Summary of Inhalation Carcinogenicity Study

of Dichloromethane

in BDF1 Mice

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Japan Bioassay Research Center

Japan Industrial Safety and Health Association

PREFACE

The tests were contracted and supported by the Ministry of 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 March 31, 2000.

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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 Crj:BDF1 mice. 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 mice. 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: 3700 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 and body weights of both males and females exposed to 2000 and 4000 ppm dichloromethane were significantly decreased: survival rates of the controls, 1000, 2000 and 4000 ppm-exposed groups were 76%, 70%, 52% and 40% for males and 52%, 52%, 34% and 42% for females; terminal body weights of the 1000, 2000 and 4000 ppm-exposed groups were 90%, 88% and 70% of the controls for males and 96%, 92% and 83% of the controls for females. The decreased survival rates were causally related to an increase in death rate due to lung and liver tumors. Bronchiolar-alveolar adenomas and carcinomas of the lung and hepatocellular adenomas and carcinomas were markedly increased in both males and females. Hemangioma in the liver and pheochromocytoma in the adrenal gland were increased in males and the combined incidences of hemangioma and hemangiosarcoma in the liver was increased in females. These tumors could not be negligible the effect on dichloromethane exposure. Hyperplasia in the terminal bronchiole of the lung (this lesion is classified as a preneoplastic lesion capable of developing into bronchiolar-alveolar adenoma and carcinoma) and peripheral vacuolic change in the liver were increased in males and females exposed to 4000 ppm dichloromethane.

Conclusions

There was clear evidence of carcinogenic activity of dichloromethane in male and female mice, based on the increased incidences of bronchiolar-alveolar adenomas and carcinomas of the lung and hepatocellular adenomas and carcinomas.

Incidences of selected neoplastic lesions of male mice 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							
lung	bronchiolar-alveolar adenoma	7	3	4	14	↑ ↑	↑
liver	hepatocellular adenoma	10	13	14	15	↑	
	hemangioma	0	4	3	5 *	↑	
adrenal	pheochromocytoma	1	0	1	3	↑	
malignant tumor							
lung	bronchiolar-alveolar carcinoma	1	14 **	22 **	39 **	↑ ↑	↑ ↑
lymph node	malignant lymphoma	14	13	16	9		
liver	hepatocellular carcinoma	10	9	14	20 *	↑ ↑	↑ ↑
	hepatoblastoma	0	0	0	1		
	hemangiosarcoma	1	0	1	1		
spleen	malignant lymphoma	2	1	5	2		
	mastcytoma:malignant	2	4	0	0		
combined analysis							
lung	(bronchiolar-alveolar adenoma+ bronchiolar-alveolar carcinoma)	8	17 *	26 **	42 **	↑ ↑	↑ ↑
liver	(hepatocellular adenoma+ hepatocellular carcinoma+ hepatoblastoma)	15	20	25 *	29 **	↑ ↑	↑ ↑
	(hemangioma+ hemangiosarcoma)	1	4	4	6	↑ ↑	
all organ	hemangioma	1	5	6	7 *	↑ ↑	
	histiocytic sarcoma	5	2	3	0 *		↓

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)

Incidences of selected neoplastic lesions of female mice 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	49	50		
benign tumor							
lung	bronchiolar-alveolar adenoma	2	4	5	12 **	↑ ↑	↑ ↑
liver	hepatocellular adenoma	1	7[a)] *	4	16 **	↑ ↑	↑ ↑
	hemangioma	2	2[a)]	0	5		
subcutis	hemangioma	2	0	0	0		
pituitary	adenoma	13	9	6	6		
ovary	cystadenoma	0	2	1	3		
malignant tumor							
lung	bronchiolar-alveolar carcinoma	3	1	8	20 **	↑ ↑	↑ ↑
	adenosquamous carcinoma	0	0	0	1		
liver	hepatocellular carcinoma	1	1[a)]	5	19 **	↑ ↑	↑ ↑
	hemangiosarcoma	1	0[a)]	0	2		
lymph node	malignant lymphoma	23	31	19	15		↓
subcutis	hemangiosarcoma	1	1	0	0		
mammary gland	adenocarcinoma	2	0	1	3		
uterus	histiocytic sarcoma	15	10	15	13		
combined analysis							
lung	(bronchiolar-alveolar adenoma+ bronchiolar-alveolar carcinoma+ adenosquamous carcinoma)	5	5	12 *	30 **	↑ ↑	↑ ↑
liver	(hepatocellular adenoma+ hepatocellular carcinoma)	2	8[a)] *	9 *	30 **	↑	↑ ↑
	(hemangioma+ hemangiosarcoma)	3	2[a)]	0	7	↑ ↑	
subcutis	(hemangioma+ hemangiosarcoma)	3	1	0	0		↓
all organ	malignant lymphoma	25	33	21	17		↓

a) : Number of examined animals is 49.

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
Crj:BDF1 mouse
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
Temperature: 23±2℃
Fluorescent light 12h/d
Chamber Environment
Temperature: 22±2℃
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,
Glutamic oxaloacetic transaminase (GOT),
Glutamic pyruvic transaminase (GPT),
Lactate dehydrogenase (LDH),
Alkaline phosphatase (ALP),
Creatine phosphokinase (CPK),
Urea nitrogen,
Sodium, Potassium, Chloride,
Calcium, Inorganic phosphorus.

<Urinalysis>
pH, Protein, Glucose, Ketone body,
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,gall bladder, 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 MICE 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	21.8 (50)	50/50	21.8 (50)	100	50/50	21.8 (50)	100	50/50	21.8 (50)	100	50/50
1	21.6 (50)	50/50	21.6 (50)	100	50/50	21.6 (50)	100	50/50	21.6 (50)	100	50/50
1	23.1 (50)	50/50	23.0 (50)	100	50/50	23.2 (50)	100	50/50	22.8 (50)	99	50/50
2	24.3 (50)	50/50	24.2 (50)	100	50/50	24.3 (50)	100	50/50	24.7 (49)	102	49/50
3	24.9 (50)	50/50	24.9 (50)	100	50/50	25.1 (50)	101	50/50	25.4 (49)	102	49/50
4	25.8 (50)	50/50	25.5 (50)	99	50/50	26.0 (50)	101	50/50	26.4 (49)	102	49/50
5	26.4 (50)	50/50	26.1 (50)	99	50/50	26.6 (50)	101	50/50	26.9 (49)	102	49/50
6	27.0 (50)	50/50	26.7 (50)	99	50/50	27.2 (50)	101	50/50	27.4 (49)	101	49/50
7	27.7 (50)	50/50	27.0 (50)	97	50/50	27.8 (50)	100	50/50	28.1 (49)	101	49/50
8	28.2 (50)	50/50	27.6 (50)	98	50/50	28.2 (50)	100	50/50	28.4 (49)	101	49/50
9	28.8 (50)	50/50	28.2 (50)	98	50/50	28.9 (50)	100	50/50	29.2 (49)	101	49/50
10	29.5 (50)	50/50	28.8 (50)	98	50/50	29.5 (50)	100	50/50	29.8 (49)	101	49/50
11	30.4 (50)	50/50	29.2 (50)	96	50/50	30.0 (50)	99	50/50	30.2 (49)	99	49/50
12	31.0 (50)	50/50	29.6 (50)	95	50/50	30.7 (50)	99	50/50	30.7 (49)	99	49/50
13	31.9 (50)	50/50	30.3 (50)	95	50/50	31.6 (49)	99	49/50	31.3 (49)	98	49/50
14	32.6 (50)	50/50	30.8 (50)	94	50/50	32.5 (49)	100	49/50	31.9 (49)	98	49/50
18	34.0 (50)	50/50	32.6 (50)	96	50/50	33.9 (49)	100	49/50	33.5 (49)	99	49/50
22	36.0 (50)	50/50	34.4 (50)	96	50/50	35.6 (49)	99	49/50	34.4 (49)	96	49/50
26	38.6 (49)	49/50	36.2 (50)	94	50/50	38.0 (49)	98	49/50	36.6 (49)	95	49/50
30	40.3 (49)	49/50	37.8 (50)	94	50/50	39.4 (49)	98	49/50	37.3 (49)	93	49/50
34	42.0 (49)	49/50	39.0 (50)	93	50/50	40.9 (49)	97	49/50	38.5 (49)	92	49/50
38	43.5 (49)	49/50	40.5 (50)	93	50/50	42.5 (49)	98	49/50	40.0 (49)	92	49/50
42	44.7 (49)	49/50	41.2 (50)	92	50/50	43.4 (49)	97	49/50	40.6 (49)	91	49/50
46	45.5 (49)	49/50	42.3 (50)	93	50/50	44.2 (49)	97	49/50	41.1 (48)	90	48/50
50	46.3 (49)	49/50	42.7 (50)	92	50/50	44.3 (49)	96	49/50	41.2 (48)	89	48/50
54	46.9 (49)	49/50	43.3 (49)	92	49/50	44.9 (49)	96	49/50	42.0 (48)	90	48/50
58	47.0 (49)	49/50	43.7 (49)	93	49/50	45.3 (49)	96	49/50	41.8 (48)	89	48/50
62	48.2 (48)	48/50	44.7 (48)	93	48/50	46.2 (49)	96	49/50	43.3 (47)	90	47/50
66	49.2 (48)	48/50	45.6 (48)	93	48/50	46.4 (49)	94	49/50	43.5 (45)	88	45/50
70	49.9 (47)	48/50	46.8 (48)	94	48/50	47.2 (49)	95	49/50	43.8 (44)	88	44/50
74	50.4 (47)	47/50	46.2 (48)	92	48/50	47.2 (48)	94	48/50	41.9 (44)	83	44/50
78	50.3 (47)	47/50	46.6 (47)	93	47/50	47.2 (47)	94	47/50	42.9 (42)	85	42/50
82	51.9 (47)	47/50	47.2 (47)	91	47/50	47.5 (44)	92	44/50	41.9 (41)	81	41/50
86	51.6 (47)	47/50	46.5 (47)	90	47/50	46.8 (42)	91	42/50	40.5 (40)	78	40/50
90	51.0 (45)	45/50	45.9 (45)	90	45/50	47.3 (38)	93	38/50	39.0 (39)	76	39/50
94	49.8 (43)	43/50	44.2 (44)	89	44/50	45.8 (37)	92	37/50	37.0 (34)	74	34/50
98	48.9 (42)	42/50	44.6 (39)	91	39/50	43.6 (36)	89	36/50	36.6 (28)	75	28/50
102	47.8 (40)	40/50	44.3 (36)	93	36/50	42.1 (32)	88	31/50	34.1 (23)	71	22/50
104	48.1 (38)	38/50	43.5 (35)	90	35/50	42.5 (27)	88	26/50	33.6 (20)	70	20/50

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

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

Weeks on Study	Control Av.Wt.	Control No.of Surviv. <50>	1000ppm Av.Wt.	1000ppm % of cont.	1000ppm No.of Surviv. <50>	2000ppm Av.Wt.	2000ppm % of cont.	2000ppm No.of Surviv. <49>	4000ppm Av.Wt.	4000ppm % of cont.	4000ppm No.of Surviv. <50>
0	18.3 (50)	50/50	18.3 (50)	100	50/50	18.3 (49)	100	50/50	18.3 (50)	100	50/50
1	17.5 (50)	50/50	17.7 (50)	101	50/50	17.8 (49)	102	49/49	17.7 (50)	101	50/50
1	18.8 (50)	50/50	19.1 (50)	102	50/50	19.0 (49)	101	49/49	18.9 (50)	101	50/50
2	19.9 (50)	50/50	20.0 (50)	101	50/50	20.3 (49)	102	49/49	20.3 (50)	102	50/50
3	20.7 (50)	50/50	20.8 (50)	100	50/50	20.9 (49)	101	49/49	21.3 (50)	103	50/50
4	21.3 (50)	50/50	21.6 (50)	101	50/50	21.9 (49)	103	49/49	22.1 (50)	104	50/50
5	21.9 (50)	50/50	21.9 (50)	100	50/50	22.2 (49)	101	49/49	22.5 (50)	103	50/50
6	22.4 (50)	50/50	22.5 (50)	100	50/50	22.8 (49)	102	49/49	22.9 (50)	102	50/50
7	22.7 (50)	50/50	22.9 (50)	101	50/50	23.0 (49)	101	49/49	22.9 (50)	101	50/50
8	23.3 (50)	50/50	23.6 (50)	101	50/50	23.7 (49)	102	49/49	23.7 (50)	102	50/50
9	23.5 (50)	50/50	23.7 (50)	101	50/50	23.5 (49)	100	49/49	23.6 (50)	100	50/50
10	23.8 (50)	50/50	23.7 (50)	100	50/50	24.2 (49)	102	49/49	24.1 (50)	101	50/50
11	24.2 (50)	50/50	24.0 (50)	99	50/50	24.3 (49)	100	49/49	24.3 (50)	100	50/50
12	24.6 (50)	50/50	24.5 (50)	100	50/50	24.6 (49)	100	49/49	24.5 (50)	100	50/50
13	25.0 (50)	50/50	24.8 (50)	99	50/50	24.9 (49)	100	49/49	25.0 (50)	100	50/50
14	25.0 (50)	50/50	24.9 (50)	100	50/50	24.8 (49)	99	49/49	25.2 (50)	101	50/50
18	26.3 (50)	50/50	25.9 (49)	98	49/50	26.0 (49)	99	49/49	25.5 (50)	97	50/50
22	27.1 (50)	50/50	26.8 (49)	99	49/50	26.4 (49)	97	49/49	26.0 (50)	96	50/50
26	27.8 (50)	50/50	27.2 (49)	98	49/50	27.2 (49)	98	49/49	26.8 (50)	96	50/50
30	28.9 (50)	50/50	27.7 (49)	96	49/50	27.4 (49)	95	49/49	26.7 (50)	92	50/50
34	29.5 (50)	50/50	28.4 (49)	96	49/50	28.1 (49)	95	49/49	27.2 (50)	92	50/50
38	29.9 (50)	50/50	29.0 (49)	97	49/50	28.3 (49)	95	49/49	27.6 (50)	92	50/50
42	30.2 (50)	50/50	29.0 (49)	96	49/50	28.6 (49)	95	49/49	27.5 (50)	91	50/50
46	30.8 (50)	50/50	29.5 (49)	96	49/50	28.8 (49)	94	49/49	27.8 (50)	90	50/50
50	30.8 (50)	50/50	29.6 (49)	96	49/50	28.9 (49)	94	49/49	27.7 (50)	90	50/50
54	31.2 (49)	49/50	29.4 (47)	94	47/50	29.1 (49)	93	49/49	27.8 (50)	89	50/50
58	21.7 (49)	49/50	29.8 (47)	94	47/50	29.6 (49)	93	49/49	27.9 (50)	88	50/50
62	32.2 (49)	49/50	30.7 (47)	95	47/50	30.2 (47)	94	47/49	28.3 (49)	88	49/50
66	33.0 (48)	48/50	31.5 (46)	95	46/50	30.5 (46)	92	46/49	28.1 (49)	85	49/50
70	33.5 (47)	46/50	31.8 (44)	95	44/50	31.6 (45)	94	45/49	28.7 (49)	86	49/50
74	33.6 (44)	44/50	31.5 (44)	94	44/50	31.0 (41)	92	41/49	28.3 (49)	84	49/50
78	34.1 (41)	41/50	31.6 (44)	93	44/50	31.4 (37)	92	37/49	28.9 (46)	85	46/50
82	35.7 (41)	41/50	32.2 (43)	90	43/50	31.4 (34)	88	34/49	28.3 (41)	79	41/50
86	35.7 (40)	40/50	32.9 (37)	92	37/50	32.0 (28)	90	28/49	28.6 (39)	80	39/50
90	35.7 (38)	38/50	32.4 (32)	91	32/50	32.6 (26)	91	26/49	28.1 (34)	79	34/50
94	34.3 (34)	34/50	32.0 (30)	93	30/50	32.0 (21)	93	21/49	27.8 (31)	81	31/50
98	34.0 (29)	29/50	32.6 (28)	96	28/50	31.8 (19)	94	19/49	27.9 (29)	82	29/50
102	33.8 (28)	28/50	32.3 (26)	96	26/50	31.1 (18)	92	18/49	28.0 (22)	83	22/50
104	33.3 (26)	26/50	32.0 (26)	96	26/50	30.8 (17)	92	17/49	27.5 (21)	83	21/50

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

TABLE 4 INCIDENCE OF EXTERNAL AND INTERNAL MASS IN CLINICAL OBSERVATION OF MALE MICE 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	0/49	0/49	0/49	1/48	1/47	3/44	3/50(1/12)
	1000ppm	0/50	0/50	0/50	0/50	0/50	0/48	1/47	6/45	6/50(2/15)
	2000ppm	0/50	0/49	0/49	0/49	0/49	0/49	1/46	1/37	2/50(2/24)
	4000ppm	0/50	0/49	0/49	0/49	0/48	0/45	0/42	2/35	2/50(1/30)
Internal mass										
	Control	0/50	1/50	0/49	0/49	0/49	0/48	4/47	10/44	11/50(5/12)
	1000ppm	0/50	0/50	0/50	0/50	0/50	0/48	5/47	7/45	7/50(4/15)
	2000ppm	0/50	0/49	0/49	0/49	1/49	4/49	8/46	7/37	10/50(8/24)
	4000ppm	0/50	2/49	2/49	3/49	3/48	1/45	6/42	8/35	13/50(11/30)

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 animal

TABLE 5 INCIDENCE OF EXTERNAL AND INTERNAL MASS IN CLINICAL OBSERVATION OF FEMALE MICE 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	1/48	0/41	2/36	3/50(2/24)
	1000ppm	0/50	0/50	0/49	0/49	0/48	0/46	3/44	2/31	4/50(2/24)
	2000ppm	0/49	0/49	0/49	0/49	0/49	0/46	3/37	2/23	3/49(3/32)
	4000ppm	0/50	0/50	0/50	0/50	1/50	1/49	5/45	3/31	8/50(7/29)
Internal mass										
	Control	0/50	0/50	0/50	1/50	3/50	4/48	6/41	13/36	20/50(11/24)
	1000ppm	0/50	0/50	0/49	2/49	2/48	2/46	10/44	5/31	16/50(13/24)
	2000ppm	0/49	0/49	1/49	1/49	1/49	10/46	11/37	6/23	19/49(17/32)
	4000ppm	0/50	0/50	0/50	0/50	0/50	4/49	11/45	10/31	17/50(11/29)

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 animal

TABLE 6 FOOD CONSUMPTION CHANGES OF MALE MICE
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	3.9 (50)	50/50	3.9 (50)	100	50/50	3.9 (50)	100	50/50	3.8 (50)	97	50/50
2	3.8 (50)	50/50	3.8 (50)	100	50/50	3.8 (50)	100	50/50	4.1 (50)	108	49/50
3	3.9 (50)	50/50	4.0 (50)	103	50/50	4.0 (50)	103	50/50	4.3 (49)	110	49/50
4	4.1 (50)	50/50	4.1 (50)	100	50/50	4.2 (50)	102	50/50	4.5 (49)	110	49/50
5	4.1 (50)	50/50	4.1 (50)	100	50/50	4.2 (50)	102	50/50	4.6 (49)	112	49/50
6	4.2 (50)	50/50	4.2 (50)	100	50/50	4.2 (50)	100	50/50	4.4 (49)	105	49/50
7	4.2 (50)	50/50	4.2 (50)	100	50/50	4.3 (50)	102	50/50	4.5 (49)	107	49/50
8	4.3 (50)	50/50	4.2 (50)	98	50/50	4.2 (50)	98	50/50	4.6 (49)	107	49/50
9	4.3 (50)	50/50	4.4 (50)	102	50/50	4.4 (50)	102	50/50	4.7 (49)	109	49/50
10	4.3 (50)	50/50	4.2 (50)	98	50/50	4.2 (50)	98	50/50	4.3 (49)	100	49/50
11	4.4 (50)	50/50	4.2 (50)	95	50/50	4.2 (50)	95	50/50	4.5 (49)	102	49/50
12	4.3 (50)	50/50	4.3 (50)	100	50/50	4.3 (50)	100	50/50	4.7 (49)	109	49/50
13	4.3 (50)	50/50	4.2 (50)	98	50/50	4.3 (50)	100	50/50	4.5 (49)	105	49/50
14	4.3 (50)	50/50	4.3 (50)	100	50/50	4.4 (49)	102	49/50	4.8 (49)	112	49/50
18	4.5 (50)	50/50	4.5 (50)	100	50/50	4.6 (49)	102	49/50	4.9 (49)	109	49/50
22	4.5 (50)	50/50	4.5 (50)	100	50/50	4.5 (49)	100	49/50	4.9 (49)	109	49/50
26	4.6 (49)	49/50	4.6 (50)	100	50/50	4.7 (49)	102	49/50	5.2 (49)	113	49/50
30	4.7 (49)	49/50	4.6 (50)	98	50/50	4.6 (49)	98	49/50	5.1 (48)	119	49/50
34	4.7 (49)	49/50	4.7 (50)	100	50/50	4.8 (49)	102	49/50	5.2 (49)	111	49/50
38	5.0 (49)	49/50	5.1 (50)	102	50/50	5.1 (49)	102	49/50	5.5 (49)	110	49/50
42	4.8 (49)	49/50	4.7 (50)	98	50/50	5.0 (49)	104	49/50	5.4 (49)	113	49/50
46	4.7 (49)	49/50	4.7 (50)	100	50/50	4.8 (49)	102	49/50	5.1 (48)	109	48/50
50	4.9 (49)	49/50	4.8 (50)	98	50/50	5.0 (49)	102	49/50	5.2 (48)	106	48/50
54	4.9 (49)	49/50	4.9 (49)	100	49/50	5.0 (49)	102	49/50	5.2 (48)	106	48/50
58	5.1 (49)	49/50	5.0 (49)	98	49/50	5.1 (49)	100	49/50	5.3 (48)	104	48/50
62	5.1 (48)	48/50	5.0 (48)	98	48/50	5.1 (49)	100	49/50	5.2 (47)	102	47/50
66	5.3 (48)	48/50	5.2 (48)	98	48/50	5.3 (49)	100	49/50	5.3 (45)	100	45/50
70	5.4 (48)	48/50	5.5 (48)	102	48/50	5.6 (49)	104	49/50	5.7 (44)	106	44/50
74	5.3 (47)	47/50	5.2 (48)	98	48/50	5.4 (48)	102	48/50	5.4 (44)	102	44/50
78	5.4 (46)	47/50	5.3 (47)	98	47/50	5.5 (47)	102	47/50	5.5 (42)	102	42/50
82	5.3 (47)	47/50	5.2 (47)	98	47/50	5.5 (44)	104	44/50	5.5 (41)	104	41/50
86	5.2 (47)	47/50	5.1 (47)	98	47/50	5.3 (42)	102	42/50	5.3 (40)	102	40/50
90	5.2 (45)	45/50	5.0 (45)	96	45/50	6.8 (38)	131	38/50	6.6 (39)	127	39/50
94	5.1 (43)	43/50	4.9 (44)	96	44/50	5.2 (37)	102	37/50	5.0 (34)	98	34/50
98	5.0 (42)	42/50	5.0 (39)	100	39/50	5.0 (36)	100	36/50	4.5 (28)	90	28/50
102	5.1 (40)	40/50	5.1 (36)	100	36/50	5.0 (32)	98	31/50	4.8 (23)	94	22/50
104	5.1 (38)	38/50	5.0 (35)	96	35/50	4.9 (27)	94	26/50	4.7 (20)	90	20/50

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

TABLE 7 FOOD CONSUMPTION CHANGES OF FEMALE MICE 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	3.4 (49)	50/50	3.4 (50)	100	50/50	3.3 (49)	97	49/49	3.3 (50)	97	50/50
2	3.3 (50)	50/50	3.4 (50)	103	50/50	3.4 (49)	103	49/49	3.5 (50)	106	50/50
3	3.6 (49)	50/50	3.7 (50)	103	50/50	3.6 (49)	100	49/49	3.9 (50)	108	50/50
4	3.8 (49)	50/50	3.8 (50)	100	50/50	3.8 (49)	100	49/49	4.1 (50)	108	50/50
5	4.0 (50)	50/50	3.9 (50)	98	50/50	4.0 (49)	100	49/49	4.2 (50)	105	50/50
6	4.2 (50)	50/50	4.1 (50)	98	50/50	4.1 (49)	98	49/49	4.2 (50)	100	50/50
7	4.1 (49)	50/50	4.2 (50)	102	50/50	4.1 (49)	100	49/49	4.1 (50)	100	50/50
8	4.2 (50)	50/50	4.2 (50)	100	50/50	4.2 (49)	100	49/49	4.5 (50)	107	50/50
9	4.2 (50)	50/50	4.2 (50)	100	50/50	4.2 (49)	100	49/49	4.4 (50)	105	50/50
10	4.2 (50)	50/50	4.1 (50)	98	50/50	3.9 (49)	93	49/49	4.1 (50)	98	50/50
11	4.1 (50)	50/50	4.1 (50)	100	50/50	4.0 (49)	98	49/49	4.2 (50)	102	50/50
12	4.1 (50)	50/50	4.1 (50)	100	50/50	4.1 (49)	100	49/49	4.3 (50)	105	50/50
13	4.2 (50)	50/50	4.1 (50)	98	50/50	4.0 (49)	95	49/49	4.2 (50)	100	50/50
14	4.0 (50)	50/50	4.0 (50)	100	50/50	4.0 (49)	100	49/49	4.3 (50)	108	50/50
18	4.4 (50)	50/50	4.3 (49)	98	49/50	4.2 (49)	95	49/49	4.4 (50)	100	50/50
22	4.3 (50)	50/50	4.3 (49)	100	49/50	4.2 (49)	98	49/49	4.4 (50)	102	50/50
26	4.4 (50)	50/50	4.5 (49)	102	49/50	4.4 (49)	100	49/49	4.8 (50)	109	50/50
30	4.5 (50)	50/50	4.4 (49)	98	49/50	4.3 (49)	96	49/49	4.6 (50)	102	50/50
34	4.6 (50)	50/50	4.5 (49)	98	49/50	4.4 (49)	96	49/49	4.8 (50)	104	50/50
38	4.7 (50)	50/50	4.7 (49)	100	49/50	4.7 (49)	100	49/49	4.9 (50)	104	50/50
42	4.5 (50)	50/50	4.5 (49)	100	49/50	4.5 (49)	100	49/49	4.7 (50)	104	50/50
46	4.4 (50)	50/50	4.4 (49)	100	49/50	4.7 (49)	95	49/49	4.5 (50)	102	50/50
50	4.4 (50)	50/50	4.3 (49)	98	49/50	4.3 (49)	98	49/49	4.4 (50)	100	50/50
54	4.4 (49)	49/50	4.3 (47)	98	47/50	4.4 (49)	100	49/49	4.3 (50)	98	50/50
58	4.7 (49)	49/50	4.4 (47)	94	47/50	4.5 (49)	96	49/49	4.5 (50)	96	50/50
62	4.6 (49)	49/50	4.5 (47)	98	47/50	4.5 (47)	98	47/49	4.5 (49)	98	49/50
66	4.6 (48)	48/50	4.6 (46)	100	46/50	4.5 (46)	98	46/49	4.5 (49)	98	49/50
70	4.7 (46)	46/50	4.8 (44)	102	44/50	4.9 (45)	104	45/49	4.9 (49)	104	49/50
74	4.5 (44)	44/50	4.5 (44)	100	44/50	4.6 (41)	102	41/49	4.7 (49)	104	49/50
78	4.6 (41)	41/50	4.6 (44)	100	44/50	4.7 (37)	102	37/49	4.6 (46)	100	46/50
82	4.8 (41)	41/50	4.6 (43)	96	43/50	4.8 (34)	100	34/49	4.7 (41)	98	41/50
86	4.5 (40)	40/50	4.7 (37)	104	37/50	4.8 (28)	107	28/49	4.6 (39)	102	39/50
90	4.5 (38)	38/50	4.6 (32)	102	32/50	6.2 (26)	138	26/49	5.9 (34)	131	34/50
94	4.5 (34)	34/50	4.6 (30)	102	30/50	4.8 (21)	107	21/49	4.6 (31)	102	31/50
98	4.5 (29)	29/50	4.7 (28)	104	28/50	4.8 (19)	107	19/49	4.4 (29)	98	29/50
102	4.7 (28)	28/50	4.8 (26)	102	26/50	4.8 (18)	102	18/49	4.6 (22)	98	22/50
104	4.7 (26)	26/50	4.7 (26)	100	26/50	4.8 (17)	102	17/49	4.5 (21)	96	21/50

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

TABLE 8 SELECTED NON NEOPLASTIC LESIONS OF MALE MICE 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
nasal cavit			<50> b)				<50>				<50>				<50>		
	eosinophilic change: olfactory epithelium	19	0	0	0	8	1	0	0*	6	1	0	0**	5	0	0	0**
lung			<50>				<50>				<50>				<50>		
	bronchiolar-alveolar cell hyperplasia	2	0	0	0	1	1	0	0	3	0	0	0	5	0	0	0
	hyperplasia: terminal bronchiole	0	0	0	0	1	0	0	0	5	0	0	0	13	0	0	0**
	hyperplasia:epithelium, alveolar duct	1	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0
tooth			<50>				<50>				<50>				<50>		
	dysplasia	39	5	0	0	31	3	1	0	26	4	0	0**	15	1	0	0**
stomach			<50>				<50>				<50>				<50>		
	hyperplasia: glandular stomach	12	21	9	0	9	22	7	0	14	26	6	0	30	11	0	0**
liver			<50>				<50>				<50>				<50>		
	granulation	27	0	0	0	16	2	0	0*	13	4	1	0**	9	1	0	0**
	clear cell focus	1	0	0	0	0	0	0	0	0	2	0	0	1	0	0	0
	acidophilic cell focus	0	1	0	0	0	2	0	0	4	2	0	0	2	0	0	0
	basophilic cell focus	1	2	0	0	6	2	0	0	2	2	1	0	5	2	0	0
	vacuolated cell focus	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0
	mixed cell focus	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	vacuolic change: peripheral	0	0	0	0	0	0	0	0	0	0	0	0	14	1	0	0**
kidney			<50>				<50>				<50>				<50>		
	basophilic change	36	1	0	0	24	3	1	0	21	1	0	0**	16	0	0	0**
	lymphocytic infiltration	20	0	0	0	2	0	0	0**	3	1	0	0**	1	0	0	0**
	vacuolization of proximal tubule	38	0	0	0	6	0	0	0**	0	0	0	0**	0	0	0	0**
adrenal			<50>				<50>				<50>				<50>		
	focal fatty change:cortex	8	0	0	0	0	0	0	0**	0	0	0	0**	1	0	0	0*
testis			<50>				<50>				<50>				<50>		
	atrophy	21	15	12	0	16	16	16	0	5	34	11	0**	5	28	16	0**
	mineralization	23	6	7	0	14	26	5	0**	15	26	6	0**	10	25	10	0**
brain			<50>				<50>				<50>				<50>		
	mineralization	31	1	0	0	28	0	0	0	27	2	0	0	11	0	0	0**

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

b) : Number of animals examined at the site

Significant difference ; * : $P \leqq 0.05$ ** : $P \leqq 0.01$ Test of Chi Square

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

Organ	Findings	Group Name / No. of Animals / Grade a)	Control 50				1000 ppm 50				2000 ppm 49				4000 ppm 50			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
nasal cavit			<50> b)				<50>				<49>				<50>			
	eosinophilic change: olfactory epithelium		23	3	0	0	15	5	0	0	5	0	0	0 **	2	1	0	0 **
	eosinophilic change: respiratory epithelium		38	6	0	0	26	7	2	0 *	24	6	1	0 *	21	1	1	0 **
	respiratory metaplasia: olfactory epithelium		22	0	0	0	30	0	0	0	4	0	0	0 **	4	0	0	0 **
lung			<50>				<50>				<49>				<50>			
	hemorrhage		1	0	0	0	1	0	0	0	3	2	0	0	1	0	0	0
	bronchiolar-alveolar cell hyperplasia		0	0	0	0	2	0	0	0	0	0	0	0	2	0	0	0
	hyperplasia:terminal bronchiole		0	0	0	0	3	0	0	0	2	0	0	0	9	0	0	0 **
	hyperplasia:epithelium, alveolar duct		0	0	0	0	0	0	0	0	1	0	0	0	3	0	0	0
tooth			<50>				<50>				<49>				<50>			
	dysplasia		8	3	0	0	9	4	0	0	12	1	0	0	29	3	0	0 **
liver			<50>				<50>				<49>				<50>			
	granulation		9	11	0	0	14	4	0	0	11	5	0	0	10	0	0	0 **
	clear cell focus		0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	acidophilic cell focus		0	1	0	0	0	1	0	0	1	2	0	0	1	1	0	0
	basophilic cell focus		0	1	0	0	1	0	0	0	3	0	0	0	3	2	0	0
	vacuolic change: peripheral		0	1	0	0	0	0	0	0	0	0	0	0	19	9	1	0 **
ovary			<50>				<50>				<48>				<50>			
	atrophy		2	25	20	0	1	24	23	0	1	20	24	0	2	9	30	0 **
uterus			<50>				<50>				<49>				<50>			
	cystic endometrial hyperplasia		27	8	0	0	27	3	0	0	24	3	0	0	25	1	0	0 *
brain			<50>				<50>				<49>				<50>			
	mineralization		12	0	0	0	11	0	0	0	10	0	0	0	2	0	0	0 **

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

b) : Number of animals examined at the site

Significant difference ; * : $P \leqq 0.05$ ** : $P \leqq 0.01$ Test of Chi Square

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

Group Name	Control	1000ppm	2000ppm	4000ppm
SITE : lung				
TUMOR : bronchiolar-alveolar adenoma				
Tumor rate				
Overall rates(a)	7/50(14.0)	3/50(6.0)	4/50(8.0)	14/50(28.0)
Adjusted rates(b)	18.42	8.57	13.79	43.48
Terminal rates(c)	7/38(18.4)	3/35(8.6)	3/26(11.5)	8/20(40.0)
Statistical analysis				
Peto test				
Standard method(d)	P=---------			
Prevalence method(d)	P=0.0006**			
Combined analysis (d)	P=---------			
Cochran-Armitage test(e)	P=0.0001*			
Fisher Exact test(e)		P=0.1590	P=0.2624	P=0.0699
SITE : lung				
TUMOR : bronchiolar-alveolar carcinoma				
Tumor rate				
Overall rates(a)	1/50(2.0)	14/50(28.0)	22/50(44.0)	39/50(78.0)
Adjusted rates(b)	2.63	31.43	50.00	86.36
Terminal rates(c)	1/38(2.6)	11/35(31.4)	13/26(50.0)	17/20(85.0)
Statistical analysis				
Peto test				
Standard method(d)	P<0.0001**			
Prevalence method(d)	P<0.0001**?			
Combined analysis (d)	P<0.0001**?			
Cochran-Armitage test(e)	P<0.0001**			
Fisher Exact test(e)		P=0.0002**	P<0.0001**	P<0.0001**
SITE : lung				
TUMOR : bronchiolar-alveolar adenoma,bronchiolar-alveolar carcinoma				
Tumor rate				
Overall rates(a)	8/50(16.0)	17/50(34.0)	26/50(52.0)	42/50(84.0)
Adjusted rates(b)	21.05	40.0	62.96	91.67
Terminal rates(c)	8/38(21.1)	14/35(40.0)	16/26(61.5)	18/20(90.0)
Statistical analysis				
Peto test				
Standard method(d)	P<0.0001**			
Prevalence method(d)	P<0.0001**?			
Combined analysis (d)	P<0.0001**?			
Cochran-Armitage test(e)	P<0.0001**			
Fisher Exact test(e)		P=0.0318*	P=0.0001**	P<0.0001**
SITE : liver				
TUMOR : hepatocellular adenoma				
Tumor rate				
Overall rates(a)	10/50(20.0)	13/50(26.0)	14/50(28.0)	15/50(30.0)
Adjusted rates(b)	21.74	30.56	37.93	50.00
Terminal rates(c)	8/38(21.1)	10/35(28.6)	9/26(34.6)	10/20(50.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.5790			
Prevalence method(d)	P=0.0188*			
Combined analysis (d)	P=0.0245*			
Cochran-Armitage test(e)	P=0.2757			
Fisher Exact test(e)		P=0.3182	P=0.2419	P=0.1783

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

Group Name	Control	1000ppm	2000ppm	4000ppm
SITE : liver				
TUMOR : hepatocellular carcinoma				
Tumor rate				
Overall rates(a)	10/50(20.0)	9/50(18.0)	14/50(28.0)	20/50(40.0)
Adjusted rates(b)	15.79	17.95	23.53	54.17
Terminal rates(c)	6/38(15.8)	5/35(14.3)	6/26(23.1)	10/20(50.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0103*			
Prevalence method(d)	P=0.0044**			
Combined analysis (d)	P=0.0002**			
Cochran-Armitage test(e)	P=0.0086**			
Fisher Exact test(e)		P=0.4994	P=0.2419	P=0.0243*
SITE : liver				
TUMOR : hepatocellular adenoma,hepatocellular carcinoma,hepatoblastoma				
Tumor rate				
Overall rates(a)	15/50(30.0)	20/50(40.0)	25/50(50.0)	29/50(58.0)
Adjusted rates(b)	28.95	41.67	55.56	80.00
Terminal rates(c)	11/38(28.9)	14/35(40.0)	14/26(53.8)	15/20(75.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0166*			
Prevalence method(d)	P=0.0002**			
Combined analysis (d)	P<0.0001**			
Cochran-Armitage test(e)	P=0.0036**			
Fisher Exact test(e)		P=0.2013	P=0.0328*	P=0.0042**
SITE : liver				
TUMOR : hemangioma				
Tumor rate				
Overall rates(a)	0/50(0.0)	4/50(8.0)	3/50(6.0)	5/50(10.0)
Adjusted rates(b)	0.0	8.57	11.54	10.00
Terminal rates(c)	0/50(0.0)	3/35(8.6)	3/26(11.5)	2/20(10.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.1682			
Prevalence method(d)	P=0.0182*			
Combined analysis (d)	P=0.0105*			
Cochran-Armitage test(e)	P=0.0700			
Fisher Exact test(e)		P=0.0587	P=0.1212	P=0.0281*
SITE : liver				
TUMOR : hemangioma,hemangiosarcoma				
Tumor rate				
Overall rates(a)	1/50(2.0)	4/50(8.0)	4/50(8.0)	6/50(12.0)
Adjusted rates(b)	2.63	8.57	15.38	10.26
Terminal rates(c)	1/38(2.6)	3/35(8.6)	4/26(15.4)	2/20(10.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0409*			
Prevalence method(d)	P=0.0366*			
Combined analysis (d)	P=0.0078**			
Cochran-Armitage test(e)	P=0.0768			
Fisher Exact test(e)		P=0.1811	P=0.1811	P=0.0559

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

Group Name	Control	1000ppm	2000ppm	4000ppm
SITE : ALL SITE				
TUMOR : hemangioma				
Tumor rate				
Overall rates(a)	1/50(2.0)	5/50(10.0)	6/50(12.0)	7/50(14.0)
Adjusted rates(b)	0.0	8.57	19.23	15.00
Terminal rates(c)	0/38(0.0)	3/35(8.6)	5/26(19.2)	3/20(15.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.1825			
Prevalence method(d)	P=0.0046**			
Combined analysis (d)	P=0.0042**			
Cochran-Armitage test(e)	P=0.0554			
Fisher Exact test(e)		P=0.1022	P=0.0559	P=0.0297*
SITE : adenal gland				
TUMOR : pheochromocytoma				
Tumor rate				
Overall rates(a)	1/50(2.0)	0/50(0.0)	1/50(2.0)	3/50(6.0)
Adjusted rates(b)	2.63	0.0	3.85	9.09
Terminal rates(c)	1/38(2.6)	0/35(0.0)	1/26(3.8)	0/20(0.0)
Statistical analysis				
Peto test				
Standard method(d)	P=---------			
Prevalence method(d)	P=0.0367*			
Combined analysis (d)	P=---------			
Cochran-Armitage test(e)	P=0.1079			
Fisher Exact test(e)		P=0.4999	P=0.2475	P=0.3086
SITE : ALL SITE				
TUMOR : histiocytic sarcoma				
Tumor rate				
Overall rates(a)	5/50(10.0)	2/50(4.0)	3/50(6.0)	0/50(0.0)
Adjusted rates(b)	7.89	0.0	2.56	0.0
Terminal rates(c)	3/38(7.9)	0/35(0.0)	0/26(0.0)	0/20(0.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.8588			
Prevalence method(d)	P=0.9551			
Combined analysis (d)	P=0.9735			
Cochran-Armitage test(e)	P=0.0372*			
Fisher Exact test(e)		P=0.2181	P=0.3576	P=0.0281*

(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 MICE IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

Group Name	Control	1000ppm	2000ppm	4000ppm
SITE : lung				
TUMOR : bronchiolar-alveolar adenoma				
Tumor rate				
Overall rates(a)	2/50(4.0)	4/50(8.0)	5/49(10.2)	12/50(24.0)
Adjusted rates(b)	7.69	11.54	17.65	36.36
Terminal rates(c)	2/26(7.7)	3/26(11.5)	3/17(17.6)	7/21(33.3)
Statistical analysis				
Peto test				
Standard method(d)	P=---------			
Prevalence method(d)	P=0.0005**			
Combined analysis (d)	P=---------			
Cochran-Armitage test(e)	P=0.0011**			
Fisher Exact test(e)		P=0.3389	P=0.2097	P=0.0038**
SITE : lung				
TUMOR : bronchiolar-alveolar carcinoma				
Tumor rate				
Overall rates(a)	3/50(6.0)	1/50(2.0)	8/49(16.3)	20/50(40.0)
Adjusted rates(b)	3.85	3.85	35.29	39.13
Terminal rates(c)	1/26(3.8)	1/26(3.8)	6/17(35.3)	7/21(33.3)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0145*			
Prevalence method(d)	P<0.0001**			
Combined analysis (d)	P<0.0001**			
Cochran-Armitage test(e)	P<0.0001**			
Fisher Exact test(e)		P=0.3086	P=0.0936	P<0.0001**
SITE : lung				
TUMOR : bronchiolar-alveolar adenoma,bronchiolar-alveolar carcinoma,adenosquamous carcinoma				
Tumor rate				
Overall rates(a)	5/50(10.0)	5/50(10.0)	12/49(24.5)	30/50(60.0)
Adjusted rates(b)	11.54	15.38	47.06	65.22
Terminal rates(c)	3/26(11.5)	4/26(15.4)	8/17(47.1)	13/21(61.9)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0145*			
Prevalence method(d)	P<0.0001**			
Combined analysis (d)	P<0.0001**?			
Cochran-Armitage test(e)	P<0.0001**			
Fisher Exact test(e)		P=0.3701	P=0.0492*	P<0.0001**
SITE : liver				
TUMOR : hepatocellular adenoma				
Tumor rate				
Overall rates(a)	1/50(2.0)	7/49(14.3)	4/49(8.2)	16/50(32.0)
Adjusted rates(b)	3.85	22.22	16.00	45.71
Terminal rates(c)	1/26(3.8)	5/26(19.2)	1/17(5.9)	9/21(42.9)
Statistical analysis				
Peto test				
Standard method(d)	P=---------			
Prevalence method(d)	P<0.0001**			
Combined analysis (d)	P=---------			
Cochran-Armitage test(e)	P<0.0001**			
Fisher Exact test(e)		P=0.0277*	P=0.1748	P<0.0001**

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

Group Name	Control	1000ppm	2000ppm	4000ppm
SITE : liver				
TUMOR : hepatocellular carcinoma				
Tumor rate				
Overall rates(a)	1/50(2.0)	1/49(2.0)	5/49(10.2)	19/50(38.0)
Adjusted rates(b)	3.85	3.85	17.65	46.15
Terminal rates(c)	1/26(3.8)	1/26(3.8)	3/17(17.6)	9/21(42.9)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0159*			
Prevalence method(d)	P<0.0001**			
Combined analysis (d)	P<0.0001**			
Cochran-Armitage test(e)	P<0.0001**			
Fisher Exact test(e)		P=0.2526	P=0.0976	P<0.0001**
SITE : liver				
TUMOR : hepatocellular adenoma,hepatocellular carcinoma				
Tumor rate				
Overall rates(a)	2/50(4.0)	8/49(16.3)	9/49(18.4)	30/50(60.0)
Adjusted rates(b)	7.69	25.93	30.0	72.73
Terminal rates(c)	2/26(7.7)	6/26(23.1)	4/17(23.5)	14/21(66.7)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0159*			
Prevalence method(d)	P<0.0001**?			
Combined analysis (d)	P<0.0001**?			
Cochran-Armitage test(e)	P<0.0001**			
Fisher Exact test(e)		P=0.0426*	P=0.0235*	P<0.0001**
SITE : liver				
TUMOR : hemangioma				
Tumor rate				
Overall rates(a)	2/50(4.0)	2/49(4.1)	0/49(0.0)	5/50(10.0)
Adjusted rates(b)	5.41	7.69	0.0	19.05
Terminal rates(c)	1/26(3.8)	2/26(7.7)	0/17(0.0)	4/21(19.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.1288			
Prevalence method(d)	P=0.1204			
Combined analysis (d)	P=0.0557			
Cochran-Armitage test(e)	P=0.1526			
Fisher Exact test(e)		P=0.3164	P=0.2525	P=0.2181
SITE : liver				
TUMOR : hemangioma,hemangiosarcoma				
Tumor rate				
Overall rates(a)	3/50(6.0)	2/49(4.1)	0/49(0.0)	7/50(14.0)
Adjusted rates(b)	8.11	7.69	0.0	19.05
Terminal rates(c)	2/26(7.7)	2/26(7.7)	0/17(0.0)	4/21(19.0)
Statistical analysis				
Peto test				
Standard method(d)	P=0.0046**			
Prevalence method(d)	P=0.2157			
Combined analysis (d)	P=0.0239*			
Cochran-Armitage test(e)	P=0.0721			
Fisher Exact test(e)		P=0.4903	P=0.1250	P=0.1590

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

Group Name	Control	1000ppm	2000ppm	4000ppm
SITE : subcutis				
TUMOR : hemangioma,hemangiosarcoma				
Tumor rate				
Overall rates(a)	3/50(6.0)	1/50(2.0)	0/49(0.0)	0/50(0.0)
Adjusted rates(b)	11.54	3.85	0.0	0.0
Terminal rates(c)	3/26(11.5)	1/26(3.8)	0/17(0.0)	0/21(0.0)
Statistical analysis				
Peto test				
Standard method(d)	P=---------			
Prevalence method(d)	P=0.9817			
Combined analysis (d)	P=---------			
Cochran-Armitage test(e)	P=0.0411*			
Fisher Exact test(e)		P=0.3086	P=0.1250	P=0.1212
SITE : lymph node				
TUMOR : malignant lymphoma				
Tumor rate				
Overall rates(a)	23/50(46.0)	31/50(62.0)	19/49(38.8)	15/50(30.0)
Adjusted rates(b)	48.15	69.23	47.06	34.78
Terminal rates(c)	12/26(46.2)	18/26(69.2)	8/17(47.1)	7/21(33.3)
Statistical analysis				
Peto test				
Standard method(d)	P=0.8474			
Prevalence method(d)	P=0.8771			
Combined analysis (d)	P=0.9387			
Cochran-Armitage test(e)	P=0.0166*			
Fisher Exact test(e)		P=0.0801	P=0.3009	P=0.0746
SITE : ALL SITE				
TUMOR : malignant lymphoma				
Tumor rate				
Overall rates(a)	25/50(50.0)	33/50(66.0)	21/49(42.9)	17/50(34.0)
Adjusted rates(b)	55.56	76.92	52.94	34.78
Terminal rates(c)	14/26(53.8)	20/26(76.9)	9/17(52.9)	7/21(33.3)
Statistical analysis				
Peto test				
Standard method(d)	P=0.6794			
Prevalence method(d)	P=0.9488			
Combined analysis (d)	P=0.9208			
Cochran-Armitage test(e)	P=0.0173*			
Fisher Exact test(e)		P=0.0780	P=0.3054	P=0.0780

(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 12 CAUSE OF DEATH OF MICE IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

Group		Male				Female			
		Control	1000ppm	2000ppm	4000ppm	Control	1000ppm	2000ppm	4000ppm
Number of dead or moribund animals		12	15	24	30	24	24	32	29
No microscopical confirmation		0	0	0	2	0	2	1	1
Respiratory system lesion		0	1	0	1	0	0	0	0
Cardiovascular lesion		0	0	1	0	0	0	0	0
Hepatic lesion		0	0	0	0	0	0	2	1
Reproductive system lesion		0	0	0	0	1	0	0	0
Hydronephrosis		1	1	2	3	0	0	0	0
Tumor death	: leukemia	5	5	6	3	9	13	8	6
	subcutis	0	1	0	0	0	0	1	1
	lung	0	2	6	14	2	0	1	4
	tooth	0	1	0	0	0	0	0	0
	liver	4	3	6	6	0	0	3	5
	urinary bladder	0	1	2	0	0	0	0	0
	pituitary	0	0	0	0	1	1	1	1
	ovary	-	-	-	-	0	0	2	0
	uterus	-	-	-	-	9	8	13	8
	peripheral nerve	1	0	0	0	0	0	0	0
	muscle	0	0	1	0	0	0	0	1
	pleura	0	0	0	0	0	0	0	1
	peritoneum	1	0	0	0	2	0	0	0
	reperitoneum	0	0	0	1	0	0	0	0

FIGURES

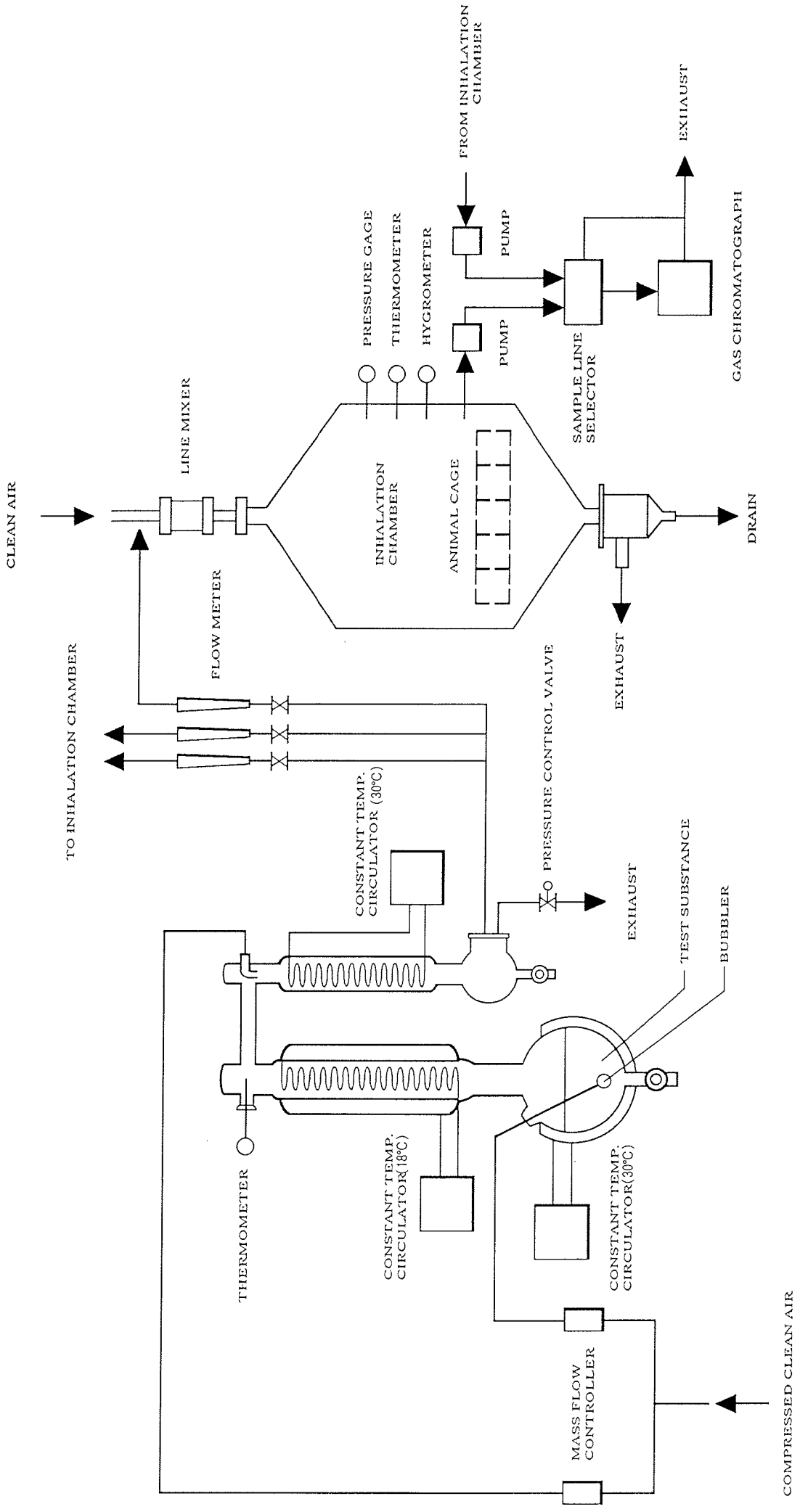


FIGURE 1 DICHLOROMETHANE VAPOR GENERATION SYSTEM AND INHALATION SYSTEM

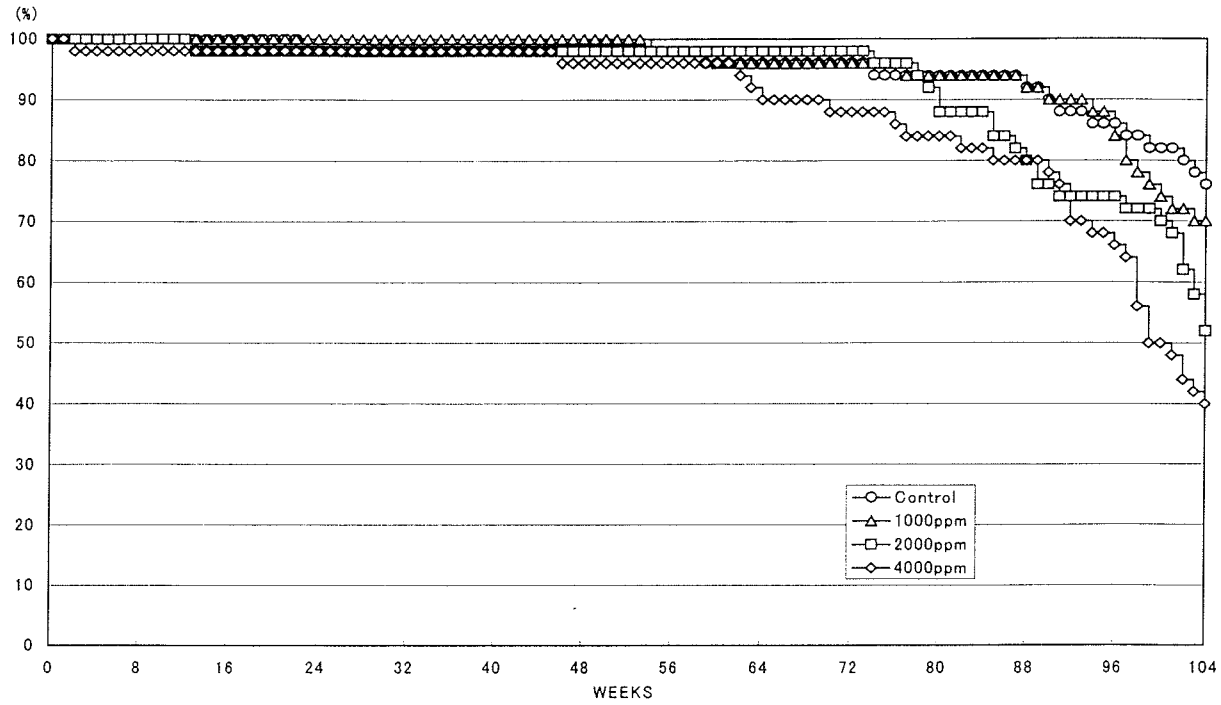


FIGURE 2 SURVIVAL ANIMAL RATE OF MALE MICE IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

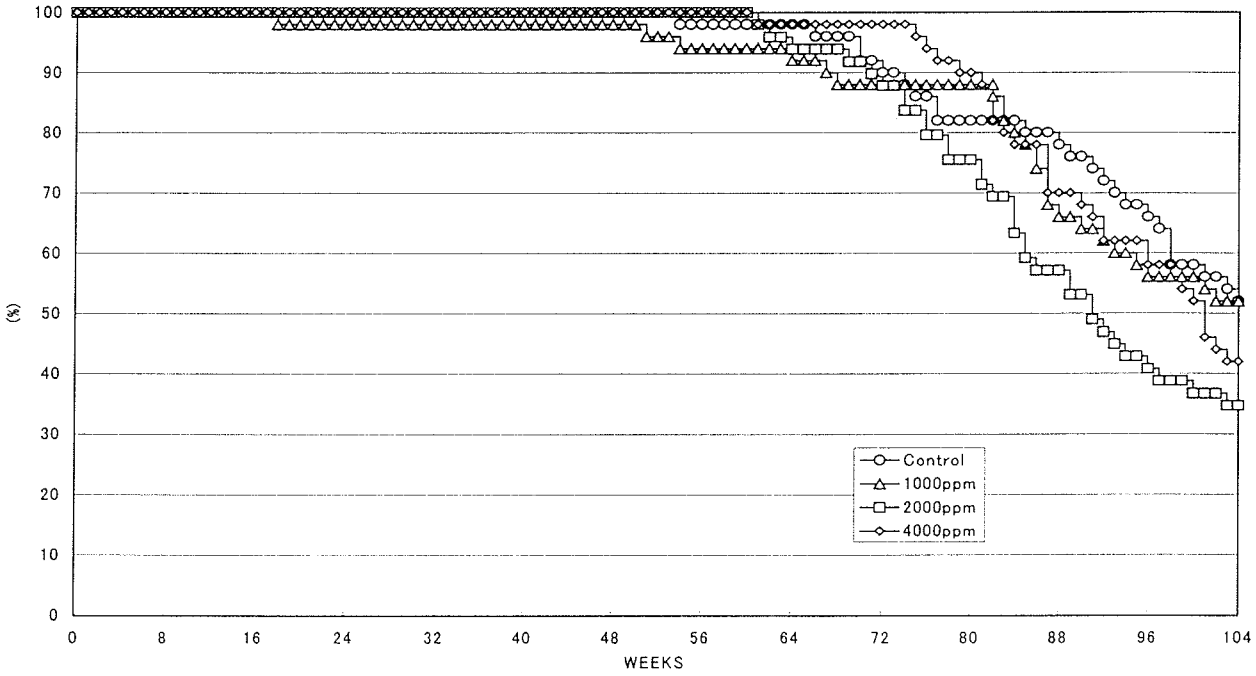


FIGURE 3 SURVIVAL ANIMAL RATE OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

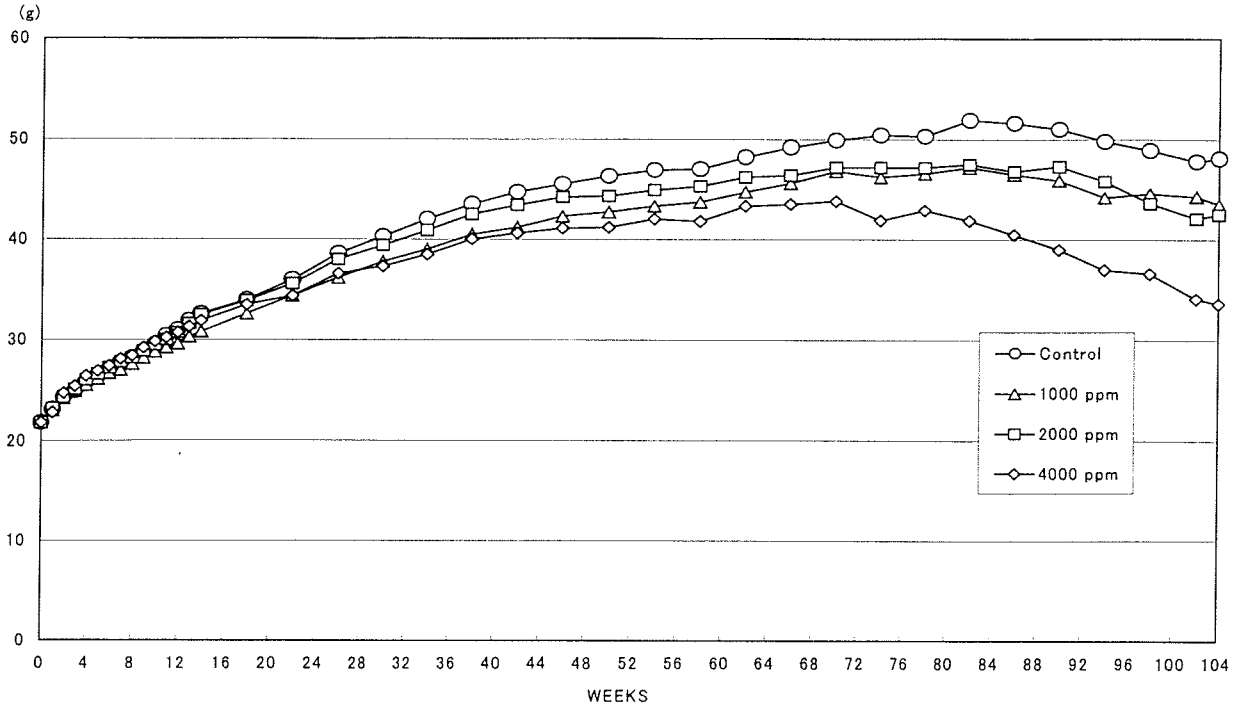


FIGURE 4 BODY WEIGHT CHANGES OF MALE MICE IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

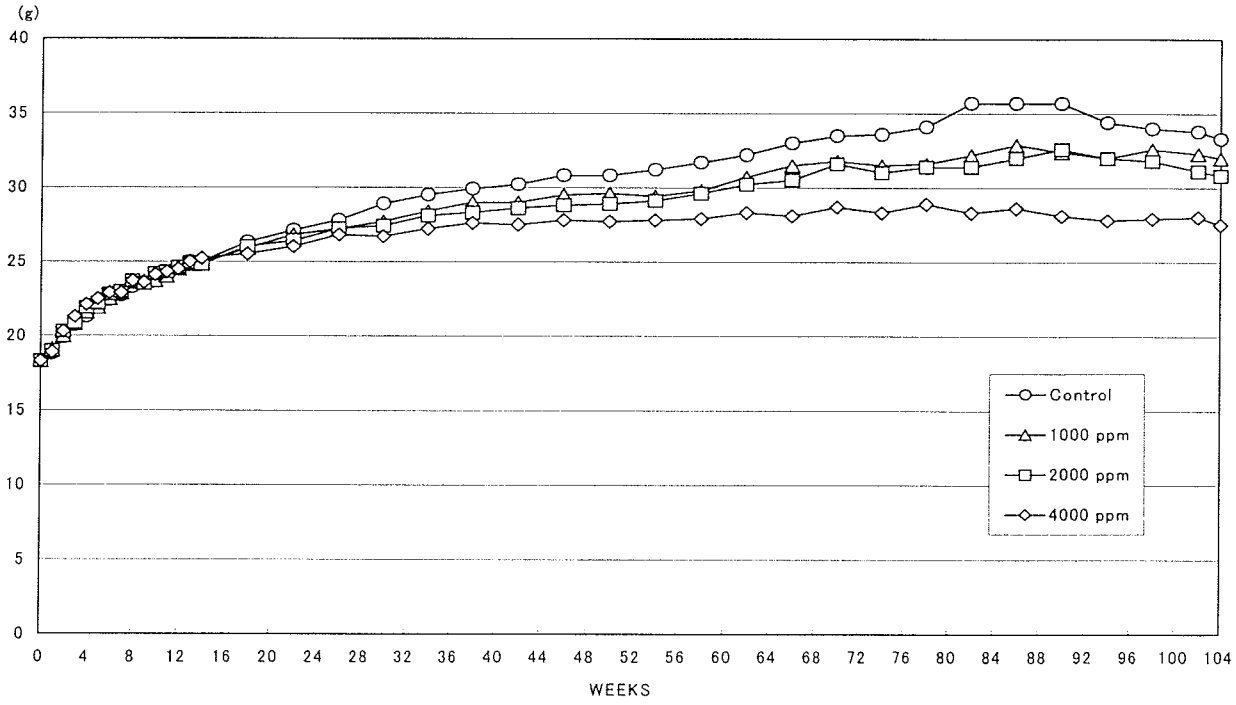


FIGURE 5 BODY WEIGHT CHANGES OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

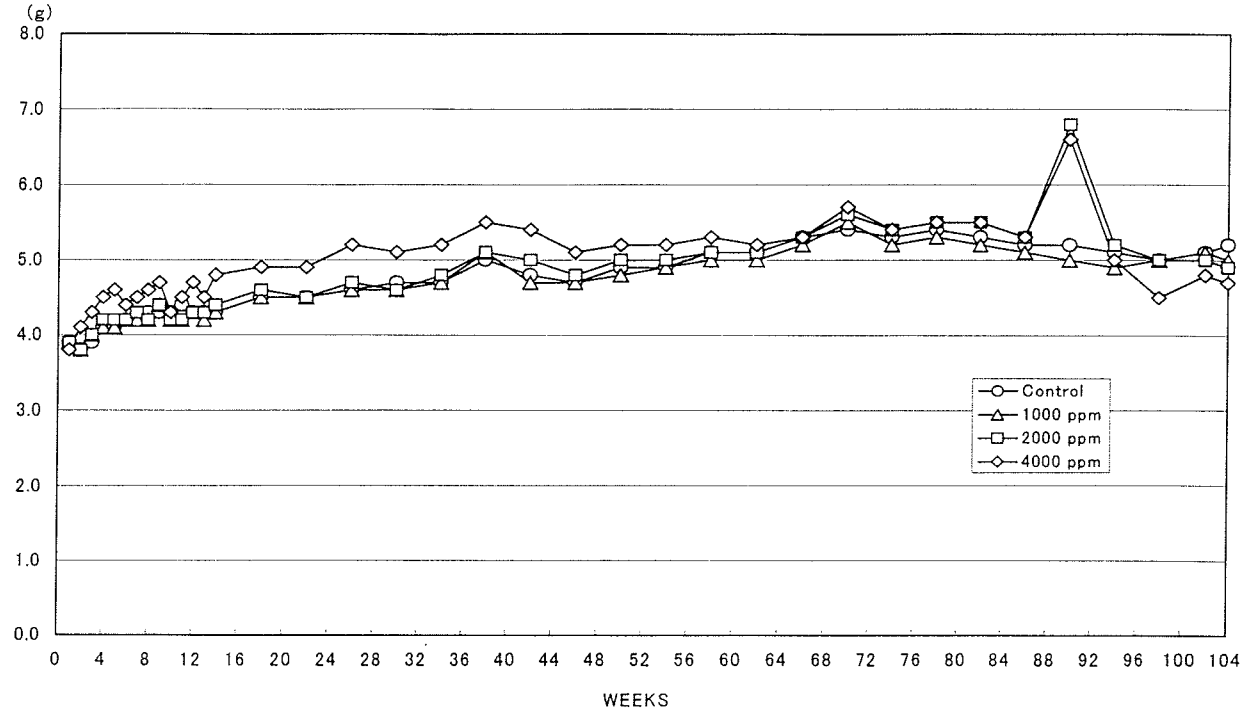


FIGURE 6 FOOD CONSUMPTION CHANGES OF MALE MICE IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

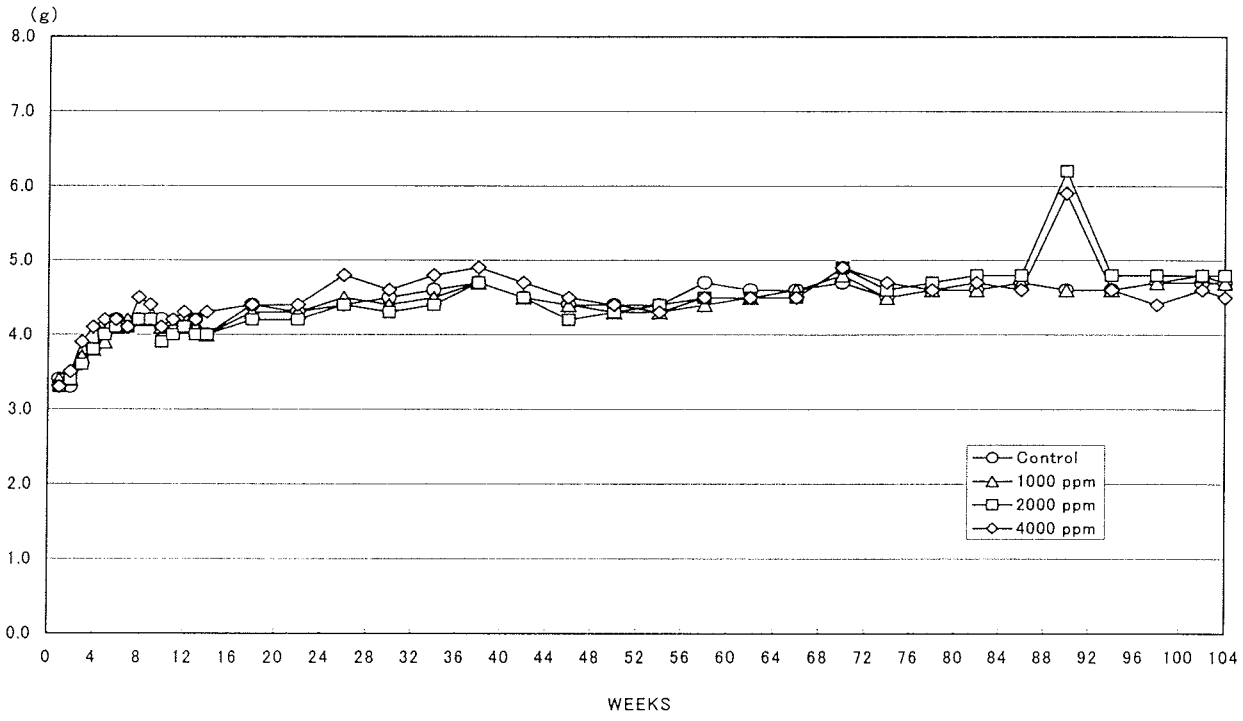


FIGURE 7 FOOD CONSUMPTION CHANGES OF FEMALE MICE IN THE 2-YEAR INHALATION STUDY OF DICHLOROMETHANE

PHOTOGRAPHS

PHOTOGRAPH 1 LUNG : BRONCHIOLAR-ALVEOLAR CARCINOMA
MALE, 4000ppm, ANIMAL No. 1303 (H&E)

PHOTOGRAPH 2 LUNG : BRONCHIOLAR-ALVEOLAR CARCINOMA
FEMALE, 4000ppm, ANIMAL No. 2305 (H&E)

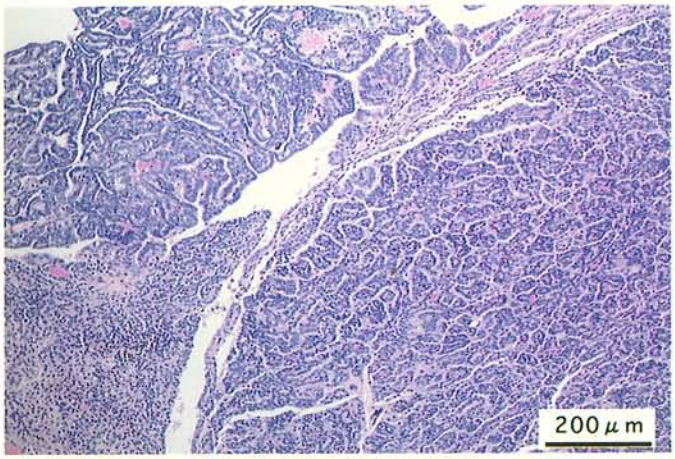
PHOTOGRAPH 3 LUNG : HYPERPLASIA : TERMINAL BRONCHIOLE
MALE, 4000ppm, ANIMAL No. 1303 (H&E)

PHOTOGRAPH 4 LIVER : HEPATOCELLULAR CARCINOMA
MALE, 4000ppm, ANIMAL No. 1313 (H&E)

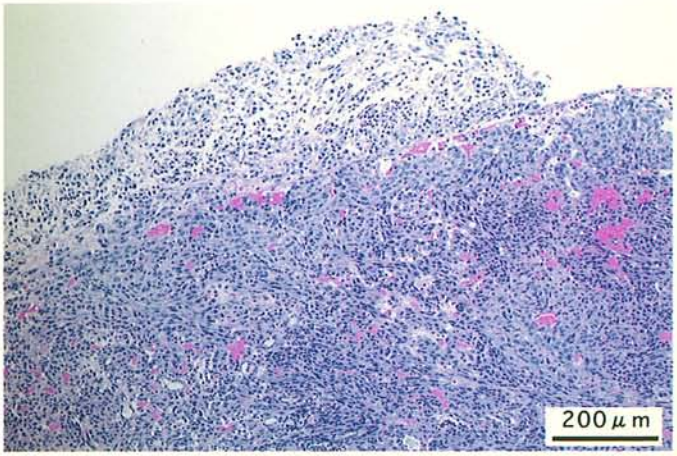
PHOTOGRAPH 5 LIVER : HEPATOCELLULAR ADENOMA
MALE, 4000ppm, ANIMAL No. 1344 (H&E)

PHOTOGRAPH 6 LIVER : HEMANGIOMA
FEMALE, 4000ppm, ANIMAL No. 2304 (H&E)

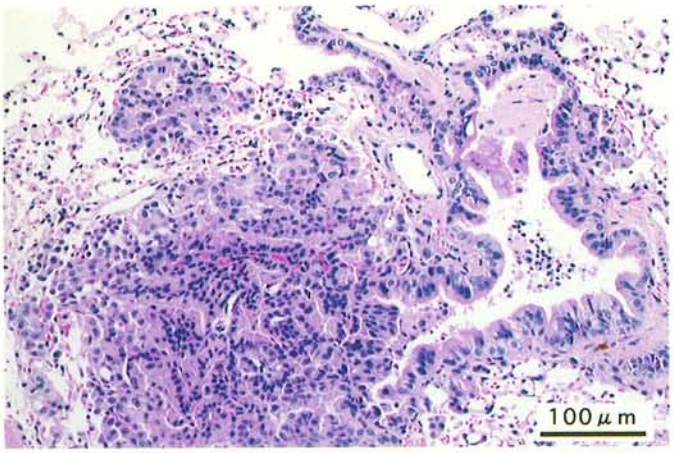
PHOTOGRAPH 7 LIVER : VACUOLIC CHANGE : PERIPHERAL(MODERATE)
MALE, 4000ppm, ANIMAL No. 1346 (H&E)



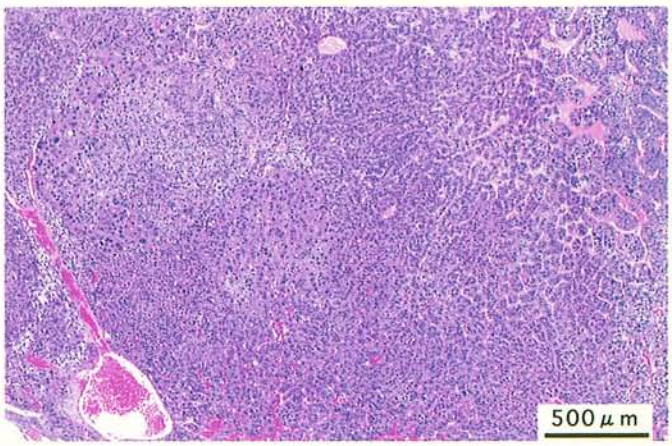
PHOTOGRAPH. 1



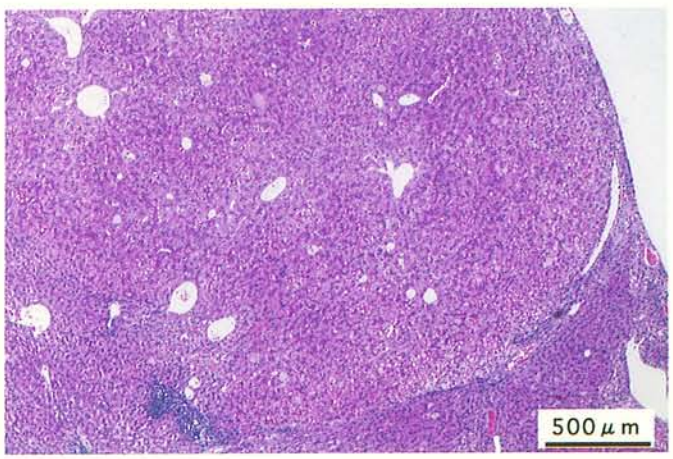
PHOTOGRAPH. 2



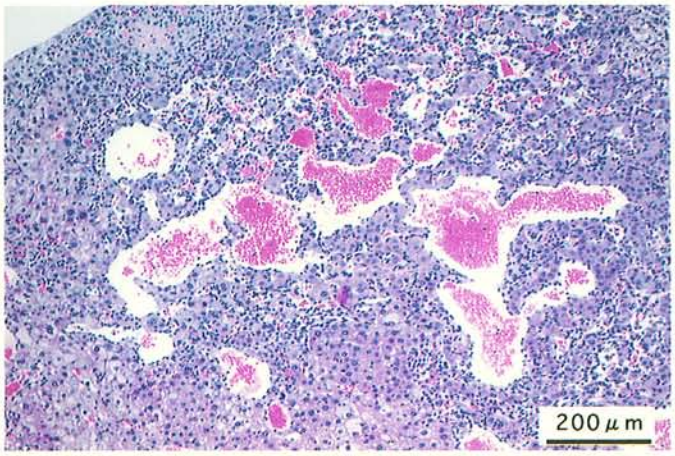
PHOTOGRAPH. 3



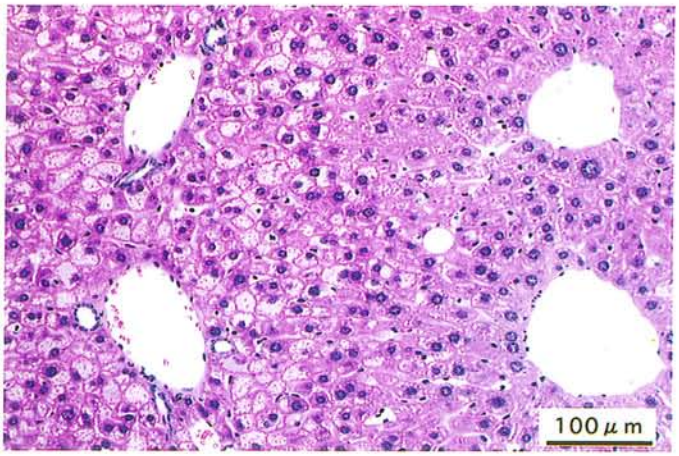
PHOTOGRAPH. 4



PHOTOGRAPH. 5



PHOTOGRAPH. 6



PHOTOGRAPH. 7