0.4895	P=0.1108

(a):Number of tumor-bearing animals/number of animals examined at the site.
(b):Kaplan-Meire estimated tumor incidence at the end of the study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill.
(d):Beneath the control incidence are the P-values associated with the trend test.
Standard method :Death analysis
Prevalence method :Incidental tumor test
Combined analysis :Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
(f):Historical incidence for 2-year studies: 8/899(0.9%); range 0% to 4%
?: The conditional probabities of the largest and smallest possible out comes can not be estimated or this P-value is beyond the estimated P-value.
------:There is no data which should be statistical analysis.
Significant difference; *:P≦0.05 **:P≦0.01

TABLE 9 NUMBER OF RATS WITH SELECTED NON-NEOPLASTIC LESIONS IN THE 2-YEAR INHALATION STUDY OF 2-METHALLYL CHLORIDE

Group name		Male				Female			
		Control	50ppm	100ppm	200ppm	Control	50ppm	100ppm	200ppm
Nasal cavity		<50>(39)	<50>(35)	<50>(33)	<50>(30)	<50>(38)	<50>(40)	<50>(45)	<50>(44)
eosinophilic change:olfactory ep.		28 (24)	46**(35)**	49**(33)**	46**(30)**	41 (32)	50**(40)**	50**(45)**	49**(44)**
	+	22 (18)	2 (0)	0 (0)	2 (0)	24 (17)	1 (1)	0 (0)	0 (0)
	2+	6 (6)	10 (6)	9 (1)	10 (6)	11 (9)	3 (2)	3 (2)	1 (0)
	3+	0 (0)	32 (28)	34 (26)	30 (21)	6 (6)	32 (24)	39 (35)	35 (32)
	4+	0 (0)	2 (1)	6 (6)	4 (3)	0 (0)	14 (13)	8 (8)	13 (12)
Kidney		<50>(39)	<50>(35)	<50>(33)	<50>(30)	<50>(38)	<50>(40)	<50>(45)	<50>(44)
chronic nephropathy		47 (39)	47 (35)	50 (33)	47 (30)	45 (38)	43 (36)	34*(30)**	27**(27)**
	+	1 (1)	0 (0)	1 (1)	2 (0)	14 (10)	18 (12)	19 (17)	22 (22)
	2+	3 (2)	1 (0)	5 (0)	2 (0)	18 (17)	11 (10)	9 (9)	3 (3)
	3+	31 (28)	36 (31)	30 (25)	31 (20)	11 (9)	14 (14)	5 (4)	2 (2)
	4+	12 (8)	10 (4)	14 (7)	12 (10)	2 (2)	0 (0)	1 (0)	0 (0)

Grade +:Slight 2+:Moderate 3+:Marked 4+:Severe
< >:Number of animals examined at the site
( ):Sacrificed animals
Significant difference *:P≦0.05 **:P≦0.01 Test of Chi square

TABLE 10 CAUSE OF DEATH OF RATS IN THE 2-YEAR INHALATION STUDY OF 2-METHALLYL CHLORIDE

	Male				Female			
Group	Control	50ppm	100ppm	200ppm	Control	50ppm	100ppm	200ppm
Number of dead or moribund animals	11	15	17	20	12	10	5	6
No microscopical confirmation	0	0	0	0	0	0	0	1
Chronic nephropathy	4	6	6	2	0	0	0	0
Urinary retention	0	1	0	0	0	0	0	0
Tumor death : leukemia	2	1	2	4	3	4	1	2
skin/apendage	0	0	0	1	0	0	0	0
subcutis	0	0	3	0	0	0	0	0
lung	0	0	0	1	0	0	0	0
thymus	0	0	0	0	0	1	0	0
oral cavity	0	1	0	0	0	0	0	0
salivary gland	0	0	0	1	0	0	0	0
small intestine	0	0	0	1	0	0	0	1
large intestine	0	0	0	1	0	0	0	0
liver	0	1	0	0	0	0	0	1
pituitary	1	0	2	2	4	2	3	1
thyroid	0	0	1	0	1	0	0	0
adrenal	0	2	0	0	3	0	0	0
prostate	1	0	0	0	-	-	-	-
uterus	-	-	-	-	0	1	1	0
mammary gland	0	0	0	0	0	2	0	0
brain	2	0	0	2	0	0	0	0
Zymbal gland	0	0	2	1	0	0	0	0
muscle	0	1	0	0	0	0	0	0
bone	0	0	0	1	1	0	0	0
mediastinum	0	0	0	1	0	0	0	0
peritoneum	1	2	1	2	0	0	0	0

FIGURES

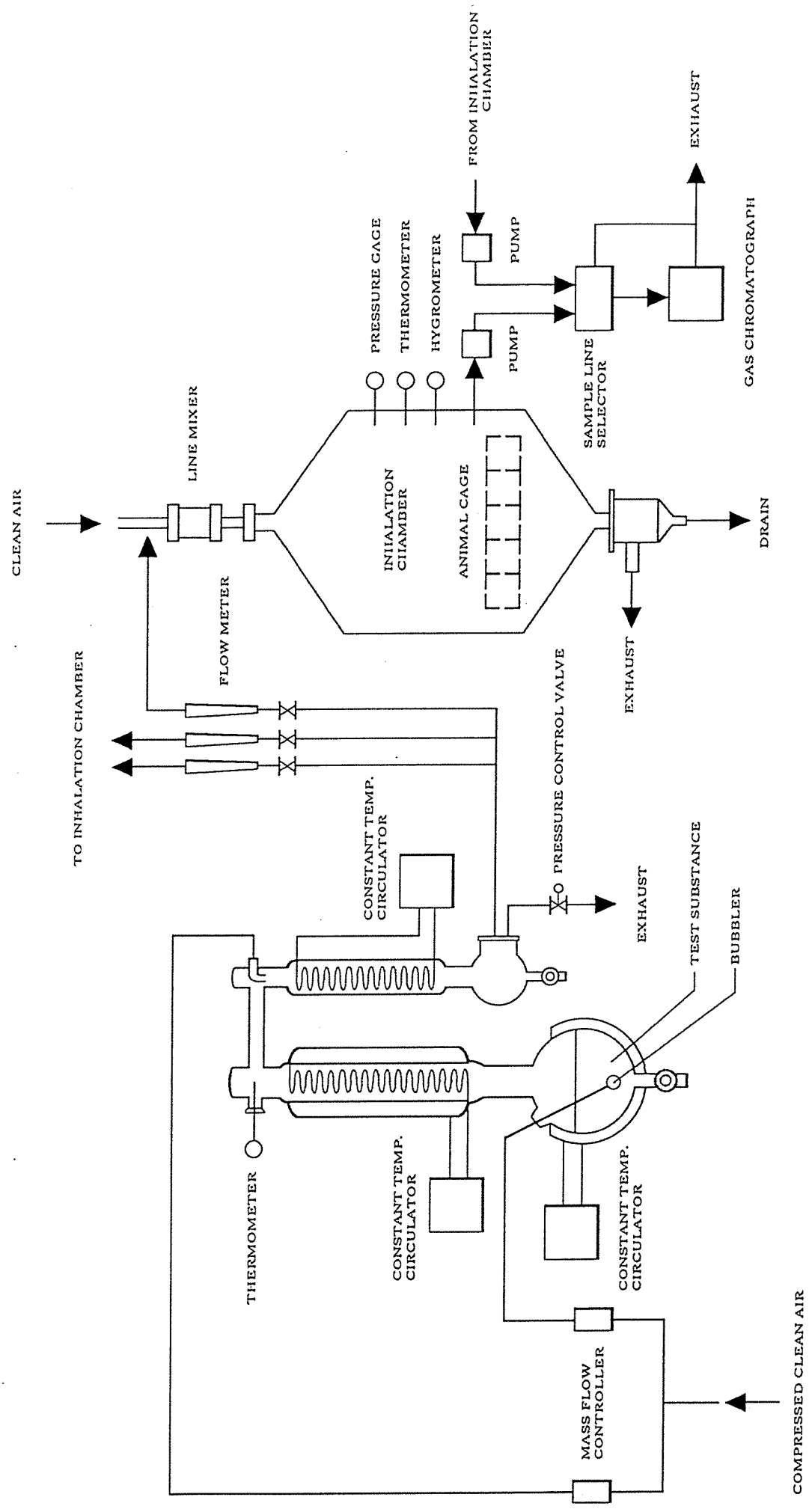


FIGURE 1 2-METHALLYL CHLORIDE VAPOR GENERATION SYSTEM AND INHALATION SYSTEM

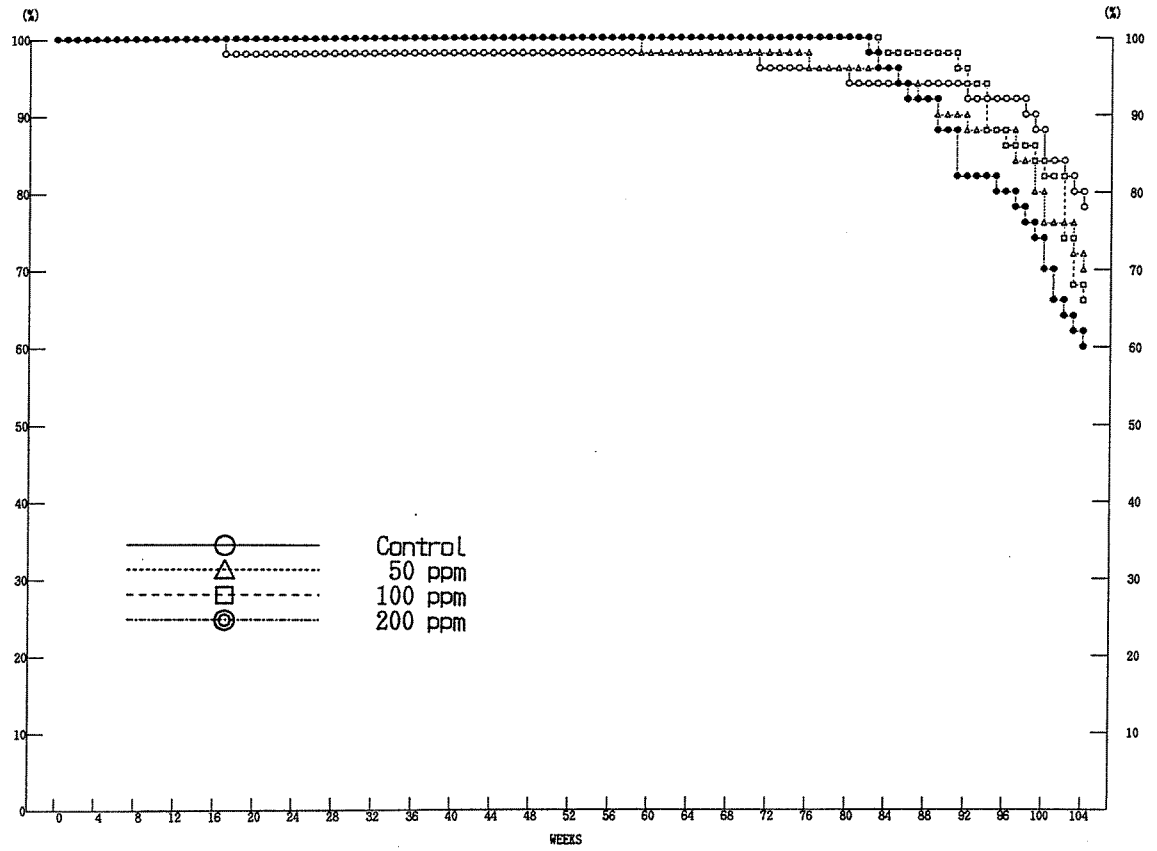


FIGURE 2 SURVIVAL ANIMAL RATE OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF 2-METHALLYL CHLORIDE

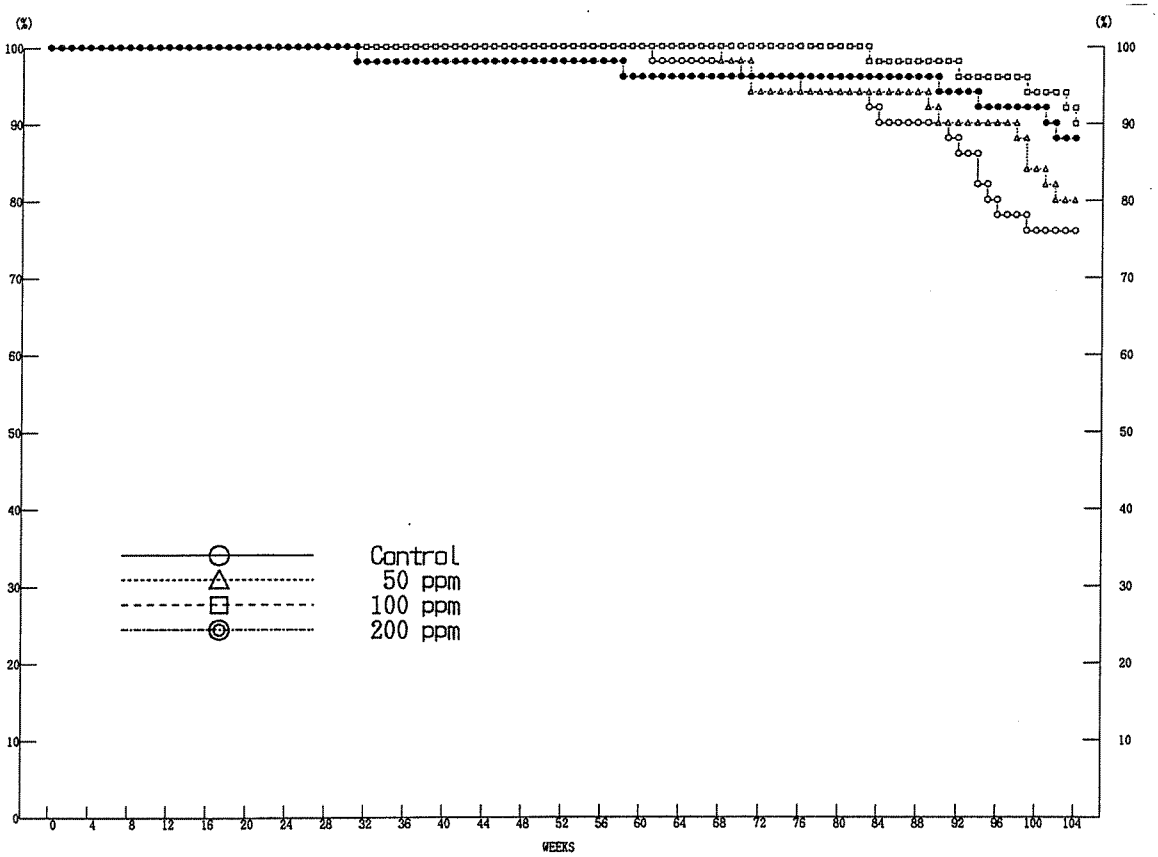


FIGURE 3 SURVIVAL ANIMAL RATE OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF 2-METHALLYL CHLORIDE

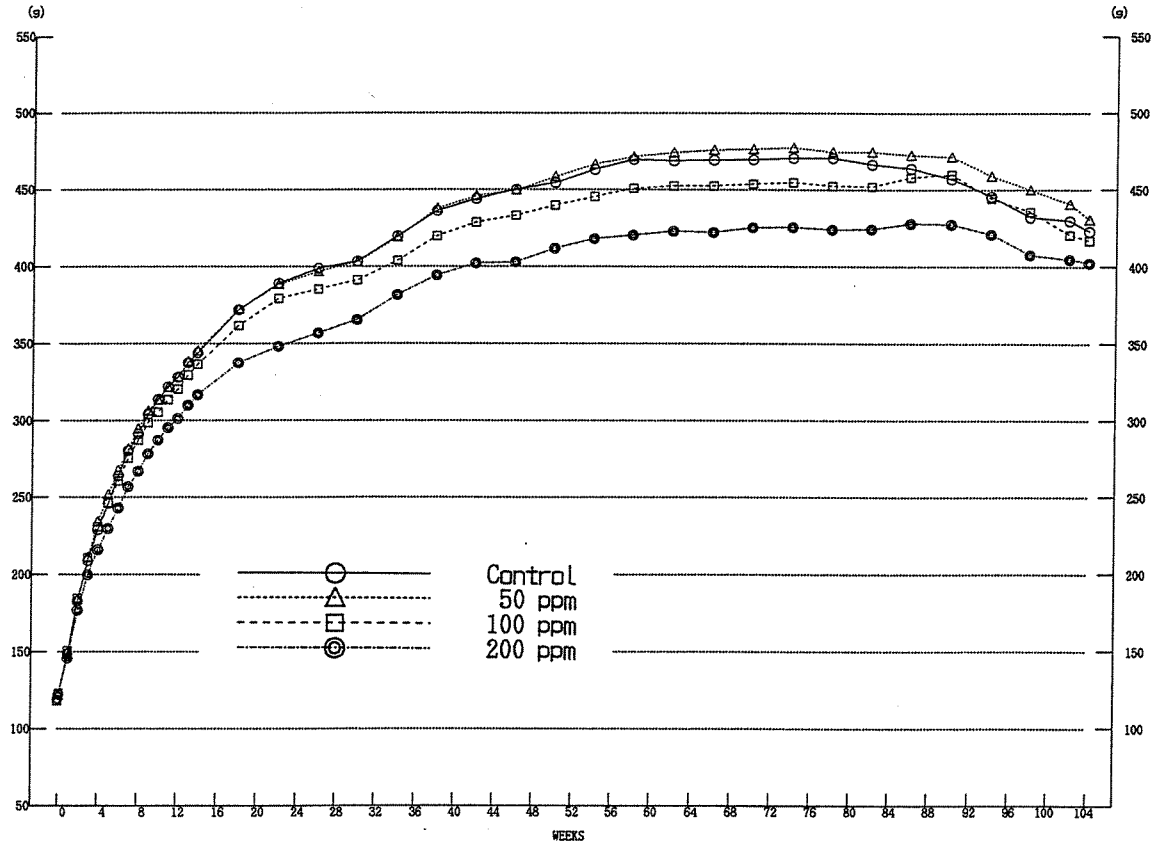


FIGURE 4 BODY WEIGHT CHANGES OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF 2-METHALLYL CHLORIDE

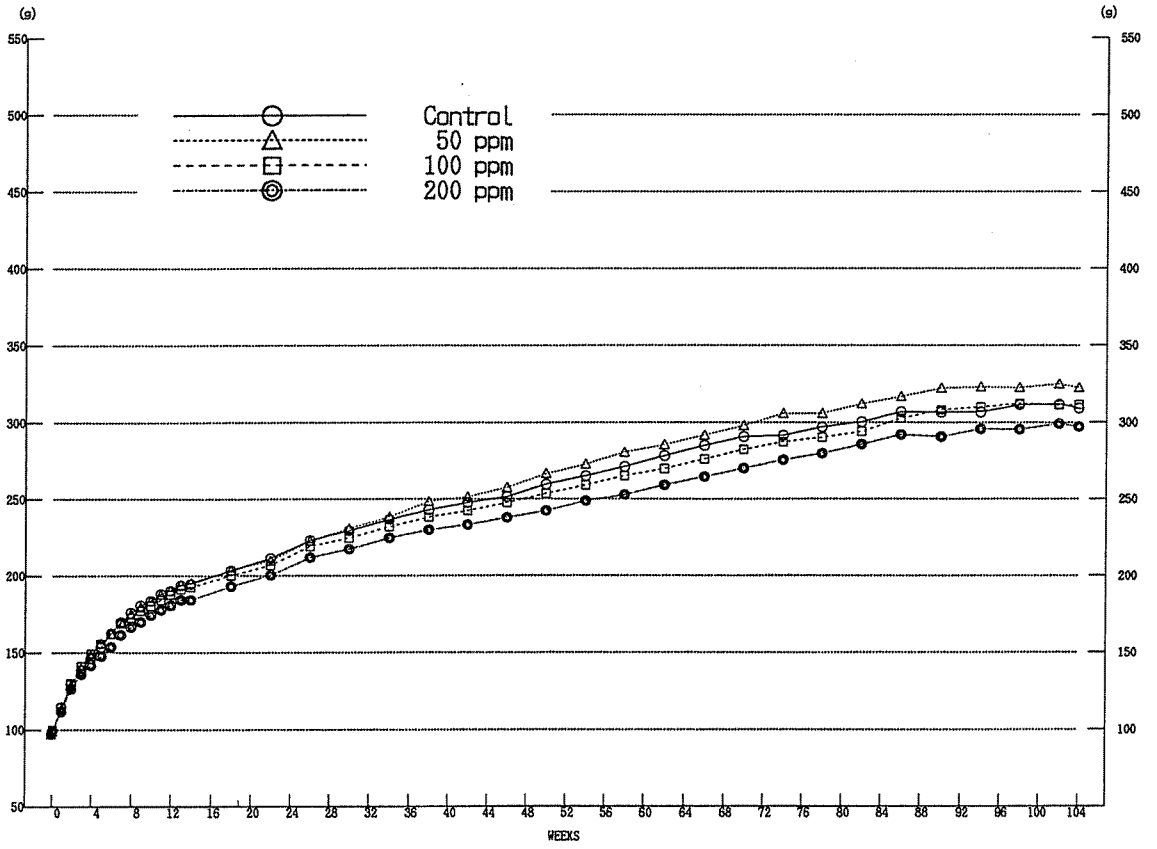


FIGURE 5 BODY WEIGHT CHANGES OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF 2-METHALLYL CHLORIDE

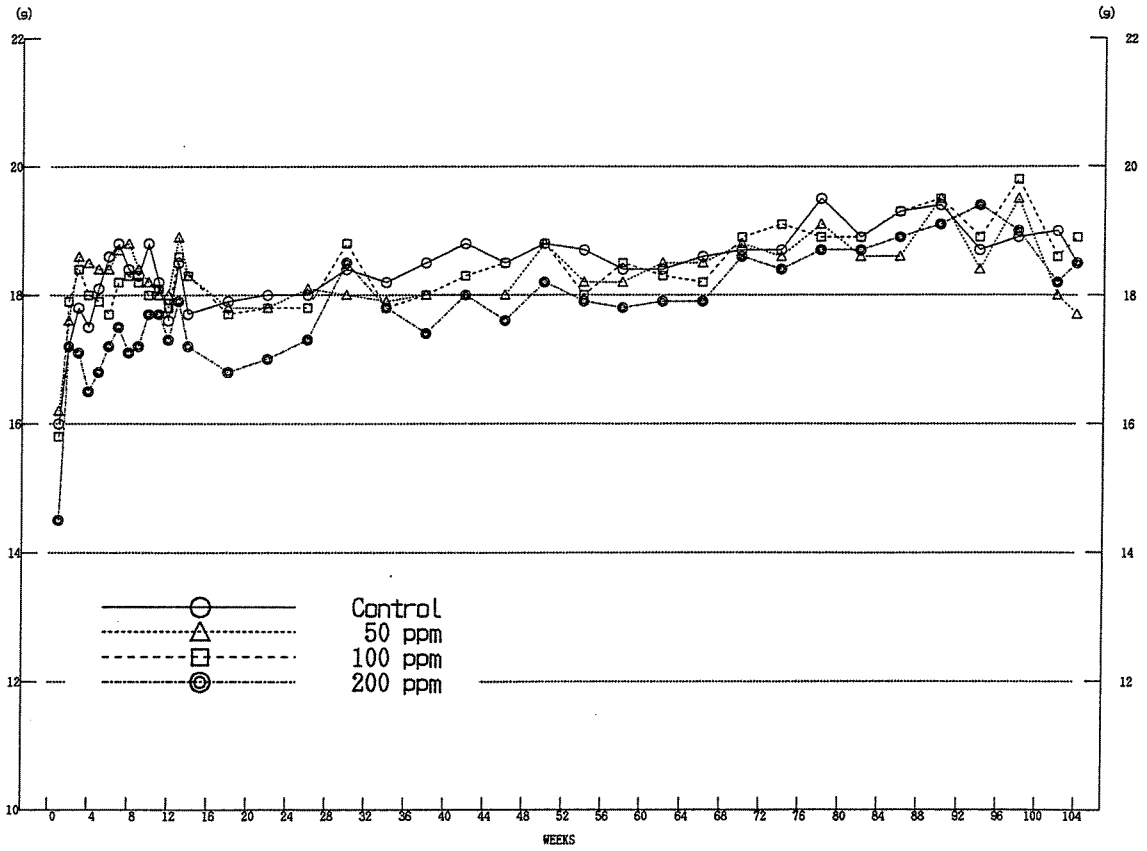


FIGURE 6 FOOD CONSUMPTION CHANGES OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF 2-METHALLYL CHLORIDE

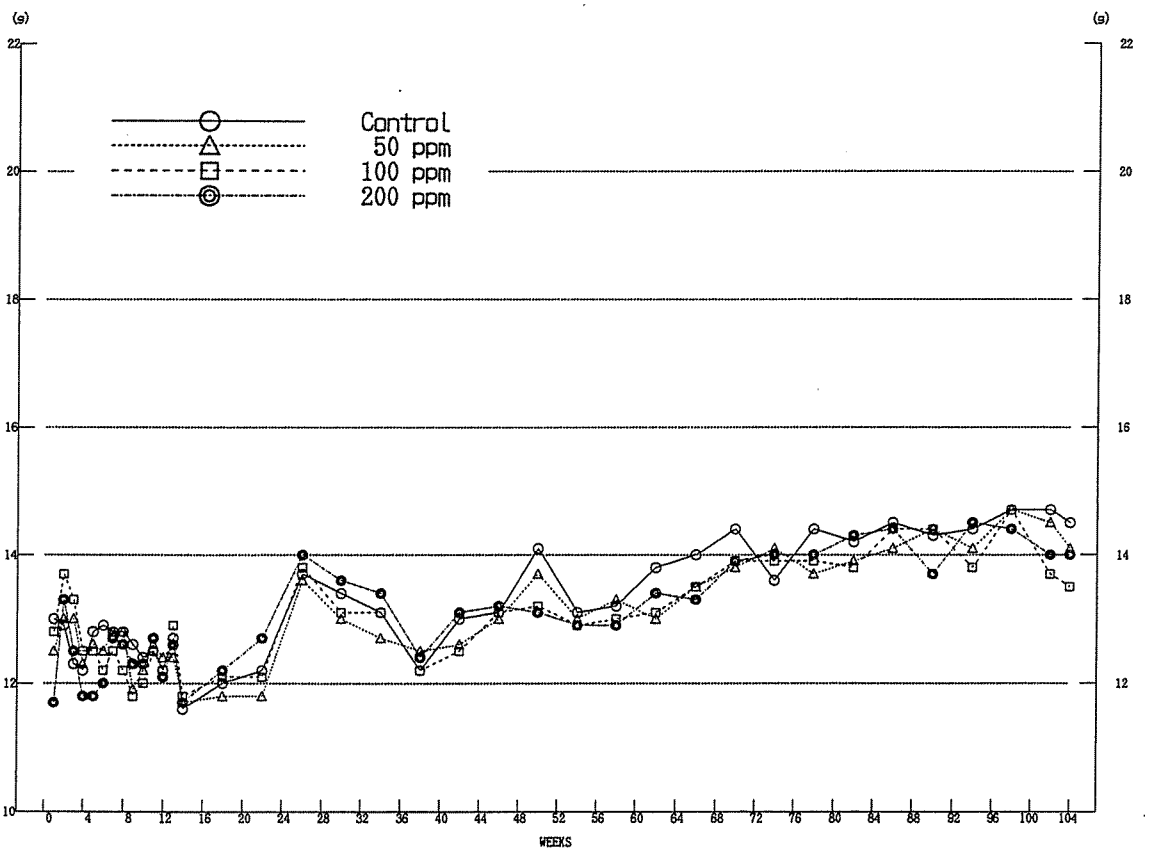


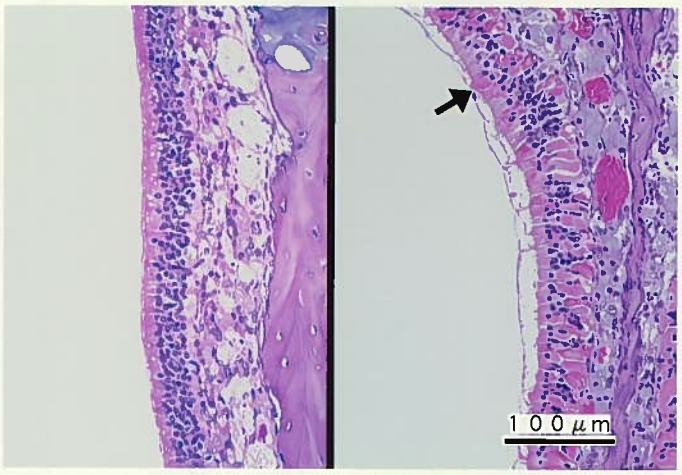
FIGURE 7 FOOD CONSUMPTION CHANGES OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF 2-METHALLYL CHLORIDE

PHOTOGRAPHS

PHOTOGRAPH 1 THYROID : FOLLICULAR ADENOMA
RAT, MALE, 200ppm, ANIMAL No. 0269-1313 (H&E)

PHOTOGRAPH 2
LEFT NASAL CAVITY : NORMAL
RAT, MALE, CONTROL, ANIMAL No. 0269-1006 (H&E)
RIGHT NASAL CAVITY : EOSINOPHILIC CHANGE : OLFACTORY EPITHELIUM (↑)
RAT, MALE, 200ppm, ANIMAL No. 0269-1306 (H&E)

PHOTOGRAPH. 1



PHOTOGRAPH. 2