JAPAN BIOASSAY RESEARCH CENTER
JAPAN INDUSTRIAL SAFETY AND HEALTH ASSOCIATION
2445 Hirasawa Hadano Kanagawa, 257 JAPAN
Tel. [81]-463-82-3911 Fax [81]-463-82-3860

September 5, 2001

Dr. Robert E. McGaughy
National Center for Health and Environmental Assessment
Office of Research and Development (Mail Code 8623D)
Environmental Protection Agency,
401 M Street, S.W., Washington, D.C. 20460
USA

Dear Dr. McGaughy:
This is reply to your e-mail and letter of August 27, 2001 regarding to our carcinogenicity studies of perchloroethylene (tetrachloroethylene).

1. These studies were conducted under the Good Laboratory Practice principles.

2. Review of final reports
Final reports were submitted to the Ministry of Labour, and evaluated in the "Expert Committee for Prevention of Occupational Cancer" which was consultative committee of the Ministry of Labour.

3. Review of histopathological data
We used internal review system.
Pathological examination data are reviewed by the following procedures;
1) Diagnose by original pathologist
2) Diagnose by review pathologist(senior pathologist in our institute)
3) Discuss the diagnosis by both original pathologist and review pathologist, and finalize the diagnosis

4. Criteria of mononuclear cell leukemia (MCL)
We know that the MCL was classified in 3 stages in the NTP study of tetrachloroethylene. In the standard criteria of the Japan Bioassay Research Center, we diagnosed as MCL when "recognized the existence of MCL cells in the congested spleen". We used this criteria also in the tetrachloroethylene study. I think that this method is better in order to compare the incidence with historical control data of MCL.

5. Historical control data
Current historical control data of the Japan Bioassay Research Center is indicated in attached table.

JAPAN BIOASSAY RESEARCH CENTER
JAPAN INDUSTRIAL SAFETY AND HEALTH ASSOCIATION
2445 Hirasawa Hadano Kanagawa, 257 JAPAN
Tel. [81]-463-82-3911 Fax. [81]-463-82-3860

I hope these informations can help your work.

Sincerely yours,

Kasuke Nagano

Kasuke Nagano, DVM, Ph.D
Division of Pathology
Japan Bioassay Research Center,
Japan Industrial Safety and Health Association
2445 Hirasawa, Hadano,
Kanagawa 257-0015, JAPAN
FAX: 81-463-82-3860
e-mail: knagano@jisha.or.jp

PS: The carcinogenicity study of carbon tetrachloride and 1,2 dichloroethane were carried out same process as tetrachloroethylene which mentioned above.

Historical Control Data of the Japan Bioassay Research Center: Crj:BDF1 Mouse: 104Week Study

1. Male

		Inhalation study + Feeding study + Drinking study (19 studies)		Inhalation study (9 studies)	
		Total incidence (%)	Range Min.–Max.	Total incidence (%)	Range Min.–Max.
Liver	Hepatocellular adenoma	165/947 (17.4%)	4.0% - 34.0%	92/448 (20.5%)	10.0% - 30.6%
	Hepatocellular carcinoma	215/947 (22.7%)	2.0% - 42.0%	105/448 (23.4%)	10.0% - 36.7%
Spleen	Hemangioma/ Hemangioendothelioma:benign	17/946 (1.8%)	0% - 10.0%	8/448 (1.8%)	0% - 8.0%
	Hemangiosarcoma/ Hemangioendothelioma	30/946 (3.2%)	0% - 8.0%	12/448 (2.7%)	0% - 6.0%
Harderian gl.	Adenoma	42/947 (4.4%)	0% - 10.0%	25/448 (5.6%)	2.0% - 10.0%
	Adenocarcinoma	1/947 (0.1%)	0% -2.0%	0/448 (0%)	

2. Female

		Inhalation study + Feeding study + Drinking study (19 studies)		Inhalation study (9 studies)	
		Total incidence (%)	Range Min.–Max.	Total incidence (%)	Range Min.–Max.
Liver	Hepatocellular adenoma	50/949 (5.3%)	2.0% - 10.0%	18/449 (4.0%)	2.0% - 6.0%
	Hepatocellular carcinoma	22/949 (2.3%)	0% - 8.0%	14/449 (3.1%)	0% - 8.0%
Spleen	Hemangioma/ Hemangioendothelioma:beiign	8/949 (0.9%)	0% - 6.0%	5/449 (1.1%)	0% - 6.0%
	Hemangiosarcoma/ Hemangioendothelioma	3/949 (0.3%)	0% - 2.0%	3/449 (0.7%)	0% - 2.0%
Harderian gl.	Adenoma	30/949 (3.2%)	0% - 12.0%	22/449 (4.9%)	0% - 12.0%
	Adenocarcinoma	1/949 (0.1%)	0% -2.0%	1/449 (0.2%)	0% - 2.0%

The terms "Hemangioendothelioma:benin" and "Hemangioendotheliom" were changed to" Hemangioma" and "Hemangiosarcoma" respectively,

Historical Control Data of the Japan Bioassay Research Center: F344DuCrj (Fishcher) Rat: 104Week Study

1. Male

		Inhalation study + Feeding study + Drinking study (23 studies)		Inhalation study (11 studies)	
		Total incidence (%)	Range Min.–Max.	Total incidence (%)	Range Min.–Max.
Mononuclear cell leukemia		147/1149 (12.8%)	6.0% - 22.0%	76/549 (13.8%)	6.0% - 22.0%
Kidney	Renal cell adenoma	2/1149 (0.2%)	0% - 2.0%	1/549 (0.2%)	0% - 2.0%
	Renal cell carcinoma	2/1149 (0.2%)	0% - 2.0%	2/549 (0.4%)	0% - 2.0%
	Nephroblastoma	3/1149 (0.3%)	0% - 2.0%	2/549 (0.4%)	0% - 2.0%
	Lipoma	1/1149 (0.1%)	0% - 2.0%	1/549 (0.2%)	0% - 2.0%

2. Female

		Inhalation study + Feeding study + Drinking study (21 studies)		Inhalation study (9 studies)	
		Total incidence (%)	Range Min.–Max.	Total incidence (%)	Range Min.–Max.
Mononuclear cell leukemia		147/1048 (14.0%)	2.0% - 26.0%	68/448 (15.2%)	8.0% - 20.0%
Kidney	Renal cell adenoma	1/1048 (0.1%)	0% - 2.0%	1/448 (0.2%)	0% - 2.0%
	Renal cell carcinoma	0/1048 (0%)		0/448 (0%)	
	Nephroblastoma	1/1048 (0.1%)	0% - 2.0%	0/549 (0%)	
	Lipoma	1/1048 (0.1%)	0% - 2.0%	0/549 (0%)	

(JISA) JAPAN

Data No. 3-1

CARCINOGENICITY STUDY OF TETRACHLOROETHYLENE BY INHALATION IN RATS AND MICE

March 31, 1993

Japan Industrial Safety Association
Japan Bioassay Research Center

Table of Contents

Abstract

A 2-year (104-week) study was conducted by general exposure using rats and mice for the purpose of investigating carcinogenicity of tetrachloroethylene by inhalation.

The laboratory animals used in the study were F344DuCrj (Fischer) rats and Crj:BDF_1 mice. Four-hundred rats and 400 mice were used in a total of 4 groups, 3 study sample treatment groups and 1 control group, of 50 males and females each.

Two-week and 13-week preliminary studies were conducted and based on these results, the concentration administered in the carcinogenicity study was set at 600 ppm, 200 ppm and 50 ppm in rats and 250 ppm, 50 ppm, and 10 ppm in mice, with these concentrations being administered for 6 hours/day, 5 days a week for 104 weeks. The observation and test items were observation of general status, body weight and food consumption determinations, urinalyses, hematological tests, blood chemistry tests, autopsy, organ weight determination, and histopathological tests.

When compared to the control groups, a significant reduction in the number of animals that survived (percentage) was seen in both rats and mice of the maximum tetrachloroethylene concentration groups, apparently the result of administration.

There was a tendency toward an increase in monocytic leukemia of the spleen among male and female rats administered tetrachloroethylene, with the incidence among males of the 600 ppm group being significantly increased when compared to the control group. This increase in monocytic leukemia apparently lead to a reduction in the survival rate among the treatment groups. Moreover, the increase in spongiosis hepatitis and hyperplasia of the liver among males, the nuclear enlargement of proximal tubules of the kidneys among males and females, and the increase in atypical tubular dilation of the proximal uriniferous tubules and exacerbation of chronic renal disease among males appear to have been due to administration of tetrachloroethylene.

There was a tendency toward an increase in the development of hepatocellular adenoma and hepatocellular carcinoma among both male and female mice administered tetrachloroethylene, and there was a significant increase in this incidence among male and females of the 250 ppm group when compared to the control. This increase in hepatocellular carcinoma lead to a reduction in the survival rate among the treatment groups. There was a tendency toward an increase in adenoma of the spleen and liver, hemangioendothelioma of all organs, and [adenoma of] Harder's gland among males. There was a tendency toward an increase in hemangioendothelioma of all organs among females. Moreover, the angiectasis and central degeneration of the liver among males and females, increase in focal necrosis of the liver among males, and the increase in nuclear enlargement of the proximal tubules and atypical tubular dilation of the kidneys among males and females apparently were due to administration of tetrachloroethylene.

An increase in the development of monocytic leukemia of the spleen was observed among male and female rats, proving that tetrachloroethylene is carcinogenic in F344/DuCrj (Fischer) rats. This tumorigenic concentration was 600 ppm in males.

An increase in hepatocellular adenoma and hepatocellular carcinoma of the liver among male and female mice and an increase in adenoma of Harder's gland among male mice was seen, proving that tetrachloroethylene is carcinogenic in Crj:BDF_1 mice. This tumorigenic concentration was 250 ppm in both females and males.

Table 1. Main tumorigenesis in carcinogenicity study of tetrachloroethylene

	(rats: male)						
	Concentration administered (ppm)		0	50	200	600	Peto's test
Male	Number of animals tested		50	50	50	50	
Benign tumors	Subcutaneous tissue	Fibroma	1	5	3	5	
	Liver	Hepatocyte adenoma	3	0	0	2	
	Pituitary	Adenoma	16	16	18	15	
	Thyroid	C-cell adenoma	6	10	7	3	
	Pancreas	Isle of Langerhans adenoma		3	4	1	3
	Adrenal glands	Melanocytoma	8	5	3	3	
	Testes	Interstitial androblastoma	47	46	45	48	
	Mammary glands	Adenoma	1	0	1	1	
		Fibroadenoma	2	2	0	0	
	Preputial glands	Adenoma	1	3	2	0	
Malignant	Spleen	Monocytic leukemia	11	14	22	27*	↑↑
	Thyroid	C-cell carcinoma	1	1	3	0	
		Follicular carcinoma	0	3	0	0	
	Pancreas	Isle of Langerhans adenoma	1	0	1	1	
	Adrenal glands	Melanocytoma	1	1	1	2	

Table 2. Main tumorigenesis in carcinogenicity study of tetrachloroethylene

	(rats: female)						
	Concentration administered (ppm)		0	50	200	600	Peto's test
Female	Number of animals tested		50	50	50	50	
Benign tumors	Pituitary	Adenoma	12	16	16	11	
	Thyroid	C-cell adenoma	4	2	1	3	
	Uterus	Endometrial stromal polyps	8	3	2	3	
	Mammary glands	Adenoma	4	0	1	0	
		Fibroadenoma	3	13*	1	0	
Malignant tumors	Spleen	Monocytic leukemia	10	17	16	19	↑
	Pituitary	Adenocarcinoma	1	2	1	0	
	Thyroid	C-cell carcinoma	1	3	0	1	
		Follicular adenocarcinoma	0	1	1	0	

Table 3. Main tumorigenesis in carcinogenicity study of tetrachloroethylene

	(mice: male)						
	Concentration		0	10	500	250	Peto's
test	administered (ppm)						
Male	Number of animals		50	50	50	50	
	tested						
Benign	Lungs	Bronchoalveolar					
tumors		epithelial adenoma	9	7	5	4	
	Spleen	Hemangioendo-					
		thelioma	1	1	0	1	
	Liver	Hepatocyte					
		adenoma	7	13	8	26**	↑↑
	Pituitary	Adenoma	0	1	2	0	
	Harder's glands	Adenoma	2	2	2	8	↑↑
Malignant	Lungs	Bronchoalveolar					
tumors		epithelial adenoma	2	3	3	0	
	Lymph nodes	Malignant					
		lymphoma	9	7	7	9	
	Spleen	Hemangioendo-					
		thelioma	1	1	3	5	↑
	Liver	Hemangioendo-					
		thelioma	1	1	5	5	↑
		Hepatocyte					
		carcinoma	7	8	12	25**	↑↑
	Testes	Histiocytic					
		sarcoma	3	0	0	1	

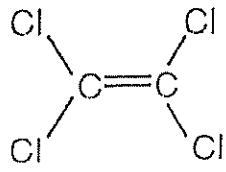
Table 4. Main tumorigenesis in carcinogenicity study of tetrachloroethylene

	(mice: female)						
	Concentration		0	10	50	250	Peto's
	administered (ppm)						test
Female	Number of animals		50	47	49	50	
	tested						
Benign	Lungs	Bronchoalveolar					
tumors		epithelial adenoma	5	2	0*	1	
	Liver	Hepatocyte					
		Adenoma	3	3	7	26**	↑↑
	Pituitary	Adenoma	9	11	4	9	↑
	Harder's glands	Adenoma	4	3	3	2	
Malignant	Lungs	Bronchoalveolar					
tumors		epithelial adenoma	0	0	2	1	
	Lymph nodes	Malignant	14	10	16	10	
		lymphoma					
	Spleen	Malignant	3	1	5	3	
		lymphoma					
	Liver	Hemangio-					
		endothelioma	0	0	0	1	
		Hepatocyte					
		carcinoma	0	0	0	14**	↑↑
	Uterus	Histiocytic	11	12	10	11	
		sarcoma					

*: Significant at level of significance of 5% or less,
**: Significant at level of significance of 1% or less (Fisher test);
↑: Significant at level of significance of 5% or less,
↑↑: Significant at level of significance of 1% or less (Peto's test).

About tetrachloroethylene

<Structural formula, molecular weight>



C_2Cl_4

Molecular formula: 165.83
Cas. No.: 127-18-4

<Generic name, secondary names>

Generic name: Tetrachloroethylene
Secondary names: Perchloroethylene
Carbon dichloride
Ethylene tetrachloride
1,1,2,2-Tetrachloroethylene
Perchlene

<Physicochemical properties, etc.>

Properties: Colorless, transparent nonflammable liquid, ether-like odor.
Boiling point: 121.20°C
Freezing point: -22.35°C
Specific gravity: $d^{2}_{4}{}^{0}$ 1.62260
Vapor pressure: 18.47 mmHg (25°C)
Solubility: Slightly soluble in water (0.015 g/100 g 25°C)
Freely soluble in ethanol, ether, chloroform, benzene
Storage conditions: Store at room temperature protected from light in an airtight container.
(References 1, 2, 3, 4, 5, 6)

<Uses>

Tetrachloroethylene is very fat soluble and nonflammable and therefore is primarily used in dry cleaning as an excellent defatting agent, as well as a metal degreasing agent and drying [agent]. It is also widely used in pesticides and insecticides, mixed solvents with cellulose ester and ether, organic synthetic intermediates, etc. (References 1, 2, 3, 4, 5, 6).

<Amount produced>

The world-wide demand for tetrachloroethylene in 1972 is assessed at approximately 600,000 tons and it is estimated to be approximately 1 million tons in 1974.

Industrial production of tetrachloroethylene in Japan began in 1952, with the amount produced in 1977 reaching approximately 55,000 tons in 1977, approximately 84,000 tons in 1987, and approximately 91,000 tons in 1989. (References 5, 6, 7)

<Allowable concentration>

The allowable concentration of tetrachloroethylene in the work environment is 50 ppm in Japan (Japan Association of Industrial Health, 1992) and 50 ppm in the US (ACGIH, 1991 ~ 1992). (References 8,9)

<Effects on humans>

When tetrachloroethylene is absorbed by inhalation, continuously contacts the skin and mucous membranes, is orally ingested, etc., it has toxic effects and induces damage to the central nervous system, lungs, skin, mucous membranes, digestive system, liver, and kidneys. Moreover, acute toxicity is induced when large amounts of vapor are inhaled over a short period of time, and these symptoms are manifested in the central nervous system. At concentrations of 2,000 ppm or greater, tetrachloroethylene manifests symptoms similar to alcohol intoxication in that it has an anesthetizing effect while simultaneously inducing eye, nose, and throat irritation, headache, lacrimation, burning sensation of the eyes, dizziness, drowsiness, stupor, nausea, eructation, vomiting, etc. Symptoms of subacute toxicity include headache, fatigue, nausea, eructation, vomiting, mental confusion, temporary deterioration of vision, etc. Repeated or long-term contact with skin induces dermatitis as a result of elimination of cutaneous fat. Moreover, if vapor or liquid comes into contact with the eyes, inflammation accompanied by lacrimation and burning pain will develop. If swallowed, initial symptoms are gastrointestinal irritation in the form of nausea, vomiting, diarrhea, bloody stools, etc. (References 7, 10, 11)
V. K. Rowe et al. (1952) conducted human exposure experiments in volunteers. The results are summarized in Table 5. (Reference 12)

Table 5. Effects of tetrachloroethylene on humans

Concentration	Exposure time	Symptoms with one exposure
106 ppm	1 hour	Mild discomfort, mild intoxication, mild
		drowsiness, odor perception, etc.
216 ppm	45 minutes ~ 2 hours	Mild mucosal irritation and mild dizziness,
		drowsiness, etc.
280 ppm	2 hours	Discomfort, mucosal irritation, eructation,
		etc., persisting even after exposure
600 ppm	10 minutes	Severe discomfort, mucosal irritation,
		dizziness, etc.

<Metabolism>

D. G. Pegg et al. (1978) investigated radioactivity after administration of ^{14}C-labeled tetrachloroethylene to rats by gavage oral administration (1 mg/kg, 500 mg/kg) and inhalation exposure (10 ppm, 600 ppm, 6 hours). At low concentrations, 70% was excreted in expired air as tetrachloroethylene, 26% was recovered in CO_2 of expired air and metabolite in feces and urine, and 3 to 4% remained *in vivo* within 72 hours after administration. On the other hand, at high concentrations, 89% was excreted in expired air as tetrachloroethylene, 9% was detected in CO_2 of expired air and feces, and 1 to 2% remained *in vivo*. Moreover, the half-life of the tetrachloroethylene excreted as expired air was approximately 7 hours, and there were no obvious differences by concentration or route of administration. It is reported that the radioactivity present *in vivo* was mainly distributed to the liver, kidneys and adipose tissue. (Reference 13)

W. Dekant et al. (1986) conducted experiments on excretion and metabolism when ^{14}C-labeled tetrachloroethylene was orally administered gavage to female rats and female mice at a dose of 800 mg/kg. It was mainly excreted in expired air as unaltered form in both species, at 91.2% in rats and 85.1% in mice, with part of the amount administered being excreted in urine (2.3% in rats and 7.1% in mice) and feces (2.0% in rats and 0.5% in mice). It is reported that urine metabolism products were identified as oxalic acid (8.0% in rats and 2.9% in mice), dichloroacetic acid (5.1% in rats and 4.4% in mice), trichloroacetic acid (54.0% in rats, 57.8% in mice), N-trichloroacetyl-aminoethanol (5.4% in rats, 5.7% in mice), trichloroethanol and its conjugates (8.7% in rats and 8.0% in mice), S-1,2,2-trichlorovinyl-N-acetylcysteine (1.6% in rats and 0.5% in mice), and other conjugates of trichloroacetic acid (1.8% in rats and 1.3% in mice). (Reference 14)

M. Mazzullo et al. (1987) researched covalent binding between tissue DNA, RNA and proteins and tetrachloroethylene by intravenous administration of ^{14}C-labeled tetrachloroethylene to rats and mice. Mice showed high polymer radioactivity in the liver, with this activity being particularly high in RNA. Rats showed high polymer radioactivity in the kidneys. Moreover, it is reported that covalent bonding of tetrachloroethylene and nucleic acids and proteins was promoted *in vitro* by the liver microsome P-450 oxidation system and that the extent of covalent binding increased when GSH was added to the liver microsome system. (Reference 15)

W. Dekant et al. (1987) conducted research on the route of metabolism using ^{14}C-labeled tetrachloroethylene and S-(1,2,2-trichlorovinyl)-L-cystein (TCVC), one of its metabolites, and report the following: Rat liver microsome fractions in the presence of NADPH confirm that tetrachloroethylene is metabolized to soluble trichloroacetic acid and oxalic acid, and the majority of this covalently binds with polymer in the microsome. Much of this polymer was identified as N-trichloroacetylated phospholipid. Moreover, the polar structures other than trichloroacetic acid and oxalic acid were estimated when tetrachloroethylene was incubated with liver microsome and cytokine (10 mM GSH add, no NADPH added) and this was identified as S-(1,2,2-trichlorovinyl)glutathione (TCVG). Furthermore, when 1-chloro-2,4-dinitrobenzene, which has strong bindability with GSH, was added, formation of TCVG was impaired and there was a reduction in TCVG formation in microsome in the presence of NADPH and GSH. TCVC, a metabolite of tetrachloroethylene, was cleaved by β–lyase of cystein conjugates in bacteria to produce dichloroacetic acid and pyruvic acid. The above-mentioned indicate that biotransformation of tetrachloroethylene proceeds in the liver, but metabolism by oxidation of tetrachloroethylene and metabolism by GSH conjugation are antagonistic reactions. (Reference 16)

//insert Figure 1//

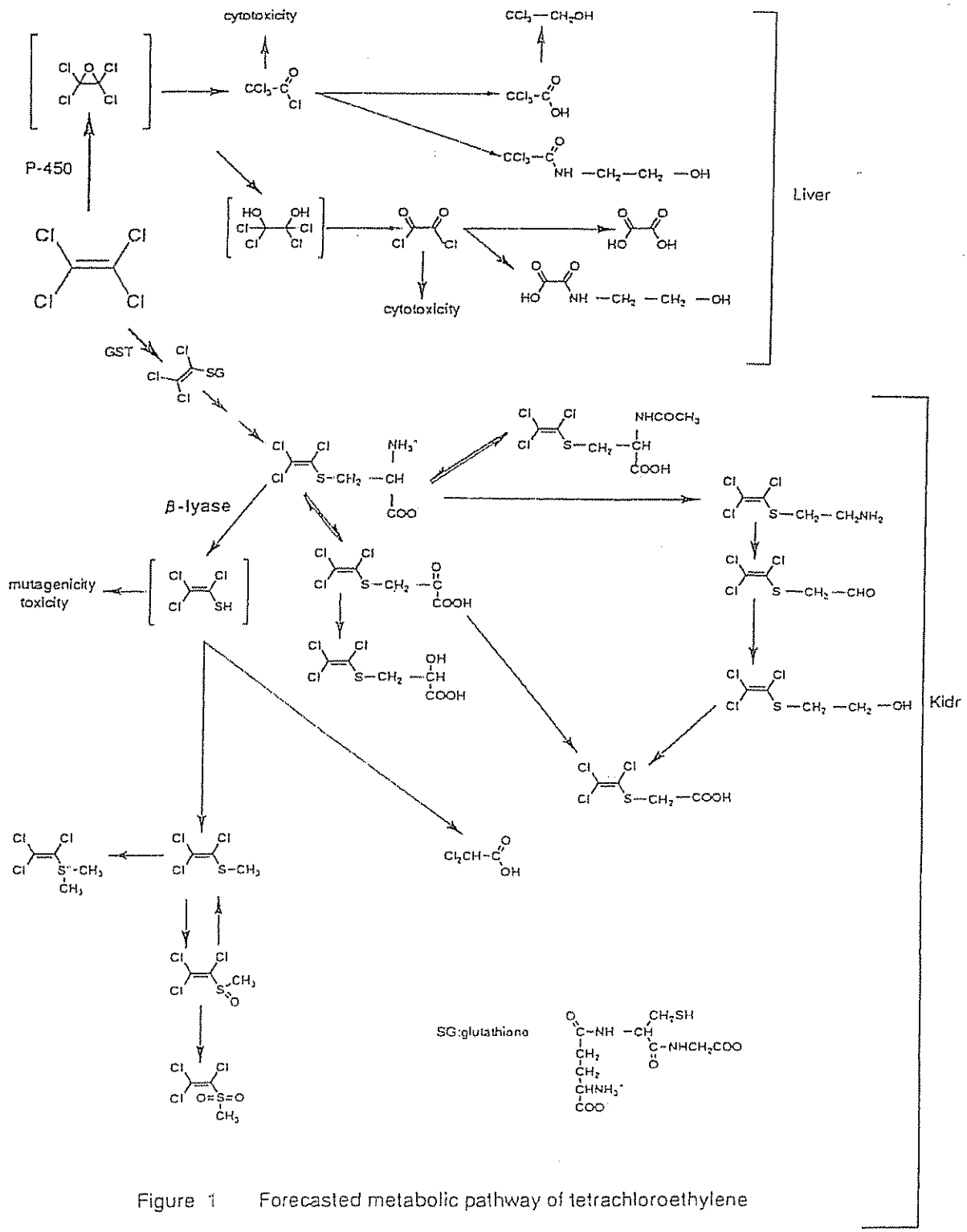


Figure 1 Forecasted metabolic pathway of tetrachloroethylene

A. M. Schumann et al. (1980) clarified the mechanism of susceptibility in mice and resistance in rats to the development of liver cancer by inhalation exposure (10 ppm) or gavage oral administration (500 mg/kg) gavage using ^{14}C-labeled tetrachloroethylene in $B6C3F_1$ mice and SD rats. As a result, the amount of metabolite per body weight (kg) in mice was 8.5-times and 1.6- times, respectively, the amount detected in rats. Moreover, it is noted that when the results in mice were compared with those in rats, there was more binding with polymer in the liver in mice. (Reference 17)

The route of metabolism as estimated based on the above-mentioned is shown in Figure 1. (Reference 18)

<Mutagenicity>

D. F. Callen (1980) et al., report that tetrachloroethylene is mutagenic by the metabolism activation method with cytochrome P-450 using *Saccharomyces cerevisiae*. (Reference 19)

R. Koch et al. (1988) report that they performed the same experiment with cytochrome P-450 using *Saccharomyces cerevisiae*, but the tetrachloroethylene was extremely toxic and they were unable to confirm its mutagenicity. (Reference 20)

S. Vamvakas et al. (1989) report the following with respect to mutagenicity of tetrachloroethylene and S-(1,2,2-trichlorovinyl)glutathione (TCVG), its GSH conjugate: Tetrachloroethylene does not show mutagenicity under exogenous metabolic activation or oxidation conditions. Moreover, TCVG shows mutagenicity in the presence of rat kidney microsome fractions containing high concentrations of γ-glutamyl transpeptidase and dipeptidase. Tetrachloroethylene produced TCVG over time and showed obvious mutagenicity by the Ames test in the presence of glutathione-S-transferase and glutathione purified from the rat liver, or when pre-cultivated with rate kidney fraction. When experiments were conducted by adding tetrachloroethylene to rat liver that had been isolated and irrigated, TCVG was excreted in bile, and this bile showed obvious antigenicity to bacteria in the presence of kidney microfraction, and this mutagenicity was weakened by adding serine borate, an inhibitor of γ-glutamyl transpeptidase, or amino oxyacetic acid, an inhibitor of β-lyase. It is noted that GSH S conjugates are severed by enzymes of the mercapturic acid route and β-lyase and this may have an effect on the occurrence of renal cancer in rats. (Reference 21)

<Acute toxicity studies>

V. K. Rowe et al. (1952) conducted inhalation exposure experiments of tetrachloroethylene using rats. The main symptom accompanying acute exposure to tetrachloroethylene was an inhibiting effect on the central nervous system. When rats were exposed, there was loss of consciousness within several minutes of exposure to 6,000 ppm or more and in several hours with exposure to 3,000 ppm, but this did not occur with exposure to 2,000 ppm. The results of these acute exposure experiments are summarized in Table 6. (Reference 12)

Table 6. Acute inhalation toxicity of tetrachloroethylene (rats)

Exposure concentration	Exposure time (hr)	Number of deaths/ number of exposed laboratory animals
20,000 ppm	1.2	30/30
	0.08	0/30
12,000 ppm	3.0	20/20
	2.5	4/20
	2.0	19/20
	1.0	16/20
	0.6	5/20
	0.4	1/20
	0.3	4/20
	0.2	0/20
6,000 ppm	8.0	17/20
	6.0	8/10
	5.0	4/5
	1.0	1/15
	0.8	1/11
	0.6	0/20
3,000 ppm	8.0	2/5
	6.0	3/10
	5.0	2/15
	4.0	0/30
2,000 ppm	14.0	0/10
	10.0	0/20

F. Friberg et al. (1953) report that the LC_{50} was 5,200 ppm when mice were exposed for 4 hours to tetrachloroethylene. (Reference 22)
J. R. Hayes et al. (1986) report that the LD_{50} was 3,835 mg/kg in males and 3,005 mg/kg in females when orally administered to rats. (Reference 23)

<Chronic toxicity studies>

V. K. Rowe et al. (1952) report that when experiments were performed by inhalation of tetrachloroethylene 7 hours/day, 5 days/week for 6 months using rats, rabbits, monkeys and guinea pigs, no effects were noted in rats, rabbits or monkeys at 400 ppm, but changes in liver tissue were noted in guinea pigs with 200 ppm. (Reference 12)

J. R. Hayes et al. (1986) performed studies of administration of tetrachloroethylene in drinking water using rats. There were no tetrachloroethylene-induced deaths when administered to rats for 90 days at a dose of 14 mg/kg, 400 mg/kg or 1,400 mg/kg body weight/day. It is reported that other than the effects of administration in the form of a significant reduction in body weight, increase in weight of the liver and kidneys relative to body weight, and dose-dependent elevation of 5'-nucleotidase activity observed among both females and males of the high-dose group, there were no changes attributed to administration in histopathological tests, blood chemistry tests or urinalyses. (Reference 23)

J. Odum et al. (1988) exposed male and female F344 rats and $B6C3F_1$ mice to inhalation of 400 ppm tetrachloroethylene for 6 hours/day for 14 days, 21 days and 28 days and to inhalation of 200 ppm for 6 hours/day for 28 days. As a result, there was an increase in liver peroxysome of the liver of mice. Elevation of activity of the cyanide-insensitive acyl CoA oxidation system of peroxysome was observed (2.6-times among males and 2.1-times among females), but there was no elevation of catalase activity of the liver. Peroxysome proliferation was not observed in the liver of rats or the kidneys of rats and mice. It is reported that trichloroacetic acid, which is known as a carcinogen and a substance that induces peroxysome proliferation of the liver, was detected as the main metabolite of tetrachloroethylene and that in comparisons of the area under concentration curve, the mice were exposed to 6-times to 7-times more than the rats. (Reference 24)

D. G. Pegg et al. (1978) report that when rats were exposed to inhalation of 600 ppm tetrachloroethylene 6 hours/day, 5 days/week for 12 months, histotoxicity was not seen at this concentration (Reference 13)

Thirteen-week inhalation studies are being performed through the NTP (National Toxicology Program TR 311: 1986) as preliminary studies of carcinogenicity of tetrachloroethylene in rats and mice. As a result of exposing rats and mice in groups of 20 males and females each to 1,600, 800, 400, 200 or 100 ppm 6 hours/day, 5 days/week for 13 weeks, deaths (4 male and 7 female rats and 2 male and 4 female mice) and a reduction in body weight of surviving animals were seen among both rats and mice in the 1,600 ppm groups. Mild to intermediate congestion of the liver was seen among both male and female rats in the 200 to 800 ppm groups. There were mild to intermediate leukocyte infiltration, central necrosis, bile stasis (400 to 1,600 ppm), and mitotic alteration (200 to 1,600 ppm) of the liver of males and females of the treated groups of mice and mild karyomegaly of the uriniferous tubules of the kidneys of male and female mice of the 200 ppm group. (Reference 25)

<Carcinogenicity studies>

Inhalation studies were conducted through the NTP (TR 311 (1986)) by exposure of F344/N rats to 200 ppm and 400 ppm and $B6C3F_1$ mice to 100 ppm and 200 ppm 6 hours/day, 5 days/week for 103 weeks. Monocytic leukemia in males and females and tumorigenesis of the uriniferous tubules of the kidneys of males were confirmed among the rats, with there being clear evidence of carcinogenicity among males and some evidence of carcinogenicity among females. It is reported that there was hepatocyte carcinoma among male and female mice and the development of hepatocyte adenoma among male mice, with there being clear evidence of carcinogenicity among male and female mice (Reference 25).

<Evaluation by IARC>

Although there is sufficient proof of carcinogenicity in laboratory animals, there is not adequate data of carcinogenicity in humans and therefore, the overall evaluation is classified as Group 2B in IARC (International Agency for Research on Cancer) Monographs (1987). (Reference 26)

I. Study Materials

1-1. Study sample lots, etc.

Lot No. used: PDL5382, PDJ5835, CTQ5124, CTN5675, CTJ4392, CTF5106, SAN5885
Manufactured by: Wako Junyaku Co., Ltd.
Grade: Special grade
Purity: 99.0%
Water content: 0.05% or less
Nonvolatile content: 0.005% or less

I-2. Identity and stability of study samples

I-2-1. Identity

It was confirmed that the tetrachloroethylene study samples that were used were the same based on determination of the mass spectrum and infrared absorption spectrum of each lot and comparison with reference values.

I-2-2. Stability

It was confirmed that the tetrachloroethylene study samples that were used were stable based on determination of the infrared absorption spectrum and gas chromatogram of each lot, on receipt and after use.

I-3. Laboratory animals

The animals were male and female F344/DuCrJ (Fischer) rats (SPF) and Crj:BDF_1 mice (SPF) from Japan Charles River. Two-hundred forty male and female rats and mice were each introduced 4 weeks *post partum* and after going through a quarantine and an acclimation period for 1 week each, 200 male and female animals close to the median body weight (body weight range when administration was started, 118 ~ 137 g for male rats, 95 ~ 107 g for female rats; 21.2 ~ 24.0 g for male mice and 17.2 ~ 20.0 g for female mice) were selected for submission to the study from among animals that grew normally and did not show anomalies in observation of their general status. Furthermore, the reasons for selecting F344/DuCrj (Fischer) rats and Crj:BDF_1 mice are as follow:

(1) Genetic stability
(2) Low incidence of spontaneous tumors
(3) Data available from previous carcinogenicity studies; known susceptibility to chemical-induced tumorigenesis.

II. Study methods

II-1. Administration

II-1-1. Route of administration, method of administration, and administration period

The route of administration was per-airway administration by general exposure.

Administration was performed by exposure of laboratory animals inside an inhalation chamber to specified concentrations of tetrachloroethylene vapor.

The administration period and exposure frequency were as follow:

6 hours/day, 5 days/week
Rats: 484 times/104 weeks
Mice: 481 times/104 weeks
(Excluding holidays)

II-1-2. Administered concentration and reason for establishing the same

The concentrations administered were set at 600 ppm, 200 ppm and 50 ppm for both male and female rats and 250 ppm, 50 ppm, and 10 ppm for both male and female mice.

Deaths were not seen among male or female rats of the treatment groups as a result of the 13-week study that had already been conducted on carcinogenicity. A change in body weight in the form of inhibition of an increase in body weight was seen among males in the 1,400 ppm group, which was the highest concentration, and there were sporadic changes as symptoms of toxicity resulting from administration in the results of hematological tests, blood chemistry tests, urinalyses, changes in organ weight, etc., among male and female rats treated with 609 ppm or more. Therefore, the maximum dose was set at 600 ppm and the minimum concentration was set at 50 ppm based on the allowable concentration (TLV). Moreover, the intermediate concentration was 4-times the minimum concentration, or 200 ppm.

Deaths were not observed among male or female mice of the treatment groups. Inhibition of an increase in body weight was found among males of groups treated with 609 ppm or more and among females of the 1,400 ppm group when compared to the control group. There were no marked changes observed as effects attributed to administration among males or females of groups treated with 265 ppm or less in terms of hematological tests, blood chemistry tests, autopsies, histopathological tests, etc. Therefore, the maximum concentration was set at 250 ppm and the intermediate concentration was set at 50 ppm, or the allowable concentration (TLV). The minimum concentration was set at 10 ppm, which is the intermediate concentration reduced by common ratio (5.0).

II-1-3 Method of generating and adjusting concentration of study sample

The method of generating the study sample involved heating tetrachloroethylene reservoir (1) in which tetrachloroethylene (liquid) had been introduced with constant temperature circulator (2) while bubbling compressed clean air (3) using bubbler (4), cooling (5) the study sample vapor that has thereby been vaporized to a specific temperature, and further heating (6) this vapor for introduction to line mixer (7) at a stable concentration, as shown in Figure 2. Next, the amount of vapor fed (8) was adjusted to the specified amount while monitoring the tetrachloroethylene concentration inside each inhalation chamber based on gas chromatograph (9).

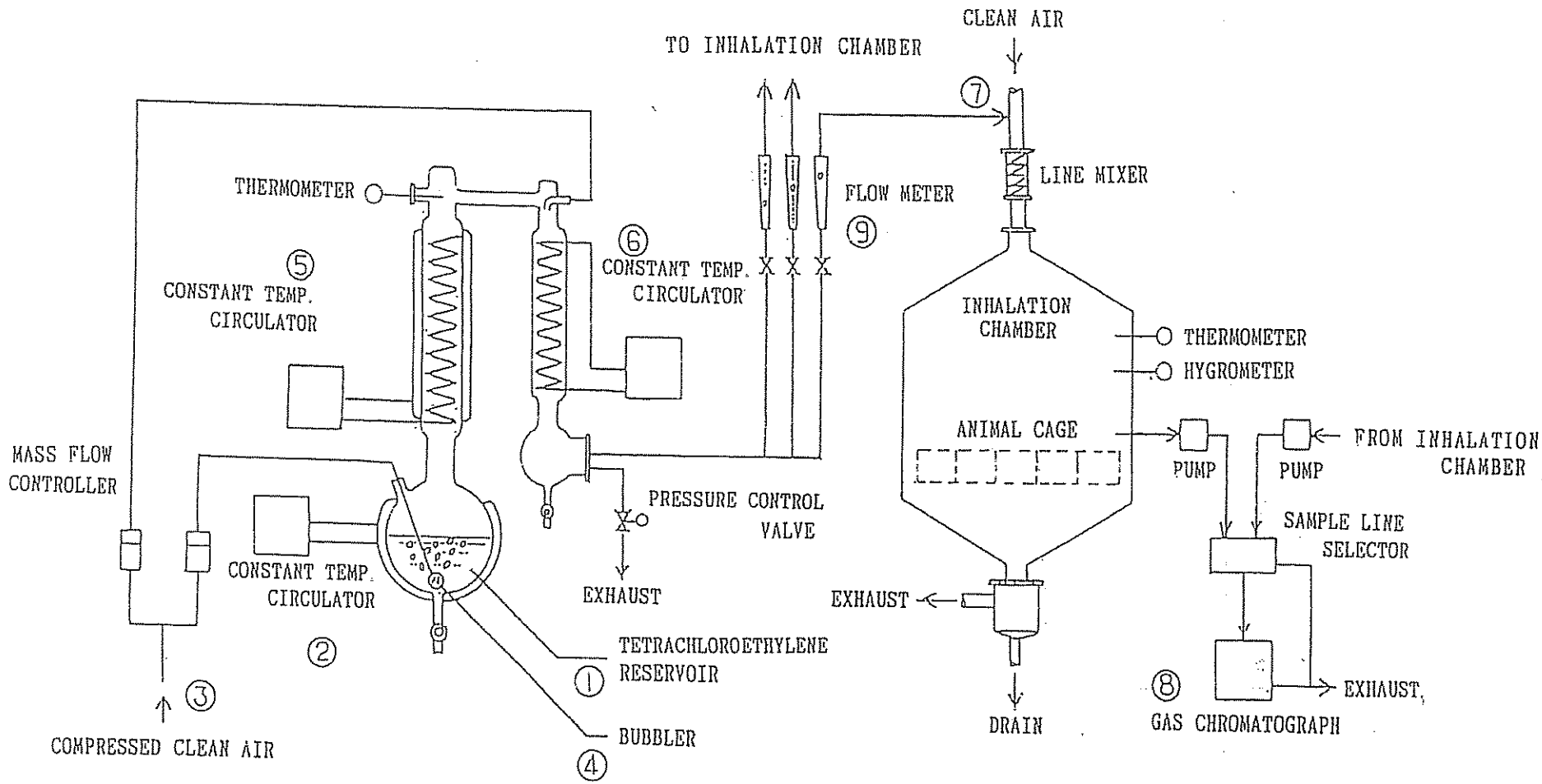


FIGURE 2 TETRACHLOROETHYLENE VAPOR GENERATION SYSTEM AND INHALATION SYSTEM

II-1-4. Determination of concentration of study sample

The concentration of tetrachloroethylene inside the inhalation chamber was determined before exposure was started and then every 15 minutes until exposure was completed using a gas chromatograph with an autosampler attached.

The average concentration administered satisfied the specified concentration. The results (average ± standard deviation) were 598.9 ± 4.9 ppm for the 600 ppm group, 199.8 ± 1.8 ppm for the 200 ppm group, and 49.9 ± 0.6 ppm for the 50 ppm group among rats and 250.2 ± 1.6 ppm for the 250 ppm group, 50.1 ± 0.3 ppm for the 50 ppm group and 10.0 ± 0.1 ppm for the 10 ppm group among mice.

II-2. Management of animals

II-2-1. Grouping and individual identification methods

Assignment of laboratory animals to each treatment group was performed by the grouping method (optimum stratification system) whereby animals are assigned one-by-one to each group on the order of heaviest body weight, total body weight of animals in each group is compared beginning the second time around, and bias between groups in terms of body weight is minimized by assigning heavier animals in order from the smaller groups. (Reference 27)

Individual identification of laboratory animals during the study period was identification with colored paint during the quarantine period and acclimation period or with an ear patch during the administration period, and by affixing individual identification No. on the cages.

Furthermore, the rats and mice were individually housed in separate chambers inside a barrier region. The study No., animal species, and animal No. were displayed on each chamber for differentiation from other studies.

II-2-2. Rearing conditions

The laboratory animals were reared inside the inhalation chambers during the acclimation period and administration period. The environmental conditions inside the inhalation chambers that were used are shown in Table 1. Moreover, rearing of the laboratory animals during the quarantine period was performed in a rearing room with an environment of temperature of 24 ± 1°C, humidity of 50 ± 5%, lighting cycle of lights on for 12 hours of light (8:00 ~ 20:00)/lights out for 12 hours (20:00 ~ 8:00), and ventilation frequency of 15 ~ 17 times/hour.

The animals were reared in groups of 5 per 1 cage ((stainless steel chain-link cage; 340 W x 294 D x 176 H mm for rats, 220 W x 212 D x 120 H mm for mice) during the quarantine period, and individually reared as 1 animal per cage during the acclimation period and administration period (acclimation period: stainless steel 6-link chain-link cages,125 W x 220 D x 176 H mm for rats, 95 W x 120 D x 120 H mm for mice; administration period: stainless steel 5-link chain-link cages, 150 W x 220 D x 176 mm for rats and 100 W x 120 D x 120 H mm for mice). Furthermore, the cages were changed every 2 weeks.

The feed was CRF-1 solid feed made by Oriental Yeast Co., Ltd. (3 Mrad = 30 Kgy - γ irradiation sterilized feed) given *ad libitum* from a solid feed feeder throughout the entire rearing period. Moreover, the drinking water was tap water (Hatano City Waterworks Bureau) that had been filtered and then disinfected with ultraviolet rays. It was provided *ad libitum* with an automatic watering device throughout the entire rearing period.

Furthermore, quality control of the feed that was used involved procuring Oriental Kobo Co., Ltd.'s own analytical data on nutrients and analytical data from Japan Food Analysis Center on impurities for each lot to confirm that there were no anomalies.

TABLE 1 EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE INHALATION STUDYS OF TETRACHLOROETHYRENE

Two-Year Studies
<Method of Administration>
Inhalation
<Number of Group>
Male 4, Female 4
<Size of Study Group>
50 males and 50 females of each groups
<Animals>
Strain and Species
F344/DuCrj(Fischer)rat
Crj:BDF1 mouse
Animal Source
Charles River Japan,Inc.
During of Time Held Before Study
2 wk
Age When Placed on Study
6 wk
Age When Killed
110 wk~111 wk
<Doses>
Rat--0,50,200,600ppm;
Mouse--0,10,50,250ppm
<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
Sterilized by ultraviolet rays
Automatic watering system
Available *ad libitum*
Animals per Cage
Single
(stainless steel wire)
Animal Room Environment
Barrier system
Temperature:22~26℃
Humidity :44~55%
Fluorescent light 12h/d
7.5~10 room air changes /h
Inhalation Chamber Environment
Temperature:24±1℃
Humidity :55±10%
Fluorescent light 12h/d
12~15 chamber air changes /h

TABLE 1 EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE INHALATION STUDYS OF TETRACHLOROETHYRENE (Continued)

Two-Year Studies
<Type and Frequency of Observation>
Clinical Sign
Observed 1/d
Body Weight
Weighed 1/wk for 14wk
Weighed 1/2wk thereafter
<Food Consumption>
Weighed 1/wk for 14wk
Weighed 1/4wk thereafter
<Hematology>
Red blood cell(RBC),
Hemoglobin,Hematocrit,
Mean corpuscular volume(MCV),
Meen corpuscular hemoglobin(MCH)
Meen corpuscular hemoglobin concentration(MCHC)
Platelet,White blood cell(WBC),
Differential WBC.
<Blood Biochemistry>
Total protein,Albumin,A/G ratio,T-bilirubin,Glucose,
T-cholesterol,Triglyceride,
Phospholipid<rat only>,
Glutamic oxaloacetic transaminase(GOT),Glutamic pyruvic transaminase(GPT),
Lactate dehydrogenase(LDH),Alkaline phosphatase(ALP),
γ-Glutamyl transpeptidase(G-GTP)<rat only>,
Creatine phosphokinase(CPK),Urea nitrogen,
Creatinine<rat only>,
Sodium,Potassium,Chloride,
Calcium,Inorganic phosphorus
<Urinalysis>
pH,Protein,Glucose,Ketone body,
Bilirubin<rat only>,
Occult blood,Urobilinogen
<Necropsy>
Necropsy performed on all animals
<Organ Weight>
Organ weight measurement performed on schedule sacrificed animals.
The following organs were weighed :brain,lung,liver,spleen,heart,kidney,adrenal,testis,ovary.
<Histopathologic Examination>
Histopathologic examination perfermed on all animals
The following organs were examind :skin,nasal cavit,trachea,lung,bone marrow,lymph node, thymus,spleen,heart,tongue,salivary gl,esophagus, stomach,small intes,large intes,liver,pancreas,kidney, urin bladd,pituitary,thyroid,adrenal,testis,epidiymis, semin ves,prostate,ovary,uterus,vagina,mammary gl, brain,spinal cord,periph nerv,eye,Harder gl,muscle,bone.

II-3. Observation and test items and methods

II-3-1. Observation of general status of animals

General status of the animals was checked once/day.

II-3-2. Body weight determination

Body weight was determined once/week for up to 14 weeks and once/2 weeks thereafter.

II-3-3. Food consumption determination

Food consumption was determined once/week for up to 14 weeks and once/4 weeks thereafter.

II-3-4. Hematological tests

Hematological tests were performed on animals surviving by the time of the scheduled dissection using blood to which EDTA-2K had been added that had been collected from the abdominal aorta under ether anesthetization immediately prior to autopsy. Furthermore, laboratory animals that served as test subjects were fasted beginning the day before the day of dissection (18 hours or longer). The test items are shown in Table 1.

II-3-5. Blood chemistry tests

Blood chemistry tests were performed on the laboratory animals surviving by the time of the scheduled dissection using plasma that had been obtained by adding lithium heparin to blood collected from the abdominal aorta under ether anesthetization and centrifugation immediately before autopsy. Furthermore, the laboratory animals that were the subject of the tests were fasted beginning the day before the day of dissection (18 hours or longer). The test items are shown in Table 1.

II-3-6. Urinalyses

Fresh urine was collected from the laboratory animals that survived up to the final week of treatment and urinalyses were conducted. The test items are shown in Table 1.

II-3-7. Pathological tests

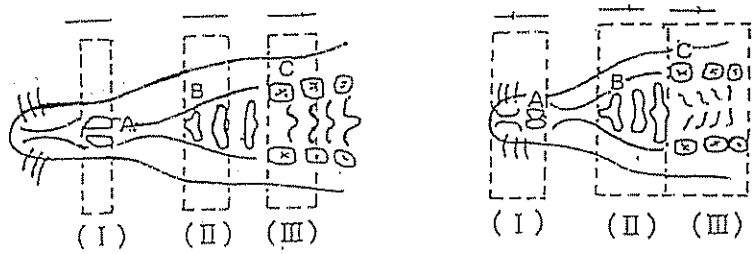
All animals were macroscopically checked at the time of dissection. After fixation of the organs of all animals with 10% neutral phosphate buffer formalin solution, the organs listed in Table 1 and those organs in which changes were macroscopically noted were wrapped with paraffin, thin slices were made, and hematoxylin eosin staining was performed for histopathological tests under a light microscope. Furthermore, the nasal cavity was cut (horizontally) in the 3 places of posterior extremity of the front teeth (level I), the incisor cusp (level II), and the anterior extremity of the first molar (level III) for the tests.

Organ weight of the animals that survived by the time of the scheduled dissection was determined as wet weight of the organs listed in Table 1. Context used in Peto's test were applied to tumorous lesions (0: tumors seen in scheduled dissections, 1: tumors seen in dead/dying animals but not directly related to the cause of death, 2: tumors that probably, but not definitely, fall under 1, 3: tumors that probably, but not definitely, fall under 4, 4:

tumors seen in dead/dying animals and directly related to the cause of death). (Reference 28)

Cutting positions

Rat **Mouse**



A Front teeth
B Incisor cusp
C Molar
→ Direction of thin slices

II-4. Numerical Treatment and Statistical Method

(1) Handling and representation of numbers

Each of the numerical data were represented together with the accuracy of the determination instrument.

Body weight was in units g. The first decimal place was rounded off to an integer in rats and the second decimal place was rounded to one decimal place in mice.

Food consumption was in units g. The amount of food consumed in 1 week (7 days) was measured up to the first decimal place and this was divided by 7 to calculate the average food consumption/day. This average second decimal places was rounded off and food consumption was represented up to the first decimal place.

Organ actual weight was in units g and was determined and represented up to the third decimal place. The organ actual weight was divided by body weight at the time of dissection to obtain units percent and the fourth decimal was rounded off to represent the organ weight/body weight ratio up to the third decimal place.

The A/G ratio of blood chemistry tests was found by calculation from albumin/(total protein – albumin). The second decimal place was rounded off and represented up to the first decimal place.

Furthermore, the average and standard deviation of each numerical data was rounded off so that the number of places would be the same as described above.

(2) Handling and representation of parameters

Body weight and food consumption were determined in all animals surviving at each determination time and missing data were excluded from the parameters.

Animals that survived by the time of the scheduled dissection served as subjects of organ weight, hematological tests, and blood chemistry tests and data that were missing were excluded from the parameters.

Animals that survived by the final week of treatment served as subjects and the number of tests served as the parameter for urinalyses.

The number of effective animals of each group (number of animals when animals excluded for reason of accident, etc., were subtracted from animals submitted for testing) served as the parameter of autopsy and histopathological test data.

However, the number of animals obtained by subtracting animals having organs that could not be studied was used as the parameter for tumorous lesions by organ.

(3) Statistical method

Preliminary tests of uniform distribution of the determinations obtained in the present experiment were first performed by the Bartlett method using the control group as the standard group. If the results showed uniform distribution, one-way variance analysis was performed and if there was a significant difference between groups, the average was tested by the multicomparison of Dunnett.

Moreover, if distribution was not uniform, the determinations were ranked through each group and the Kruskal-Wallis test was performed. If there was a significant difference between groups, Dunnet-(type) multicomparison was performed.

Bilateral tests were performed at a level of significance of 5% in the preliminary tests and bilateral tests were performed at 5% and 1% in the final tests.

Furthermore, non-tumorous lesions from the histopathological tests were grouped into dead/dying cases and cases scheduled for autopsy and χ^2 tests were performed with animals in which there were no findings as grade 0. Moreover, χ^2 tests were also conducted on urinalyses. The χ^2 tests were between the control group and each treatment group.

The items for which there were 2 or fewer numbers of tests performed on males and females in each group were excluded from the tests.

Peto's test, the Cochran-Armitage test, and the Fisher test were performed on each tumor of each organ when tumorous lesions were found at an incidence exceeding 5% in any group. Moreover, the Peto's test was conducted using context applied during histopathological tests (refer to II-3-7. Pathological tests) by the death analysis method <standard rates> (tests on tumors to which context 3 or 4 was applied), incidental tumor test <prevalence rates> (tests on tumors to which context 0, 1 or 2 was applied), and death analysis method + incidental tumor test <combined rates> (tests on total of context 0 through 4). Fisher tests were conducted between the control group and each treatment group.

III. Study Results

III-1. Carcinogenicity studies using rats (Test No. 0104)

(1) Observation of laboratory animal status

Survival

Survival by group during the administration period is shown in Figures 3 and 4.

The number of animals surviving (survival rate) during the final determination week (104 weeks) was 28/50 animals (56%) in the 600 ppm group, 30/50 animals (60%) in the 200 ppm group, 34/50 animals (68%) in the 50 ppm group, and 37/50 animals (74%) in the control group among males and 34/50 animals (68%), 34/50 animals (68%) in the 200 ppm group, 34/50 animals (68%) in the 50 ppm group, and 42/50 animals (84%) in the control group among females.

There was an increase in the number of deaths with an increase in the concentration administered.

General status

Of the results of checking the general status of the animals, the incidence of detecting external masses and internal masses is shown in Tables 2 and 3.

Judging from all animals (dead and surviving animals), there were no changes in the number of animals in which an external mass was found when each treatment group is compared with the control group, in males or females. The number of animals in which an external mass was detected was 29 cases in the 600 ppm group, 36 cases in the 200 ppm group, 30 cases in the 50 ppm group, and 27 cases in the control group among males and 8 cases in the 600 ppm group, 8 cases in the 200 ppm group, 14 cases in the 50 ppm group, and 5 in the control group. The period of occurrence of external masses was generally at least 1 year after starting exposure to tetrachloroethylene, and there was not a difference in this time of occurrence between each group.

Judging from the occurrence of internal masses in all animals, there was an increase in the number of occurrences, in both males and females, in each treatment group when compared to the control group. The number of occurrences was 13 cases in the 600 ppm group, 7 cases in the 200 ppm group, 5 cases in the 50 ppm group, and 3 cases in the control group among males and 8 cases in the 600 ppm group, 8 cases in the 200 ppm group, 10 cases in the 50 ppm group, and 4 cases in the control group among females.

Other characteristic findings in terms of general status that were attributed to tetrachloroethylene administration were not found in any of the dead or surviving animals.

TABLE 2 INCIDENCE AND TIME OF MASS OCCURRENCE (CLINICAL OBSERVATION) :RAT :MALE

Time of mass occurrence (Dosing week)	0~13	14~26	27~39	40~52	53~65	66~78	79~91	92~104	0~104
The kind of mass	No. of animals with mass (No of dead and moribund animals with mass)								
Internal mass									
Control	0	0	0	0	0	0	0	3	3 (1)
50 ppm	0	0	0	0	0	0	3	2	5 (4)
200 ppm	0	0	0	0	1	0	1	6	7 (6)
600 ppm	0	0	0	0	0	1	4	10	13 (11)
External mass									
Control	0	0	0	0	10	13	11	13	27 (8)
50 ppm	0	0	0	0	7	10	12	17	30 (15)
200 ppm	0	0	0	0	14	23	18	20	36 (13)
600 ppm	0	0	0	0	4	9	19	23	29 (11)

TABLE 3 INCIDENCE AND TIME OF MASS OCCURRENCE (CLINICAL OBSERVATION) :RAT :FEMALE

Time of mass occurrence (Dosing week)	0~13	14~26	27~39	40~52	53~65	66~78	79~91	92~104	0~104
The kind of mass	No. of animals with mass (No. of dead and moribund animals with mass)								
Internal mass									
Control	0	0	0	0	0	0	1	3	4 (3)
50 ppm	0	0	0	0	1	0	5	4	10 (8)
200 ppm	0	0	0	0	0	1	4	5	8 (6)
600 ppm	0	0	0	0	0	1	2	6	8 (7)
External mass									
Control	0	0	0	0	0	0	1	3	5 (3)
50 ppm	0	0	0	0	1	4	8	12	14 (5)
200 ppm	0	0	0	0	2	1	5	6	8 (2)
600 ppm	0	0	0	0	2	5	4	2	8 (5)

Body weight

Changes in body weight by group during the administration period are shown in Figures 5 and 6.

Inhibition of an increase in body weight when compared to the control group was seen among males of the 600 ppm almost consistently from 7 weeks to 100 weeks.

Inhibition of an increase in body weight when compared to the control group was seen among females of the groups treated with 200 ppm or more almost consistently from the 32 weeks to 104 weeks.

Food consumption

Food consumption by group during the administration period (food consumption/animal/day) is shown in Figures 7 and 8.

Food consumption dropped among males and females of the treated groups in comparison to the control group throughout almost all weeks, but this reduction was always less than 10%.

(2) Hematological tests, blood chemistry tests, urinalyses

Hematological tests

An increase in MCH was seen among females of the 600 ppm group.

Blood chemistry tests

There was an increase in GPT activity among males of the 600 ppm group and females of groups treated with 200 ppm or more, an increase in urea nitrogen among females of groups treated with 200 ppm or more, and a reduction in triglycerides and an increase in potassium among females of the 600 ppm group.

Urinalyses

There were no changes worth noting among males or females.

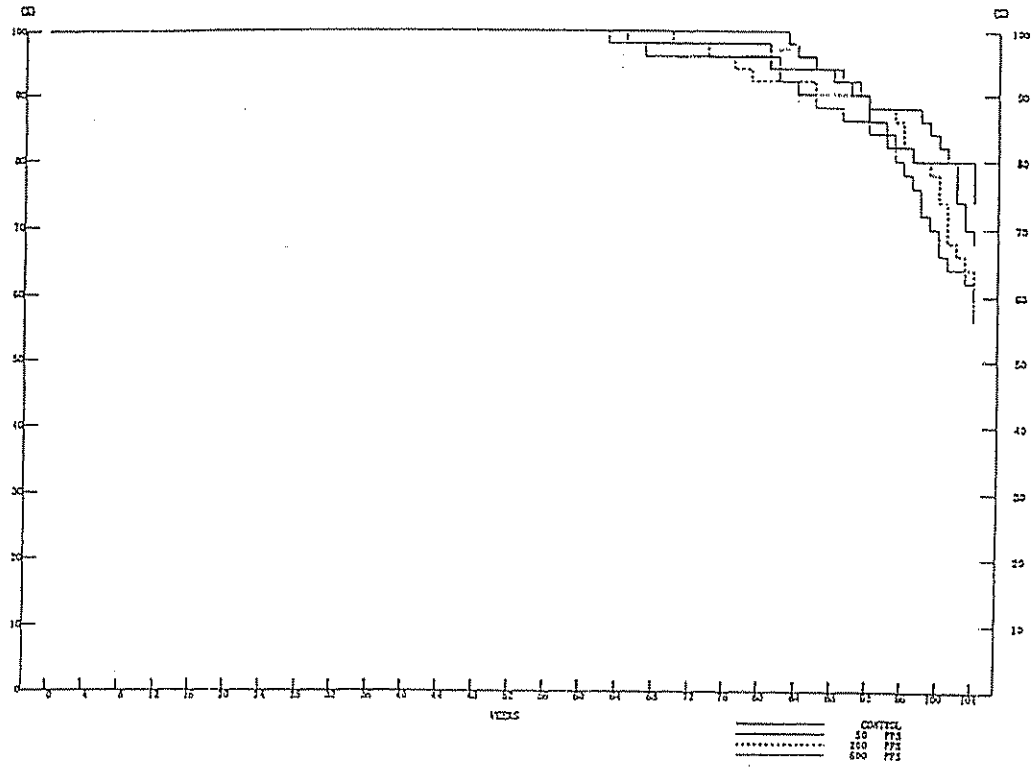


FIGURE 3 SURVIVAL ANIMAL RATE : RAT:MALE(TWO-YEAR STUDIES)

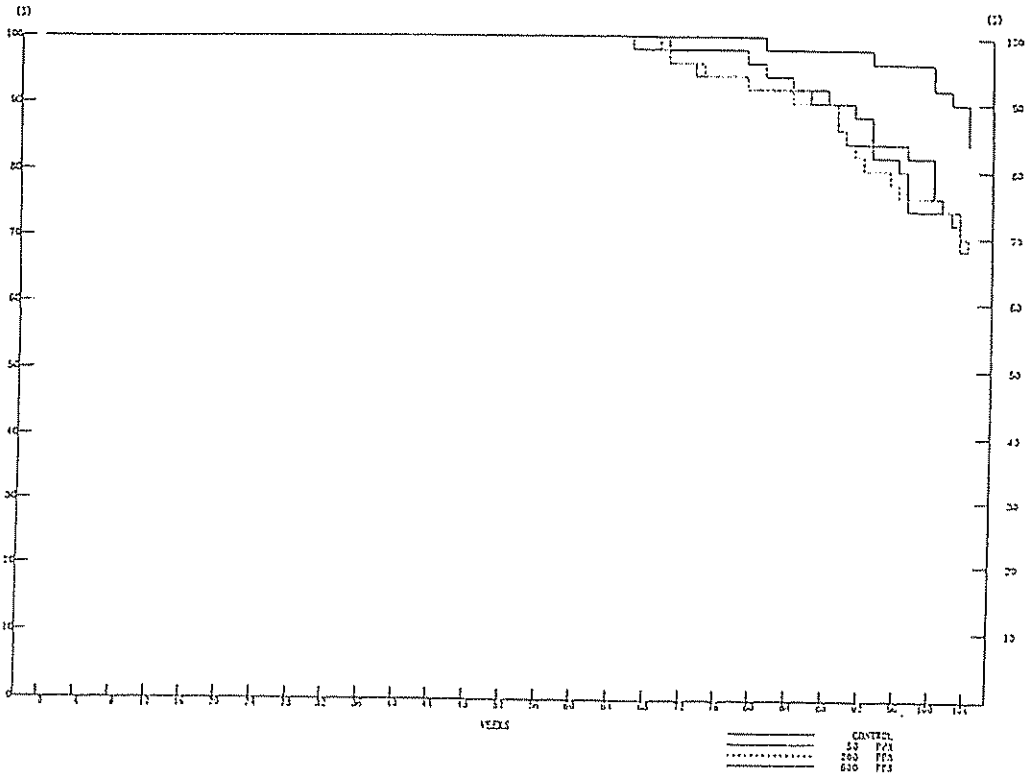


FIGURE 4 BODY WEIGHT CHANGES : RAT:FEMALE(TWO-YEAR STUDIES)

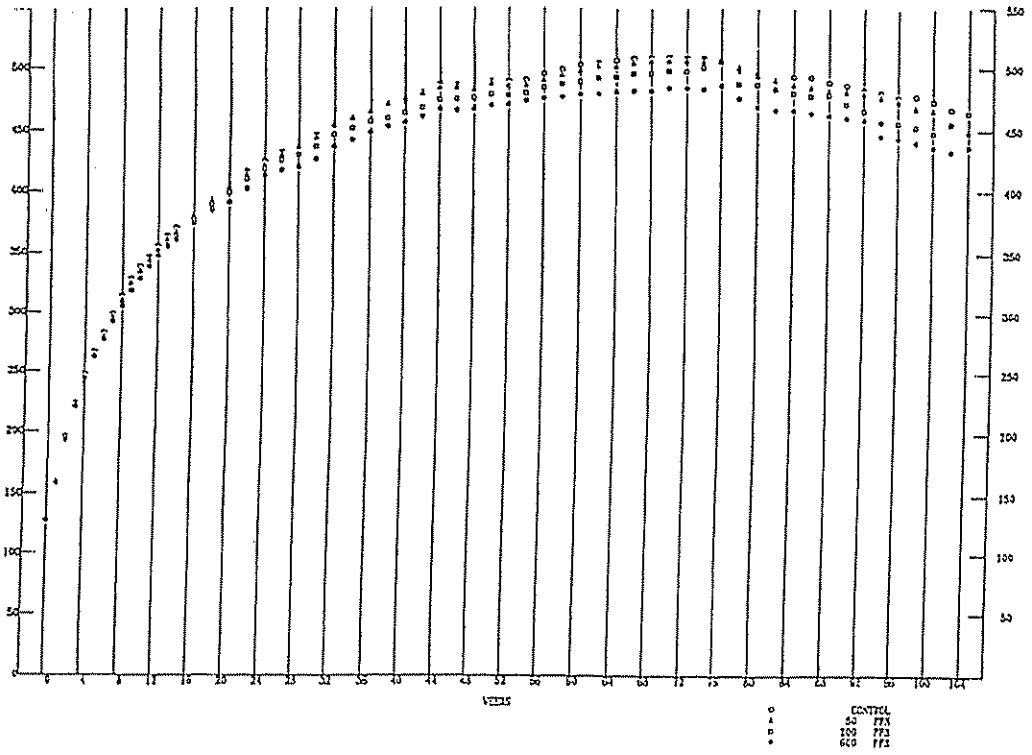


FIGURE 5 BODY WEIGHT CHANGES : RAT:MALE(TWO-YEAR STUDIES)

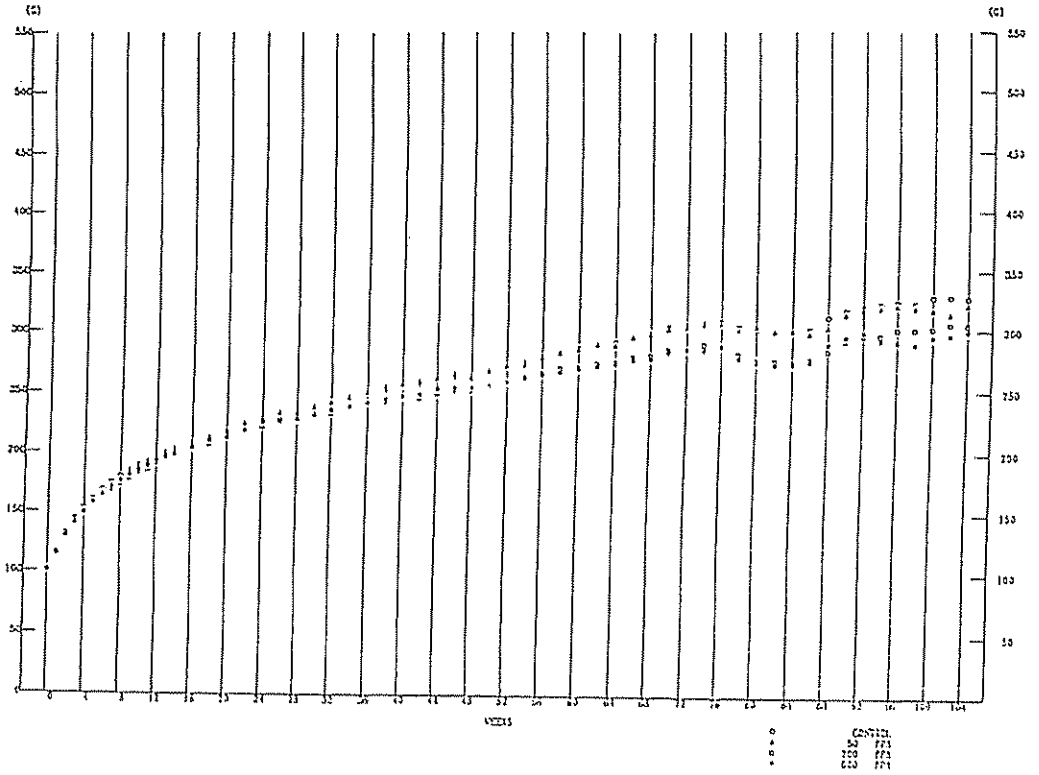


FIGURE 6 BODY WEIGHT CHANGES : RAT:FEMALE(TWO-YEAR STUDIES)

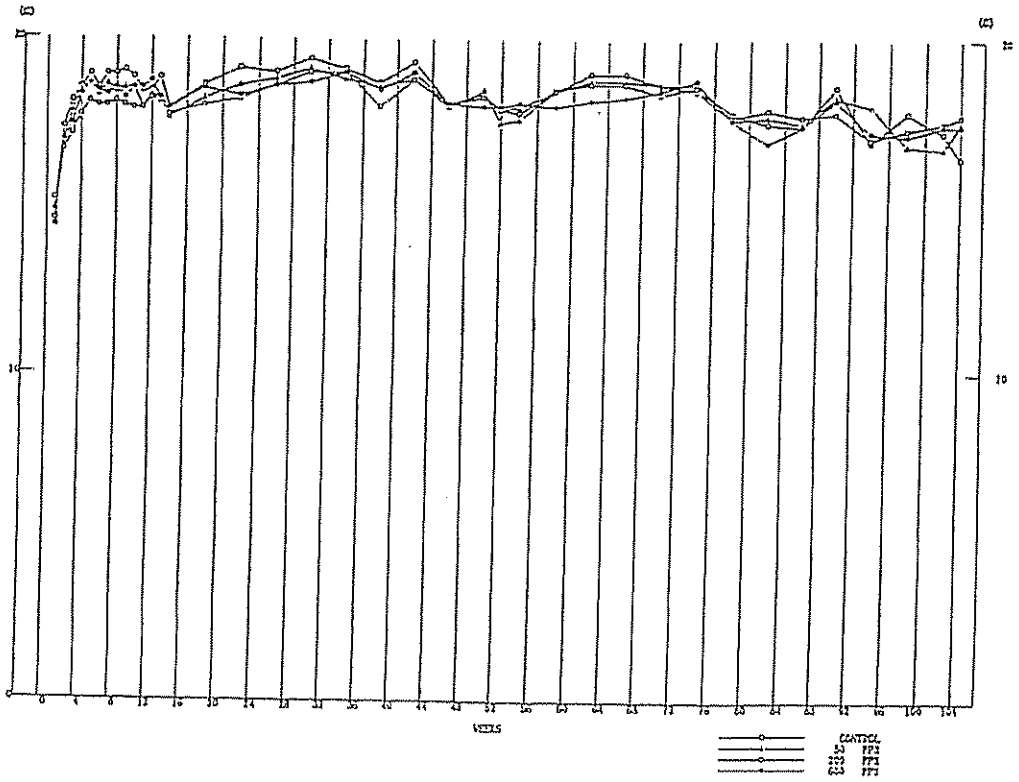


FIGURE 7 FOOD CONSUMPTION : RAT:MALE(TWO-YEAR STUDIES)

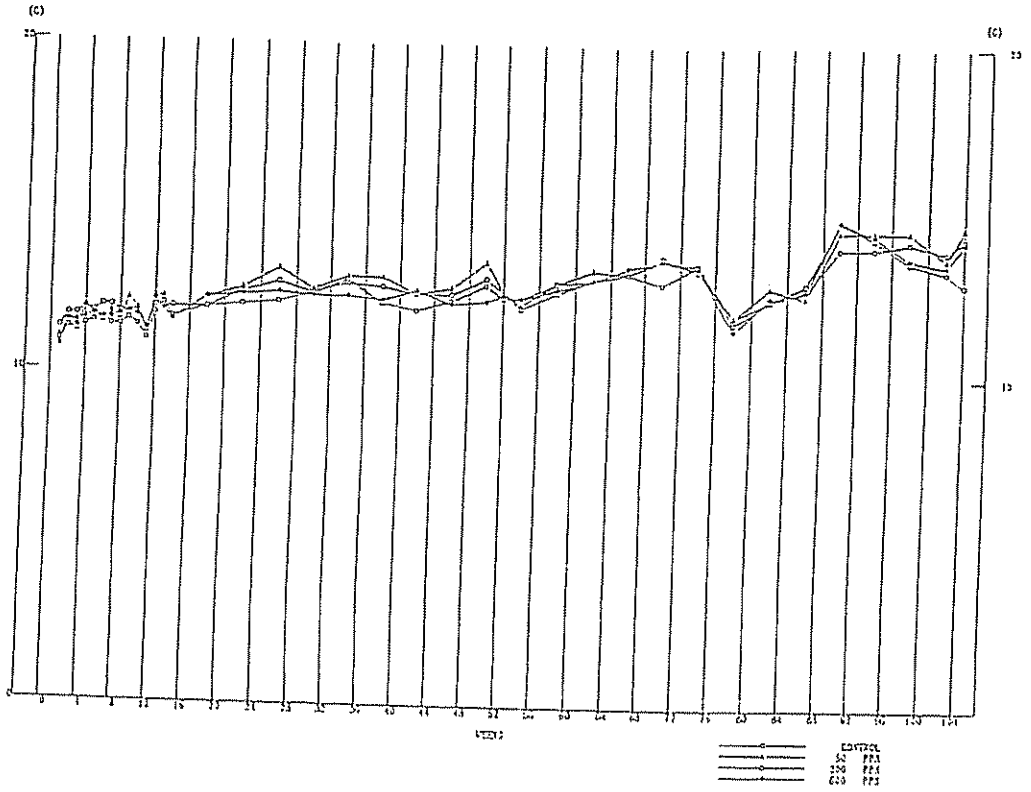


FIGURE 8 FOOD CONSUMPTION : RAT:FEMALE(TWO-YEAR STUDIES)

(3) Pathological tests

Autopsy

Of the autopsy findings observed at the time of dissection, characteristic findings or findings seen at a high incidence among treated groups when compared to the control group are listed below:

Enlargement of the spleen was reported in dead/dying cases throughout the study among males and females of all treatment groups and in scheduled dissections among males of all treatment groups, and the incidence was also high in most groups. The incidence of subcutaneous tumors and granulation of the liver was high in cases of scheduled autopsy among males of all treatment groups.

Organ weight

Kidney actual weight was high among males of groups treated with 200 ppm or more when compared to the control group. Liver actual weight was high among females treated with 50 ppm and 600 ppm.

Kidney relative weight was high among males treated with 200 ppm or more when compared to the control group. Heart, lung, kidney, and liver relative weights were high among females treated with 200 ppm or more and adrenal relative weight was high among females of the 200 ppm group.

Histopathological tests

Nasal cavity

An increase in blood clots was found in dead/dying cases among males of the 600 ppm group and a reduction in eosinophilic changes of the respiratory epithelium was found in dead/dead dying cases among females of all treatment groups.

Spleen

There was a tendency toward an increase in monocytic leukemia among males (27/50 of the 600 ppm group, 22/50 of the 200 ppm group, 14/50 of the 50 ppm group, 11/50 of the group [sic]) according to Peto's test (standard rate, prevalence rate, combined rate), and the Cochran-Armitage test, and there was a significant increase in monocytic leukemia among males of the 600 ppm group by the Fisher test as well. Moreover, there was a tendency toward an increase in monocytic leukemia among females (19/50 of the 600 ppm group, 16/50 of the 200 ppm group, 17/50 of the 50 ppm group, 10/50 of the control group) according to Peto's test (standard method (Tables 4, 5).

There was a reduction in extramedullary hematopoiesis among cases of scheduled dissection of males of groups treated with 200 ppm or more.

Liver

There was an increase in spongiosis hepatitis among cases of scheduled dissection of males in the 600 ppm group.

There was a reduction in granulation among cases of dead/dying females of the groups treated with 200 ppm or more. Furthermore, although not statistically significant, there was an increase in hyperplasia among males of the 600 ppm group. Judging from all animals when the dead/dying cases and scheduled dissections are totaled, there was an increase in spongiosis hepatitis among males of groups treated with 200 ppm or more and among males of the 600 ppm group (Table 6).

TABLE 4 NEOPLASTIC LESIONS (SPLEEN) INCIDENCE AND STATISTICAL ANALYSIS : RAT : MALE

Group Name	Control	50 ppm	200 ppm	600 ppm
SITE : spleen				
TUMOUR : mononuclear cell leukemia				
Overall Rates(a)	11/50 (22.0)	14/50 (28.0)	22/50 (44.0)	27/50 (54.0)
Adjusted Rates(b)	24.32	17.65	40.00	42.86
Terminal Rates(c)	9/37 (24.3)	6/34 (17.6)	12/30 (40.0)	12/28 (42.9)
Standard Rates(d)	P=0.0022**			
Prevalence Rates(d)	P=0.0104*			
Combind analysis(d)	P=0.0001**			
Cochran-Armitage Test(e)	P=0.0005**			
Fisher Exact Test(e)		P=0.3777	P=0.0707	P=0.0201*

TABLE 5 NEOPLASTIC LESIONS (SPLEEN) INCIDENCE AND STATISTICAL ANALYSIS : RAT : FEMALE

Group Name	Control	10 ppm	50 ppm	250 ppm
SITE : spleen				
TUMOUR : mononuclear cell leukemia				
Overall Rates(a)	10/50 (20.0)	17/50 (34.0)	16/50 (32.0)	19/50 (38.0)
Adjusted Rates(b)	14.29	20.59	22.50	20.59
Terminal Rates(c)	6/42 (14.3)	7/34 (20.6)	7/34 (20.6)	7/34 (20.6)
Standard Rates(d)	P=0.0486*			
Prevalence Rates(d)	P=0.3153			
Combind analysis(d)	P=0.0571			
Cochran-Armitage Test(e)	P=0.1397			
Fisher Exact Test(e)		P=0.1636	P=0.2039	P=0.1027

(a):Number of tumor-bearing animals/number of animals examined at the site

(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.

(c):Observed tumor incidence at terminal kill.

(d):Beneth the control incidence are the P-values associated with the trend test (Peto test)

Standard method : Death analysis

Prevalence method : Incidental tumor test

Combind analysis : Death analysis + Incidental tumor test

(e):The Cochran-Armitage and Fisher's exact test compare directly the overall incidence rates.

TABLE 6 NUMBER OF RAT WITH SELECTED LIVER LESIONS

	Male				Female			
Group	Control	50ppm	200ppm	600ppm	Control	50ppm	200ppm	600ppm
Number of examined	50	50	50	50	50	50	50	50
Spongiosis hepatis	5	4	10	16	0	0	0	0
Hyperplasia	4	2	5	13	2	1	5	1
Clear cell focus	3	1	0	1	1	0	0	1
Acidphilic cell focus	1	0	1	0	0	0	0	0
Basophilic cell focus	4	3	0	4	1	1	2	0
Vacuolic cell focus	0	2	1	1	0	0	0	2
Mixed cell focus	4	3	1	1	0	0	0	2
Hepatocellular adenoma	3	0	0	2	0	0	1	0
Cholangiocellular adenoma	1	0	0	0	1	0	0	0
Cholangiocellular carcinoma	1	0	1	0	0	0	0	0

Kidneys

Males of the 600 ppm group showed an increase in nuclear enlargement of the proximal tubule among dead/dying cases and an increase of nuclear enlargement of the proximal tubule and atypical dilation (proximal tubule) and exacerbation of chronic renal disease among scheduled dissections. Moreover, females of the 600 ppm group showed an increase in nuclear enlargement of the proximal tubule among scheduled dissections. In addition, judging from all animals when dead/dying cases and scheduled dissections are totaled, there was an increase in nuclear enlargement of the proximal tubule among males of groups treated with 200 ppm or higher and females of groups treated with 600 ppm or higher and an increase in atypical dilation (proximal tubule) among males and females of the 600 ppm groups (Table 7).

TABLE 7 NUMBER OF RAT WITH SELECTED KIDNEY LESIONS

	Male				Female			
Group	Control	50ppm	200ppm	600ppm	Control	50ppm	200ppm	600ppm
Number of examined	50	50	50	50	50	50	50	50
Nuclear enlargement: proximal tubule	0	0	23	48	0	0	1	18
Atypical tubular dilation: proximal tubule	0	0	0	24	0	0	0	6
Liposarcoma	0	0	0	1	0	0	0	0
Renal cell adenoma	1	2	1	2	1	0	0	0
Renal cell carcinima	0	0	0	0	0	0	0	1

Mammary glands

In tests of fibroadenoma in females and tests in which adenoma and fibroadenoma in females were added together, there was a tendency toward a reduction by the Cochran-Armitage test, with there being an increase by the Fisher test only among females of the 50 ppm.

Cause of death

The pathological cause of death among dead/dying cases is shown in Table 8. When males and females of the treatment groups are compared with the control groups, there was an increase in death due to leukemia.

TABLE 8 CAUSE OF DEATH :RAT

	Male				Female			
Group	Control	50ppm	200ppm	600ppm	Control	50ppm	200ppm	600ppm
Number of dead/moribund animal	13	16	20	22	8	16	16	16
Renal lesion	1	0	2	0	0	0	1	0
Chronic nephropathy	0	0	0	1	0	0	0	0
Tumor death : leukemia	3	8	9	14	4	10	7	12
: subcutis	0	1	2	1	0	0	0	1
: spleen	0	0	0	0	1	0	0	0
: small intestine	0	0	0	1	0	0	1	0
: kidney	0	0	0	2	0	0	0	0
: pituitary gland	4	2	4	1	3	1	2	3
: thyroid	0	0	0	0	0	1	0	0
: adrenal	1	1	0	1	0	1	0	0
: uterus	0	0	0	0	0	1	3	0
: mammary gland	1	0	0	0	0	1	0	0
: prep /cli. gland	1	0	0	0	0	0	1	0
: Zymbal gland	0	1	1	0	8	8	8	9
: muscle	1	0	0	0	0	0	1	0
: bone	0	1	1	1	5	2	2	2
: pleura	1	0	0	0	1	0	0	0
: mediastinum	0	1	0	0	1	2	2	1
: peritoneum	0	1	0	0	0	0	0	0

III-2. Carcinogenicity studies using mice (Study No. 0105)

(1) Observation of laboratory animal status

Survival

Survival by group during the administration period is shown in Figures 9 and 10.

The number of animals surviving (survival rate) during the final determination week (104 weeks) was 22/50 animals (44%) in the 250 ppm group, 28/50 animals (56%) in the 50 ppm group, 35/50 animals (70%) in the 10 ppm group, and 31/50 animals (62%) in the control group among males and 17/50 animals (34%) in the 250 ppm group, 22/49 animals (45%) in the 50 ppm group, 27/47 animals (57%) in the 10 ppm group, and 32/50 animals (64%) in the control group among females.

There was an increase in the number of deaths with an increase in the concentration administered

General status

Of the results of checking the general status of the animals, the incidence of detecting external masses and internal masses is shown in Tables 9 and 10.

The number of animals in which an external mass was detected was 12 cases in the 250 ppm group, 6 cases in the 50 ppm group, 5 cases in the 10 ppm group, and 5 cases in the control group among males and 5 cases in the 250 ppm group, 8 cases in the 50 ppm group, 4 cases in the 10 ppm group, and 8 in the control group among females.

The number of occurrences of internal masses was 30 cases in the 250 ppm group, 31 cases in the 50 ppm group, 26 cases in the 10 ppm group, and 18 cases in the control group among males and 22 cases among the 250 ppm group, 21 cases among the 50 ppm group, 16 cases among the 10 ppm group, and 18 cases in the control group among females.

Other characteristic findings in terms of general status that were attributed to tetrachloroethylene administration were not found in any of the dead or surviving animals.

TABLE 9 INCIDENCE AND TIME OF MASS OCCURRENCE (CLINICAL OBSERVATION) :MOUSE :MALE

Time of mass occurrence (Dosing week)	0~13	14~26	27~39	40~52	53~65	66~78	79~91	92~104	0~104
The kind of mass	No of animals with mass (No of dead and moribund animals with mass)								
Internal mass									
Control	0	0	0	1	3	8	10	11	18 (12)
10 ppm	0	0	0	0	3	14	9	16	26 (9)
50 ppm	0	0	0	1	3	17	18	20	31 (14)
250 ppm	0	0	0	1	4	11	12	20	30 (18)
External mass									
Control	0	0	0	0	0	0	4	3	5 (3)
10 ppm	0	0	0	0	0	2	2	4	5 (3)
50 ppm	0	0	0	0	2	1	5	5	6 (3)
250 ppm	0	0	0	0	1	2	7	10	12 (7)

//insert Table 10//

TABLE 10 INCIDENCE AND TIME OF MASS OCCURRENCE (CLINICAL OBSERVATION) :MOUSE :FEMALE

Time of mass occurrence (Dosing week)	0~13	14~26	27~39	40~52	53~65	66~78	79~91	92~104	0~104
The kind of mass	No of animals with mass (No. of dead and moribund animals with mass)								
Internal mass									
Control	0	0	0	1	3	7	7	8	18 (14)
10 ppm	0	0	0	1	2	7	7	8	16 (14)
50 ppm	0	0	0	2	9	9	8	7	21 (13)
250 ppm	0	0	0	1	1	7	9	13	22 (18)
External mass									
Control	0	0	0	0	0	2	4	4	8 (4)
10 ppm	0	0	0	0	0	1	1	4	4 (2)
50 ppm	0	0	0	0	2	1	5	6	8 (7)
250 ppm	0	0	0	0	1	2	3	3	5 (3)

Body weight

Changes in body weight by group during the administration period are shown in Figures 11 and 12.

Inhibition of an increase in body weight when compared to the control group was seen among males of the 250 ppm group almost consistently from 6 weeks to 104 weeks.

Inhibition of an increase in body weight when compared to the control group was seen among females of the 250 ppm group continuously from 80 weeks to 104 weeks.

Food consumption

Food consumption by group during the administration period (food consumption/animal/day) is shown in Figures 13 and 14.

There was a tendency toward a reduction in food consumption when compared to the control group from the beginning of the administration period, and a tendency toward a reduction was noted from 30 weeks to 104 weeks, among males of the 250 ppm group.

There was a tendency toward a reduction when compared to the control group beginning at 80 weeks, and a tendency toward a reduction was noted from 88 weeks to 104 weeks, among females of the 250 ppm group.

(2) Hematological tests, blood chemistry tests, urinalyses

Hematological tests

There was an increase in the erythrocyte count and hematocrit level of males and females of the 250 ppm group, an increase in the hemoglobin concentration of females of the 250 ppm group, a reduction in MCV, MCH, MCHC, and platelet count among males of the 250 ppm group, and an increase in the segmented neutrophil ratio and a reduction in the lymphocyte ratio among females of the 10 ppm group.

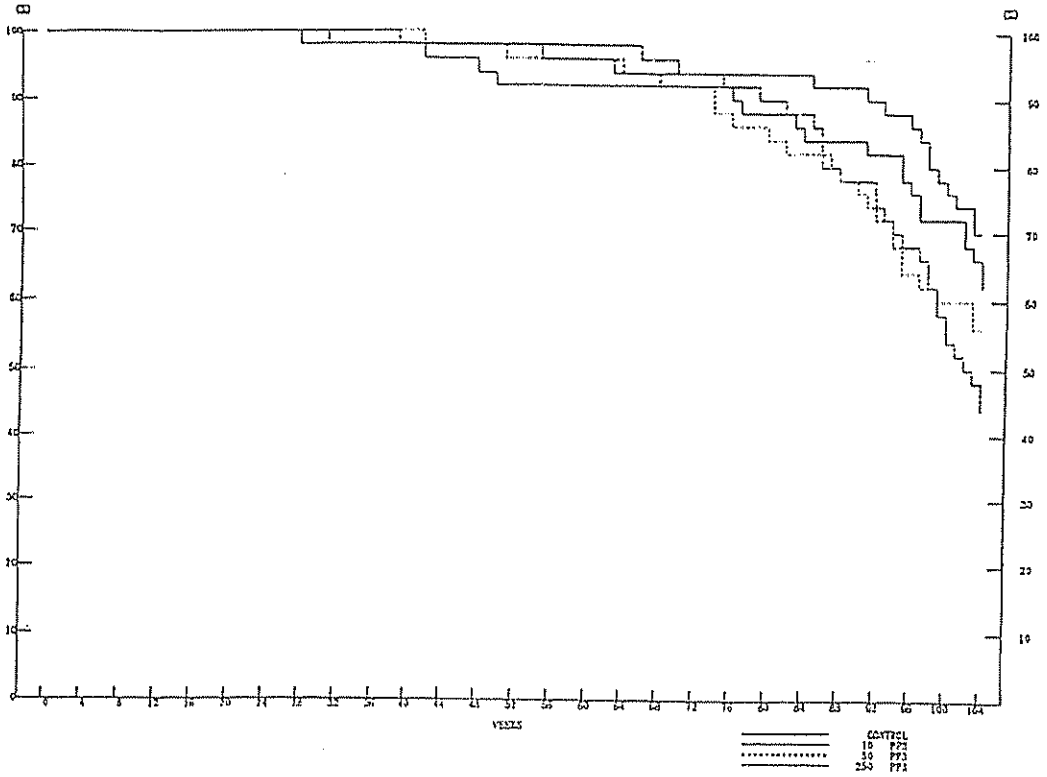


FIGURE 9 SURVIVAL ANIMAL RATE : MOUSE:MALE(TWO-YEAR STUDIES)

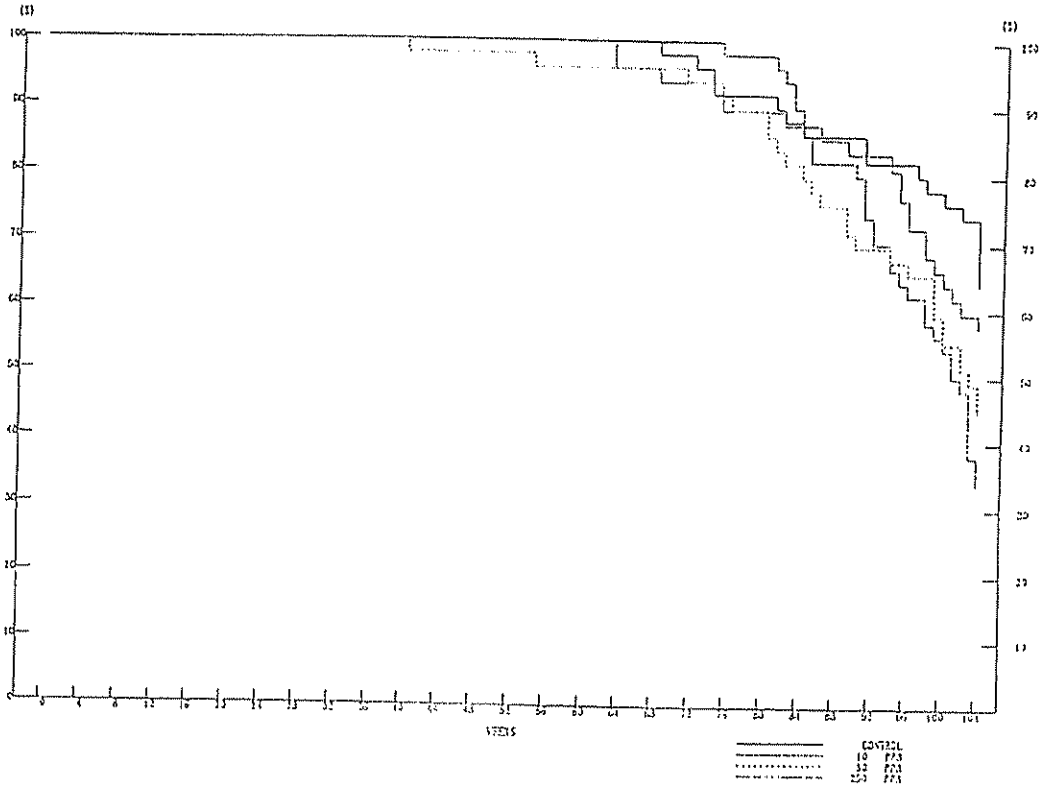


FIGURE 10 SURVIVAL ANIMAL RATE : MOUSE:FEMALE(TWO-YEAR STUDIES)

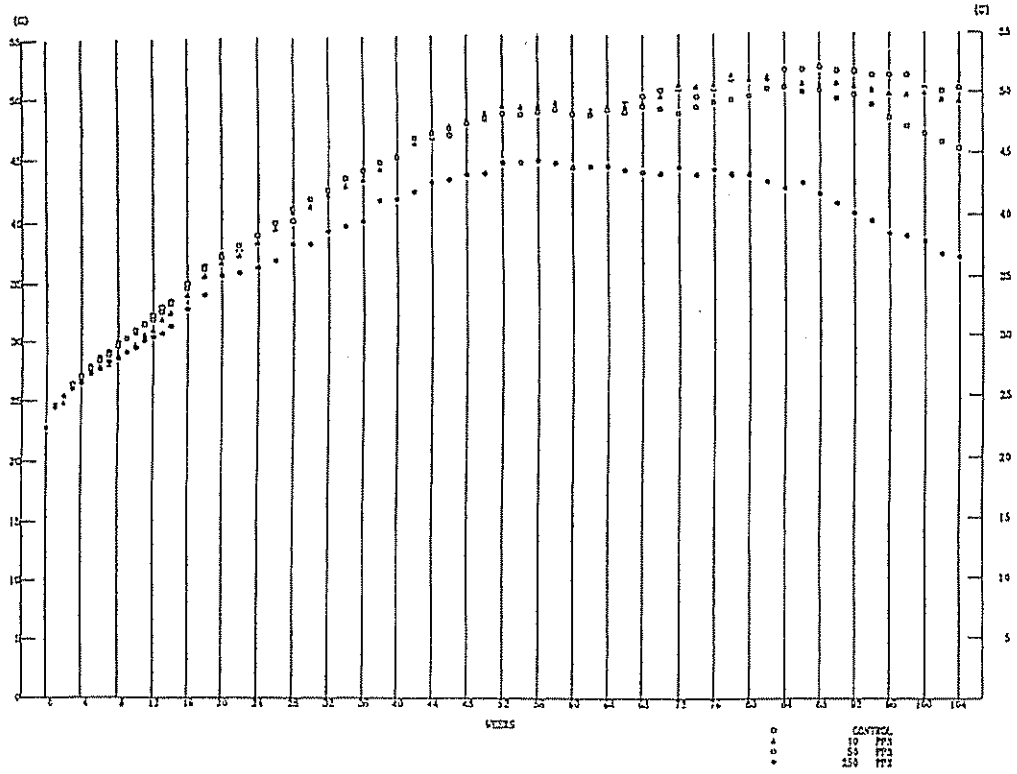


FIGURE 11 BODY WEIGHT CHANGES : MOUSE:MALE(TWO-YEAR STUDIES)

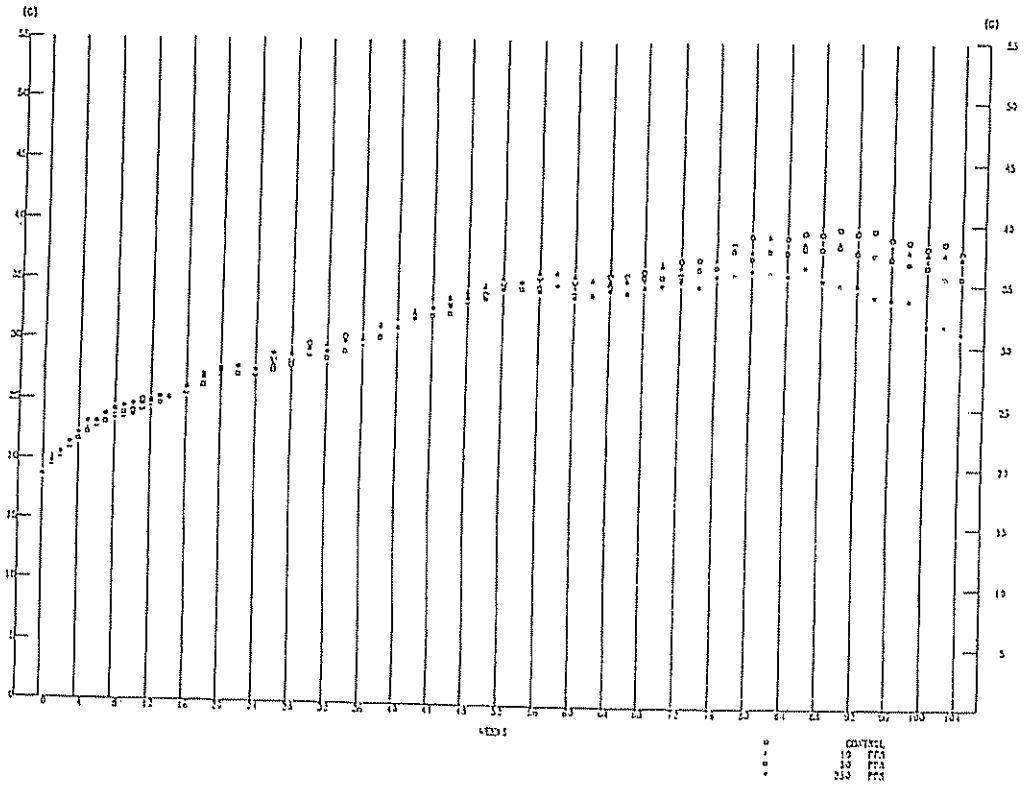


FIGURE 12 BODY WEIGHT CHANGES : MOUSE:FEMALE(TWO-YEAR STUDIES)

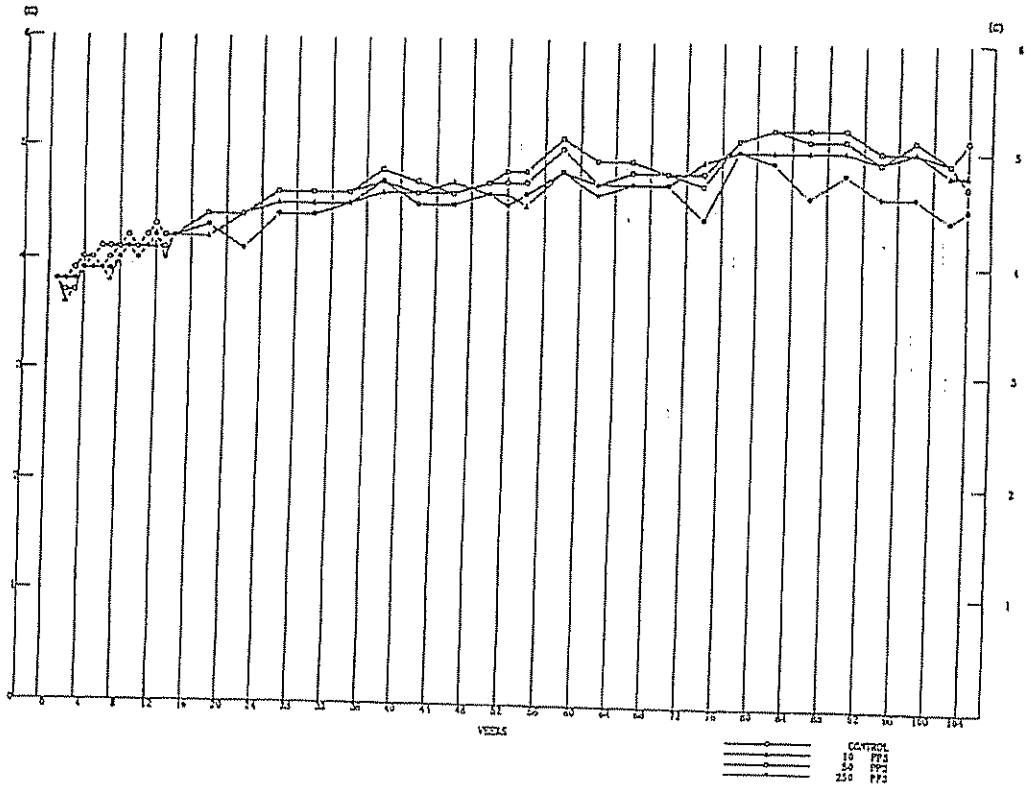


FIGURE 13 FOOD CONSUMPTION : MOUSE:MALE(TWO-YEAR STUDIES)

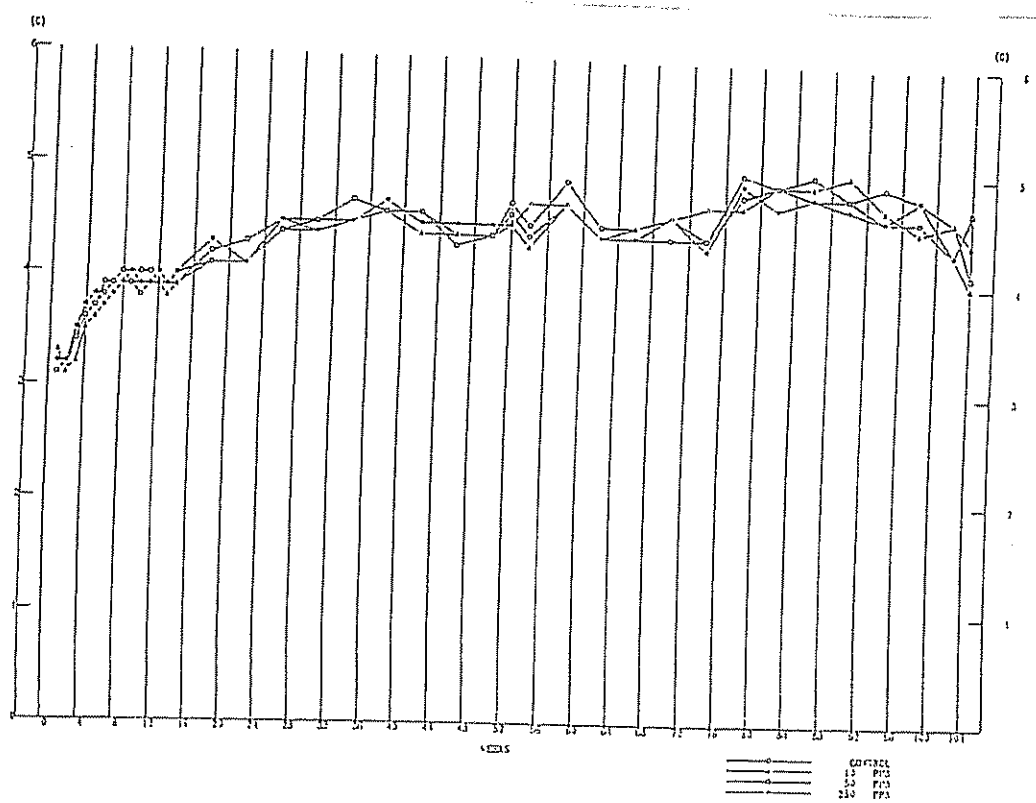


FIGURE 14 FOOD CONSUMPTION : MOUSE:FEMALE(TWO-YEAR STUDIES)

Blood chemistry tests

There was elevation of GOT and GPT activity among males of groups treated with 50 ppm or more and females of the 250 ppm group, an increase in total bilirubin, elevation of LDH and ALP activities, and a reduction in chlorides among males and females of the 250 ppm group, an increase in total protein, total cholesterol, and calcium, and a reduction in glucose, triglycerides, and urea nitrogen among males of the 250 ppm group, and a reduction in potassium among females of the 250 ppm group.

Urinalysis

There were no marked differences from the control group among males or females of any of the treatment groups.

(3) Pathological tests

Autopsy

Of the autopsy findings observed at the time of dissection, characteristic findings or findings seen at a high incidence among treated groups when compared to the control group are listed below:

Males and females of the 250 ppm group showed a high number of occurrences and incidence of hepatic nodules when compared to the control group among both dead/dying cases throughout the course of the study and in scheduled dissections.

Organ weight

In terms of actual weight, kidney weight was low and spleen and liver weights were high among males of the 250 ppm group when compared to the control group. There was not a significant difference among the females.

In terms of body weight ratio, a high value was obtained for the adrenals, testes, heart, lungs, kidneys, spleen, liver and brain among males of the 250 ppm group when compared to the control group. A high value was obtained for the heart, lungs, kidneys and brain among females of the 250 ppm group.

Histopathological tests

Nasal cavity

A reduction in eosinophilic changes of the respiratory epithelium was found among males of the 250 ppm group, both in the dead/dead dying cases and the scheduled dissections. A reduction in respiratory epithelialization of the olfactory epithelium among males of the 250 ppm group, an increase in respiratory epithelialization of the olfactory epithelium among females of the 10 ppm group, and a reduction in respiratory epithelialization of the nasal glands among females and males of the 250 ppm group were found in the scheduled dissections.

Spleen

There was a tendency toward an increase in hemangioendothelioma of males (5/50 of the 250 ppm group, 3/50 of the 50 ppm group, 1/50 of the 10 ppm group, 1/50 of the control group) according to Peto's test (prevalence rate, combined rate) and the Cochran-Armitage test. Moreover, there was a tendency toward an increase by Peto's test (prevalence method, combined method) in tests that combined benign and malignant hemangioendothelioma as well. Scheduled dissections revealed an increase in extramedullary hematopoiesis among males and females of the 250 ppm group and among females of the 10 ppm group (Table 11).

TABLE 11 NEOPLASTIC LESIONS (SPLEEN) INCIDENCE AND STATISTICAL ANALYSIS
MOUSE:MALE

Group Name	Control	10 ppm	50 ppm	250 ppm
SITE : spleen				
TUMOUR : hemangioendothelioma				
Overall Rates(a)	1/50 (2.0)	1/50 (2.0)	3/50 (6.0)	5/50 (10.0)
Adjusted Rates(b)	3.23	0.0	6.38	12.00
Terminal Rates(c)	1/31 (3.2)	0/35 (0.0)	0/28 (0.0)	2/22 (9.1)
Standard Rates(d)	P=0.5167			
Prevalence Rates(d)	P=0.0177*			
Combind analysis(d)	P=0.0340*			
Cochran-Armitage Test(e)	P=0.0420*			
Fisher Exact Test(e)		P=0.2475	P=0.3235	P=0.1210
SITE : spleen				
TUMOUR : hemangioendothelioma:benign,hemangioendothelioma				
Overall Rates(a)	2/50 (4.0)	2/50 (4.0)	3/50 (6.0)	6/50 (12.0)
Adjusted Rates(b)	6.45	2.86	6.38	12.90
Terminal Rates(c)	2/31 (6.5)	1/35 (2.9)	0/28 (0.0)	2/22 (9.1)
Standard Rates(d)	P=0.5167			
Prevalence Rates(d)	P=0.0276*			
Combind analysis(d)	P=0.0458*			
Cochran-Armitage Test(e)	P=0.0608			
Fisher Exact Test(e)		P=0.3088	P=0.4909	P=0.1606

(a):Number of tumor-bearing animals/number of animals examined at the site.
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill
(d):Beneth the control incidence are the P-values associated with the trend test.(Peto test)
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combind analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher's exact test compare directly the overall incidence rates

Liver

Male hepatocellular adenoma (26/50 in the 250 ppm group, 8/50 in the 50 ppm group, 13/50 in the 10 ppm group, and 7/50 in the control group) was significantly increased among the 250 ppm group when compared to the control group according to the Fisher test, and there was a tendency toward an increase according to Peto's test (prevalence method, combined method) and the Cochran-Armitage test as well. Moreover, female hepatocellular adenoma (26/49 in the 250 ppm group, 7/49 in the 50 ppm group, 3/47 in the 10 ppm group, 3/50 in the control group) was significantly increased in the 250 ppm group when compared to the control group according to the Fisher test and there was a tendency toward an increase according to Peto's test (prevalence method) and the Cochran-Armitage test as well. Male hepatocellular carcinoma (25/50 in the 250 ppm group, 12/50 in the 50 ppm group, 8/50 in the 10 ppm group, and 7/50 in the control group) was significantly increased in the 250 ppm group when compared to the control group according to the Fisher test and there was a tendency toward an increase according to Peto's test (standard method, prevalence method, combined method) and the Cochran-Armitage test as well. Moreover, the results of each test were the same for female hepatocellular carcinoma (14/49 of the 250 ppm group, 0/49 of the 50 ppm group, 0/47 of the 10 ppm group, 0/50 of the control group) as for males.

There was a tendency toward an increase in male hemangioendothelioma (5/50 of the 250 ppm group, 5/50 of the 50 ppm group, 1/50 of the 10 ppm group, 1/50 of the control group) according to Peto's test (prevalence method, combined method) (Tables 12 and 13).

TABLE 12 NEOPLASTIC LESIONS (LIVER) INCIDENCE AND STATISTICAL ANALYSIS
MOUSE:MALE

Group Name	Control	10 ppm	50 ppm	250 ppm
SITE : liver				
TUMOUR : hepatocellular adenoma				
Overall Rates(a)	7/50 (14.0)	13/50 (26.0)	8/50 (16.0)	26/50 (52.0)
Adjusted Rates(b)	15.22	34.29	20.59	77.27
Terminal Rates(c)	4/31 (12.9)	12/35 (34.3)	5/28 (17.9)	17/22 (77.3)
Standard Rates(d)	P=1.0000 ?			
Prevalence Rates(d)	P<0.0001**			
Combind analysis(d)	P<0.0001**			
Cochran-Armitage Test(e)	P<0.0001**			
Fisher Exact Test(e)		P=0.1634	P=0.4854	P=0.0029**
SITE : liver				
TUMOUR : hemangioendothelioma				
Overall Rates(a)	1/50 (2.0)	1/50 (2.0)	5/50 (10.0)	5/50 (10.0)
Adjusted Rates(b)	0.0	2.17	3.57	13.64
Terminal Rates(c)	0/31 (0.0)	0/35 (0.0)	1/28 (3.6)	3/22 (13.6)
Standard Rates(d)	P=0.2158			
Prevalence Rates(d)	P=0.0270*			
Combind analysis(d)	P=0.0332*			
Cochran-Armitage Test(e)	P=0.0883			
Fisher Exact Test(e)		P=0.2475	P=0.1210	P=0.1210
SITE : liver				
TUMOUR : hepatocellular carcinoma				
Overall Rates(a)	7/50 (14.0)	8/50 (16.0)	12/50 (24.0)	25/50 (50.0)
Adjusted Rates(b)	16.13	16.67	25.00	45.45
Terminal Rates(c)	5/31 (16.1)	5/35 (14.3)	7/28 (25.0)	10/22 (45.5)
Standard Rates(d)	P=0.0010**			
Prevalence Rates(d)	P=0.0002**			
Combind analysis(d)	P<0.0001**			
Cochran-Armitage Test(e)	P<0.0001**			
Fisher Exact Test(e)		P=0.4854	P=0.2119	P=0.0041**
SITE : liver				
TUMOUR : hepatocellular adenoma, hepatocellular carcinoma				
Overall Rates(a)	13/50 (26.0)	21/50 (42.0)	19/50 (38.0)	40/50 (80.0)
Adjusted Rates(b)	26.47	50.00	39.39	90.91
Terminal Rates(c)	8/31 (25.8)	17/35 (48.6)	11/28 (39.3)	20/22 (90.9)
Standard Rates(d)	P=0.0020**			
Prevalence Rates(d)	P<0.0001**			
Combind analysis(d)	P<0.0001**			
Cochran-Armitage Test(e)	P<0.0001**			
Fisher Exact Test(e)		P=0.1615	P=0.2359	P=0.0018**

(a):Number of tumor-bearing animals/number of animals examined at the site
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill.
(d):Beneth the control incidence are the P-values associated with the trend test.(Peto test)
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combind analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher's exact test compare directly the overall incidence rates.
? :The conditional probabilities of the largest and smallest possible out comes can not estimated or this P-value beyond the estimated P-value.

TABLE 13 NEOPLASTIC LESIONS (LIVER) INCIDENCE AND STATISTICAL ANALYSIS
MOUSE:FEMALE

Group Name	Control	10 ppm	50 ppm	250 ppm
SITE : liver				
TUMOUR : hepatocellular adenoma				
Overall Rates(a)	3/50 (6 0)	3/47 (6.4)	7/49 (14.3)	26/49 (53.1)
Adjusted Rates(b)	9 38	11.11	30 43	64 00
Terminal Rates(c)	3/32 (9 4)	3/27 (11.1)	6/22 (27.3)	9/17 (52 9)
Standard Rates(d)	P=------			
Prevalence Rates(d)	P<0.0001**?			
Combind analysis(d)	P=------			
Cochran-Armitage Test(e)	P<0.0001**			
Fisher Exact Test(e)		P=0 3673	P=0.1836	P=0.0001**
SITE : liver				
TUMOUR : hepatocellular carcinoma				
Overall Rates(a)	0/50 (0 0)	0/47 (0 0)	0/49 (0.0)	14/49 (28.6)
Adjusted Rates(b)	0 0	0 0	0.0	23.33
Terminal Rates(c)	0/32 (0 0)	0/27 (0 0)	0/22 (0.0)	3/17 (17.6)
Standard Rates(d)	P<0.0001**?			
Prevalence Rates(d)	P<0.0001**?			
Combind analysis(d)	P<0.0001**?			
Cochran-Armitage Test(e)	P<0.0001**?			
Fisher Exact Test(e)		P=0.5000	P=0.5000	P=0.0001**
SITE : liver				
TUMOUR : hepatocellular adenoma.hepatocellular carcinoma				
Overall Rates(a)	3/50 (6.0)	3/47 (6.4)	7/49 (14 3)	33/49 (67.3)
Adjusted Rates(b)	9 38	11.11	30.43	69.70
Terminal Rates(c)	3/32 (9.4)	3/27 (11.1)	6/22 (27.3)	10/17 (58.8)
Standard Rates(d)	P<0.0001**?			
Prevalence Rates(d)	P<0.0001**?			
Combind analysis(d)	P<0.0001**?			
Cochran-Armitage Test(e)	P<0.0001**?			
Fisher Exact Test(e)		P=0.3673	P=0.1836	P<0.0001**

(a):Number of tumor-bearing animals/number of animals examined at the site.
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality.
(c):Observed tumor incidence at terminal kill.
(d):Beneth the control incidence are the P-values associated with the trend test.(Peto test)
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combind analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher's exact test compare directly the overall incidence rates.
? :The conditional probabilities of the largest and smallest possible out comes can not estimated or this P-value beyond the estimated P-value.
------:There is no data which should be statistic analysis

With respect to non-tumorous lesions, males and females of the 250 ppm group showed an increase in angiectasis and central degeneration when compared to the control, both among dead/dying cases and scheduled dissections, and males of the 50 ppm group showed an increase in angiectasis among scheduled dissections. Furthermore, although not statistically significant, there was an increase in focal necrosis of the males of the 250 ppm group, both among dead/dying cases and scheduled dissections. Moreover, judging from all animals when dead/dying cases and scheduled dissections are totaled, there was an increase in angiectasis and central degeneration among males and females of the 250 ppm group and an increase in focal necrosis among males of the 250 ppm group (Table 14).

TABLE 14 NUMBER OF MOUSE WITH SELECTED LIVER LESIONS

	Male				Female			
Group	Control	10ppm	50ppm	250ppm	Control	10ppm	50ppm	250ppm
Number of examined	50	50	50	50	50	47	49	50
Anigectasis	1	3	12	30	6	9	8	26
Degeneration:central	1	1	4	37	0	1	2	30
Necrosis:central	0	0	2	3	1	1	1	0
Necrosis:focal	3	4	8	13	1	1	1	0
Hyperplasia	1	0	3	3	0	1	0	0
Clear cell focus	2	2	3	5	1	1	0	4
Acidophilic cell focus	1	1	0	0	0	0	0	1
Basophilic cell focus	2	3	4	5	0	0	0	1
Vacuolic cell focus	0	0	0	0	1	0	0	1
Mixed cell focus	0	0	0	0	2	1	1	0
Hepatocellular adenoma	7	13	8	26	3	3	7	26
Cholangiocellular adenoma	0	0	0	0	0	1	0	0
Histiocytic sarcoma	2	0	1	1	1	1	1	0
Hemangioendothelioma	1	1	5	5	0	0	0	1
Hepatocellular carcinoma	7	8	12	25	0	0	0	14

Stomach

There was a reduction in hyperplasia of the ventriculus when compared to the control among scheduled dissections of males and females of the 250 ppm group and males of the 10 ppm group.

Kidneys

There was an increase in nuclear enlargement of the proximal tubules when compared to the control group among males and females of the 250 ppm group in both dead/dying animals and scheduled dissections and in scheduled dissections of males of the 50 ppm group. Furthermore, although not statistically significant, atypical dilation (proximal tubule) was seen among males and females of the 250 ppm group, and judging from all animals when dead/dying cases and scheduled dissections are totaled, there was an increase in nuclear enlargement of the proximal tubule and atypical tubular dilation (proximal tubule) among males and females of the 250 ppm group (Table 15).

TABLE 15 NUMBER OF MOUSE WITH SELECTED KIDNEY LESIONS

	Male				Female			
Group	Control	10ppm	50ppm	250ppm	Control	10ppm	50ppm	250ppm
Number of examined	50	50	50	50	50	47	49	50
Nuclear enlargement:								
proximal tubule	0	0	6	38	0	0	1	49
Atypical tubular dilation:								
proximal tubule	0	0	0	1	0	0	0	6
Renal cell adenoma	0	1	0	0	0	0	0	0
Renal cell carcinoma	0	0	1	0	0	0	0	0

Harder's gland

There was a tendency toward an increase in male adenoma (8/50 in the 250 ppm group, 2/50 of the 50 ppm group, 2/50 of the 10 ppm group, and 2/50 of the control group) according to Peto's test (prevalence method) and the Cochran-Armitage test (Table 16).

TABLE 16 NEOPLASTIC LESIONS (HARDERIAN GLAND) INCIDENCE AND STATISTICAL ANALYSIS : MOUSE:MALE

Group Name	Control	10 ppm	50 ppm	250 ppm
SITE : Harderian gland				
TUMOUR : adenoma				
Overall Rates(a)	2/50 (2 0)	2/50 (4 0)	2/50 (4.0)	8/50 (16.0)
Adjusted Rates(b)	6.45	5.26	6.45	23.08
Terminal Rates(c)	2/31 (6.5)	1/35 (2 9)	0/28 (0 0)	4/22 (18.2)
Standard Rates(d)	P=------			
Prevalence Rates(d)	P=0.0024**			
Combind analysis(d)	P=------			
Cochran-Armitage Test(e)	P=0 0046**			
Fisher Exact Test(e)		P=0.3088	P=0.3088	P=0.0671

(a):Number of tumor-bearing animals/number of animals examined at the site
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality
(c):Observed tumor incidence at terminal kill
(d):Beneth the control incidence are the P-values associated with the trend test (Peto test)
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combind analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher's exact test compare directly the overall incidence rates.
------:There is no data which should be statistic analysis.

Pituitary

There was a tendency toward an increase in female adenoma (9/50 of the 250 ppm group, 4/48 of the 50 ppm group, 11/47 of the 10 ppm group, and 9/49 of the control group) by Peto's test (standard method) (Table 17)

//insert Table 17//

TABLE 17 NEOPLASTIC LESIONS (PITUITARY GLAND) INCIDENCE AND STATISTICAL ANALYSIS : MOUSE:FEMALE

Group Name	Control	10 ppm	50 ppm	250 ppm
SITE : pituitary gland				
TUMOUR : adenoma				
Overall Rates(a)	9/49 (18.4)	11/47 (23.4)	4/48 (8.3)	9/50 (18.0)
Adjusted Rates(b)	23.08	31.03	13.64	27.78
Terminal Rates(c)	7/32 (21.9)	8/27 (29.6)	3/22 (13.6)	4/17 (23.5)
Standard Rates(d)	P=0.0154*			
Prevalence Rates(d)	P=0.6810			
Combind analysis(d)	P=0.2327			
Cochran-Armitage Test(e)	P=0.9190			
Fisher Exact Test(e)		P=0.4032	P=0.1655	P=0.3839

(a):Number of tumor-bearing animals/number of animals examined at the site
(b):Kaplan-Meire estimate tumor incidence at the end of study after adjusting for intercurrent mortality
(c):Observed tumor incidence at terminal kill
(d):Beneth the control incidence are the P-values associated with the trend test (Peto test)
Standard method : Death analysis
Prevalence method : Incidental tumor test
Combind analysis : Death analysis + Incidental tumor test
(e):The Cochran-Armitage and Fisher's exact test compare directly the overall incidence rates

All organs

Judging from statistical treatment when male hemangioendothelioma is tabulated for all organs (8/50 in the 250 ppm group, 6/50 in the 50 ppm group, 1/50 in the 10 ppm group, and 2/50 in the control group), there was a tendency toward an increase by Peto's test (standard method) and the Cochran-Armitage test. Moreover, when female hemangioendothelioma is similarly tabulated for all organs (3/50 in the 250 ppm group, 2/49 in the 50 ppm group, 0/47 in the 10 ppm group, and 1/50 in the control group), there was a tendency toward an increase by Peto's test (standard method, combined method).

Others

Scheduled dissections revealed reduction in dysplasia of the teeth in males of groups treated with 50 ppm or more, an increase in vitreous deposits of the brain among males of the 50 ppm group, and an increase in osteosclerosis of the bones among females of the 250 ppm group when compared to the control groups

Cause of death

The pathological cause of death among dead/dying cases is shown in Table 18. When males and females of the treatment groups are compared with the control groups, there was an increase in death due to tumors of the liver.

TABLE 18 CAUSE OF DEATH : MOUSE

	Male				Female			
Group	Control	10ppm	50ppm	250ppm	Control	10ppm	50ppm	250ppm
Number of dead/moribund animal	19	15	22	28	18	20	27	33
No microscopical confirmation	2	2	1	0	0	1	2	0
Cardiovascular lesion	0	0	0	0	0	1	2	0
Respiratory sy. lesion	1	0	0	1	0	0	0	0
Hepatic lesion	0	1	1	4	0	0	0	1
Urinary sy. lesion	0	0	0	1	0	0	0	0
Renal lesion	3	1	4	1	1	2	0	1
Urinary retention	1	3	2	0	0	0	0	0
Amyloidosis	0	1	0	0	0	0	0	0
Arteritis	2	0	1	0	0	0	0	0
Tumor death : leukemia	4	3	3	4	6	4	13	10
: subcutis	0	0	0	1	0	2	0	1
: stomach	0	0	0	0	1	0	0	0
: lung	1	0	1	0	0	0	0	0
: lymph node	0	0	0	1	0	0	0	0
: spleen	0	1	0	0	0	0	0	0
: liver	4	3	8	13	0	1	1	7
: pituitary gland	0	0	0	0	1	2	2	4
: ovary	0	0	0	0	0	0	0	1
: uterus	0	0	0	0	8	8	8	9
: mammary gland	0	0	0	0	0	0	1	0
: epididymis	0	0	0	1	5	2	2	2
: seminal vesicle	1	0	0	1	1	0	0	0
: bone	0	0	1	0	1	2	2	1

IV. Discussion

Carcinogenicity of tetrachloroethylene was studied by inhalation administration of tetrachloroethylene for 104 weeks to F344/DuCrj (Fischer) rats and Crj:BDF_1 mice (0, 50, 200 or 600 ppm to rats, 0, 10, 50 or 250 ppm to mice). The concentration to be administered for the carcinogenicity study was determined from the results of 2-week (0, 200, 400, 800, 1,600, 3,200 ppm in rats and mice) and 13-week (0, 50, 115, 265, 609, and 1,400 ppm) studies in rats and mice.

<<Carcinogenicity preliminary studies>>

<Two-week study>

There were 5 deaths among male rats and 7 deaths among female rats in the 3,200 ppm group, which was the maximum concentration for both males and females, and toxic effects such as inhibition of an increase in body weight, etc., were even seen among the surviving animals. Toxic effects other than inhibition of an increase in body weight, etc., were not seen among males of groups treated with 800 ppm or less or among females treated with 1,600 ppm or less.

There were 9 deaths among male mice and 7 deaths among female mice in the 3,2000 ppm group, the maximum concentration for both males and females, and toxic effects such as inhibition of an increase in body weight, etc., were even seen among the surviving animals. Although there was no inhibition of an increase in body weight among males or females of groups treated with 1,600 ppm or less, fading of the liver was seen at the time of scheduled dissections among groups treated with 400 ppm or more, and findings of central enlargement of the liver among males and females treated with 1,600 ppm or more, necrosis and regeneration of the proximal tubule, indicating an effect on the kidneys, among males and females of groups treated with 800 ppm or more, etc., were often reported.

Toxic effects were seen with up to 1,600 ppm among rats and therefore, the concentrations of the 13-week study were set at a maximum concentration that was slightly less at 1,400 ppm and a minimum concentration of 50 ppm, or the allowable concentration, with 609 ppm, 265 ppm, and 115 pm in between at a nominal ratio of 2.3 based on these results.

Findings of toxic effects were seen in the kidneys among females and males of the 800 ppm group and in the liver among males and females of the 1,600 ppm group in mice, etc., and therefore, it was concluded that the maximum concentration is between 800 and 1,400 ppm and that studies would be performed with the same concentration as in rats (1,400, 609, 265, 115, 50, 0 ppm).

<13-week study>

Although there were no deaths associated with administration among male or female rats, there was inhibition of an increase in body weight among males in the 1,400 ppm group, or the maximum concentration group. Moreover, toxic effects were sporadically seen in terms of hematological tests, blood chemistry tests, urinalyses, and liver weight among males and females of groups treated with 609 ppm or more. Based on these results, the maximum concentration of the carcinogenicity study was set at 600 ppm, or approximately the same as the minimum concentration at which changes were seen, while

the minimum concentration was set at the allowable concentration of 50 ppm and the intermediate concentration was set at 4-times the minimum concentration, or 200 ppm.

Although there were no deaths attributed to administration among male or female mice, inhibition of an increase in body weight was seen among males of groups treated with 609 ppm or more and females of the 1,400 ppm. There were changes in the liver in terms of organ weight among males and females of the groups treated with 265 ppm or more and changes were also seen among groups treated with 609 ppm or more in terms of hematological tests and blood chemistry tests. Central enlargement of the liver and changes in the proximal tubules of the kidneys among groups treated with 609 ppm or more were seen in pathology findings. Based on these results, the maximum concentration of the carcinogenicity study was set at 250 ppm, which is approximately the same as the maximum concentration at which there were changes in the 13-week study, the intermediate concentration was set at the current allowable concentration of 50 ppm, and the minimum concentration was set at 10 ppm by a nominal ratio of 5.0.

<<Carcinogenicity study>>
<Survival>

The carcinogenicity study revealed inhibition of an increase in body weight among both female and male rats and mice in the maximum concentration groups (600 ppm in rats and 250 ppm in mice) when compared to the control group. There was further a reduction in the survival rate when compared to that of the control group. These were considered to be the effect of tetrachloroethylene.

<Correlation between study sample and tumorigenesis>
Rats

Of the tumors observed in the current carcinogenicity study, tumors that showed a tendency toward an increase in tendency tests were monocytic leukemia of the spleen of both males and females, and this was judged to be due to administration of tetrachloroethylene. Based on the Fisher test, the concentration at which the occurrence of monocytic leukemia is exacerbated was judged to be 600 ppm among males.

Mice

Of the tumors observed in the current carcinogenicity study, tumors that showed a tendency toward an increase in tendency tests included hemangioendothelioma of the spleen among males, hepatocellular adenoma and hepatocellular carcinoma among males and females, hemangioendothelioma of the liver and adenoma of Harder's gland among males, and adenoma of the pituitary among females. Of these, the adenoma of the Harder's gland among males (2/50 in the control group, 2/50 of the 10 ppm group, 2/50 of the 50 ppm group, and 8/50 of the 250 ppm group) hepatocellular adenoma (males: 7/50 of the control group, 13/50 of the 10 ppm group, 8/50 of the 50 ppm group, 26/50 of the 250 ppm group; females: 3/50 of the control group, 3/47 of the 10 ppm group, 7/49 of the 50 ppm group, 26/49 of the 250 ppm group) and hepatocellular carcinoma (males: 7/50 of the control group, 8/50 of the 10 ppm group, 12/50 of the 50 ppm group, 25/50 of the 250 ppm group; females: 0/50 of the control group, 0/47 of the 10 ppm group, 0/49 of the 50 ppm group, 14/49 of the 250 ppm group) of the liver of males and females appeared to increase with administration of the tetrachloroethylene. Moreover, there was a significant increase in the number of occurrences of hepatocellular adenoma and hepatocellular carcinoma of the liver among females and males in the 250 ppm group when compared to the control group

according to the Fisher test and therefore, it appears that the concentration at which both tumors are induced by tetrachloroethylene is 250 ppm in both males and females. With respect to hemangioendothelioma of the spleen among males (1/50 in the control group, 1/50 in the 10 ppm group, 3/50 in the 50 ppm group, 5/50 in the 250 ppm group), the number of occurrences of was 0/50 to 4/50 in the control groups of previous studies of this center, with there being almost no difference from the 5/50 occurrences in the 250 ppm group, and therefore, it could not be concluded that there was an increase in tumors as a result of tetrachloroethylene administration. Moreover, with respect to the hemangioendothelioma of the liver among males (1/50 in the control group, 1/50 in the 10 ppm group, 5/50 in the 50 ppm group, and 5/50 in the 250 ppm group), the number of occurrences in control groups of previous studies of this center has been 0/50 to 3/50 and although there were a few more occurrences [in the present study], it could not be concluded that the incidence of tumors increased as a result of administration of tetrachloroethylene. With respect to pituitary adenoma in females (9/49 in the control groups, 11/47 in the 10 ppm group, 4/48 in the 50 ppm group, 9/50 in the 250 ppm group), the number of occurrences in control groups of previous studies of this center was 7/50 to 9/50 and therefore, it cannot be said that the 9/50 occurrences in the 250 ppm is a high percentage, and it cannot be concluded that there was an increase in tumors by administration of tetrachloroethylene.

<Correlation between study sample and non-tumorous lesions, etc.>

Rats

In relation to the monocytic leukemia of the spleen, infiltration of the lungs, liver, etc., of the treatment groups by leukemia was often seen. Moreover, the reduction in extramedullary hematopoiesis of the spleen of males among scheduled dissections and the increase in blood clots in the nasal cavity of dead/dying cases were apparently due to exacerbation of the leukemia. As shown in Table 6, there was not characteristic tumorigenesis of the liver, but there was an increase in hyperplasia or spongiosis hepatitis, indicating that tetrachloroethylene does affect the liver. An increase in nuclear enlargement of the proximal tubules among males and females, an increase in atypical tubular dilation of the proximal tubules and exacerbation of chronic renal disease among males, etc., were found in the kidneys, indicating that tetrachloroethylene does affect the kidneys (Table 7).

Mice

There was an increase in angiectasis and central degeneration of the liver among males and females and an increase in focal necrosis, etc., of the liver among males, indicating that tetrachloroethylene affects the liver (Table 14).

There was an increase in nuclear enlargement of proximal tubules and atypical tubular dilation of the kidneys among males and females, indicating that tetrachloroethylene had an effect on the kidneys (Table 15).

<Monocytic leukemia>

Administration of tetrachloroethylene apparently promoted the leukemia that is prevalent among F344 rats. Moreover, similar results were obtained by the NTP (TR 311). A comparison is shown in Table 7.

Table 7. Comparison of the occurrence of monocytic leukemia in F344 rats

	Japan Bioassay Research Center				N T P		
	Control	50 ppm	200 ppm	600 ppm	Control	200 ppm	400 ppm
Males	11/50	14/50	22/50	27/50	28/50	37/50	37/50
Females	10/50	17/50	16/50	19/50	18/50	30/50	29/50

<On hepatocellular adenoma, hepatocellular carcinoma and liver damage>

Although there was not an increase in tumors of the liver, which did increase [in the NTP study, with administration of tetrachloroethylene to rats, there was an increase in the occurrence of mixed cell focus and hyperplasia, which are pre-tumorous changes, indicating that there was some type of damage to the liver. However, these findings were not seen in the NTP (TR 311) study.

An increase in the occurrence of hepatocellular adenoma and hepatocellular carcinoma was seen in both male and female mice. Central enlargement (hepatocellular enlargement) of the liver was seen in the 2-week study and 13-week study and central hepatocellular degeneration of the liver was seen in the carcinogenicity study as non-tumorous changes. These changes are findings indicating liver damage by tetrachloroethylene and these forms of liver dysfunction appear to have promoted the increase in liver tumors in mice. Similar results were obtained by NTP (TR311), and a comparison is shown in Tables 8 and 9.

Table 8. Comparison of hepatocellular adenoma in mice

	Japan Bioassay Research Center < BDF_1 >				N T P < $B6C3F_1$ >		
	Control	10 ppm	50 ppm	250 ppm	Control	200 ppm	400 ppm
Males	7/50	13/50	8/50	26/50	12/49	8/49	19/50
Females	3/50	3/47	7/49	26/49	3/48	6/50	2/50

Table 9. Comparison of occurrence of hepatocellular carcinoma in mice

	Japan Bioassay Research Center <BDF$_1$>				N T P <B6C3F$_1$>		
	Control	10 ppm	50 ppm	250 ppm	Control	200 ppm	400 ppm
Males	7/50	8/50	12/50	25/50	7/49	2549	26/50
Females	0/50	0/47	0/49	14/49	1/48	13/50	36/50

<Kidney damage>

Nuclear enlargement (findings indicating an increase in the size of the nucleus) of the proximal tubules was seen in rats and mice This change was obvious in the straight part of the proximal tubule and was a result similar to those of the NTP (TR 311) study. There are reports of this finding with other substances as well (example of substances that induce nuclear enlargement of uriniferous tubules: nitrosourea, ochratoxin, bromodichloromethane, tri(2,3-dibromopropyl)phosphate) and it is accompanied by distension of the uriniferous tubules in cases of severe change. Necrosis, regeneration, and nuclear regeneration were observed in the 2-week study, while regeneration and nuclear enlargement of proximal tubules were observed in the 13 -week study in the mice of the present study (the effect on the kidneys with administration was not chronic impairment occurring from the early stages). The meaning of the nuclear enlargement is unclear, but substances that are the cause of nuclear enlargement reportedly inhibit cellular fission. (Reference 29)

With respect to the occurrence of renal tumors, it is concluded that there is a correlation between nuclear enlargement and tumorigenesis in the NTP and examples of other substances are given. However, although there are few occurrences of tumors in the data of the present study, there was no increase in the occurrence of tumors (Tables 10 and 11), and nuclear enlargement occurred from the early stages. Therefore, a correlation between nuclear enlargement and tumorigenesis could not be confirmed and other findings indicating that there is a correlation were not obtained.

Table 10. Tumorigenesis in rat kidneys

KIDNEY : RAT	Male				Female			
	Control	50 ppm	200 ppm	600 ppm	Control	50 ppm	200 ppm	600 ppm
Renal Cell Adenoma	1	2	1	2	1	0	0	0
Renal Cell Carcinoma	0	0	0	0	0	0	0	1
Liposacroma	0	0	0	1	0	0	0	0

Table 11. Tumorigenesis in mouse kidney

KIDNEY : MOUSE	Male				Female			
	Control	10 ppm	50 ppm	250 ppm	Control	10 ppm	50 ppm	250 ppm
Renal Cell Adenoma	1	1	0	0	0	0	0	0
Renal Cell Carcinoma	0	0	1	0	0	0	0	0

V. **Conclusion**

Carcinogenicity studies were performed by inhalation of tetrachloroethylene over a 2-year-period (104 weeks) using F344/DuCrj (Fischer) rats and Crj:BDF_1 mice.

An increase in the occurrence of monocytic leukemia of the spleen was seen among male and female rats, proving that tetrachloroethylene is carcinogenic in F344/DuCrj (Fischer) rats. This tumorigenic concentration was 600 ppm in males.

An increase in the occurrence of hepatocellular adenoma and hepatocellular carcinoma among males and females and an increase in the occurrence of adenoma of Harder's gland among males was seen in mice, proving that tetrachloroethylene is carcinogenic in Crj:DBF_1 mice. This tumorigenic concentration was 250 ppm in both males and females.

VI. References

1. Asahara, T., Togura, N., Okawara, N., Kumanoya, T., Senoo, G., editors (1980), Solvent Handbook, pp. 277-279, Koensha, Tokyo.

2. Goto, S., Ikeda, M., Hara, I., editors (1981), Industrial Poisons Catalog (Revised Edition), pp. 619-620, Ishiyaku Shuppansha, Tokyo.

3. Chemical Dictionary Editing Committee, editors (1978), Kagaku Jiten No. 6, pp. 109, Kyoritsu Shuppansha, Tokyo.

5. Kagaku Kogyo Nipposha (1989), Chemical Products 10889, pp. 670-671, Kagaku Kogyo Nipposha, Tokyo.

6. Kagaku Kogyo Nipposha (1991), Chemical Products 11691, pp. 702, Kagaku Kogyo, Nipposha, Tokyo.

8. Japan Association of Industrial Health (1992), Recommendations on Allowable Concentrations (1992), *Sangyo Igaku*, 24, 363-384.

9. American Conference of Governmental Industrial Hygienists (1991), ACGIH Chemical Substances and TLV of Physical Factors, BEI of Chemical Substances (for 1991 ~ 1992), *Nihon Sokuryo Kyokai Shiryo*, No. 30, pp. 39, Japanese Association of Industrial and Environmental Surveyors.

18. Tomizawa, H., Tateishi, M. (1990): New Route of Metabolism of Glutathione Conjugates and Relationship with Manifestation of Toxicity of the Same, *Eisei Kagaku*, 36, 359-372.

27. Kawabe, M. (1986): Establishment of Appropriate Stratification Formula for Grouping by Analysis of Body Weight Changes in Rats and Mice in Long-Term Toxicity Studies, *Yakuri to Chiryo*, 14, 7285-7302.

Photo. 1 Spleen, Monocytic Leukemia
2-year study, rat, male, 600 ppm group, Animal No. 0104-1302
(HE staining, 152 x)

Photo. 2 Harder's Gland, Adenenoma: A
2-year study, rat, male, 600 ppm group, Animal No. 0104-1327
(HE staining, 60 x)

Photo. 3 Liver, Spongiosis Cirrhosis: A
2-year study, rat, male, 600 ppm group, Animal No. 0104-1305
(HE staining, 60 x)

Photo. 4 Liver, Hyperplasia: A
2-year study, rat, male, 600 ppm group, Animal No. 0104-1307
(HE staining, 60 x)

Photo. 5 Kidney, Nuclear Enlargement of Proximal Tubule: A and Atypical Tubular Dilation (Proximal Tubule): B
2-year study, rat, male, 600 ppm group, Animal No. 0104-1333
(HE staining, 304 x)

Photo. 6 Kidney, Chronic Renal Disease
2-year study, rat, male, 600 ppm group, Animal No. 0104-1334
(HE staining, 60 x)

Photo. 7 Liver, Hepatocellular adenoma: A
2-year study, mouse, male, 250 ppm group, Animal No. 0105-1307
(HE staining, 152 x)

Photo. 8 Liver, Hepatocellular carcinoma
2-year study, mouse, male, 250 ppm group, Animal No. 0105-1346
(HE staining, 152 x)

Photo. 9 Liver, Hemangioendothelioma
2-year study, mouse, male, 250 ppm group, Animal No. 0105-1345
(HE staining, 152 x)

Photo. 10 Liver, Angiectasis: A
2-year study, mouse, male, 250 ppm group, Animal No. 0105-1307
(HE staining, 60 x)

Photo. 11 Spleen, Hemangioendothelioma: A
2-year study, mouse, male, 250 ppm group, Animal No. 0105-1347
(HE staining, 152 x)

Photo. 12 Kidney, Nuclear Enlargement: Proximal Tubule: A
2-year study, mouse, male, 250 ppm group, Animal No. 0105-1306
(HE staining, 304 x)