Summary of Inhalation Carcinogenicity Study

of Dichloromethane

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 Labou 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 March 31, 2000.

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Summary of Inhalation Carcinogenicity Study of Dichloromethane in F344 Rats

Purpose, materials and methods

Dichloromethane (CAS No. 75-09-2) is a colorless liquid with a boiling point of 39.95°C. It is soluble in ethanol and ether and poorly soluble in water.

The carcinogenicity and chronic toxicity of dichloromethane (greater than 99.9% pure) were examined by inhalation exposure using F344/DuCrj (Fischer) rats. Groups of test animals were exposed to dichloromethane vapor at target concentrations of 0 (clean air), 1000, 2000 or 4000 ppm (v/v) for 6 hours/day, 5 days/week for 2 years (104 weeks). Each group of test animals consisted of either 50 male or 50 female rats. Both sexes were exposed to each concentration of dichloromethane vapor. The highest dose level was chosen so as not to exceed the maximum tolerated dose (MTD), based on both growth rate and toxicity in a previous 13-week toxicity study. The identity of the dichloromethane used in these experiments was confirmed by both infrared spectrometry and mass spectrometry, and it was analyzed by gas chromatography before and after its use to affirm its stability. Stainless-steel inhalation exposure chambers (volume: 7600 L) were used throughout the 2-year exposure period. Dichloromethane vapor-air mixtures were generated by bubbling clean air through dichloromethane liquid and the mixtures supplied to the inhalation exposure chambers. Air concentrations of dichloromethane 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. All animals, including those found dead or in a moribund state as well as those 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 at the terminal necropsy, surviving animals were fasted overnight and bled under deep ether anesthesia. Organs and tissues were removed, weighed and examined for macroscopic lesions at necropsy. The organs and tissues were then fixed and embedded in paraffin. Five μm thick tissue sections were prepared and stained with hematoxylin and eosin and examined microscopically. Incidences of neoplastic lesions were statistically analyzed by Fisher's exact test. Any positive dose-response trends of dichloromethane induction of neoplastic lesions were analyzed by Peto's test. Incidences of non-neoplastic lesions and urinalysis were analyzed by the 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

Survival rates of females exposed to 4000 ppm dichloromethane were decreased compared with the control; in this group, death due to uterine tumors was increased compared with the other female groups. There were more palpable internal masses and these masses arose earlier in the 4000 ppm-exposed females compared with the other female groups. The occurrence of skin and subcutis masses increased in a dose-related manner in exposed male groups. Body weights of the 4000 ppm-exposed males and females were slightly suppressed. The red blood cell and hematcrit values were increased in females exposed to 2000 ppm and 4000 ppm dichloromethane; sugesting to be related to an increase in carboxyhemoglobin levels after exposure to dichloromethane, which has been previously reported.

Exposure to dichloromethane resulted in an increased incidence of fibromas of the subcutis, mammary gland fibroadenomas and peritoneal mesotheliomas in males and mammary gland fibroadenomas in females. In 4000 ppm exposed males, there was an increased incidence of fibrosis in the spleen, a decrease in the incidence of respiratory metaplasia in the olfactory epithelium of the nasal cavity, and a decrease in mineralization in the cornea of the eye. In females, basophilic cell foci in the liver was increased in all exposed groups, and acidophilic cell foci and vacuolated cell foci in the liver were increased in the females exposed to 2000 ppm and above.

Conclusions

There was some evidence of carcinogenic activity of dichloromethane in male and female rats, based on the increased incidences of fibromas of the subcutis, mammary gland fibroadenomas and peritoneal mesotheliomas in males and mammary gland fibroadenomas in females.

Incidences of selected neoplastic lesions of male rats in the 2-year inhalation carcinogenicity study of dichloromethane

Dose (ppm)		0	1000	2000	4000	Peto test	Cochran-Armitage test
	Number of examined animals	50	50	50	50		
benign tumor							
subcutis	fibroma	1	4	7 *	12 **	↑ ↑	↑ ↑
	lipoma	0	0	4	1		
mammary	fibroadenoma	1	2	3	8 *	↑ ↑	↑ ↑
gland	adenoma	1	0	0	0		
liver	hepatocellular adenoma	0	0	1	1		
pituitary	adenoma	18	7 **	10	12		
thyroid	C-cell adenoma	9	5	7	4		
	follicular adenoma	0	4	0	1		
pancreas	islet cell adenoma	6	2	4	3		
adrenal	pheochromocytoma	8	13	14	9		
testis	interstitial cell tumor	45	49	48	46		
preputial gland	adenoma	0	2	4	1		
skin/appendage	keratoacanthoma	2	4	1	2		
malignant tumor							
subcutis	fibrosarcoma	0	0	1	0		
mammary gland	adenocarcinoma	1	0	0	0		
peritoneum	mesothelioma	3	1	0	7	↑	↑
liver	hepatocellular carcinoma	1	0	1	2		
spleen	mononuclear cell leukemia	3	3	8	4		
thyroid	follicular adenocarcinoma	2	2	0	4		
combined analysis							
subcutis	(fibroma+ fibrosarcoma)	1	4	8 *	12 **	↑ ↑	↑ ↑
mammary	(fibroadenoma+adenoma)	2	2	3	8 *	↑ ↑	↑
gland	(fibroadenoma+adenoma+ adenocarcinoma)	3	2	3	8	↑ ↑	↑
liver	(hepatocellular adenoma+ hepatocellular carcinoma)	1	0	2	3	↑	
pituitary	(adenoma+adenocarcinoma)	19	7 **	10 *	12		

Significant difference

* : p≦0.05		** : p≦0.01		(Fisher test)
↑ : p≦0.05	increase	↑ ↑ : p≦0.01	increase	(Peto, Cochran-Armitage test)
↓ : p≦0.05	decrease	↓ ↓ : p≦0.01	decrease	(Cochran-Armitage test)

Table 2 Incidences of selected neoplastic lesions of female rats in the 2-year inhalation carcinogenicity study of dichloromethane

Dose (ppm)		0	1000	2000	4000	Peto test	Cochran-Armitage test
Number of examined animals		50	50	50	50		
benign tumor							
mammary gland	fibroadenoma	7	7	9	14	↑ ↑	↑
	adenoma	0	2	1	0		
uterus	endometrial stromal polyp	8	11	6	9	↑	
	leiomyoma	0	0	0	1		
liver	hepatocellular adenoma	2	0	0	3		
pituitary	adenoma	18	28 *	24	19		
thyroid	C-cell adenoma	5	3	7	6		
adrenal	pheochromocytoma	4	2	4	2		
malignant tumor							
mammary gland	adenocarcinoma	0	2	0	1		
uterus	leiomyosarcoma	0	0	0	1		
	endometrial stromal sarcoma	0	0	0	2		
spleen	mononuclear cell leukemia	2	4	8 *	7	↑	
combined analysis							
mammary gland	(fibroadenoma+ adenoma)	7	8	10	14	↑ ↑	
	(fibroadenoma+ adenoma+ adenocarcinoma)	7	9	10	14	↑ ↑	
uterus	(leiomyosarcoma + endometrial stromal sarcoma)	0	0	0	3		↑ ↑
pituitary	(adenoma+ adenocarcinoma)	19	29 *	24	19		

Significant difference

* : p≦0.05		** : p≦0.01		(Fisher test)
↑ : p≦0.05	increase	↑ ↑ : p≦0.01	increase	(Peto, Cochran-Armitage test)
↓ : p≦0.05	decrease	↓ ↓ : p≦0.01	decrease	(Cochran-Armitage test)

TABLES

TABLES (CONTINUED)

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

<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 and Female
0, 1000, 2000, or 4000ppm
<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
Temperatu: : 23±3°C
Fluorescent light 12h/d
Chamber Environment
Temperatu: : 22±2°C
Humidity : 55±15%
Pressure : 0～-15mmAq
12±1 chamber air changes/h
(6±1 chamber air changes/h during exposure)
<Type and Frequency of Observation>
Clinical Sign
Observed 1 per d
Body Weight
Weighed first exposure and 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 DICHLOROMETHANE

<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, nasopharynx, larynx, 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, parathyroid, 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 DICHLOROMETHANE

Weeks on Study	Control		1000ppm			2000ppm			4000ppm		
	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	117 (50)	50/50	117 (50)	100	50/50	117 (50)	100	50/50	117 (50)	100	50/50
1	120 (50)	50/50	120 (50)	100	50/50	120 (50)	100	50/50	120 (50)	100	50/50
1	145 (50)	50/50	144 (50)	99	50/50	146 (50)	101	50/50	142 (50)	98	50/50
2	175 (50)	50/50	173 (50)	99	50/50	175 (50)	100	50/50	168 (50)	96	50/50
3	202 (50)	50/50	201 (50)	100	50/50	200 (50)	99	50/50	192 (50)	95	50/50
4	226 (50)	50/50	224 (50)	99	50/50	222 (50)	98	50/50	214 (50)	95	50/50
5	246 (50)	50/50	244 (50)	99	50/50	241 (50)	98	50/50	232 (50)	94	50/50
6	263 (50)	50/50	260 (50)	99	50/50	257 (50)	98	50/50	248 (50)	94	50/50
7	279 (50)	50/50	276 (50)	99	50/50	272 (50)	97	50/50	262 (50)	94	50/50
8	293 (50)	50/50	290 (50)	99	50/50	286 (50)	98	50/50	275 (50)	94	50/50
9	306 (50)	50/50	303 (50)	99	50/50	298 (50)	97	50/50	287 (50)	94	50/50
10	316 (50)	50/50	312 (50)	99	50/50	308 (50)	97	50/50	297 (50)	94	50/50
11	326 (50)	50/50	321 (50)	98	50/50	317 (50)	97	50/50	306 (50)	94	50/50
12	334 (50)	50/50	328 (50)	98	50/50	324 (50)	97	50/50	312 (50)	93	50/50
13	343 (50)	50/50	337 (50)	98	50/50	332 (50)	97	50/50	322 (50)	94	50/50
14	350 (50)	50/50	344 (50)	98	50/50	340 (50)	97	50/50	328 (50)	94	50/50
18	373 (50)	50/50	367 (50)	98	50/50	362 (50)	97	50/50	351 (50)	94	50/50
22	389 (50)	50/50	386 (50)	99	50/50	381 (50)	98	50/50	369 (50)	95	50/50
26	402 (50)	50/50	398 (50)	99	50/50	394 (50)	98	50/50	381 (50)	95	50/50
30	417 (50)	50/50	412 (50)	99	50/50	410 (50)	98	50/50	397 (50)	95	50/50
34	429 (50)	50/50	426 (50)	99	50/50	423 (50)	99	50/50	408 (50)	95	50/50
38	438 (50)	50/50	434 (50)	99	50/50	431 (50)	98	50/50	417 (50)	95	50/50
42	446 (50)	50/50	443 (50)	99	50/50	441 (50)	99	50/50	424 (50)	95	50/50
46	452 (50)	50/50	448 (50)	99	50/50	447 (50)	99	50/50	431 (50)	95	50/50
50	459 (50)	50/50	455 (50)	99	50/50	455 (50)	99	50/50	436 (50)	95	50/50
54	461 (50)	50/50	460 (50)	100	50/50	452 (50)	98	50/50	440 (50)	95	50/50
58	465 (50)	50/50	463 (50)	100	50/50	456 (50)	98	50/50	440 (50)	95	50/50
62	467 (50)	50/50	467 (50)	100	50/50	461 (50)	99	50/50	442 (50)	95	50/50
66	471 (50)	50/50	471 (50)	100	50/50	462 (50)	98	50/50	444 (50)	94	50/50
70	467 (50)	50/50	470 (50)	101	50/50	461 (50)	99	50/50	440 (49)	94	49/50
74	466 (48)	48/50	472 (49)	101	49/50	459 (50)	98	50/50	440 (48)	94	48/50
78	463 (48)	48/50	466 (49)	101	49/50	464 (49)	100	49/50	444 (46)	96	46/50
82	463 (46)	46/50	473 (48)	102	48/50	460 (49)	99	48/50	448 (45)	97	45/50
86	448 (46)	46/50	475 (48)	106	48/50	462 (48)	103	48/50	445 (43)	99	43/50
90	455 (42)	42/50	468 (48)	103	48/50	462 (47)	102	47/50	443 (43)	97	43/50
94	446 (39)	39/50	463 (48)	104	48/50	454 (47)	102	47/50	438 (41)	98	41/50
98	436 (37)	37/50	459 (44)	105	44/50	437 (44)	100	44/50	430 (37)	99	36/50
102	419 (36)	36/50	448 (43)	107	43/50	423 (42)	101	41/50	424 (30)	101	30/50
104	412 (32)	32/50	437 (43)	106	43/50	416 (39)	101	38/50	417 (29)	101	28/50

< > : 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 DICHLOROMETHANE

Weeks on Study	Control		1000ppm			2000ppm			4000ppm		
	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	96 (50)	50/50	96 (50)	100	50/50	96 (50)	100	50/50	96 (50)	100	50/50
1	97 (50)	50/50	97 (50)	100	50/50	97 (50)	100	50/50	97 (50)	100	50/50
1	110 (50)	50/50	109 (50)	99	50/50	110 (50)	100	50/50	107 (50)	97	50/50
2	124 (50)	50/50	122 (50)	98	50/50	123 (50)	99	50/50	118 (50)	95	50/50
3	135 (50)	50/50	133 (50)	99	50/50	134 (50)	99	50/50	128 (50)	95	50/50
4	144 (50)	50/50	142 (50)	99	50/50	143 (50)	99	50/50	135 (50)	94	50/50
5	154 (50)	50/50	152 (50)	99	50/50	151 (50)	98	50/50	144 (50)	94	50/50
6	161 (50)	50/50	160 (50)	99	50/50	158 (50)	98	50/50	150 (50)	93	50/50
7	168 (50)	50/50	166 (50)	99	50/50	164 (50)	98	50/50	155 (50)	92	50/50
8	174 (50)	50/50	171 (50)	98	50/50	168 (50)	97	50/50	160 (50)	92	50/50
9	179 (50)	50/50	177 (50)	99	50/50	174 (50)	97	50/50	166 (50)	93	50/50
10	184 (50)	50/50	181 (50)	98	50/50	178 (50)	97	50/50	170 (50)	92	50/50
11	189 (50)	50/50	187 (50)	99	50/50	185 (50)	98	50/50	177 (50)	94	50/50
12	193 (50)	50/50	189 (50)	98	50/50	187 (50)	97	50/50	179 (50)	93	50/50
13	197 (50)	50/50	194 (50)	98	50/50	191 (50)	97	50/50	184 (50)	93	50/50
14	199 (50)	50/50	196 (50)	98	50/50	194 (50)	97	50/50	186 (50)	93	50/50
18	209 (50)	50/50	206 (50)	99	50/50	204 (50)	98	50/50	196 (50)	94	50/50
22	215 (50)	50/50	214 (50)	100	50/50	210 (50)	98	50/50	205 (50)	95	50/50
26	222 (50)	50/50	219 (50)	99	50/50	217 (50)	98	50/50	210 (50)	95	50/50
30	233 (50)	50/50	231 (50)	99	50/50	228 (50)	98	50/50	222 (50)	95	50/50
34	240 (50)	50/50	237 (50)	99	50/50	234 (50)	98	50/50	227 (50)	95	50/50
38	242 (50)	50/50	241 (50)	100	50/50	236 (50)	98	50/50	230 (50)	95	50/50
42	248 (50)	50/50	248 (50)	100	50/50	243 (50)	98	50/50	239 (50)	96	50/50
46	253 (50)	50/50	251 (50)	99	50/50	248 (50)	98	50/50	244 (50)	96	50/50
50	263 (50)	50/50	261 (50)	99	50/50	259 (50)	98	50/50	251 (50)	95	50/50
54	268 (50)	50/50	266 (50)	99	50/50	260 (50)	97	50/50	257 (50)	96	50/50
58	271 (50)	50/50	273 (50)	101	50/50	264 (50)	97	50/50	259 (50)	96	50/50
62	274 (50)	50/50	276 (50)	101	50/50	269 (50)	98	50/50	264 (49)	96	49/50
66	282 (50)	50/50	284 (50)	101	50/50	277 (50)	98	50/50	270 (47)	96	47/50
70	288 (50)	50/50	291 (50)	101	50/50	284 (50)	99	50/50	278 (47)	97	47/50
74	295 (49)	49/50	300 (49)	102	49/50	291 (50)	99	50/50	281 (47)	95	47/50
78	301 (49)	49/50	305 (49)	101	49/50	298 (50)	99	50/50	286 (44)	95	44/50
82	308 (48)	48/50	312 (49)	101	49/50	303 (50)	98	50/50	293 (44)	95	44/50
86	314 (48)	48/50	321 (48)	102	48/50	316 (48)	101	48/50	298 (42)	95	42/50
90	323 (46)	46/50	320 (48)	99	48/50	322 (48)	100	48/50	306 (39)	95	39/50
94	326 (46)	46/50	323 (45)	99	45/50	329 (47)	101	47/50	307 (39)	94	39/50
98	324 (46)	46/50	319 (43)	98	42/50	324 (45)	100	45/50	309 (36)	95	36/50
102	324 (45)	45/50	320 (41)	99	41/50	322 (43)	99	43/50	301 (32)	93	31/50
104	315 (45)	45/50	312 (40)	99	40/50	315 (43)	100	43/50	293 (31)	93	30/50

< > : 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 INHALATION STUDY OF DICHLOROMETHANE

Time of mass occurrence (weeks)	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	1/50	2/50	2/50	4/50	6/47	7/40	10/50(5/18)
1000ppm	0/50	0/50	0/50	1/50	1/50	2/50	6/49	16/48	18/50(2/ 7)
2000ppm	0/50	0/50	0/50	5/50	4/50	6/50	10/49	14/47	18/50(6/12)
4000ppm	0/50	0/50	0/50	1/50	2/50	4/50	17/46	22/42	24/50(8/22)
Internal mass									
Control	0/50	0/50	0/50	0/50	0/50	0/50	1/47	1/40	2/50(2/18)
1000ppm	0/50	0/50	0/50	0/50	0/50	0/50	0/49	1/48	1/50(1/ 7)
2000ppm	0/50	0/50	0/50	0/50	0/50	0/50	0/49	3/47	3/50(2/12)
4000ppm	0/50	0/50	0/50	0/50	0/50	2/50	1/46	2/42	5/50(5/22)

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 INHALATION STUDY OF DICHLOROMETHANE

Time of mass occurrence (weeks)	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	1/50	5/49	10/46	11/50(1/ 5)
1000ppm	0/50	0/50	0/50	1/50	1/50	1/50	3/49	6/46	7/50(1/10)
2000ppm	0/50	0/50	0/50	0/50	0/50	0/50	1/50	9/47	9/50(1/ 7)
4000ppm	0/50	0/50	0/50	0/50	2/50	2/47	2/44	12/39	12/50(2/20)
Internal mass									
Control	0/50	0/50	0/50	0/50	0/50	0/50	0/49	0/46	0/50(0/ 5)
1000ppm	0/50	0/50	0/50	0/50	0/50	0/50	0/49	2/46	2/50(2/10)
2000ppm	0/50	0/50	0/50	0/50	0/50	0/50	2/50	0/47	2/50(1/ 7)
4000ppm	0/50	0/50	0/50	1/50	1/50	0/47	5/44	3/39	9/50(9/20)

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

Weeks on Study	Control Av.FC.	Control No.of Surviv. <50>	1000ppm Av.FC.	1000ppm % of cont.	1000ppm No.of Surviv. <50>	2000ppm Av.FC.	2000ppm % of cont.	2000ppm No.of Surviv. <50>	4000ppm Av.FC.	4000ppm % of cont.	4000ppm No.of Surviv. <50>
1	15.0 (50)	50/50	15.0 (50)	100	50/50	15.0 (50)	100	50/50	14.3 (50)	95	50/50
2	16.5 (50)	50/50	16.3 (50)	99	50/50	16.2 (50)	98	50/50	15.0 (50)	91	50/50
3	17.8 (50)	50/50	17.5 (50)	98	50/50	17.0 (50)	96	50/50	16.1 (50)	90	50/50
4	18.7 (50)	50/50	18.3 (50)	98	50/50	17.8 (50)	95	50/50	17.0 (50)	91	50/50
5	19.0 (50)	50/50	18.9 (50)	99	50/50	18.1 (50)	95	50/50	17.6 (50)	93	50/50
6	18.9 (50)	50/50	18.6 (50)	98	50/50	18.3 (50)	97	50/50	17.5 (50)	93	50/50
7	18.5 (50)	50/50	18.2 (50)	98	50/50	17.8 (50)	96	50/50	17.2 (50)	93	50/50
8	18.7 (50)	50/50	18.5 (50)	99	50/50	18.0 (50)	96	50/50	17.4 (50)	93	50/50
9	19.2 (50)	50/50	19.0 (50)	99	50/50	18.4 (50)	96	50/50	17.8 (50)	93	50/50
10	19.0 (50)	50/50	18.8 (50)	99	50/50	18.3 (50)	96	50/50	17.9 (50)	94	50/50
11	19.1 (50)	50/50	18.6 (50)	97	50/50	18.2 (50)	95	50/50	18.2 (50)	95	50/50
12	18.8 (50)	50/50	18.2 (50)	97	50/50	17.6 (50)	94	50/50	17.4 (50)	93	50/50
13	18.9 (50)	50/50	18.2 (50)	96	50/50	18.0 (50)	95	50/50	17.9 (50)	95	50/50
14	18.6 (50)	50/50	18.1 (50)	97	50/50	17.8 (50)	96	50/50	17.5 (50)	94	50/50
18	18.6 (50)	50/50	18.2 (50)	98	50/50	17.8 (50)	96	50/50	17.6 (50)	95	50/50
22	18.7 (50)	50/50	18.4 (50)	98	50/50	18.4 (50)	98	50/50	17.9 (50)	96	50/50
26	18.7 (50)	50/50	18.3 (50)	98	50/50	18.3 (50)	98	50/50	18.1 (50)	97	50/50
30	18.8 (50)	50/50	18.3 (50)	97	50/50	18.2 (50)	97	50/50	18.4 (50)	98	50/50
34	18.7 (50)	50/50	18.5 (50)	99	50/50	18.4 (50)	98	50/50	18.5 (50)	99	50/50
38	18.9 (50)	50/50	18.3 (50)	97	50/50	18.2 (50)	96	50/50	18.3 (50)	97	50/50
42	18.8 (50)	50/50	18.4 (50)	98	50/50	18.4 (50)	98	50/50	18.0 (50)	96	50/50
46	18.5 (50)	50/50	18.1 (50)	98	50/50	18.3 (50)	99	50/50	18.2 (50)	98	50/50
50	18.6 (50)	50/50	18.1 (50)	97	50/50	18.5 (50)	99	50/50	18.1 (50)	97	50/50
54	18.1 (50)	50/50	18.5 (50)	102	50/50	17.3 (50)	96	50/50	18.2 (50)	101	50/50
58	18.9 (50)	50/50	19.0 (50)	101	50/50	18.6 (50)	98	50/50	18.4 (50)	97	50/50
62	18.6 (50)	50/50	18.8 (50)	101	50/50	18.5 (50)	99	50/50	18.7 (50)	101	50/50
66	18.7 (50)	50/50	18.8 (50)	101	50/50	18.5 (50)	99	50/50	18.1 (50)	97	50/50
70	18.5 (50)	50/50	18.5 (50)	100	50/50	18.2 (50)	98	50/50	18.1 (49)	98	49/50
74	19.0 (48)	48/50	18.9 (49)	99	49/50	18.6 (50)	98	50/50	19.0 (48)	100	48/50
78	19.0 (48)	48/50	18.4 (49)	97	49/50	19.0 (49)	100	49/50	18.9 (46)	99	46/50
82	19.3 (46)	46/50	18.7 (48)	97	48/50	18.2 (49)	94	48/50	18.8 (45)	97	45/50
86	17.8 (44)	46/50	19.5 (47)	110	48/50	18.6 (48)	104	48/50	20.1 (43)	113	43/50
90	18.5 (42)	42/50	18.8 (48)	102	48/50	19.4 (47)	105	47/50	20.0 (43)	108	43/50
94	18.3 (39)	39/50	18.3 (48)	100	48/50	18.0 (47)	98	47/50	18.6 (41)	102	41/50
98	17.9 (37)	37/50	18.0 (43)	101	44/50	17.9 (44)	100	44/50	19.5 (37)	109	36/50
102	17.2 (34)	36/50	18.3 (43)	106	43/50	17.5 (42)	102	41/50	19.0 (27)	110	30/50
104	17.3 (32)	32/50	17.4 (43)	101	43/50	16.6 (39)	96	38/10	18.6 (28)	108	28/50

< > : 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 DICHLOROMETHANE

Weeks on Study	Control		1000ppm			2000ppm			4000ppm		
	Av.FC.	No.of Surviv. <50>	Av.FC.	% of cont.	No.of Surviv. <50>	Av.FC.	% of cont.	No.of Surviv. <50>	Av.FC.	% of cont.	No.of Surviv. <50>
1	12.0 (50)	50/50	12.0 (50)	100	50/50	12.0 (50)	100	50/50	11.2 (50)	93	50/50
2	12.2 (50)	50/50	12.1 (50)	99	50/50	11.9 (50)	98	50/50	11.0 (50)	90	50/50
3	12.0 (50)	50/50	12.1 (50)	101	50/50	12.0 (50)	100	50/50	11.4 (50)	95	50/50
4	12.4 (50)	50/50	12.5 (50)	101	50/50	12.3 (50)	99	50/50	11.4 (50)	92	50/50
5	13.1 (50)	50/50	13.0 (50)	99	50/50	12.6 (50)	96	50/50	12.1 (50)	92	50/50
6	12.9 (50)	50/50	12.6 (50)	98	50/50	12.4 (50)	96	50/50	11.8 (50)	91	50/50
7	12.6 (50)	50/50	12.5 (50)	99	50/50	11.8 (50)	94	50/50	11.3 (50)	90	50/50
8	12.6 (50)	50/50	12.4 (50)	98	50/50	11.8 (50)	94	50/50	11.4 (50)	90	50/50
9	13.1 (50)	50/50	12.7 (50)	97	50/50	12.3 (50)	94	50/50	11.9 (50)	91	50/50
10	12.9 (50)	50/50	12.8 (50)	99	50/50	12.5 (50)	97	50/50	11.9 (50)	92	50/50
11	12.8 (50)	50/50	13.0 (49)	102	50/50	12.8 (50)	100	50/50	12.3 (50)	96	50/50
12	13.2 (50)	50/50	12.7 (50)	96	50/50	12.3 (50)	93	50/50	11.9 (50)	90	50/50
13	13.2 (50)	50/50	12.8 (50)	97	50/50	12.5 (50)	95	50/50	12.5 (50)	95	50/50
14	12.8 (50)	50/50	12.9 (50)	101	50/50	12.3 (50)	96	50/50	12.1 (50)	95	50/50
18	12.4 (50)	50/50	12.7 (50)	102	50/50	12.3 (50)	99	50/50	12.1 (50)	98	50/50
22	12.9 (50)	50/50	12.8 (50)	99	50/50	12.3 (50)	95	50/50	12.7 (50)	98	50/50
26	12.5 (50)	50/50	12.9 (50)	103	50/50	12.4 (50)	99	50/50	12.6 (50)	101	50/50
30	13.5 (50)	50/50	13.7 (50)	101	50/50	13.2 (50)	98	50/50	13.3 (50)	99	50/50
34	12.9 (50)	50/50	13.3 (50)	103	50/50	12.7 (50)	98	50/50	13.1 (50)	102	50/50
38	12.6 (50)	50/50	12.8 (50)	102	50/50	12.2 (50)	97	50/50	13.0 (50)	103	50/50
42	13.3 (50)	50/50	13.2 (50)	99	50/50	12.8 (50)	96	50/50	13.2 (50)	99	50/50
46	13.2 (50)	50/50	13.0 (50)	98	50/50	12.8 (50)	97	50/50	13.1 (50)	99	50/50
50	13.7 (50)	50/50	13.5 (45)	99	50/50	13.3 (50)	97	50/50	13.5 (50)	99	50/50
54	12.9 (50)	50/50	13.1 (50)	102	50/50	12.2 (50)	95	50/50	13.3 (50)	103	50/50
58	13.2 (50)	50/50	13.9 (50)	105	50/50	12.7 (50)	96	50/50	12.9 (50)	98	50/50
62	13.2 (50)	50/50	13.1 (50)	99	50/50	13.0 (50)	98	50/50	13.6 (49)	103	49/50
66	13.7 (50)	50/50	13.9 (50)	101	50/50	13.6 (50)	99	50/50	14.0 (47)	102	47/50
70	13.7 (50)	50/50	14.0 (50)	102	50/50	13.7 (50)	100	50/50	14.3 (47)	104	47/50
74	14.2 (49)	49/50	14.9 (49)	105	49/50	14.3 (50)	101	50/50	14.4 (47)	101	47/50
78	14.3 (49)	49/50	14.7 (49)	103	49/50	14.8 (50)	103	50/50	14.8 (44)	103	44/50
82	14.3 (48)	48/50	14.6 (49)	102	49/50	13.8 (50)	97	50/50	14.7 (44)	103	44/50
86	14.8 (47)	48/50	15.1 (48)	102	48/50	15.1 (48)	102	48/50	15.6 (42)	105	42/50
90	15.1 (46)	46/50	14.3 (48)	95	48/50	15.4 (48)	102	48/50	15.6 (39)	103	39/50
94	14.5 (46)	46/50	14.6 (45)	101	45/50	15.0 (47)	103	47/50	14.6 (39)	101	39/50
98	14.4 (46)	46/50	13.6 (43)	94	42/50	13.7 (45)	95	45/50	15.2 (36)	106	36/50
102	15.2 (45)	45/50	14.0 (41)	92	41/50	14.5 (42)	95	43/50	14.5 (32)	95	31/50
104	13.6 (45)	45/50	13.3 (40)	98	40/50	13.7 (43)	101	43/50	13.9 (31)	102	30/50

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

TABLE 8 SELECTED NON NEOPLASTIC LESIONS OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

Group Name		Control				1000 ppm				2000 ppm				4000 ppm			
No. of Animals		50				50				50				50			
Organ	Findings Grade a)	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
spleen		<50> b)				<50>				<50>				<50>			
	fibrosis	1	0	0	0	1	1	0	0	3	2	0	0	6	5	0	0 **
eye		<50>				<50>				<50>				<50>			
	mineralization:cornea	33	0	0	0	25	0	0	0	26	0	0	0	17	0	0	0 **
liver		<50>				<50>				<50>				<50>			
	acidophilic cell focus	2	1	0	0	8	0	0	0	9	5	0	0 *	13	10	0	0 **
	basophilic cell focus	18	0	0	0	28	8	1	0 **	15	25	0	0 **	11	23	2	0 **
	vacuolated cell focus	2	0	0	0	7	0	0	0	12	1	0	0 **	8	3	0	0 *

a) 1 : Slight 2 : Moderate 3 : Marked 4 : Severe

b) : Number of animals examined at the site

Significant difference ; * : P ≦ 0.05 ** : P ≦ 0.01 Test of Chi Square

TABLE 9 SELECTED NON NEOPLASTIC LESIONS OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

Group Name		Control				1000 ppm				2000 ppm				4000 ppm			
No. of Animals		50				50				50				50			
Organ	Findings Grade a)	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
kidney		<50> b)				<50>				<50>				<50>			
	chronic nephropathy	8	23	14	3	22	21	2	0 **	19	17	5	0 **	27	6	5	0 **

a) 1 : Slight 2 : Moderate 3 : Marked 4 : Severe

b) : Number of animals examined at the site

Significant difference ; * : P ≦ 0.05 ** : P ≦ 0.01 Test of Chi Square

TABLE 10 NEOPLASTIC LESIONS INCIDENCE AND STATISTICAL ANALYSIS IN MALE RATS IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

Group Name	Control	1000ppm	2000ppm	4000ppm
SITE : subcutis				
TUMOR : fibroma				
Tumor rate				
Overall rates(a)	1/50(2.0)	4/50(8.0)	7/50(14.0)	12/50(24.0)
Adjusted rates(b)	3.13	9.30	15.56	36.67
Terminal rates(c)	1/32(3.1)	4/43(9.3)	5/38(13.2)	10/28(35.7)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0799 ?			
Prevalence method(d)	P=0.0001**			
Combined analysis (d)	P<0.0001**			
Cochran-Armitage test(e)	P=0.0004**			
Fisher Exact test(e)		P=0.1811	P=0.0297*	P=0.0009**
SITE : subcutis				
TUMOR : fibroma, fibrosarcoma				
Tumor rate				
Overall rates(a)	1/50(2.0)	4/50(8.0)	8/50(16.0)	12/50(24.0)
Adjusted rates(b)	3.13	9.30	17.78	36.67
Terminal rates(c)	1/32(3.1)	4/43(9.3)	6/38(15.8)	10/28(35.7)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0799 ?			
Prevalence method(d)	P=0.0001**			
Combined analysis (d)	P<0.0001**			
Cochran-Armitage test(e)	P=0.0005**			
Fisher Exact test(e)		P=0.1811	P=0.0154*	P=0.0009**
SITE : mammary gland				
TUMOR : fibroadenoma				
Tumor rate				
Overall rates(a)	1/50(2.0)	2/50(4.0)	3/50(6.0)	8/50(16.0)
Adjusted rates(b)	3.13	4.65	6.12	25.00
Terminal rates(c)	1/32(3.1)	2/43(4.7)	2/38(5.3)	7/28(25.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0923			
Prevalence method(d)	P=0.0020**			
Combined analysis (d)	P=0.0007**			
Cochran-Armitage test(e)	P=0.0037**			
Fisher Exact test(e)		P=0.5000	P=0.3086	P=0.0154*
SITE : mammary gland				
TUMOR : adenoma, fibroadenoma				
Tumor rate				
Overall rates(a)	2/50(4.0)	2/50(4.0)	3/50(6.0)	8/50(16.0)
Adjusted rates(b)	6.25	4.65	6.12	25.00
Terminal rates(c)	2/32(6.3)	2/43(4.7)	2/38(5.3)	7/28(25.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0923			
Prevalence method(d)	P=0.0068**			
Combined analysis (d)	P=0.0026**			
Cochran-Armitage test(e)	P=0.0126*			
Fisher Exact test(e)		P=0.3088	P=0.4999	P=0.0458*

TABLE 10 NEOPLASTIC LESIONS INCIDENCE AND STATISTICAL ANALYSIS IN MALE RATS IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE(continued)

Group Name	Control	1000ppm	2000ppm	4000ppm
SITE : mammary gland				
TUMOR : adenoma, fibroadenoma, adenocarcinoma				
Tumor rate				
Overall rates(a)	3/50(6.0)	2/50(4.0)	3/50(6.0)	8/50(16.0)
Adjusted rates(b)	6.25	4.65	6.12	25.00
Terminal rates(c)	2/32(6.3)	2/43(4.7)	2/38(5.3)	7/28(25.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.3854			
Prevalence method(d)	P=0.0068**			
Combined analysis (d)	P=0.0086**			
Cochran-Armitage test(e)	P=0.0345*			
Fisher Exact test(e)		P=0.4999	P=0.3388	P=0.0999
SITE : peritoneum				
TUMOR : mesothelioma				
Tumor rate				
Overall rates(a)	3/50(6.0)	1/50(2.0)	0/50(0.0)	7/50(14.0)
Adjusted rates(b)	5.13	0.0	0.0	14.29
Terminal rates(c)	1/32(3.1)	0/43(0.0)	0/38(0.0)	4/28(14.3)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0960			
Prevalence method(d)	P=0.0568			
Combined analysis (d)	P=0.0198*			
Cochran-Armitage test(e)	P=0.0409*			
Fisher Exact test(e)		P=0.3086	P=0.1212	P=0.1590
SITE : liver				
TUMOR : hepatocellular adenoma, hepatocellular carcinoma				
Tumor rate				
Overall rates(a)	1/50(2.0)	0/50(0.0)	2/50(4.0)	3/50(6.0)
Adjusted rates(b)	3.13	0.0	5.26	10.71
Terminal rates(c)	1/32(3.1)	0/43(0.0)	2/38(5.3)	3/28(10.7)
Statistical analysis				
Peto test				
Standard method(d)	P=---------			
Prevalence method(d)	P=0.0371*			
Combined analysis (d)	P=---------			
Cochran-Armitage test(e)	P=0.1232			
Fisher Exact test(e)		P=0.4999	P=0.5000	P=0.3086

(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 metho :Incidental tumor test
Combined analysi :Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
? :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 11 NEOPLASTIC LESIONS INCIDENCE AND STATISTICAL ANALYSIS IN FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

Group Name	Control	1000ppm	2000ppm	4000ppm
SITE : mammary gland				
TUMOR : fibroadenoma				
Tumor rate				
Overall rates(a)	7/50(14.0)	7/50(14.0)	9/50(18.0)	14/50(28.0)
Adjusted rates(b)	15.56	15.00	20.93	43.33
Terminal rates(c)	7/45(15.6)	6/40(4.7)	9/43(20.9)	13/30(43.3)
Statistical analysis				
Peto test				
Standard method(d)	P=---------			
Prevalence method(d)	P=0.0029**			
Combined analysis (d)	P=---------			
Cochran-Armitage test(e)	P=0.0454**			
Fisher Exact test(e)		P=0.3866	P=0.3932	P=0.0699
SITE : mammary gland				
TUMOR : adenoma, fibroadenoma				
Tumor rate				
Overall rates(a)	7/50(14.0)	8/50(16.0)	10/50(20.0)	14/50(28.0)
Adjusted rates(b)	15.56	17.50	23.26	43.33
Terminal rates(c)	7/45(15.6)	7/40(17.5)	10/43(23.3)	13/30(43.3)
Statistical analysis				
Peto test				
Standard method(d)	P=---------			
Prevalence method(d)	P=0.0035**			
Combined analysis (d)	P=---------			
Cochran-Armitage test(e)	P=0.0574			
Fisher Exact test(e)		P=0.4996	P=0.2980	P=0.0699
SITE : mammary gland				
TUMOR : adenoma, fibroadenoma, adenocarcinoma				
Tumor rate				
Overall rates(a)	7/50(14.0)	9/50(18.0)	10/50(20.0)	14/50(28.0)
Adjusted rates(b)	15.56	18.18	23.26	43.33
Terminal rates(c)	7/45(15.6)	7/40(17.5)	10/43(23.3)	13/30(43.3)
Statistical analysis				
Peto test				
Standard method(d)	P=0.5814			
Prevalence method(d)	P=0.0038**			
Combined analysis (d)	P=0.0056**			
Cochran-Armitage test(e)	P=0.0730			
Fisher Exact test(e)		P=0.3932	P=0.2980	P=0.0699
SITE : uterus				
TUMOR : endometrial stromal polyp				
Tumor rate				
Overall rates(a)	8/50(16.0)	11/50(22.0)	6/50(12.0)	9/50(18.0)
Adjusted rates(b)	17.39	21.28	13.95	20.00
Terminal rates(c)	7/45(15.6)	8/40(20.0)	6/43(14.0)	6/30(20.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0152*			
Prevalence method(d)	P=0.6589			
Combined analysis (d)	P=0.3162			
Cochran-Armitage test(e)	P=0.9493			
Fisher Exact test(e)		P=0.3059	P=0.3875	P=0.4995

TABLE 11 NEOPLASTIC LESIONS INCIDENCE AND STATISTICAL ANALYSIS IN FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE(continued)

Group Name	Control	1000ppm	2000ppm	4000ppm
SITE : uterus				
TUMOR : endometrial stromal sarcoma, leiomyosarcoma				
Tumor rate				
Overall rates(a)	0/50(0.0)	0/50(0.0)	0/50(0.0)	3/50(6.0)
Adjusted rates(b)	0.0	0.0	0.0	0.0
Terminal rates(c)	0/50(0.0)	0/40(0.0)	0/43(0.0)	0/30(0.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0034**?			
Prevalence method(d)	P=---------			
Combined analysis (d)	P=0.0034**?			
Cochran-Armitage test(e)	P=0.0079**			
Fisher Exact test(e)		P=0.5000	P=0.5000	P=0.1212
SITE : spleen				
TUMOR : mononclear cell lukemia				
Tumor rate				
Overall rates(a)	2/50(4.0)	4/50(8.0)	8/50(16.0)	7/50(14.0)
Adjusted rates(b)	2.22	7.50	9.30	10.00
Terminal rates(c)	1/45(2.2)	3/40(7.5)	4/43(9.3)	3/30(10.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0375*			
Prevalence method(d)	P=0.1001			
Combined analysis (d)	P=0.0151*			
Cochran-Armitage test(e)	P=0.0793			
Fisher Exact test(e)		P=0.3389	P=0.0458*	P=0.0798
SITE : pituitary gland				
TUMOR : adenoma				
Tumor rate				
Overall rates(a)	18/50(36.0)	28/50(56.0)	24/50(48.0)	19/50(38.0)
Adjusted rates(b)	37.78	53.19	51.16	42.42
Terminal rates(c)	17/45(37.8)	19/40(47.5)	22/43(51.2)	12/30(40.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.1991			
Prevalence method(d)	P=0.5048			
Combined analysis (d)	P=0.3817			
Cochran-Armitage test(e)	P=0.7183			
Fisher Exact test(e)		P=0.0353*	P=0.1558	P=0.4991

TABLE 11 NEOPLASTIC LESIONS INCIDENCE AND STATISTICAL ANALYSIS IN FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE(continued)

Group Name	Control	1000ppm	2000ppm	4000ppm
SITE : pituitary gland				
TUMOR : adenoma, adenocarcinoma				
Tumor rate				
Overall rates(a)	19/50(38.0)	29/50(58.0)	24/50(48.0)	19/50(38.0)
Adjusted rates(b)	37.78	55.32	51.16	42.42
Terminal rates(c)	17/45(37.8)	20/40(50.0)	22/43(51.2)	12/30(40.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.3387			
Prevalence method(d)	P=0.5659			
Combined analysis (d)	P=0.4988			
Cochran-Armitage test(e)	P=0.5485			
Fisher Exact test(e)		P=0.0356*	P=0.2101	P=0.4173

(a):Number of tumor-bearing animals/number of animals examined at the si
(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 metho :Incidental tumor test
Combined analysi :Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates.
? :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 12 CAUSE OF DEATH OF RATS IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

	Male				Female			
Group	Control	1000ppm	2000ppm	4000ppm	Control	1000ppm	2000ppm	4000ppm
Number of dead or moribund animals	18	7	12	22	5	10	7	20
No microscopical confirmation	0	0	0	1	0	0	0	0
Cardiovascular lesion	0	1	0	0	0	0	0	1
Digestive system lesion	2	0	1	0	0	0	0	1
Renal lesion	0	0	0	2	0	0	1	2
Thrombosis	0	0	0	0	0	1	0	0
Hemorrhage	0	0	0	0	0	1	0	0
Urinary retention	0	0	1	0	0	0	0	0
Chronic nephropathy	2	0	2	3	0	0	0	0
Tumor death : leukemia	3	1	5	4	2	3	5	4
subcutis	1	0	1	3	0	0	0	0
lung	1	0	0	0	0	0	0	0
oral cavity	0	0	0	0	1	0	0	0
stomach	1	0	0	0	0	0	0	0
liver	1	0	0	0	0	0	0	0
pancreas	0	1	0	0	0	0	0	0
kidney	0	1	0	1	0	0	0	0
urinary bladder	0	0	0	2	0	0	0	0
pituitary	3	0	1	1	2	3	0	4
thyroid	0	1	0	0	0	0	0	1
ovary	-	-	-	-	0	0	0	1
uterus	-	-	-	-	0	1	0	6
brain	2	0	0	2	0	0	0	0
mamary gland	0	0	0	1	0	1	0	0
brain	1	0	1	0	0	0	0	0
Zymbal gland	1	1	0	0	0	0	0	0
bone	0	0	0	0	0	0	1	0
peritoneum	2	1	0	3	0	0	0	0
retroperit	0	0	0	1	0	0	0	0

FIGURES

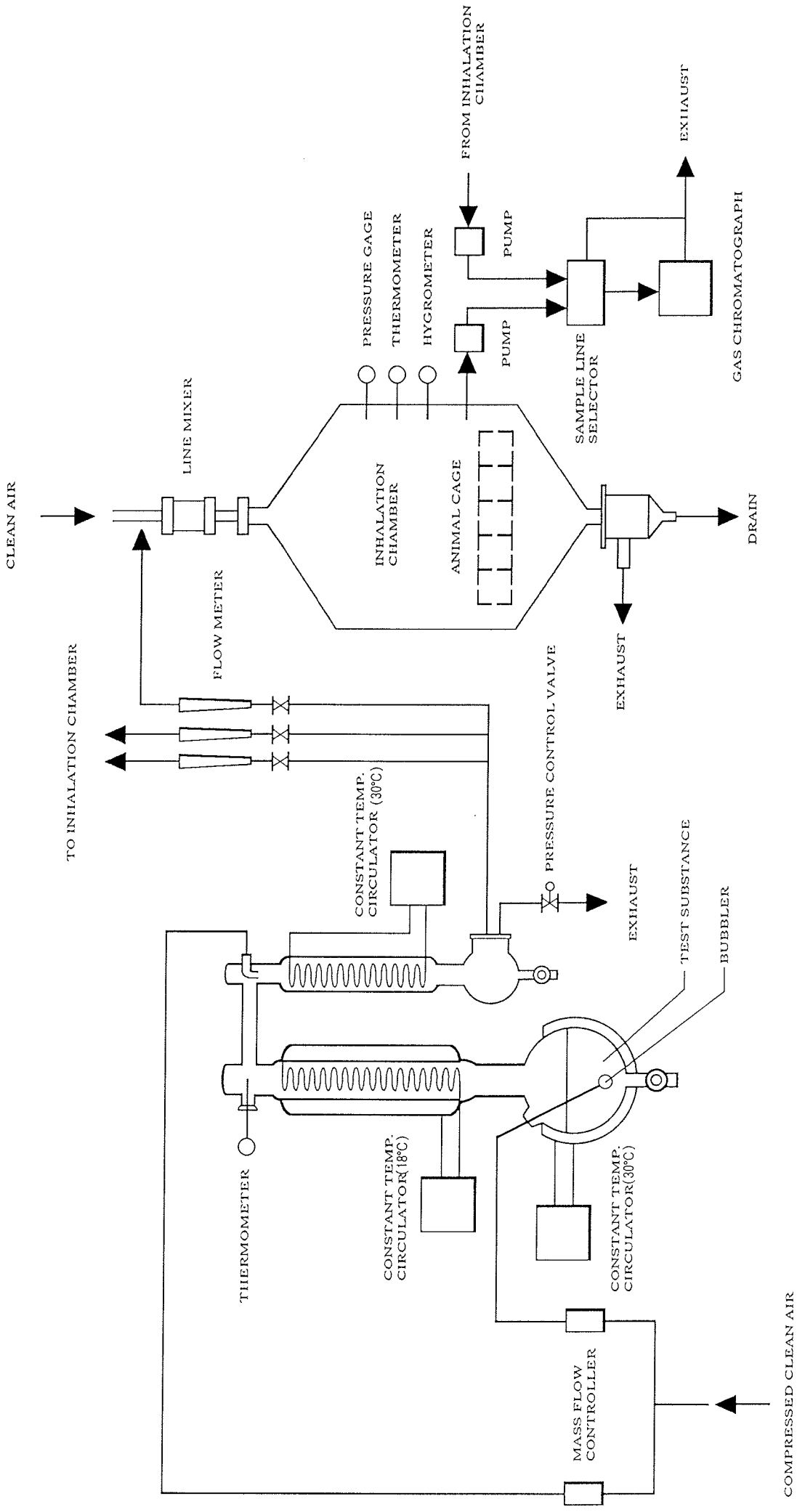


FIGURE 1 DICHLOROMETHANE VAPOR GENERATION SYSTEM AND INHALATION SYSTEM

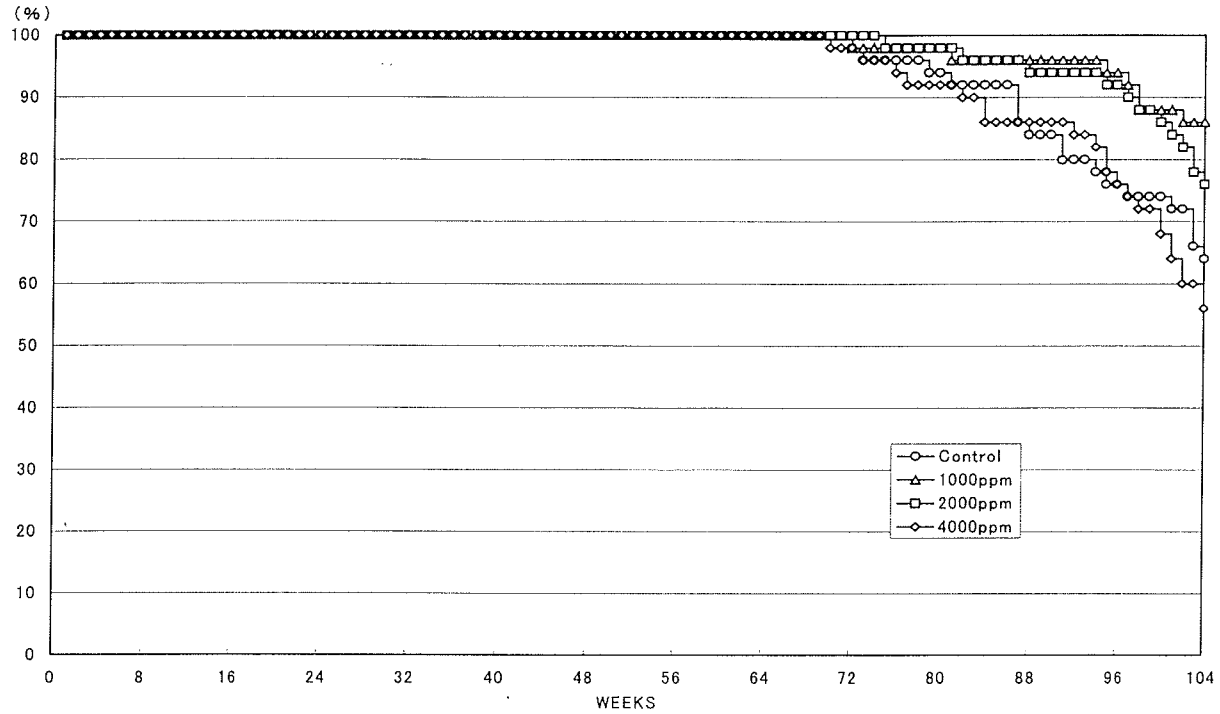


FIGURE 2 SURVIVAL ANIMAL RATE OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

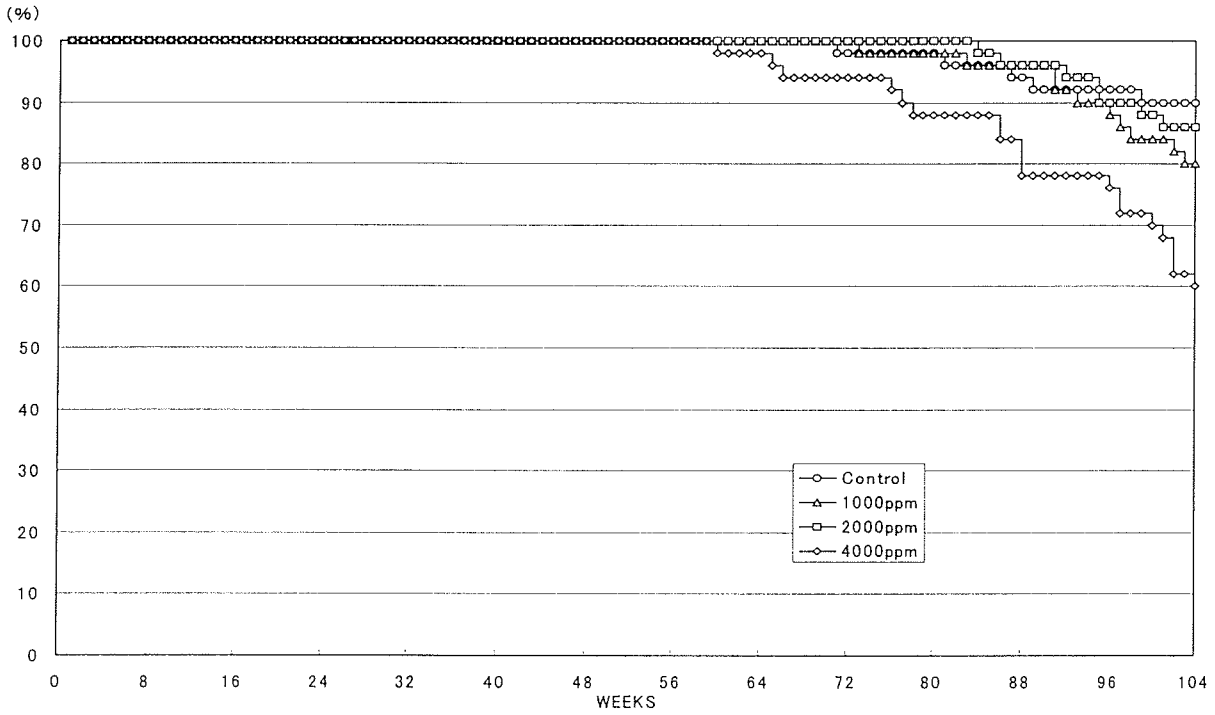


FIGURE 3 SURVIVAL ANIMAL RATE OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

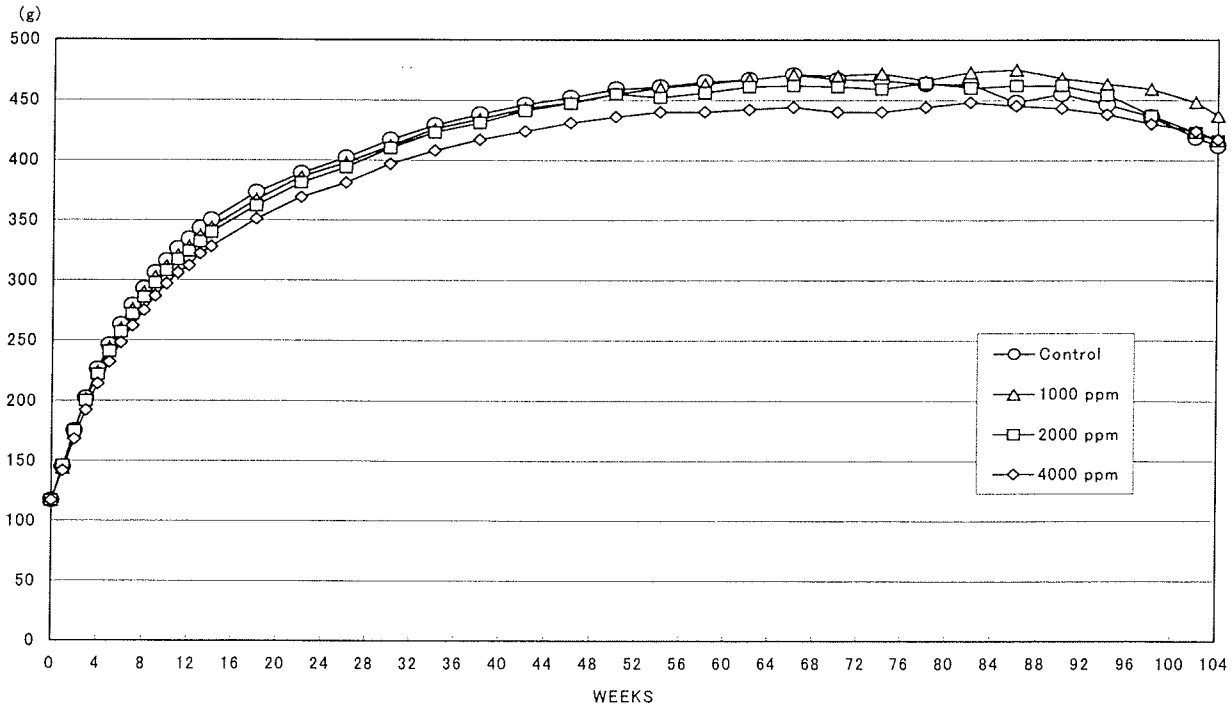


FIGURE 4 BODY WEIGHT CHANGES OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

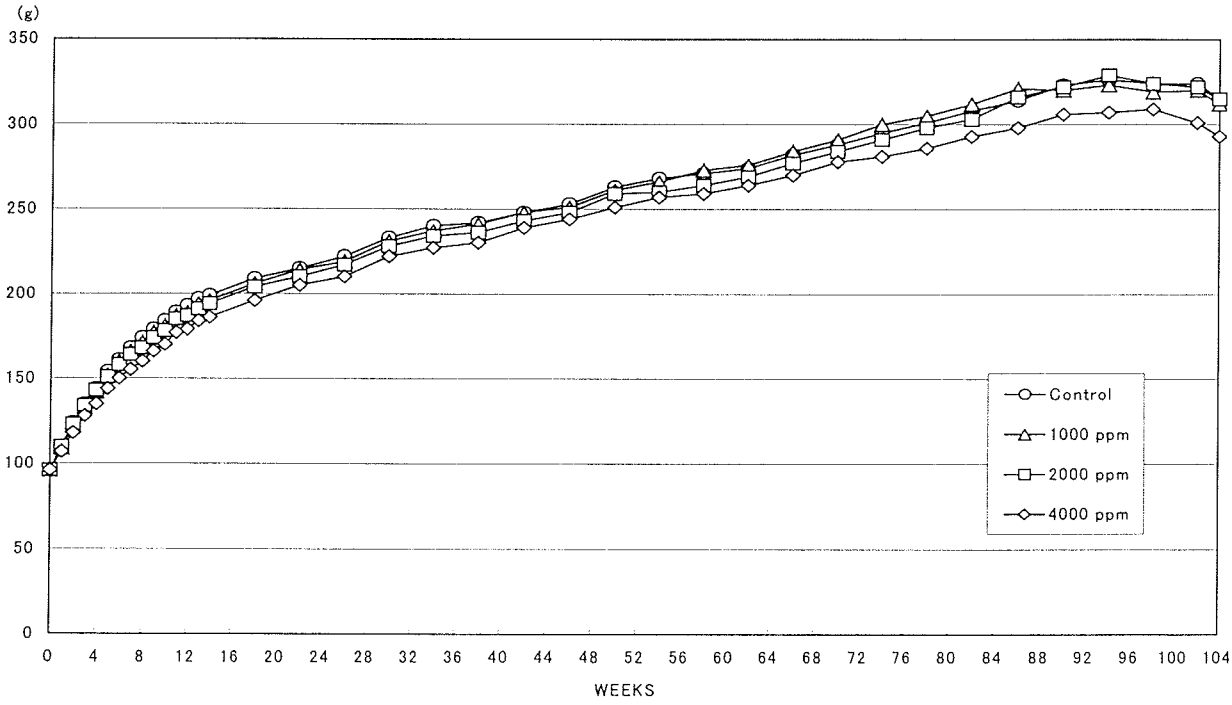


FIGURE 5 BODY WEIGHT CHANGES OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

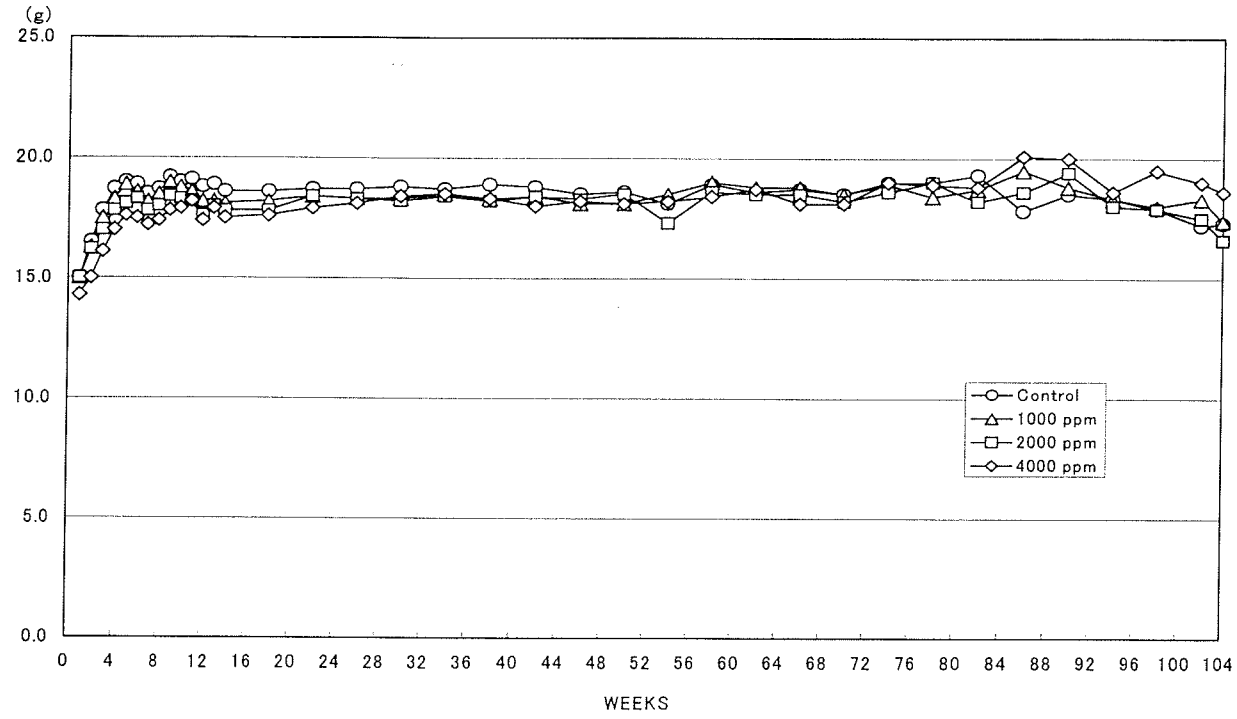


FIGURE 6 FOOD CONSUMPTION CHANGES OF MALE RATS IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

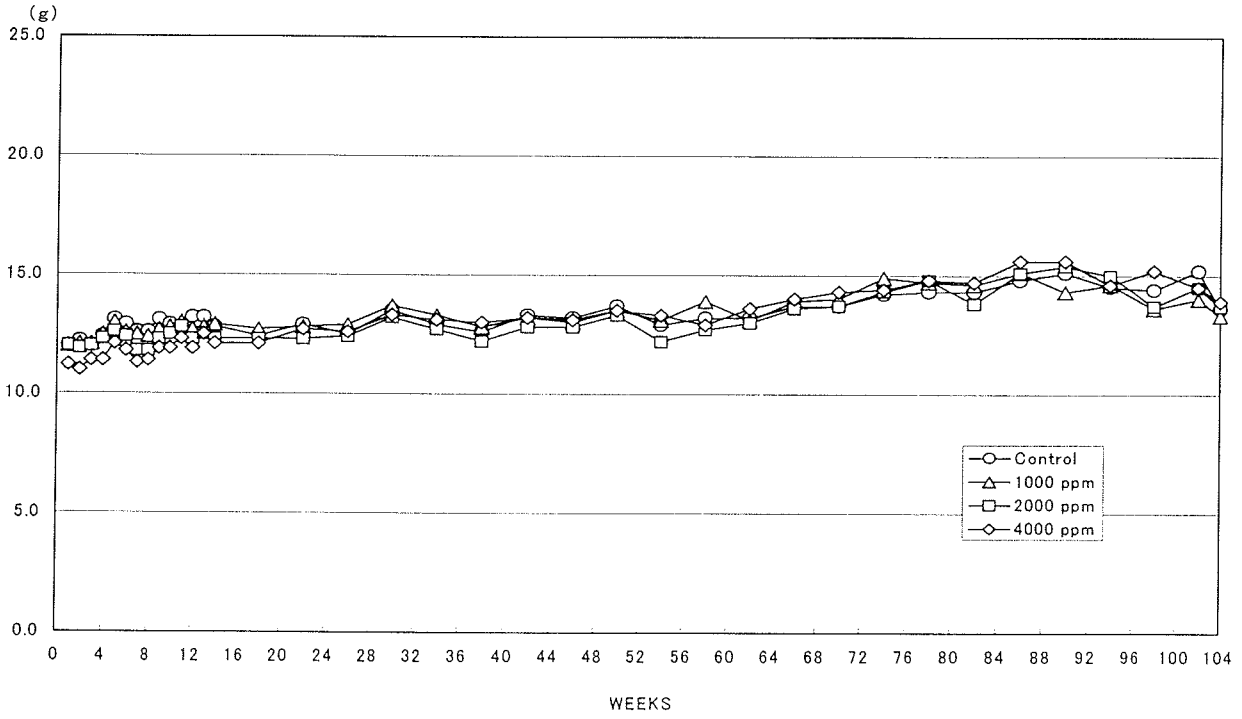


FIGURE 7 FOOD CONSUMPTION CHANGES OF FEMALE RATS IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

PHOTOGRAPHS

PHOTOGRAPH 1 SUBCUTIS : FIBROMA
MALE, 4000ppm, ANIMAL No. 1324 (H&E)

PHOTOGRAPH 2 MAMMARY GLAND : FIBROADENOMA
MALE, 4000ppm, ANIMAL No. 1333 (H&E)

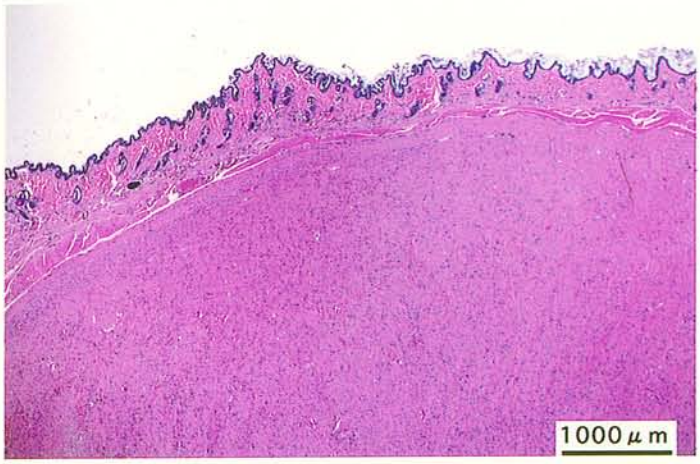
PHOTOGRAPH 3 PERITONEUM : MESOTHELIOMA
MALE, 4000ppm, ANIMAL No. 1307 (H&E)

PHOTOGRAPH 4 LIVER : ACIDOPHILIC CELL FOCUS
FEMALE, 4000ppm, ANIMAL No. 2304 (H&E)

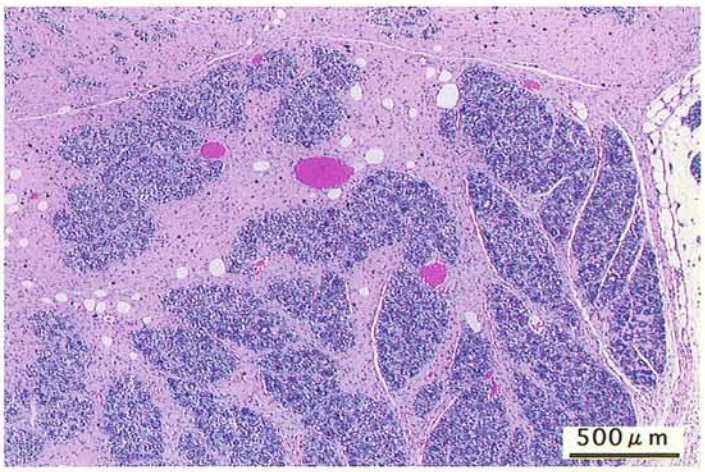
PHOTOGRAPH 5 LIVER : BASOPHILIC CELL FOCUS
FEMALE, 4000ppm, ANIMAL No. 2304 (H&E)

PHOTOGRAPH 6 LIVER : VACUOLATED CELL FOCUS
FEMALE, 4000ppm, ANIMAL No. 2309 (H&E)

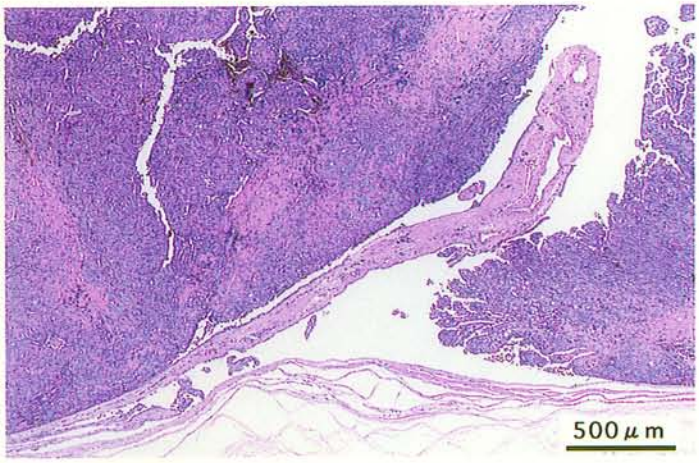
PHOTOGRAPH 7 SPLEEN : FIBROSIS
MALE, 4000ppm, ANIMAL No. 1322 (H&E)



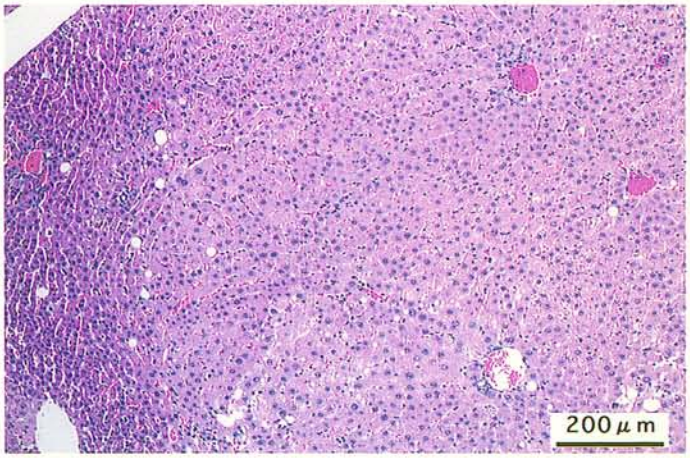
PHOTOGRAPH. 1



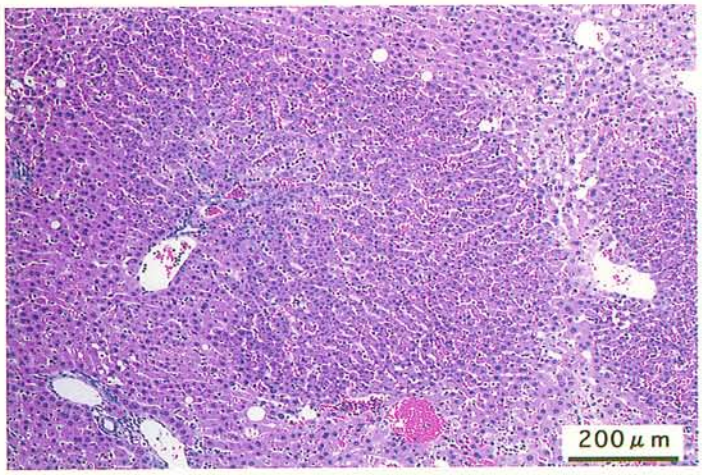
PHOTOGRAPH. 2



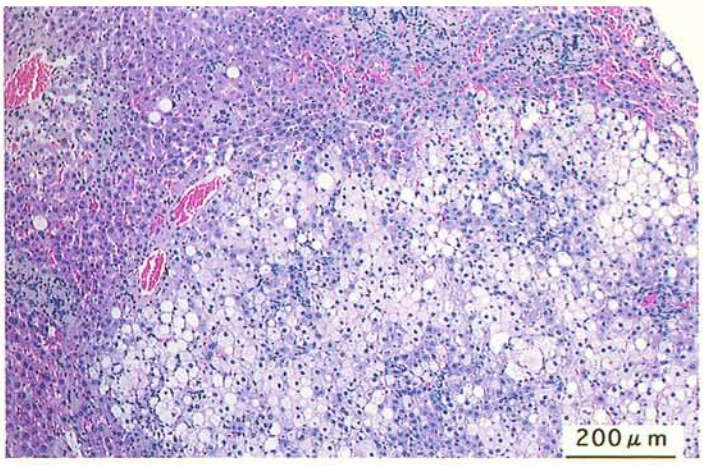
PHOTOGRAPH. 3



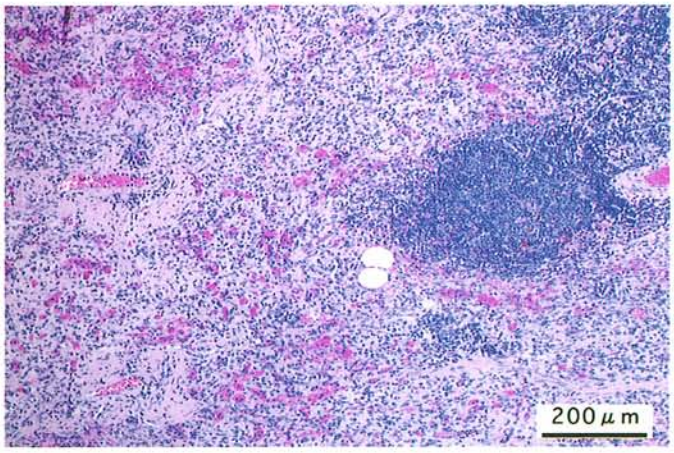
PHOTOGRAPH. 4



PHOTOGRAPH. 5



PHOTOGRAPH. 6



PHOTOGRAPH. 7