Report of Carcinogenicity Study by Oral Administration of 1,4-Dioxane (Mixed with Water) to Rats and Mice

December 28, 1990

Japan Industrial Safety and Health Association
Japan Bioassay Research Center

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Title

Report of Carcinogenicity Study by Oral Administration of 1,4-Dioxane (Mixed with Water) to Rats and Mice

Study Objective

The study was conducted to investigate carcinogenicity of 1,4-dioxane by 2 years continuous oral administration to rats and mice.

Name and Address of Study Facility

Japan Industrial Safety and Health Association, Japan Bioassay Research Center
2445 Hirasawa, Hadano, Kanagawa, Japan

Study Schedules

Animal Introduction	Rats	February 5, 1985
	Mice	February 26, 1985
Study Group Assignment	Rats	February 19, 1985
	Mice	March 12, 1985
Beginning Administration	Rats	February 19, 1985
	Mice	March 12, 1985
Ending Administration	Rats	February 17, 1987
	Mice	March 10, 1987
Scheduled Necropsy	Rats	February 18 – 24, 1987
	Mice	March 11 – 17, 1987

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Statement by the Chief Investigator

Study Title : Report of Carcinogenicity Study by Oral Administration of 1,4-Dioxane (Mixed with Water) to Rats and Mice

The study was conducted in accordance with the Study Protocol. This report was prepared based on the study results. It is true and accurate to the best of my knowledge and belief.

Japan Industrial Safety and Health Association
Japan Bioassay Research Center

Chief Investigator: *Seigo, YAMAMOTO* 

December 28, 1990

<Form-QAP-3 -3>

Proof of Quality Assurance

Study Numbers: 0063, 0064

Title: Carcinogenicity Study by Oral Administration of 1,4-Dioxane (Mixed with Water) to Rats and Mice

Name of the Substance Studied: 1,4-Dioxane

I certify that the study was conducted accurately in accordance with the Study Protocol, and that the raw data collected was accurately reflected in the final report.

Date: *December 28, 1990*

Quality Assurance Supervisor

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Japan Bioassay Research Center

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Report of Carcinogenicity Study by Oral Administration of 1,4-Dioxane (Mixed with Water) to Rats and Mice

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Abstract

A 104-week study was conducted to investigate carcinogenicity of 1,4-dioxane in F344/DuCrj (Fischer) rats and Crj: BDF_1 mice with drinking water.

In the study, four study groups were created, with each group consisting of 50 males and 50 females of rats and 50 males and 50 females of mice, totaling 400 rats and 400 mice. The 1,4-dioxane solution which was mixed with drinking water at concentrations of 5,000, 1,000, or 200 ppm for rats, and 8,000, 2,000, or 500 ppm for mice was provided ad libitum for 104 weeks. The following was performed: observations of general symptoms, measurement of body weight, food and water consumption, urinalysis, hematological and blood biochemistry examinations, necropsy, measurement of organ weight, and histopathological examinations.

The number of surviving rats in the 5,000 ppm groups of both sexes at the ending of administration (Week 104) was significantly lower than the control groups. The common causes of deaths among the male rats were peritoneal mesothelioma, tumors and nonneoplastic lesions of the nasal cavity and those among the female rats were tumors and nonneoplastic lesions of the nasal cavity and liver. It was considered that the administration of 1,4-dioxane caused to increase the number of lesions resulting in the increased incidences of death. On the other hand, the number of surviving mice in the 8,000 and 2,000 ppm female groups at the ending of administration (Week 104) was significantly lower than the control groups. The common causes of deaths among the female mice were hepatic tumors in the 8,000 ppm group, and hepatic tumors and leukemia (primarily malignant lymphoma) in the 2,000 ppm group. It was considered that the administration of 1,4-dioxane caused to increase the number of lesions resulting in the increased incidences of death. As for changes in body weight during the administration period, suppression of body weight gain was observed in the 5,000 ppm rat groups of both sexes, compared with the control groups. Suppression of body weight gain among the mice was observed in the 8,000 ppm groups of both sexes during the entire administration period and in the 2,000 ppm groups of both sexes at the end of administration, compared with the control groups.

As for neoplastic lesions in rats, an increased incidence of nasal tumors was noted in the 5,000 ppm groups of both sexes, which indicated 1,4-dioxane caused to increase an incidence of nasal tumors. The most common type of nasal tumors was squamous cell carcinoma. Though there were a few incidences, rhabdomyosarcoma, sarcoma NOS, and esthesioneuroepithelioma among the males and esthesioneuroepithelioma among the females were observed. In the livers, incidences of hepatocellular adenoma and hepatoma were increased in the 5,000 ppm groups of both sexes, which indicated 1,4-dioxane caused to increase incidences of hepatocellular adenoma and hepatoma. Incidences of peritoneal mesothelioma were increased only in the 5,000 ppm male group. It is known that there is a gender difference in spontaneous occurrence of peritoneal mesothelioma in rats and that the tumor occurs more commonly in males. It was indicated that 1,4-dioxane further caused to increase incidences of this tumor in males.

As for neoplastic lesions in mice, the study groups of both sexes had an increasing trend in incidence of hepatoma, which indicated 1,4-dioxane caused to increase incidences of hepatoma. Increased incidences of hepatoma occurred more noticeably in females than males and even the lowest dosage female group of 500 ppm showed the increased incidences. Among the tumors found in the nasal cavity in rats, one female had an adenocarcinoma and one male had an esthesioneuroepithelioma in the 8,000 ppm groups.

The following nonneoplastic lesions of the nasal cavity were observed in the 5,000 ppm groups of both sexes in rats: squamous metaplasia of the respiratory epithelium, adhesion of the nasal concha, respiratory epithelial metaplasia of the olfactory epithelium, hydropic degeneration and hardening of the lamina propria, atrophy of the olfactory epithelium, nuclear enlargement of the olfactory epithelium (the supporting cells) and respiratory epithelium, calcinosis, multiplication of the nasal glands, inflammation of the squamous epithelium, and acute rhinitis. There were increased incidences of hyperplasia or cell focus in the livers which could be considered as a preneoplastic change in the ≧ 1,000 ppm male groups and the 5,000 ppm female group. There were increased incidences of spongiosis hepatis in the ≧ 1,000 ppm male groups and the 5,000 ppm female group, and nuclear enlargement of the proximal renal tubule in the 5,000 ppm groups of both sexes in rats. These effects were considered to be caused by 1,4-dioxane.

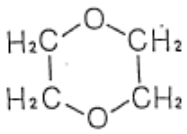
The following were observed in the 8,000 ppm groups of both sexes in the nasal cavity of mice: rhinitis, atrophy of the olfactory epithelium, and nuclear enlargement of the respiratory epithelium and olfactory epithelium (the supporting cells). An increased incidence of nuclear enlargement of the olfactory epithelium (the supporting cells) was also observed in the 2,000 ppm groups. An increased incidence of vasodilatation in the livers, and nuclear enlargement of the proximal renal tubule were observed in the 8,000 ppm male group. Nuclear enlargement of the trachea in the 8,000 ppm male group, and nuclear enlargement of the bronchial tube in the ≧ 2,000 ppm groups of both sexes were observed, and these changes were considered to be caused by 1,4-dioxane.

Blood biochemistry examinations revealed that rats in the 5,000 ppm groups of both sexes had increases in GOT, GPT, LDH, ALP, and γ-GTP, and mice in the 8,000 and 2,000 ppm groups of both sexes had increases in GOT, GPT, LDH, and ALP. These changes were considered to be caused by the administration of 1,4-dioxane.

To summarize the two-year carcinogenicity study of 1,4-dioxane, it was found that F344/DuCrj (Fischer) rats in the 5,000 ppm groups showed increased incidences of squamous cell carcinoma (primarily) in the nasal cavity, hepatoma, and peritoneal mesothelioma. Crj: BDF_1 mice in the ≧ 500 ppm groups showed increased incidences of hepatoma. It was indicated that 1,4-dioxane was carcinogenic to both rats and mice.

About 1,4-Dioxane

<Formula, Molecular Weight>



$C_4H_8O_2$

Molecular Weight :88.1

CAS.No. :123-91-1

<Nomenclature and Other Names>

Nomenclature : 1,4-Dioxane
Other Names : Diethylene dioxide
1,4-Diethylene dioxide
Diethylene ether
Di(ethylene oxide)
1,4-Dioxacyclohexane

<Physicochemical Characteristics>

Characteristics : Colorless, flammable liquid
Boiling Point : 101℃
Melting Point : 11.8℃
Specific Gravity : d_4^{20} 1.0329
Vapor Pressure : 37mmHg(25℃)
Solubility : Soluble in water, ethanol, ether, and other common organic solvents
Storage : Store in a sealed container in a dark place at room temperature

<Usage>

1,4-dioxane is used as a solvent for surface treatment of leathers, transistors, paints, pharmaceutical products, and a reaction solvent, as well as a stabilizer for trichloroethane. (References 6 and 7)

In the United States, 1,4-dioxane is used primarily as a stabilizer for a chlorinated solvent and a solvent for cellulose, ethylcellulose, benzyl cellulose, resin, oil, wax, dye, etc. It is also used as a solvent in the electronic industry and an intermediate of pesticide and biochemical product, adhesives, cosmetics, drugs, and a surface coating agent for a chemical rubber. (References 8, 9 and 10)

<Production Volume>

The production volumes of 1,4-dioxane in Japan were 600 metric tons in 1968, 2,200 tons in 1972, 2,300 tons in 1978, and 7,000 tons in 1988. Approximately 60 – 70 tons in 1974 and approximately 100 tons in 1975 were exported primarily to Great Britain and Australia. (References 6, 11, 12, and 13)

The production volumes of 1,4-dioxane in the United States were 6,300 tons in 1972, 7,400 tons in 1973. (References 14)

<Allowable Concentration>

The allowable concentration of 1,4-dioxane in a workplace is 10 ppm (The Japanese Society of Occupational Health, 1990) in Japan which is considered to cause systemic effects if it enters via the skin. It is 25 ppm (ACGIH) in the United States. (References 15 and 16)

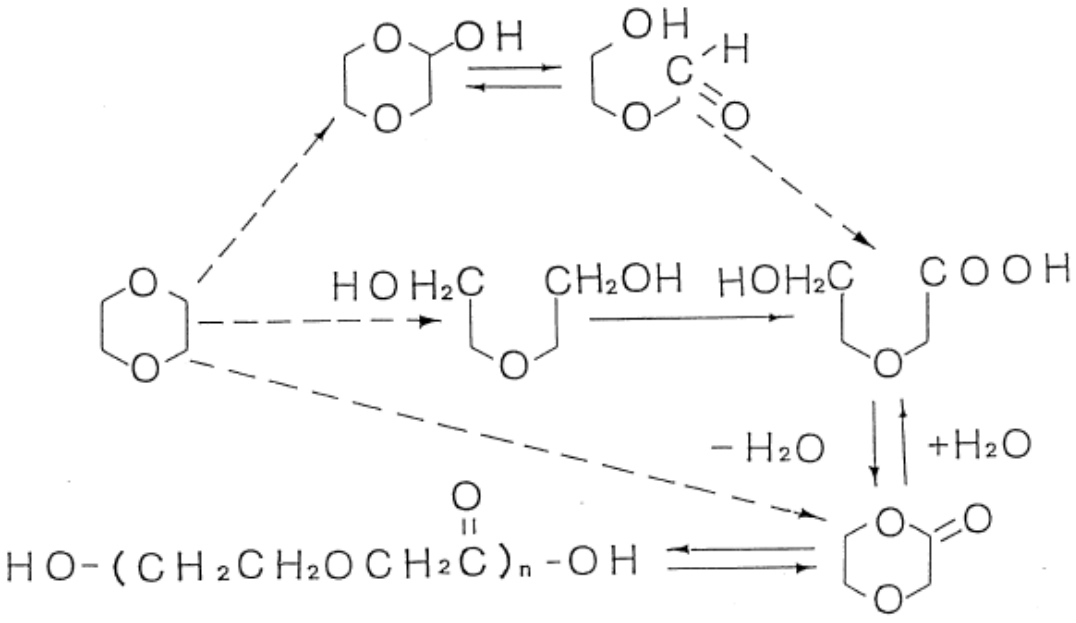
<Effects on Humans>

The atmospheric concentration of 300 ppm causes irritation of the eyes, nose, and throat (Reference 19). People exposed to high concentrations experienced the following symptoms in the digestive system: loss of appetite, nausea, and vomiting. People who died from high exposure had complained of pain in the abdominal to waist area, irritation of the respiratory system, oliguria, and lethargy. They passed away within two weeks after experiencing deterioration of consciousness. A necropsy concluded the cause of death as hemorrhagic nephritis. Histopathological examinations indicated necrosis and hemorrhage of the renal cortex. (Reference 20)

<Metabolism>

Yin-Tak Woo et. al. (1977 to 1978) reported on metabolism of 1,4-dioxane. According to the report, when 1,4-dioxane was administered to rats, 1,4-dioxane-2-one was identified in urine as a metabolite and it was excreted within approximately 48 hours (Reference 17). The oral LD50 value of 1,4-dioxane-2-one for rats was 0.79 ± 0.15 g/kg, which was higher in toxicity than the oral LD50 value of 1,4-dioxane of 5.3 ± 0.1 g/kg. The report also suggested that an enzyme involving the metabolism of 1,4-dioxane was cytochrome P450 enzyme (Reference 18).

K. T. Kitchin et. al. (1990) reported a study on female rats which were given 0, 168, 840, 2,550 or 4,200 mg/kg of 1,4-dioxane 21 and 4 hours before sacrifice. 1,4-dioxane significantly increased hepatic DNA damage and cytochrome P-450 content at doses of 2,550 and 4,200 mg/kg. A significant increase in the activity of ODC was observed at doses of ≧ 840 mg/kg of 1,4-dioxane (Reference 31).



Tentative metabolic pathway of 1,4-dioxane (Reference 39)

<Mutagenicity>

Results of mutagenicity tests for 1,4-dioxane is reported in Table 1. Mutagenicity of 1,4-dioxane was negative in the Ames test, weak positive in sister chromatid exchanges (SCE) assay with Chinese hamster ovary (CHO) cells, and positive for rat liver DNA damages.

TABLE 1 EXPERIMENTAL FINDINGS GERMANE TO THE CARCINOGENESIS OF 1,4-DIOXANE

Test system	Endpoint	1,4-Dioxane	Result	Reference
[Genotoxicity parametersu]				
Salmonella	Mutation	5000mg/l*	−	22
Salmonella	Mutation	51500mg/l	−	21
Yeast	Aneuploidy	47500mg/l	−	23
Wheat	Chromosome aberation	10000mg/l	+	25
Chinese hamuster(CHO)	Chromosome aberation	10.5mg/ml	−	30
	SCE	10.5mg/ml	+(weak)	30
Dorsophila	Mutation	35000mg/l	−	26
Rat hepatocytes	DNA damage	26.40mg/l	+(at cytotoxic conc.)	24
		2.64mg/l	−(below cytotoxic conc.)	24
Rat liver	DNA alkylation	1000mg/kg	−	27
Rat liver	DNA repair	1000mg/kg	−	27
Rat liver	DNA damage	2500mg/kg	+	31
[Promation of carcinogenesis parameters]				
Mouse skin	Cancer promotion	1%	+	28
Rat liver	Liver weight/body weight	1000mg/kg	+	21
Rat liver	DNA synthesis	1000mg/kg	+	21
Rat liver	GGT foci number	1000mg/kg	+	29
Rat liver	GGT foci volume	1000mg/kg	+	29
Rat liver	ODC induction	840mg/kg	+	31
Rat liver	Cytochrome P-450 induction	2550mg/kg	+	31

*Using the molecular weight of 88.11mg/mmol,5000mg/l is 56.8mM 1,4-dioxane

<Acute Toxicity (Oral)>

Laug, et al. (1939) reported oral LD50 values of 5.66 g/kg of 1,4-dioxane in mice, 5.17 g/kg in rats, and 3.90 g/kg in guinea pigs (Reference 32). As shown in Table 2, there are several reports published.

TABLE 2 LD_{50} VALUES OF SEVERAL EXPERIMENTS

Species	LD_{50} (g/kg)	Refference
Rat	6.2	33
Rat	7.35	33
Rat	7.12	34
Rat	5.6	35
Guinea pig	1.27	33
Guinea pig	3.15	34
Rabbit	2.1	33
Mouse	5.7	34
Cat	2.0	34

<Long Term Studies (in Drinking Water)>

Hoch-Ligeti et. al. (1970) published a report on a 13-month study in rats that a long-term oral administration of 1,4-dioxane in drinking water induced increased incidences of nasal tumors and hepatoma. Increased incidences of nasal tumors were found in one rat in both of the 0.75 and 1.0% groups, and two rats in both of the 1.4 and 1.8% groups. Increased incidences of hepatoma was found in all of the concentration groups (Reference 36). The same group of researchers conducted a re-test in the same manner and reported incidences of early hepatic tumors, proliferation of epithelial cells in Bowman's, capsule, fibrosis of the areas surrounding the glomus bodies, and renal tubular dilatation (Reference 37).

R.J. Kociba et. al. (1974) conducted a 2-year study in rats (at dosage levels of 0, 1.0, 0.1, and 0.01%). They reported a decrease in number of survivors (a shortened lifespan) and suppressed body weight gain in the 1.0% group, degeneration and necrosis of the hepatic cells and tubular epithelium, and regeneration trend in the 0.1 and 1.0% groups, and hepatoma and squamous cell carcinoma in the nasal cavity in the 1.0% group (Reference 38).

NIC (National Cancer Inst.) (1978, NCI-CG-TR-80) conducted studies in rats (at dosage levels of 0, 0.5, and 1.0% for 110-week) and mice (at dosage levels of 0, 0.5, and 1.0% for 90-week) and reported the results that squamous cell carcinoma in the nasal cavity in rats of both sexes, hepatocellular adenoma in female rats, and hepatoma in mice of both sexes in the 0.5 and 1.0% groups occurred (Reference 35).

<IARC Monograph>

International Agency for Research on Cancer (IARC) Monograph (1987) evaluated that there was inadequate evidence in humans for the carcinogenicity of 1,4-dioxane. As for carcinogenicity of 1,4-dioxane in experimental animals, tests in rats and guinea pigs by oral administration in drinking water produced adenoma and cancers of the liver and cancers of the nasal cavity in rats of both sexes, hepatoma in male guinea pigs, and cancers of the gallbladder in guinea pigs of both sexes. No carcinogenic effect was observed in an inhalation study in rats. 1,4-dioxane was active as a promoter in a two-stage skin carcinogenesis study in mice. Based on the above facts, there was sufficient evidence in experimental animals for the carcinogenicity of 1,4-dioxane. IARC concluded the evidence was sufficient and classified 1,4-dioxane as Group 2B (Reference 40).

I Study Materials

I-1 Lots Used for the Study Articles, etc.

Lot Numbers	:	B846524 (February 19, 1985–February 18, 1986) B51248 (February 19, 1986–March 13, 1987)
Manufacturer	:	Dojindo Laboratories
Grade	:	Pure solvent for absorption spectrum
Purity	:	≧ 99%
Moisture	:	≦ 0.3%
Nonvolatile Matter	:	≦ 0.01%

I-2 Identity and Stability of the Study Articles

I-2-1 Identity

It was verified that each lot of 1,4-dioxane was identical by measuring the boiling point and the infrared absorption spectrum and comparing them against the literature values. (Appendix O 1)

I-2-2 Stability

It was verified that each lot was stable by measuring the boiling point and the infrared absorption spectrum and by acquiring the gas chromatogram at the time of lot acceptance and usage completion. (Appendix O 2)

I-3 Laboratory Animals

F344/DuCrj (Fischer) rats (SPF) and Crj: BDF_1 mice (SPF) of both sexes by Charles River Japan, Inc. were used. 240 male and 240 female rats and 240 male and 240 female mice at 4 weeks old were introduced (the range of body weights at the time of acceptance: 50–75 g for male rats, 50–65 g for female rats, 13–21 g for male mice, and 12–20 g for female mice). After one week of quarantine and habituation, 200 male and 200 female rats and 200 male and 200 female mice with normal development, no abnormal general symptoms, and body weights close to the median weight were selected and used for the study (the range of body weights at the beginning of administration: 125–145 g for male rats, 99–110 g for female rats, 22.1–25.7 g for male mice, and 18.1–20.6 g for female mice).

The reasons why F344/DuCrj (Fischer) rats (SPF) and Crj: BDF_1 mice (SPF) were used for the study are as follows:

a) Genetic stability;
b) Fewer spontaneous tumor incidences; and
c) The date availability from the past carcinogenicity studies and the known tumor sensitivity to substances.

II Study Method

II-1 Administration

II-1-1 Administration Route, Method, and Duration

Oral administration was selected. The drinking water dissolved with 1,4-dioxane was provided ad libitum to the laboratory animals. The administration duration was 104 weeks. The drinking water was changed twice a week.

II-1-2 Concentration Selection and Reason

The highest concentration for rats was set at 5,000 ppm and the lower concentrations were set at 1,000 or 200 ppm (with a common ratio of 5.0). The highest concentration for mice was set at 8,000 ppm and the lower concentrations were set at 2,000 or 500 ppm (with a common ratio of 4.0). Deionized water was given to the control groups. These concentrations were determined based on the results from the 13-week preliminary studies using rats and mice.

II-1-3 Study Solution Preparation

The solutions were prepared by mixing 1,4-dioxane with deionized water to a concentration of 5%. The prepared solution was used to make the other solutions by adding deionized water in order to dilute them to the set concentrations. The solutions were prepared twice a week.

II-1-4 Concentration Measurement at Time of Preparation

The concentrations of the 1,4-dioxane solutions were measured using a gas chromatography method every two to three months during the administration period to ensure that the concentrations of the solutions would be measured in the range of the set concentration of 90–114%. (Appendix O 3)

II-1-5 Stability of the Study Articles under Administration Conditions

Stability of 1,4-dioxane in drinking water was measured using a gas chromatography method to ensure appropriate stability for four days. (Appendix O 4)

II-1-6 Amount of the Study Articles Consumed

The ingested amounts of 1,4-dioxane were calculated from the measured body weights, the amounts of water consumed, and the set concentrations. (Appendices A 1 to 4)

TABLE 3 EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE DRINKING WATER STUDIES OF 1,4-DIOXANE

	RATS	MICE
Method of Administration	Drinking water	Drinking water
Number of Groups	Male 4, Female 4	Male 4, Female 4
Size of Study Groups	50 males and 50 females of each group	50 males and 50 females of each group
Animals		
Strain and Species	F344/DuCrj(Fischer)rats	Crj:BDF_1 mice
Animal Source	Charles River Japan,Inc	Charles River Japan,Inc
During of Quarantine	2 wk	2 wk
Age When Placed on Study	6 wk	6 wk
Age When Killed	110～111 wk	110～111 wk
Doses	0,200,1000,5000ppm	0,500,2000,8000ppm
Duration of Dosing	7d/wk for 104 wk	7d/wk for 104 wk
Animal Maintenance		
Feed	CRF-1 (Oriental Yeast Co.,Ltd) Sterilized by γ-ray Available ad libitum	CRF-1 (Oriental Yeast Co.,Ltd) Sterilized by γ-ray Available ad libitum
Water	Formulated water Deionized water sterilized by filter and ultraviolet ray Available ad libitum by water bottles	Formulated water Deionized water sterilized by filter and ultraviolet ray Available ad libitum by water bottles
Animals per Cage	Single	Single
Animal Room Environment	Barrier system Temp:24±2℃ Hum :55±10% Fluorescent light 12h/d 15-17 room air changes /h	Barrier system Temp:24±2℃ Hum :55±10% Fluorescent light 12h/d 15-17 room air changes /h
Type and Frequency of Observation		
Clinical Sign	Observed 1×d	Observed 1×d
Body Weight	Weighed 1×wk for 14wk Weighed 1×2wk thereafter	Weighed 1×wk for 14wk Weighed 1×2wk thereafter
Food Consumption	Weighed 1×wk for 14wk Weighed 1×4wk thereafter	Weighed 1×wk for 14wk Weighed 1×4wk thereafter

TABLE 3 EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE DRINKING WATER STUDIES OF 1,4-DIOXANE (Continued)

	RATS	MICE
Hematology	Red blood cell(RBC) Hemoglobin Hematocrit Mean corpuscular volume(MCV) Platelet White blood cell(WBC) Differential WBC	Red blood cell(RBC) Hemoglobin Hematocrit Mean corpuscular volume(MCV) Platelet White blood cell(WBC) Differential WBC
Blood Biochemistry	Total protein Albumin A/G ratio T-bilirubin Glucose T-cholesterol Triglyceride Phospholipid Glutamic oxaloacetic transaminase(GOT) Glutamic pyruvic transaminase(GPT) Lactate dehydrogenase(LDH) Alkaline phosphatase(ALP) Leucine aminopeptidase(LAP) γ-Gultamyl transpeptidase(G-GTP) Creatine phosphokinase(CPK) Urea nitrogen Creatinine Sodium Potassium Chloride Calcium Inorganic phosphorus	Total protein Albumin A/G ratio T-bilirubin Glucose T-cholesterol Triglyceride Phospholipid Glutamic oxaloacetic transaminase(GOT) Glutamic pyruvic transaminase(GPT) Lactate dehydrogenase(LDH) Alkaline phosphatase(ALP) Leucine aminopeptidase(LAP) Creatine phosphokinase(CPK) Urea nitrogen Sodium Potassium Chloride Calcium Inorganic phosphorus
Urinalysis	pH Protein Glucose Ketone body Bilirubin Occult blood Urobilinogen	pH Protein Glucose Ketone body Occult blood Urobilinogen
Necropsy	Necropsy performed on all animals.	Necropsy performed on all animals.
Organ Weight	Organ weight measurement performed on schedule sacrified animals. The following organs were weighed: brain,lung,liver,spleen,heart, kidney,adrenal,testis,ovary.	Organ weight measurement performed on schedule sacrifiled animals. The following organs were weighed: brain,lung,liver,spleen,heart, kidney,adrenal,testis,ovary.
Histopathologic Examination	Histopathologic examination perf-ormed on all animals. The following organs were examined :brain,lung,liver,spleen,heart, kidney,adrenal,testis,ovary, thyroid,pancreas,stomach, small intestine,large intestine, thymus,lymph nodes(axilla,inguinal), pituitary,urinary bladder,eye, Harder gland,tongue,spinal cord, peripheral nerve(sciatic), esophagus,bone marrow,epididimys, seminal vesicle,prostate,salivary gland,skin,uterus,vagina, mammry gland,muscle,treachea, nasal cavity,bone.	Histopathologic examination perf-ormed on all animals. The following organs were examined :brain,lung,liver,spleen,heart, kidney,adrenal,testis,ovary, thyroid,pancreas,stomach, small intestine,large intestine, thymus,lymph nodes(axilla,inguinal), pituitary,urinary bladder,eye, Harder gland,tongue,spinal cord, peripheral nerve(sciatic), esophargus,bone marrow,epididimys, seminal vesicle,prostate,salivary gland,skin,uterus,vagina, mammry gland,muscle,treachea, nasal cavity,bone.

II-2 Animal Management

II-2-1 Group Assignment and Individual Identification Method

A method for assigning the laboratory animals into each group to reduce weight bias (a stratified technique) was used (Reference 1).

The individual identification of the laboratory animals was achieved by placing the individual identification numbers on the cages.

The rats and mice were kept in an independent chamber within a barrier area. Each chamber was distinguished from other studies by labeling with the study number, species, and animal numbers.

II-2-2 Rearing Conditions

The rats and mice were reared in a barrier-system animal room with a temperature of 24 ± 1 °C, humidity of 50 ± 5%, lighting cycle of a 12-hour lighted period (8:00–20:00) and a 12-hour darkened period (20:00–8:00), and ventilations of 15–17 times/hour. During the period of quarantine, each stainless net cage hosted a group of five animals. The stainless net cage had dimensions of 340 (W) x 294 (D) x 176 (H) mm for rats and 220 (W) x 212 (D) x 120 (H) mm for mice. During the periods of habituation and administration, each cage hosted one animal. The stainless net cage had dimensions of 170 (W) x 294 (D) x 176 (H) mm for rats and 112 (W) x 212 (D) x 120 (H) mm for mice. The rearing racks and cages were replaced every two weeks.

CRF-1 solid feeds (3Mrad = 30KGy-gamma radiation sterilized feed provided by Oriental Yeast Co., Ltd.) was provided ad libitum from a solid food feeder during the study periods of all the studies. The drinking water was provided by Hadano City Waterworks Bureau. The drinking water was prepared by filtering and deionizing the city water before UV sterilization and further filtering. The drinking water was provided ad libitum via a water bottle.

The quality control of the animal feed was achieved by verifying the nutritional compositions against analysis data provided by Oriental Yeast Co., Ltd. and by checking each lot for impurities against analysis data provided by the Japan Food Research Laboratories. The quality control data is summarized in Appendices P 1 and 2.

II-3 Observation, Examinations, and Their Methods

II-3-1 Observation of General Symptoms in Animals

The observation of general symptoms in the animals was performed in the morning and evening twice daily throughout the rearing period.

II-3-2 Measurement of Body Weight

The body weight was measured once weekly after the start of the administration up to Week 14 and thereafter once in every two weeks. The body weight was also measured at time of death and sacrifice.

II-3-3 Measurement of Food Consumption

The food consumption was measured once weekly after the start of the administration up to Week 14 and thereafter once in every four weeks.

II-3-4 Measurement of Water Consumption

The water consumption was measured once weekly after the start of the administration up to Week 14 and thereafter once in every two weeks.

II-3-5 Hematological Examination

Blood samples were collected from the abdominal aorta of all the animals which were alive at the time of the scheduled necropsy and put under etherization immediately before necropsy. Hematological examinations were performed on EDTA-2K added blood samples. A blood smear was prepared with the EDTA-2K added blood sample to measure a differential leukocyte count.

The animals subjected to the examinations were fasted starting from the day before necropsy (≧ 18 hours).

The examination items and methods are described in Table 3 and Appendix Q 1.

II-3-6 Blood Biochemistry Examination

Blood samples were collected from the abdominal aorta of all the animals which were alive at the time of the scheduled necropsy and put under etherization immediately before necropsy. Heparin lithium was added to the blood samples which were centrifuged (at 3,000 rpm for 30 minutes) to extract the plasmas. The resulting plasmas were used to perform blood biochemistry examinations.

The animals subjected to the examinations were fasted starting from the day before necropsy (≧ 18 hours).

The examination items and methods are described in Table 3 and Appendix Q 1.

II-3-7 Urinalysis

Urinalysis was performed in fresh urine samples collected from all the animals which were alive by the week of the final administration.

The examination items and methods are described in Table 3 and Appendix Q 1.

II-3-8 Pathological Examination

All organs were visually observed at the time of necropsy and fixed using 10% neutral buffered formalin solution. The organs listed in Table 3 (histopathological examination) and the ones with visually observed changes were embedded in paraffin, sliced thinly and stained with hematoxylin-eosin, and studied histopathologically with an optical microscope. Tissue sections of the nasal cavity were cut out (transversely) at the following three regions: the posterior end of the incisor (Level 1), the incisive papilla (Level 2), and the anterior end of the first molar (Level 3). The wet weight (actual weight) of organs listed in Table 3 was measured for all animals surviving up to the scheduled necropsy.

The following contexts of observation used in the Peto tests (Reference 2) were added to neoplastic lesions.

0: Tumor found at the time of the scheduled necropsy

1: Tumor found in a dead / moribund animal, and tumor which was not directly related to the cause of death

2: Tumor considered to be 1, but it was not confirmed

3: Tumor considered to be 4, but it was not confirmed

4: Tumor found in a dead / moribund animal, and tumor which was directly related to the cause of death

II-4 Numerical Processing and Statistical Methodology

(1) Handling and Presentation of Data

All numerical data was presented based on the precision of the measurement devices.

Body weight was expressed in grams. The values were rounded to an integer for rats and to one decimal place for mice.

The amount of food consumed was expressed in grams. The food consumption during a week was measured to one decimal place and divided by 7 (days) to calculate a daily average with the resulting value rounded to one decimal place.

The amount of water consumed was expressed in grams. The water consumption for four days in a week was measured to one decimal place and divided by 7 (days) to calculate a daily average with the resulting value rounded to one decimal place.

The daily average of 1,4-dioxane consumed in each group was derived by multiplying the water consumption by the 1,4-dioxane concentration, dividing the result by the body weight and by days. It was expressed in g / kg (body weight)/ day and the value was rounded to three decimal places.

The actual organ weight was measured to three decimal places and was expressed in grams. Organ-body weight ratio, obtained by dividing the actual organ weight by the body weight at the time of necropsy, was expressed in percentage with the value rounded to three decimal places.

Accuracy in hematology and blood biochemistry examinations is listed in Appendix Q 2. The A/G ratio was calculated by the formula albumin / (total protein - albumin) and the result was rounded to one decimal place.

The mean and standard deviation corresponding to each item noted above were rounded so that they contained the same number of decimal places as the corresponding item.

(2) Handling and Presentation of Population Size

The number of the animals in each group (population) with a variety of statistical verification is listed in the Summary Tables.

The body weight and the amount of food were measured for any animals surviving at the time of measurement. Any missing data were excluded from the population size.

The measurement of organ weights and hematology and blood biochemistry examinations were conducted for any animals surviving at the time of the scheduled necropsy. Any missing data were excluded from the population size.

Urinalysis was performed on any animals surviving until the final week of administration. The number of urinalyses performed was used as the population size.

The effective number of animals in each group (the number of animals used in the study minus the number of animals removed because of an accident or other reasons) was used as the population size for necropsy and histopathological data.

The Peto test was performed by each organ and those organs which could not be histopathologically examined were excluded from the population size.

(3) Statistical Methodology

With the control groups used as the standard groups, all measurements obtained in the study were first tested for homoscedasticity using a Bartlett's test. If the result was homoscedastic, a one-way ANOVA analysis was performed. If a statistically significant difference was recognized between the groups, the mean values were tested with a Dunnett's multiple comparison test.

If unequal distribution was identified, the measurements were ranked throughout the groups and a Kruskal-Wallis test was performed. A Dunnett's multiple comparison test was performed if a statistically significant difference was noted between the groups.

In a preliminary testing, two-sided tests were performed at a significance level of 5%, and in a final testing, two-sided tests at significance levels of 1% and 5% were performed.

A grade of zero was assigned to animals not noted to have a nonneoplastic lesion in histopathologic examinations, and a χ^2 test was performed. A χ^2 test was also performed for the number of surviving animals and urinalysis.

Among neoplastic lesions, the Cochran-Armitage test and Fisher's exact test were performed on the total number of tumor-bearing organs per tumor in each dosage group. Using the contexts (see "II-3-8: Pathological Examination") added at the time of histopathologic examinations, the mortality method (tumors with the contexts 3 and 4), the prevalence method (tumors with the contexts 0, 1, and 2) and the mortality method + the prevalence method (the total number of tumors with the context 0–4) of the Peto tests were performed.

A χ^2 test and Fisher's exact test were performed for pair wise comparisons between the control group and each dosage group.

II-5 Archive of Study Records and Materials

The Study Protocol, samples, raw data, records, final report, Proof of Quality Assurance, study materials, and any other materials related to the study will be stored in a study material archival facility according to the Standard Operating Procedures established by the Japan Bioassay Research Center for ten years from the submission of the final report.

III Study Results

III-1 Carcinogenicity Study in Rats

III-1-1 Observation of Animal Conditions

(1) Survival

Survival data in each group during the administration period is listed in Tables 4 and 5, and Figures 1 and 2.

Compared with the control group, a significant decrease in number of survivors was observed in the 5,000 ppm male group starting from Week 90 and in the 1,000 ppm male group starting from Weeks 92–96 after the start of the administration.

Compared with the control group, a significant decrease in number of survivors was observed in the 5,000 ppm female group starting from Week 102.

The first death in all male groups occurred in Week 66, and that in a female occurred in Week 74 in the 5,000 ppm group, Week 58 in the 1,000 ppm group, Week 84 in the 200 ppm and Week 72 in the control group.

(2) General Symptoms

The observed symptoms of the non-survivors in each group from the start of administration to death, and those of the survivors in the late stage of administration (Weeks 92–104) are listed in Tables 6 and 7.

Compared with the control group, a high incidence rate of tumor mass was noted in the nose (5/26), chest (2/26) and abdomen (6/26) in the 5,000 ppm female group. Abnormal nasal noises were also observed in the 5,000 ppm groups of both sexes (5/28 in males and 5/26 in females).

There were no differences of incidence of tumor mass in the survivors between the control groups and each dosed group. Abnormal nasal noises were observed only in the 5,000 ppm male group (2/22).

(3) Body Weight

Changes in body weight in each group during the administration period are listed in Tables 4 and 5 and Figures 3 and 4.

Compared with the control group, the males in the 5,000 ppm group showed 2–10% of suppression of body weight gain throughout the administration period.

Compared with the control group, the females in the 5,000 ppm group showed 2–19% of suppression of body weight gain throughout the administration period.

(Appendices B 1 and 2)

TABLE 4 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN MALE RAT

Week-Day on Study	Control Av.Wt.	No.of Surviv.	200 ppm Av.Wt.	% of cont.	No.of Surviv.	1000 ppm Av.Wt.	% of cont.	No.of Surviv.	5000 ppm Av.Wt.	% of cont.	No.of Surviv.
0-0	134 (50)	50	134 (50)	100	50	134 (50)	100	50	135 (50)	101	50
1-7	173 (50)	50	172 (50)	99	50	171 (50)	99	50	171 (50)	99	50
2-7	206 (50)	50	205 (50)	100	50	204 (50)	99	50	202 (50)	98	50
3-7	233 (50)	50	231 (50)	99	50	231 (50)	99	50	229 (50)	98	50
4-7	253 (50)	50	251 (50)	99	50	250 (50)	99	50	248 (50)	98	50
5-7	271 (50)	50	268 (50)	99	50	268 (50)	99	50	264 (50)	97	50
6-7	283 (50)	50	279 (50)	99	50	281 (50)	99	50	276 (50)	98	50
7-7	296 (50)	50	293 (50)	99	50	293 (50)	99	50	287 (50)	97	50
8-7	309 (50)	50	305 (50)	99	50	305 (50)	99	50	297 (50)	96	50
9-7	320 (50)	50	316 (50)	99	50	317 (50)	99	50	310 (50)	97	50
10-7	327 (50)	50	322 (50)	98	50	323 (50)	99	50	316 (50)	97	50
11-7	334 (50)	50	330 (50)	99	50	331 (50)	99	50	323 (50)	97	50
12-7	340 (50)	50	335 (50)	99	50	336 (50)	99	50	328 (50)	96	50
13-7	346 (50)	50	342 (50)	99	50	343 (50)	99	50	334 (50)	97	50
14-7	350 (50)	50	345 (50)	99	50	347 (50)	99	50	338 (50)	97	50
16-7	359 (50)	50	353 (50)	98	50	357 (50)	99	50	347 (50)	97	50
18-7	367 (50)	50	361 (50)	98	50	365 (50)	99	50	355 (50)	97	50
20-7	375 (50)	50	369 (50)	98	50	373 (50)	99	50	363 (50)	97	50
22-7	384 (50)	50	377 (50)	98	50	380 (50)	99	50	370 (50)	96	50
24-7	390 (50)	50	383 (50)	98	50	386 (50)	99	50	376 (50)	96	50
26-7	395 (50)	50	390 (50)	99	50	393 (50)	99	50	381 (50)	96	50
28-7	400 (50)	50	394 (50)	99	50	398 (50)	100	50	385 (50)	96	50
30-7	407 (50)	50	401 (50)	99	50	404 (50)	99	50	392 (50)	96	50
32-7	413 (50)	50	407 (50)	99	50	412 (50)	100	50	399 (50)	97	50
34-7	420 (50)	50	412 (50)	98	50	417 (50)	99	50	404 (50)	96	50
36-7	425 (50)	50	417 (50)	98	50	421 (50)	99	50	406 (50)	96	50
38-7	429 (50)	50	422 (50)	98	50	426 (50)	99	50	410 (50)	96	50
40-7	433 (50)	50	425 (50)	98	50	430 (50)	99	50	414 (50)	96	50
42-7	436 (50)	50	430 (50)	99	50	434 (50)	100	50	417 (50)	96	50
44-7	443 (50)	50	436 (50)	98	50	440 (50)	99	50	423 (50)	95	50
46-7	449 (50)	50	442 (50)	98	50	445 (50)	99	50	430 (50)	96	50
48-7	453 (50)	50	445 (50)	98	50	449 (50)	99	50	432 (50)	95	50
50-7	456 (50)	50	450 (50)	99	50	453 (50)	99	50	435 (50)	95	50
52-7	460 (50)	50	453 (50)	98	50	456 (50)	99	50	439 (50)	95	50
54-7	464 (50)	50	457 (50)	98	50	460 (50)	99	50	443 (50)	95	50
56-7	468 (50)	50	462 (50)	99	50	465 (50)	99	50	448 (50)	96	50
58-7	473 (50)	50	464 (50)	98	50	468 (50)	99	50	452 (50)	96	50
60-7	477 (50)	50	469 (50)	98	50	473 (50)	99	50	456 (50)	96	50
62-7	480 (50)	50	472 (50)	98	50	475 (50)	99	50	459 (50)	96	50
64-7	483 (50)	50	472 (50)	98	50	476 (50)	99	50	459 (50)	95	50
66-7	486 (49)	49	475 (49)	98	49	478 (49)	98	49	461 (49)	95	49
68-7	488 (49)	49	481 (48)	99	48	479 (49)	98	49	462 (48)	95	48
70-7	490 (49)	49	482 (48)	98	48	481 (48)	98	48	461 (48)	94	48
72-7	490 (49)	49	484 (48)	99	48	481 (48)	98	48	461 (47)	94	47
74-7	492 (49)	49	484 (48)	98	48	481 (48)	98	48	459 (47)	93	47
76-7	492 (49)	49	484 (48)	98	48	481 (48)	98	48	459 (47)	93	47
78-7	493 (49)	49	484 (48)	98	48	479 (48)	97	48	456 (47)	92	47
80-7	493 (49)	49	484 (48)	98	48	479 (46)	97	46	454 (47)	92	47
82-7	492 (49)	49	483 (48)	98	48	478 (45)	97	45	451 (47)	92	47
84-7	489 (49)	49	479 (48)	98	48	480 (44)	98	44	451 (44)	92	44
86-7	488 (48)	48	481 (47)	99	47	479 (44)	98	44	445 (42)	91	42
88-7	486 (48)	48	480 (47)	99	47	477 (44)	98	44	442 (42)	91	42
90-7	484 (48)	48	478 (47)	99	47	476 (43)	98	43	441 (41)	91	41 *
92-7	479 (48)	48	478 (47)	100	47	472 (41)	99	41 *	442 (40)	92	40 *
94-7	473 (48)	48	477 (47)	101	47	465 (40)	98	40 *	440 (38)	93	38 **
96-7	468 (47)	47	475 (45)	101	45	463 (39)	99	39 *	434 (37)	93	37 **
98-7	460 (45)	44	471 (45)	102	45	450 (39)	98	39	428 (33)	93	33 **
100-7	462 (43)	42	468 (45)	101	45	451 (37)	98	37	423 (28)	92	28 **
102-7	463 (41)	41	466 (45)	101	45	443 (37)	96	37	416 (25)	90	24 **
104-7	458 (40)	40	462 (45)	101	45	440 (35)	96	35	413 (23)	90	22 **

No. of Survivars : Significant difference ; * : P≦0.05 ** : P≦0.05 Test of CHI SQUARE

TABLE 5 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN FEMALE RAT

Week-Day on Study	Control Av.Wt.	No.of Surviv.	200 ppm Av.Wt.	% of cont.	No.of Surviv.	1000 ppm Av.Wt.	% of cont.	No.of Surviv.	5000 ppm Av.Wt.	% of cont.	No.of Surviv.
0-0	105 (50)	50	105 (50)	100	50	105 (50)	100	50	105 (50)	100	50
1-7	123 (50)	50	123 (50)	100	50	123 (50)	100	50	121 (50)	98	50
2-7	138 (50)	50	138 (50)	100	50	137 (50)	99	50	135 (50)	98	50
3-7	149 (50)	50	149 (50)	100	50	149 (50)	100	50	146 (50)	98	50
4-7	159 (50)	50	159 (50)	100	50	158 (50)	99	50	155 (50)	97	50
5-7	167 (50)	50	168 (50)	101	50	167 (50)	100	50	164 (50)	98	50
6-7	173 (50)	50	173 (50)	100	50	173 (50)	100	50	170 (50)	98	50
7-7	179 (50)	50	180 (50)	101	50	180 (50)	101	50	175 (50)	98	50
8-7	185 (50)	50	184 (50)	99	50	183 (50)	99	50	179 (50)	97	50
9-7	189 (50)	50	190 (50)	101	50	190 (50)	101	50	184 (50)	97	50
10-7	193 (50)	50	193 (50)	100	50	192 (50)	99	50	187 (50)	97	50
11-7	197 (50)	50	197 (50)	100	50	197 (50)	100	50	191 (50)	97	50
12-7	200 (50)	50	199 (50)	100	50	199 (50)	100	50	192 (50)	96	50
13-7	202 (50)	50	202 (50)	100	50	202 (50)	100	50	195 (50)	97	50
14-7	203 (50)	50	203 (50)	100	50	203 (50)	100	50	196 (50)	97	50
16-7	207 (50)	50	208 (50)	100	50	207 (50)	100	50	199 (50)	96	50
18-7	211 (50)	50	212 (50)	100	50	211 (50)	100	50	202 (50)	96	50
20-7	215 (50)	50	215 (50)	100	50	214 (50)	100	50	204 (50)	95	50
22-7	217 (50)	50	218 (50)	100	50	217 (50)	100	50	207 (50)	95	50
24-7	220 (50)	50	222 (50)	101	50	220 (50)	100	50	209 (50)	95	50
26-7	222 (50)	50	224 (50)	101	50	223 (50)	100	50	212 (50)	95	50
28-7	226 (50)	50	226 (50)	100	50	224 (50)	99	50	215 (50)	95	50
30-7	229 (50)	50	229 (50)	100	50	228 (50)	100	50	218 (50)	95	50
32-7	232 (50)	50	231 (50)	100	50	231 (50)	100	50	220 (50)	95	50
34-7	235 (50)	50	235 (50)	100	50	234 (50)	100	50	223 (50)	95	50
36-7	238 (50)	50	238 (50)	100	50	235 (50)	99	50	226 (50)	95	50
38-7	240 (50)	50	240 (50)	100	50	238 (50)	99	50	229 (50)	95	50
40-7	242 (50)	50	242 (50)	100	50	241 (50)	100	50	231 (50)	95	50
42-7	247 (50)	50	245 (50)	99	50	243 (50)	98	50	233 (50)	94	50
44-7	252 (50)	50	250 (50)	99	50	248 (50)	98	50	237 (50)	94	50
46-7	257 (50)	50	255 (50)	99	50	253 (50)	98	50	244 (50)	95	50
48-7	259 (50)	50	259 (50)	100	50	255 (50)	98	50	246 (50)	95	50
50-7	263 (50)	50	263 (50)	100	50	260 (50)	99	50	250 (50)	95	50
52-7	268 (50)	50	266 (50)	99	50	263 (50)	98	50	253 (50)	94	50
54-7	273 (50)	50	271 (50)	99	50	268 (50)	98	50	260 (50)	95	50
56-7	278 (50)	50	277 (50)	100	50	275 (50)	99	50	265 (50)	95	50
58-7	282 (50)	50	281 (50)	100	50	278 (49)	99	49	269 (50)	95	50
60-7	287 (50)	50	285 (50)	99	50	283 (49)	99	49	273 (50)	95	50
62-7	291 (50)	50	289 (50)	99	50	286 (49)	98	49	278 (50)	96	50
64-7	296 (50)	50	294 (50)	99	50	291 (49)	98	49	281 (50)	95	50
66-7	300 (50)	50	297 (50)	99	50	295 (49)	98	49	284 (50)	95	50
68-7	303 (50)	50	301 (50)	99	50	298 (49)	98	49	286 (50)	94	50
70-7	306 (50)	50	304 (50)	99	50	300 (49)	98	49	288 (50)	94	50
72-7	311 (49)	49	307 (50)	99	50	301 (49)	97	49	287 (50)	92	50
74-7	313 (49)	49	308 (50)	98	50	303 (49)	97	49	291 (48)	93	48
76-7	316 (49)	49	310 (50)	98	50	306 (47)	97	47	291 (48)	92	48
78-7	319 (49)	49	312 (50)	98	50	307 (47)	96	47	294 (48)	92	48
80-7	321 (49)	49	312 (50)	97	50	309 (47)	96	47	294 (48)	92	48
82-7	323 (49)	49	314 (50)	97	50	312 (46)	97	46	292 (48)	90	47
84-7	323 (49)	49	319 (49)	99	49	315 (45)	98	45	297 (45)	92	45
86-7	323 (49)	49	321 (49)	99	49	317 (45)	98	45	293 (44)	91	44
88-7	322 (47)	47	320 (49)	99	49	317 (45)	98	45	290 (44)	90	44
90-7	330 (44)	44	321 (48)	97	48	317 (45)	96	45	290 (43)	88	43
92-7	329 (43)	43	323 (47)	98	47	319 (44)	97	44	283 (42)	86	42
94-7	329 (43)	43	320 (46)	97	46	320 (44)	97	44	282 (39)	86	39
96-7	329 (42)	41	318 (45)	97	45	319 (43)	97	43	278 (39)	84	39
98-7	330 (41)	41	320 (43)	97	43	319 (42)	97	42	275 (35)	83	35
100-7	332 (39)	39	315 (43)	95	43	321 (41)	97	41	270 (31)	81	31
102-7	331 (39)	39	314 (42)	95	41	325 (39)	98	39	270 (27)	82	27 *
104-7	326 (39)	38	320 (37)	98	37	317 (38)	97	38	264 (24)	81	24 **

No. of Survivars : Significant difference ; * : P≦0.05 ** : P≦0.05 Test of CHI SQUARE

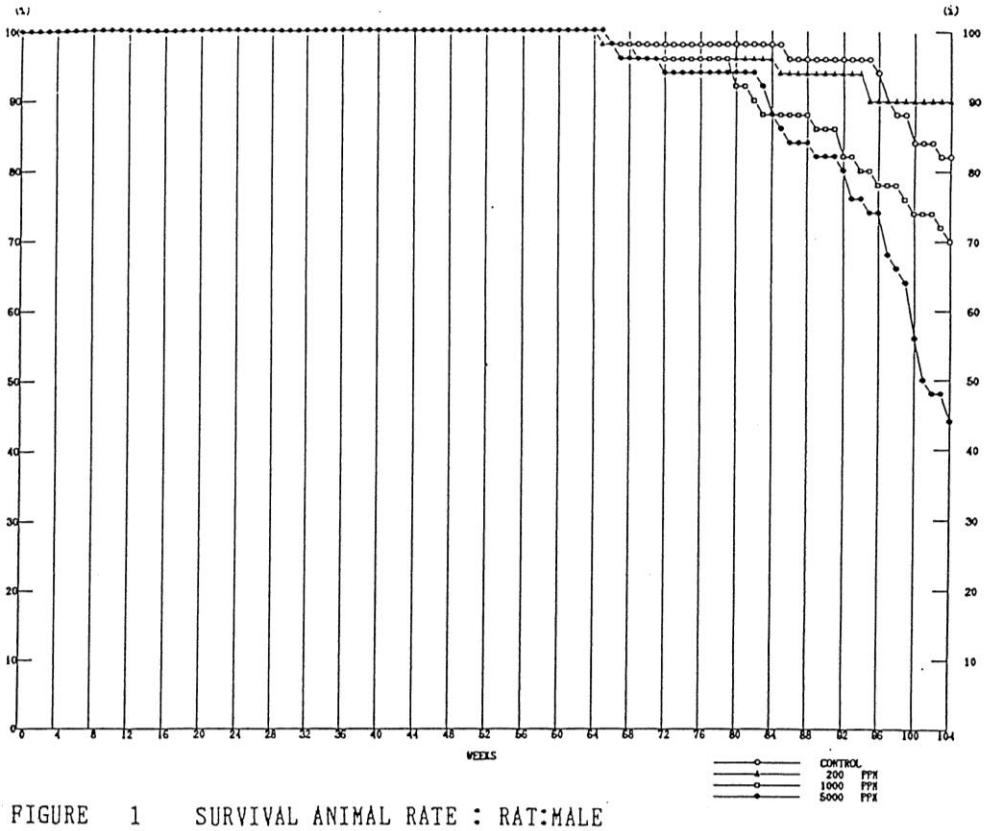


FIGURE 1 SURVIVAL ANIMAL RATE : RAT:MALE

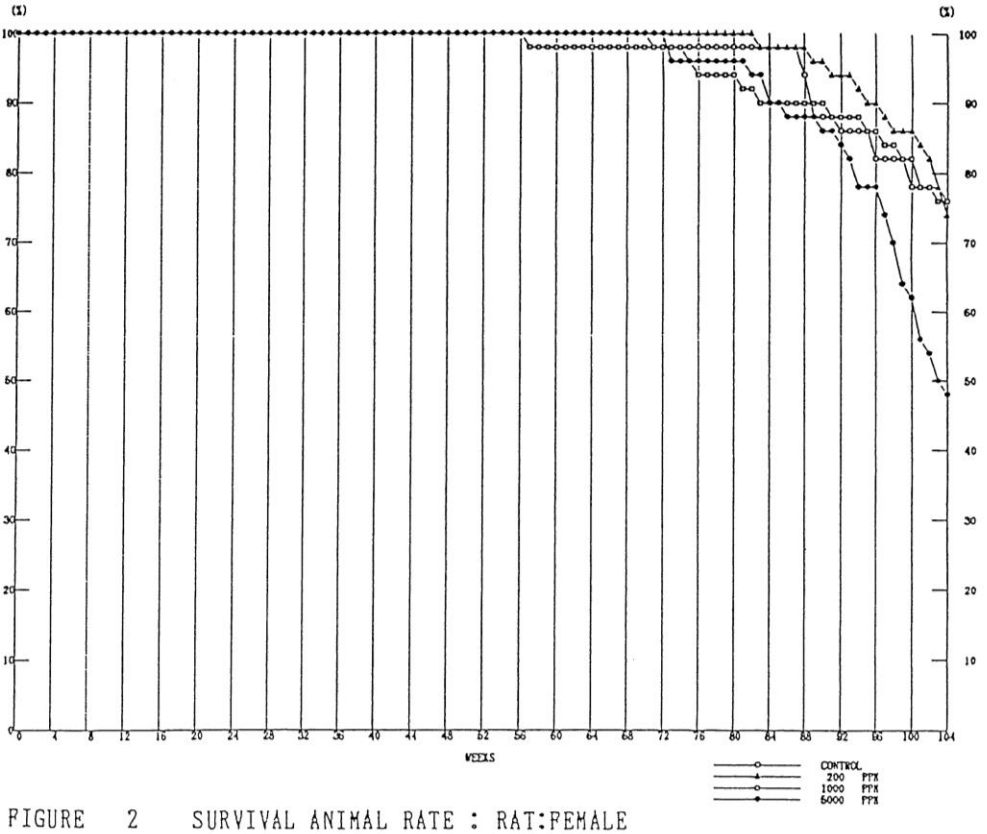


FIGURE 2 SURVIVAL ANIMAL RATE : RAT:FEMALE

TABLE 6 CLINICAL OBSERVATION OF DEAD AND MORIBUND RAT (0-104W SUMMARY)

	MALE				FEMALE			
Clinical sign	Control	200ppm	1000ppm	5000ppm	Control	200ppm	1000ppm	5000ppm
(DEAD AND MORIBUND ANIMAL NUMBERS)	(10)	(5)	(15)	(28)	(12)	(13)	(12)	(26)
LOCOMOTOR MOVEMENT DECR	6	2	5	8	4	9	6	12
HUNCHBACK POSITION	4	2	5	8	2	9	5	15
WASTING	3	1	3	5	2	5	4	12
PILOERECTION	9	4	6	10	5	12	7	18
FROG BELLY	0	1	0	2	0	1	2	0
ANEMIA	6	3	6	14	8	7	7	16
JAUNDICE	0	1	0	1	0	0	0	2
CYANOSIS	1	0	0	2	0	0	0	2
ASCITES	1	1	1	2	0	1	0	0
ABNORMAL TESTIS	6	2	7	22	-	-	-	-
ABNORMAL RESPIRATION	6	2	5	8	3	11	7	17
RESPIRATORY SOUND ABNOR	1	0	0	2	0	1	1	1
NOISY	1	0	0	5	0	0	0	5
SUBNORMAL TEMP	3	1	0	4	2	3	3	8
INTERNAL MASS	0	0	0	0	0	1	4	1
EXTERNAL MASS								
M.NOSE	0	0	0	1	0	0	0	5
M.EYE	0	0	1	0	0	0	0	1
M.MANDIBULAR	1	0	0	0	0	1	0	0
M.EAR	0	0	1	0	0	0	0	0
M.PERI EAR	0	0	1	0	0	0	0	0
M.NECK	0	0	1	0	0	1	0	0
M.BREAST	0	0	1	0	1	0	3	7
M.ABDOMEN	0	1	0	4	0	2	2	6
M.ANTERIOR.DORSUM	0	0	1	1	0	0	0	0
M.POSTERIOR DORSUM	1	0	1	0	1	0	1	0
M.HINDLIMB	0	0	1	0	1	0	0	0
M.GENITALIA	0	0	0	0	0	2	0	2
M.TAIL	0	0	1	0	0	0	0	0

TABLE 7 CLINICAL OBSERVATION OF SURVIVAL RAT (92-104W SUMMARY)

	MALE				FEMALE			
Clinical sign	Control	200ppm	1000ppm	5000ppm	Control	200ppm	1000ppm	5000ppm
(SURVIVAL ANIMAL NUMBERS)	(40)	(45)	(35)	(22)	(38)	(37)	(38)	(24)
LOCOMOTOR MOVEMENT DECR	0	0	1	1	0	1	0	3
HUNCHBACK POSITION	1	0	3	1	1	2	1	2
WASTING	1	0	3	1	1	1	0	3
PILOERECTION	4	3	3	2	3	3	1	9
FROG BELLY	0	0	0	1	0	0	0	0
ANEMIA	1	2	4	2	3	5	1	7
JAUNDICE	0	0	0	0	0	0	0	1
ASCITES	0	0	0	1	0	0	0	0
ABNORMAL TESTIS	15	27	21	21	-	-	-	-
ABNORMAL RESPIRATION	3	0	1	3	2	5	2	6
RESPIRATORY SOUND ABNOR	0	0	1	0	0	0	0	0
NOISY	0	0	0	2	0	0	0	0
SUBNORMAL TEMP	0	0	0	0	0	0	1	1
INTERNAL MASS	0	0	0	0	0	0	0	0
EXTERNAL MASS								
M.NOSE	1	0	0	0	0	0	0	0
M.PERI MOUTH	0	1	1	0	0	1	1	0
M.MANDIBULAR	1	0	0	0	0	0	0	0
M.EAR	0	0	0	0	0	0	1	0
M.PERI EAR	0	1	0	1	0	0	0	0
M.NECK	0	0	0	0	1	0	0	0
M.FORLIMB	0	0	0	0	1	0	0	0
M.BREAST	1	2	1	3	0	2	4	2
M.ABDOMEN	6	3	7	1	2	1	1	2
M.ANTERIOR.DORSUM	1	2	0	2	0	0	2	1
M.POSTERIOR DORSUM	4	1	2	1	0	0	0	0
M.HINDLIMB	1	0	0	1	0	1	0	0
M.GENITALIA	1	0	0	0	5	5	5	1
M.ANUS	0	0	1	0	0	0	0	0
M.SCROTUM	0	0	1	0	-	-	-	-
M.TAIL	1	2	1	0	0	0	0	0

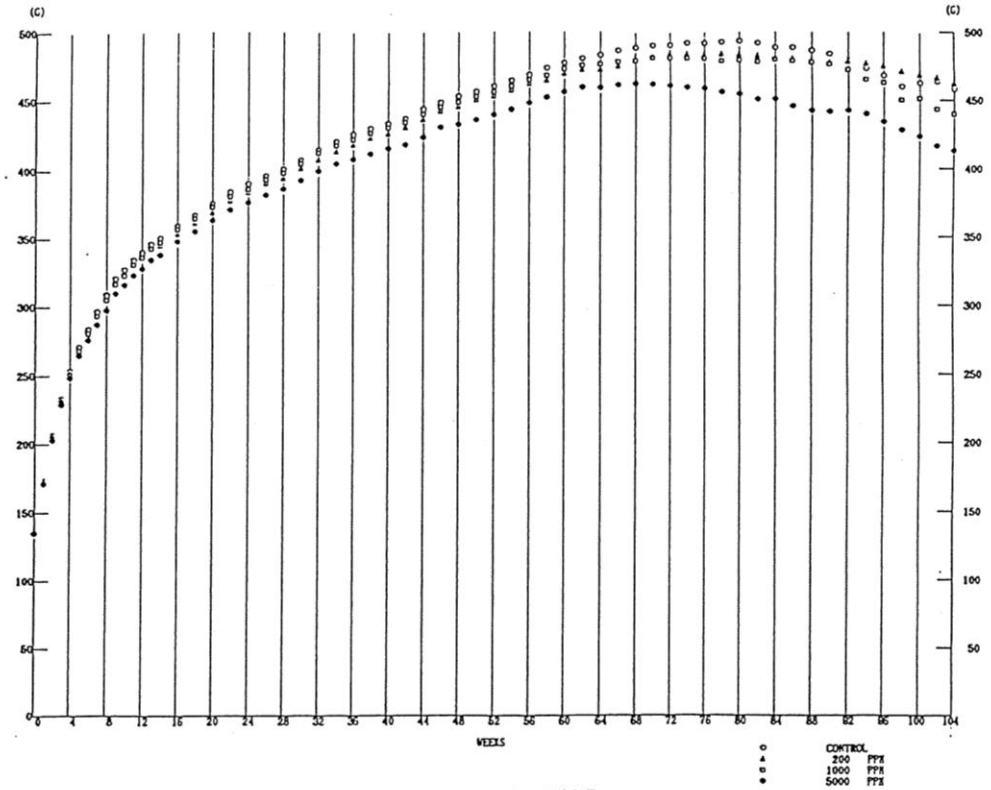


FIGURE 3 BODY WEIGHT CHANGES : RAT:MALE

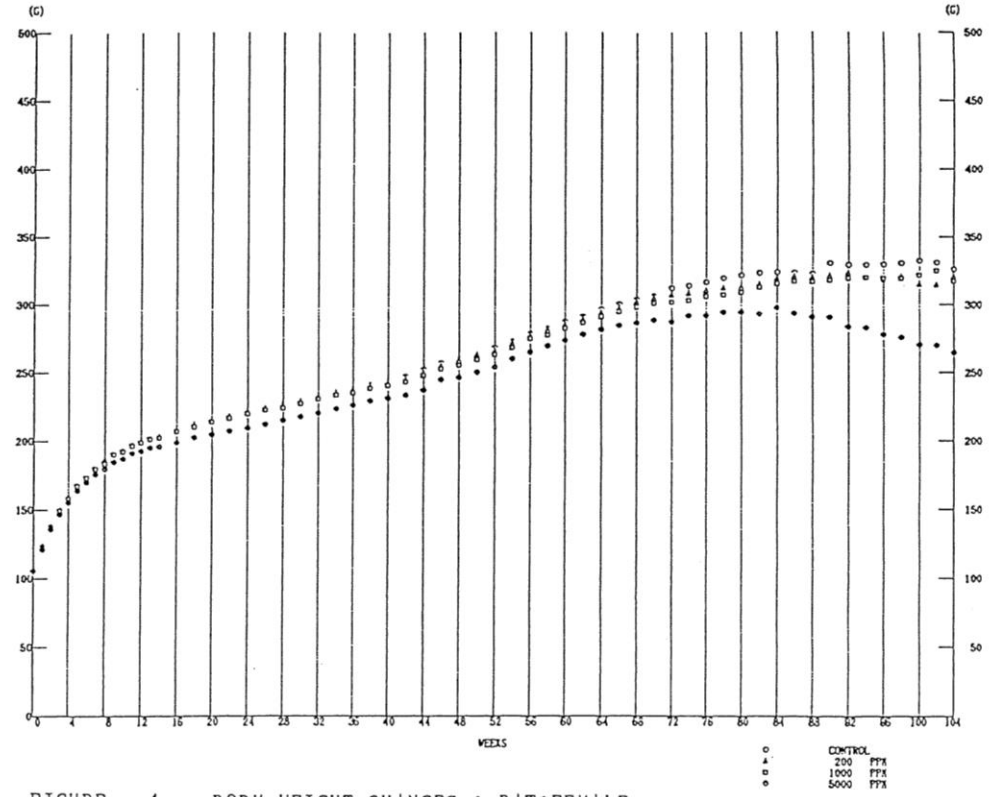


FIGURE 4 BODY WEIGHT CHANGES : RAT:FEMALE

(4) Food Consumption

Food consumption (per animal per day) in each group during the administration period is listed in Tables 8 and 9 and Figures 5 and 6.

Compared with the control group, the males in the 5,000 ppm group had decreased food consumption by 2–4% in the early stage (up to Week 8) and the middle stage of administration (around Week 50).

Compared with the control group, the females in the 5,000 ppm female group had decreased food consumption by 4–9% in the late stage of administration (Week 90 and later).

(Appendices C 1 and 2)

(5) Water Consumption

Water consumption (per animal per day) in each group during the administration period is listed in Tables 10 and 11 and Figures 7 and 8.

Compared with the control group, the males in the 5,000 ppm male group had decreased water consumption by 3–6% in many weeks in the first half of administration (up to Week 36).

(Appendices D 1 and 2)

TABLE 8 FOOD CONSUMPTION IN MALE RAT

Week-Day on Study	Control Av.FC.	No.of Surviv.	200 ppm Av.FC.	% of cont.	No.of Surviv.	1000 ppm Av.FC.	% of cont.	No.of Surviv.	5000 ppm Av.FC.	% of cont.	No.of Surviv.
1-7	16.4 (50)	50	16.4 (50)	100	50	16.3 (50)	99	50	15.9 (50)	97	50
2-7	17.5 (50)	50	17.4 (50)	99	50	17.3 (50)	99	50	16.9 (50)	97	50
3-7	17.7 (50)	50	17.6 (50)	99	50	17.6 (50)	99	50	17.0 (50)	96	50
4-7	17.9 (50)	50	17.7 (50)	99	50	18.0 (50)	101	50	17.4 (50)	97	50
5-7	17.7 (50)	50	17.5 (50)	99	50	17.6 (50)	99	50	17.3 (50)	98	50
6-7	17.5 (50)	50	17.2 (50)	98	50	17.7 (50)	101	50	17.0 (50)	97	50
7-7	17.5 (50)	50	17.3 (50)	99	50	17.4 (50)	99	50	17.0 (50)	97	50
8-7	17.5 (50)	50	17.3 (50)	99	50	17.4 (50)	99	50	16.9 (50)	97	50
9-7	17.6 (50)	50	17.5 (50)	99	50	17.6 (50)	100	50	17.4 (50)	99	50
10-7	17.1 (50)	50	16.8 (50)	98	50	17.2 (50)	101	50	17.0 (50)	99	50
11-7	16.8 (50)	50	16.6 (50)	99	50	17.0 (50)	101	50	16.7 (50)	99	50
12-7	16.5 (50)	50	16.4 (50)	99	50	16.7 (50)	101	50	16.3 (50)	99	50
13-7	16.3 (50)	50	16.2 (50)	99	50	16.5 (50)	101	50	16.2 (50)	99	50
14-7	16.1 (50)	50	16.0 (50)	99	50	16.3 (50)	101	50	16.0 (50)	99	50
18-7	16.3 (50)	50	16.1 (50)	99	50	16.5 (50)	101	50	16.0 (50)	98	50
22-7	15.8 (50)	50	15.8 (50)	100	50	16.0 (50)	101	50	15.6 (50)	99	50
26-7	16.0 (50)	50	16.2 (50)	101	50	16.2 (50)	101	50	16.1 (50)	101	50
30-7	16.4 (50)	50	16.3 (50)	99	50	16.4 (50)	100	50	16.2 (50)	99	50
34-7	16.4 (50)	50	16.3 (50)	99	50	16.5 (50)	101	50	16.2 (50)	99	50
38-7	16.1 (50)	50	16.1 (50)	100	50	16.3 (50)	101	50	15.8 (50)	98	50
42-7	16.1 (50)	50	16.0 (50)	99	50	16.2 (50)	101	50	15.6 (50)	97	50
46-7	16.4 (50)	50	16.2 (50)	99	50	16.4 (50)	100	50	16.0 (50)	98	50
50-7	16.4 (50)	50	16.3 (50)	99	50	16.9 (50)	103	50	16.0 (50)	98	50
52-7	16.5 (50)	50	16.1 (50)	98	50	16.6 (50)	101	50	15.8 (50)	96	50
54-7	16.6 (50)	50	16.4 (50)	99	50	16.9 (50)	102	50	16.2 (50)	98	50
58-7	16.6 (50)	50	16.2 (50)	98	50	16.7 (50)	101	50	16.3 (50)	98	50
62-7	17.0 (50)	50	16.9 (50)	99	50	16.9 (50)	99	50	16.7 (50)	98	50
66-7	17.0 (49)	49	17.2 (49)	101	49	17.1 (49)	101	49	16.8 (49)	99	49
70-7	17.1 (49)	49	16.8 (48)	98	48	17.1 (48)	100	48	16.7 (48)	98	48
74-7	16.9 (49)	49	16.6 (48)	98	48	16.6 (48)	98	48	16.5 (47)	98	47
78-7	16.7 (49)	49	16.4 (48)	98	48	16.1 (48)	96	48	16.4 (47)	98	47
82-7	16.7 (49)	49	16.5 (48)	99	48	16.3 (45)	98	45	15.8 (47)	95	47
86-7	16.4 (48)	48	16.6 (47)	101	47	16.9 (44)	103	44	16.2 (42)	99	42
90-7	16.4 (48)	48	16.2 (47)	99	47	16.5 (43)	101	43	15.7 (41)	96	41
94-7	15.7 (48)	48	15.9 (47)	101	47	15.8 (40)	101	40	15.4 (38)	98	38
98-7	15.9 (45)	44	16.4 (45)	103	45	15.7 (39)	99	39	15.9 (33)	100	33
102-7	16.5 (41)	41	16.4 (45)	99	45	16.0 (37)	97	37	16.4 (24)	99	24
104-7	16.2 (40)	40	16.4 (45)	101	45	16.0 (35)	99	35	15.4 (23)	95	22

TABLE 9 FOOD CONSUMPTION IN FEMALE RAT

Week-Day on Study	Control Av.FC.	No.of Surviv.	200 ppm Av.FC.	% of cont.	No.of Surviv.	1000 ppm Av.FC.	% of cont.	No.of Surviv.	5000 ppm Av.FC.	% of cont.	No.of Surviv.
1-7	12.3 (50)	50	12.5 (50)	102	50	12.4 (50)	101	50	12.0 (50)	98	50
2-7	12.4 (50)	50	12.5 (50)	101	50	12.5 (50)	101	50	12.2 (50)	98	50
3-7	12.3 (50)	50	12.4 (50)	101	50	12.2 (50)	99	50	12.1 (50)	98	50
4-7	12.2 (50)	50	12.4 (50)	102	50	12.4 (50)	102	50	12.1 (50)	99	50
5-7	12.2 (50)	50	12.4 (50)	102	50	12.3 (50)	101	50	12.2 (50)	100	50
6-7	12.2 (50)	50	12.2 (50)	100	50	12.4 (50)	102	50	12.0 (50)	98	50
7-7	12.1 (50)	50	12.3 (50)	102	50	12.4 (50)	102	50	12.2 (50)	101	50
8-7	11.9 (50)	50	11.9 (50)	100	50	12.0 (50)	101	50	11.9 (50)	100	50
9-7	12.3 (50)	50	12.5 (50)	102	50	12.5 (50)	102	50	12.2 (50)	99	50
10-7	12.1 (50)	50	12.4 (50)	102	50	12.3 (50)	102	50	12.1 (50)	100	50
11-7	12.0 (50)	50	12.3 (50)	103	50	12.2 (50)	102	50	12.2 (50)	102	50
12-7	12.0 (50)	50	12.2 (50)	102	50	12.0 (50)	100	50	11.8 (50)	98	50
13-7	11.8 (50)	50	12.1 (50)	103	50	12.1 (50)	103	50	12.0 (50)	102	50
14-7	11.6 (50)	50	11.9 (50)	103	50	11.8 (49)	102	50	11.7 (50)	101	50
18-7	11.8 (50)	50	12.0 (50)	102	50	11.8 (50)	100	50	11.9 (50)	101	50
22-7	11.2 (50)	50	11.7 (50)	104	50	11.5 (50)	103	50	11.5 (50)	103	50
26-7	11.5 (50)	50	11.9 (50)	103	50	11.8 (50)	103	50	11.8 (50)	103	50
30-7	11.8 (50)	50	12.1 (50)	103	50	11.8 (50)	100	50	12.0 (50)	102	50
34-7	12.0 (50)	50	12.3 (50)	103	50	11.9 (50)	99	50	12.2 (50)	102	50
38-7	11.7 (50)	50	12.0 (50)	103	50	11.9 (50)	102	50	12.1 (50)	103	50
42-7	12.0 (50)	50	12.0 (50)	100	50	11.7 (50)	98	50	11.8 (50)	98	50
46-7	12.1 (50)	50	12.4 (50)	102	50	12.3 (50)	102	50	13.0 (50)	107	50
50-7	12.1 (50)	50	12.4 (50)	102	50	12.1 (50)	100	50	12.5 (50)	103	50
52-7	12.3 (49)	50	12.5 (50)	102	50	12.1 (50)	98	50	12.6 (50)	102	50
54-7	12.6 (50)	50	12.8 (50)	102	50	12.5 (50)	99	50	13.1 (50)	104	50
58-7	12.3 (50)	50	12.7 (50)	103	50	12.4 (49)	101	49	12.8 (50)	104	50
62-7	12.9 (50)	50	13.2 (50)	102	50	12.9 (49)	100	49	13.2 (50)	102	50
66-7	13.1 (50)	50	13.2 (50)	101	50	13.0 (49)	99	49	13.6 (50)	104	50
70-7	13.2 (50)	50	13.5 (50)	102	50	13.0 (49)	98	49	13.5 (49)	102	50
74-7	13.3 (49)	49	13.2 (50)	99	50	12.6 (49)	95	49	13.2 (47)	99	48
78-7	13.8 (49)	49	14.0 (50)	101	50	13.9 (47)	101	47	14.0 (48)	101	48
82-7	13.7 (49)	49	13.8 (50)	101	50	13.4 (46)	98	46	13.6 (48)	99	47
86-7	13.4 (49)	49	14.1 (49)	105	49	13.7 (45)	102	45	13.7 (44)	102	44
90-7	14.1 (44)	44	13.9 (48)	99	48	13.7 (45)	97	45	12.9 (44)	91	43
94-7	13.8 (43)	43	13.6 (46)	99	46	13.5 (44)	98	44	13.3 (39)	96	39
98-7	14.3 (41)	41	13.9 (43)	97	43	13.9 (42)	97	42	13.5 (35)	94	35
102-7	14.4 (39)	39	13.7 (42)	95	41	14.3 (39)	99	39	13.3 (27)	92	27
104-7	14.3 (39)	38	14.0 (37)	98	37	14.4 (38)	101	38	13.3 (24)	93	24

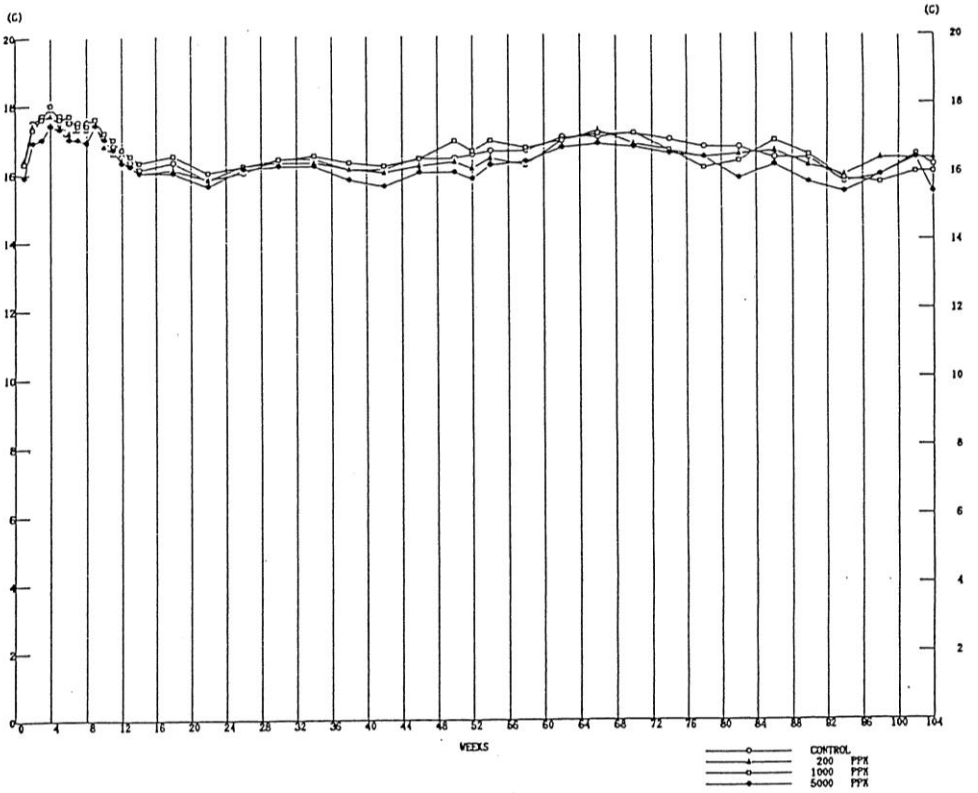


FIGURE 5 FOOD CONSUMPTION : RAT:MALE

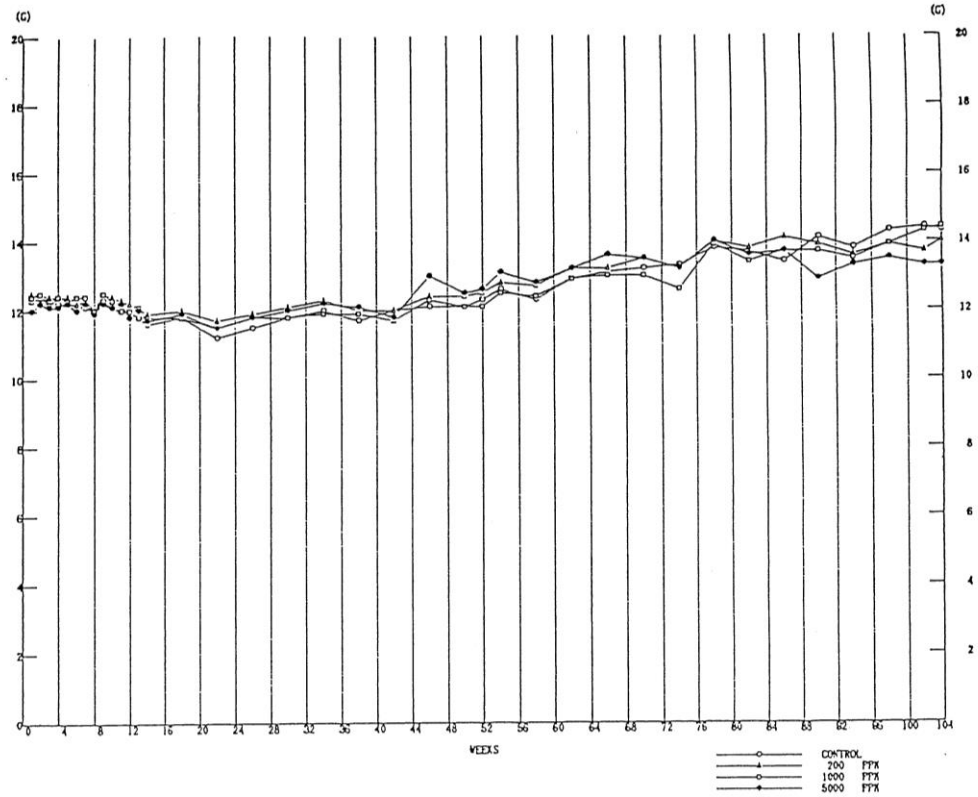


FIGURE 6 FOOD CONSUMPTION : RAT:FEMALE

TABLE 10 WATER CONSUMPTION IN MALE RAT

Week-Day on Study	Control Av.WC.	No.of Surviv.	200 ppm Av.WC.	% of cont.	No.of Surviv.	1000 ppm Av.WC.	% of cont.	No.of Surviv.	5000 ppm Av.WC.	% of cont.	No.of Surviv.
1-7	20.3 (50)	50	20.4 (50)	100	50	20.8 (50)	102	50	20.0 (50)	99	50
2-7	22.0 (50)	50	21.9 (50)	100	50	22.3 (50)	101	50	21.1 (50)	96	50
3-7	22.9 (50)	50	23.1 (50)	101	50	23.4 (50)	102	50	22.5 (50)	98	50
4-7	22.6 (50)	50	23.0 (50)	102	50	23.4 (50)	104	50	22.3 (50)	99	50
5-7	22.2 (50)	50	22.4 (50)	101	50	23.0 (50)	104	50	22.2 (50)	100	50
6-7	21.9 (50)	50	21.9 (50)	100	50	22.7 (50)	104	50	21.6 (50)	99	50
7-7	21.4 (50)	50	21.4 (50)	100	50	22.2 (50)	104	50	20.9 (50)	98	50
8-7	21.7 (50)	50	21.8 (50)	100	50	22.5 (50)	104	50	21.0 (50)	97	50
9-7	20.9 (50)	50	21.0 (50)	100	50	21.5 (50)	103	50	20.5 (50)	98	50
10-7	20.6 (50)	50	20.5 (50)	100	50	21.3 (50)	103	50	20.3 (50)	99	50
11-7	20.2 (50)	50	20.5 (50)	101	50	20.9 (50)	103	50	19.5 (50)	97	50
12-7	20.0 (49)	50	20.5 (50)	103	50	20.7 (50)	104	50	19.3 (50)	97	50
13-7	19.6 (49)	50	20.0 (50)	102	50	20.4 (50)	104	50	18.8 (50)	96	50
14-7	19.7 (49)	50	19.8 (50)	101	50	20.0 (50)	102	50	18.6 (50)	94	50
16-7	19.5 (50)	50	19.1 (50)	98	50	19.9 (50)	102	50	18.5 (50)	95	50
18-7	19.1 (50)	50	19.1 (50)	100	50	19.7 (50)	103	50	18.5 (50)	97	50
20-7	19.2 (50)	50	19.2 (50)	100	50	19.7 (50)	103	50	18.3 (50)	95	50
22-7	19.5 (50)	50	19.1 (50)	98	50	19.4 (50)	99	50	18.4 (50)	94	50
24-7	19.3 (50)	50	19.1 (50)	99	50	19.6 (50)	102	50	18.4 (50)	95	50
26-7	19.0 (50)	50	19.0 (50)	100	50	19.0 (50)	100	50	18.1 (50)	95	50
28-7	19.7 (50)	50	19.2 (50)	97	50	19.7 (50)	100	50	18.6 (50)	94	50
30-7	19.3 (50)	50	19.2 (50)	99	50	19.5 (50)	101	50	18.5 (50)	96	50
32-7	19.3 (50)	50	19.1 (50)	99	50	19.4 (50)	101	50	18.7 (50)	97	50
34-7	19.2 (50)	50	19.2 (50)	100	50	19.4 (50)	101	50	18.4 (50)	96	50
36-7	19.2 (50)	50	19.1 (50)	99	50	19.3 (50)	101	50	18.4 (50)	96	50
38-7	19.0 (50)	50	19.1 (50)	101	50	19.5 (50)	103	50	18.6 (50)	98	50
40-7	19.2 (50)	50	19.0 (50)	99	50	19.4 (50)	101	50	19.0 (50)	99	50
42-7	19.1 (50)	50	19.0 (50)	99	50	19.1 (50)	100	50	18.8 (50)	98	50
44-7	19.2 (50)	50	19.0 (50)	99	50	19.4 (50)	101	50	18.9 (50)	98	50
48-7	19.6 (50)	50	19.3 (50)	98	50	19.5 (50)	99	50	19.3 (50)	98	50
50-7	19.6 (50)	50	19.4 (50)	99	50	19.9 (50)	102	50	19.5 (50)	99	50
52-7	20.1 (50)	50	19.5 (50)	97	50	20.1 (50)	100	50	19.7 (50)	98	50
54-7	19.3 (50)	50	19.2 (50)	99	50	19.7 (50)	102	50	19.2 (50)	99	50
56-7	19.8 (50)	50	19.5 (50)	98	50	19.6 (48)	99	50	19.5 (50)	98	50
58-7	19.1 (50)	50	18.7 (50)	98	50	19.5 (50)	102	50	19.7 (50)	103	50
60-7	19.5 (50)	50	19.2 (50)	98	50	19.3 (50)	99	50	19.1 (50)	98	50
62-7	19.8 (50)	50	19.5 (50)	98	50	19.9 (50)	101	50	19.8 (50)	100	50
64-7	19.8 (50)	50	19.6 (50)	99	50	19.6 (50)	99	50	19.4 (50)	98	50
66-7	19.6 (49)	49	19.5 (48)	99	49	19.7 (49)	101	49	19.4 (49)	99	49
68-7	19.5 (49)	49	19.4 (48)	99	48	19.6 (49)	101	49	19.3 (48)	99	48
70-7	19.7 (49)	49	19.1 (48)	97	48	19.7 (48)	100	48	19.4 (48)	98	48
72-7	20.2 (49)	49	20.1 (48)	100	48	20.0 (48)	99	48	20.1 (47)	100	47
74-7	20.7 (49)	49	20.3 (48)	98	48	20.6 (48)	100	48	20.1 (46)	97	47
76-7	20.8 (48)	49	20.4 (48)	98	48	20.5 (48)	99	48	20.6 (47)	99	47
78-7	20.9 (49)	49	20.3 (48)	97	48	20.2 (48)	97	48	20.3 (47)	97	47
80-7	21.3 (49)	49	20.1 (48)	94	48	20.0 (46)	94	46	20.6 (47)	97	47
82-7	21.2 (49)	49	20.6 (48)	97	48	21.1 (45)	100	45	20.9 (47)	99	47
84-7	21.6 (49)	49	21.0 (48)	97	48	21.2 (44)	98	44	21.4 (44)	99	44
86-7	21.6 (46)	48	21.2 (47)	98	47	22.1 (44)	102	44	21.7 (42)	100	42
88-7	22.4 (47)	48	22.1 (47)	99	47	23.2 (44)	104	44	22.2 (42)	99	42
90-7	22.6 (48)	48	22.1 (47)	98	47	23.1 (42)	102	43	22.1 (41)	98	41
92-7	23.0 (48)	48	22.7 (47)	99	47	22.9 (40)	100	41	22.4 (40)	97	40
94-7	22.5 (47)	48	22.2 (47)	99	47	23.2 (40)	103	40	22.5 (38)	100	38
96-7	22.6 (46)	47	22.4 (45)	99	45	23.0 (38)	102	39	22.8 (36)	101	37
100-7	23.5 (43)	42	23.8 (45)	101	45	24.5 (37)	104	37	24.7 (28)	105	28
102-7	24.5 (41)	41	23.7 (44)	97	45	24.4 (36)	100	37	24.2 (24)	99	24
104-7	23.5 (39)	40	23.5 (44)	100	45	24.8 (35)	106	35	24.3 (22)	103	22

TABLE 11 WATER CONSUMPTION IN FEMALE RAT

Week-Day on Study	Control Av.WC.	No.of Surviv.	200 ppm Av.WC.	% of cont.	No.of Surviv.	1000 ppm Av.WC.	% of cont.	No.of Surviv.	5000 ppm Av.WC.	% of cont.	No.of Surviv.
1-7	17.6 (49)	50	17.9 (49)	102	50	18.2 (49)	103	50	17.4 (50)	99	50
2-7	19.1 (49)	50	19.0 (49)	99	50	18.3 (48)	96	50	17.7 (50)	93	50
3-7	19.1 (47)	50	19.9 (48)	104	50	19.7 (49)	103	50	18.1 (50)	95	50
4-7	19.7 (48)	50	20.1 (49)	102	50	19.4 (49)	98	50	17.4 (47)	88	50
5-7	18.4 (48)	50	20.7 (48)	113	50	19.5 (48)	106	50	18.6 (48)	101	50
6-7	19.5 (48)	50	20.4 (47)	105	50	19.1 (47)	98	50	18.3 (47)	94	50
7-7	19.2 (45)	50	21.2 (47)	110	50	19.0 (45)	99	50	19.5 (48)	102	50
8-7	20.4 (45)	50	21.4 (47)	105	50	19.7 (45)	97	50	19.8 (48)	97	50
9-7	18.6 (44)	50	21.9 (45)	118	50	19.9 (46)	107	50	19.1 (47)	103	50
10-7	18.9 (45)	50	21.4 (45)	113	50	19.2 (44)	102	50	19.0 (48)	101	50
11-7	19.2 (46)	50	21.3 (44)	111	50	18.2 (43)	95	50	18.2 (49)	95	50
12-7	19.6 (45)	50	22.3 (46)	114	50	19.3 (45)	98	50	18.9 (49)	96	50
13-7	20.7 (48)	50	22.6 (48)	109	50	20.2 (46)	98	50	18.4 (48)	89	50
14-7	18.8 (46)	50	20.8 (44)	111	50	19.7 (45)	105	50	18.7 (48)	99	50
16-7	19.2 (49)	50	20.9 (44)	109	50	19.9 (45)	104	50	19.2 (49)	100	50
18-7	18.7 (47)	50	21.4 (47)	114	50	19.1 (46)	102	50	18.5 (48)	99	50
20-7	19.3 (47)	50	21.2 (44)	110	50	20.1 (46)	104	50	19.0 (46)	98	50
22-7	18.1 (46)	50	21.0 (46)	116	50	19.6 (47)	108	50	19.2 (48)	106	50
24-7	19.1 (46)	50	20.5 (45)	107	50	19.8 (47)	104	50	19.3 (48)	101	50
26-7	17.6 (46)	50	20.3 (44)	115	50	18.6 (46)	106	50	19.6 (47)	111	50
28-7	18.2 (47)	50	20.5 (47)	113	50	19.0 (47)	104	50	19.5 (46)	107	50
30-7	17.9 (48)	50	19.3 (44)	108	50	19.8 (49)	111	50	18.7 (48)	104	50
32-7	18.2 (48)	50	21.0 (47)	115	50	20.2 (49)	111	50	19.5 (48)	107	50
34-7	17.7 (47)	50	19.2 (46)	108	50	18.9 (47)	107	50	18.4 (47)	104	50
36-7	17.9 (47)	50	19.9 (48)	111	50	18.4 (50)	103	50	19.4 (49)	108	50
38-7	17.9 (48)	50	20.4 (48)	114	50	18.7 (48)	104	50	20.0 (47)	112	50
40-7	17.6 (47)	50	19.8 (49)	113	50	18.9 (50)	107	50	19.3 (48)	110	50
42-7	18.4 (48)	50	19.2 (47)	104	50	18.2 (49)	99	50	18.8 (48)	102	50
44-7	17.9 (47)	50	19.3 (48)	108	50	17.9 (49)	100	50	19.3 (47)	108	50
48-7	17.8 (49)	50	19.0 (47)	107	50	17.6 (49)	99	50	19.0 (48)	107	50
50-7	17.5 (49)	50	18.4 (49)	105	50	17.3 (49)	99	50	19.1 (49)	109	50
52-7	17.7 (49)	50	18.3 (48)	103	50	18.2 (50)	103	50	19.4 (49)	110	50
54-7	16.6 (49)	50	18.3 (48)	110	50	17.6 (50)	106	50	18.7 (49)	113	50
56-7	16.7 (49)	50	18.0 (49)	108	50	17.7 (50)	106	50	17.8 (48)	107	50
58-7	16.1 (50)	50	17.1 (50)	106	50	16.6 (49)	103	49	17.5 (50)	109	50
60-7	16.4 (49)	50	17.3 (49)	105	50	16.4 (49)	100	49	17.1 (50)	104	50
62-7	16.3 (50)	50	17.9 (50)	110	50	16.8 (49)	103	49	17.3 (50)	106	50
64-7	16.6 (50)	50	18.6 (50)	112	50	16.9 (49)	102	49	17.4 (50)	105	50
66-7	17.0 (50)	50	17.7 (49)	104	50	17.1 (49)	101	49	17.6 (50)	104	50
68-7	17.1 (50)	50	17.9 (48)	105	50	16.7 (49)	98	49	17.9 (50)	105	50
70-7	17.2 (50)	50	18.4 (50)	107	50	16.9 (49)	98	49	17.6 (50)	102	50
72-7	18.1 (49)	49	18.9 (50)	104	50	17.3 (49)	96	49	18.1 (50)	100	50
74-7	18.6 (49)	49	19.3 (50)	104	50	18.0 (49)	97	49	18.2 (48)	98	48
76-7	18.7 (49)	49	19.6 (50)	105	50	19.1 (47)	102	47	18.8 (48)	101	48
78-7	18.7 (48)	49	19.3 (49)	103	50	18.2 (46)	97	47	18.8 (48)	101	48
80-7	18.6 (48)	49	19.3 (50)	104	50	18.5 (47)	99	47	18.3 (48)	98	48
82-7	19.4 (49)	49	20.3 (49)	105	50	18.8 (46)	97	46	18.9 (48)	97	47
84-7	20.0 (49)	49	21.6 (47)	108	49	19.3 (45)	97	45	19.3 (45)	97	45
86-7	20.1 (49)	49	20.9 (47)	104	49	19.9 (45)	99	45	19.3 (44)	96	44
88-7	20.7 (47)	47	21.6 (47)	104	49	20.5 (45)	99	45	20.6 (44)	100	44
90-7	21.3 (43)	44	22.3 (47)	105	48	21.2 (44)	100	45	19.6 (44)	92	43
92-7	21.2 (43)	43	22.3 (47)	105	47	20.7 (44)	98	44	19.9 (41)	94	42
94-7	21.5 (43)	43	23.5 (44)	109	46	21.1 (43)	98	44	20.8 (39)	97	39
96-7	21.5 (42)	41	22.3 (45)	104	45	21.6 (43)	100	43	20.0 (39)	93	39
100-7	24.3 (39)	39	21.9 (41)	90	43	22.4 (41)	92	41	19.8 (31)	81	31
102-7	24.7 (37)	39	22.9 (40)	93	41	24.0 (38)	97	39	20.5 (26)	83	27
104-7	23.6 (39)	38	23.1 (36)	98	37	23.3 (36)	99	38	20.6 (24)	87	24

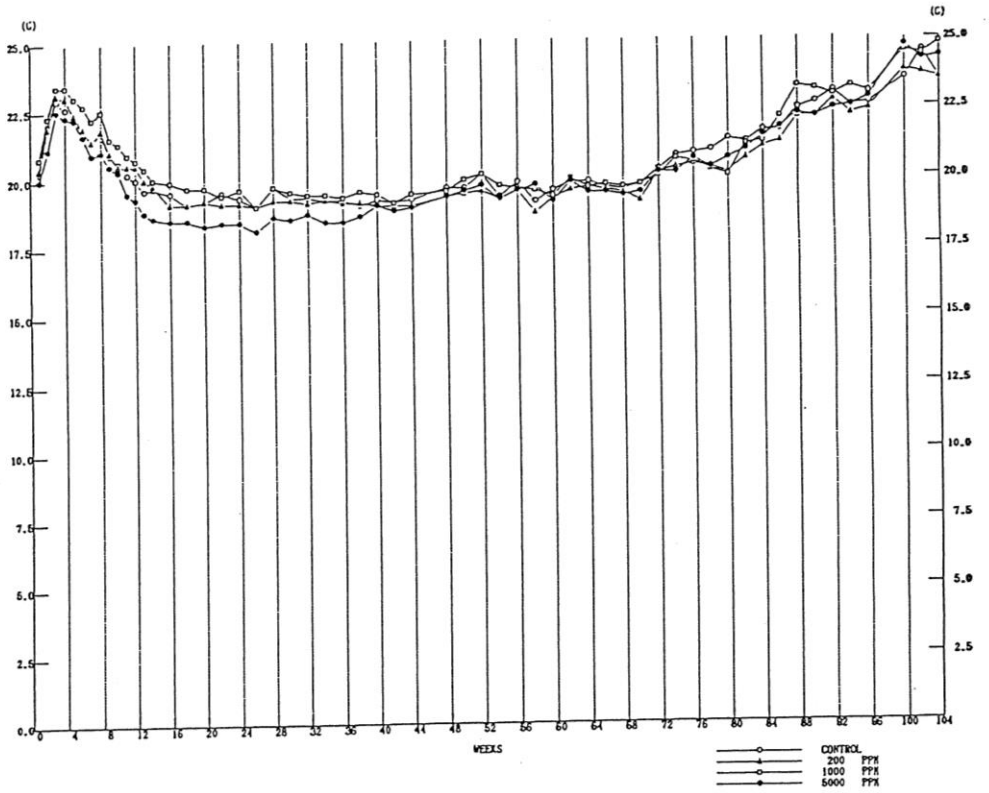


FIGURE 7 WATER CONSUMPTION : RAT:MALE

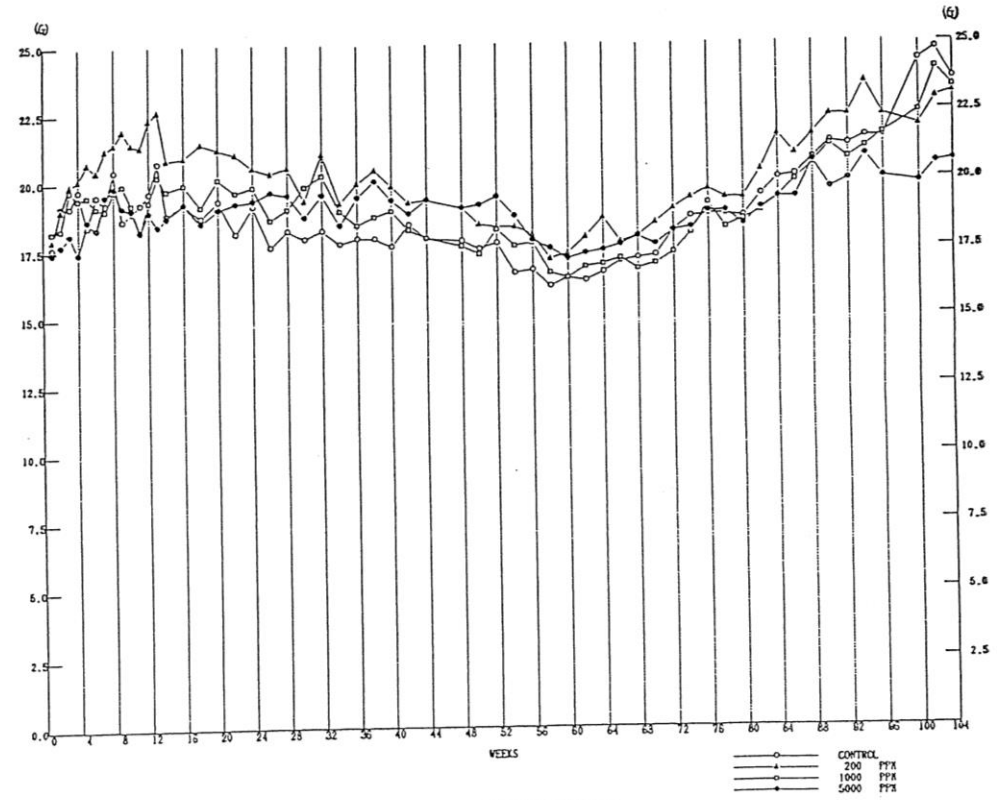


FIGURE 8 WATER CONSUMPTION : RAT:FEMALE

III-1-2 Hematology and Blood Biochemistry Examinations, and Urinalysis

(1) Hematology Examination

The results are listed in Appendices E 1 and 2 (the Summary Tables) and Appendices R 1 and 2 (the Individual Tables).

Among the males, the following was observed in the 5,000 ppm group: decreases in the red blood cell counts, hemoglobin concentration, hematocrit level, and the mean corpuscular volume, and an increase in platelet counts. The following was observed in the 1,000 ppm group: decreases in the red blood cell counts, hemoglobin concentration, and hematocrit level, and an increase in platelet counts. An increase in eosinophil percentage was observed in the 200 ppm group.

Among the females, the following was observed in the 5,000 ppm group: decreases in the hemoglobin concentration, hematocrit level, and the mean corpuscular volume, and an increase in platelet counts. A decrease in eosinophil percentage was observed in the 5,000 and 1,000 ppm groups.

(2) Blood Biochemistry Examination

The results are listed in Appendices F 1 and 2 (the Summary Tables) and Appendices S 1 and 2 (the Individual Tables).

Among the males in the 5,000 ppm group, the following was observed: increases in GOT, GPT, LDH, ALP, γ-GTP, CPK, phospholipid, potassium, and inorganic phosphorus, and decreases in glucose, total protein, and albumin.

Among the females in the 5,000 ppm group, the following was observed: increases in GOT, GPT, LDH, ALP, LAP, γ-GTP, CPK, total bilirubin, total cholesterol, phospholipid, and potassium, and a decrease in glucose and A/G ratio. In the 1,000 ppm group, a decrease in creatinine was observed.

(3) Urinalysis

The results are listed in Appendices G 1 and 2 (the Summary Tables) and Appendices T 1 and 2 (the Individual Tables).

Among the males, a decrease in pH level was noted in the 5,000 ppm group.

Among the females, a decrease in pH level and increased incidences of positive occult blood were noted in the 5,000 ppm group. A change in pH level and increased incidences of positive occult blood were also noted in the 1,000 ppm group.

III-1-3 Pathological Examination

(1) Necropsy

Findings observed at the time of necropsy are listed in Appendices H 1 to 4 (the Summary Tables) and Appendices U 1 and 2 (the Individual Tables). The findings characteristic to the administered groups in comparison to the control group and the findings of high incidence are described below.

Many non-surviving / moribund males had white dots / macules and nodules on the livers and peritoneal nodules. Males surviving at the time of the scheduled necropsy had high incidence rate of subcutaneous masses, liver nodules and cysts, coarse surface of the livers, peritoneal nodules, and ascitic fluid. Many non-surviving / moribund females had reddening of the lungs, white dots / macules on the livers, liver nodules, and ascitic fluid. Many females surviving at the time of the scheduled necropsy had subcutaneous masses, liver nodules, and renal granulation.

(2) Organ Weight

The actual weights of the organs and their ratios to body weight measured for all animals surviving until the time of the scheduled necropsy are listed in Appendices I 1 and 2 (the Summary Tables of the actual weights), Appendices J 1 and 2 (the Summary Tables of the actual weight to body weight ratio), Appendices V 1 and 2 (the Individual Tables of the actual weights), and Appendices W 1 and 2 (the Individual Tables of the actual weight to body weight ratio).

Among the males in the 5,000 ppm group, compared with the control group, the actual liver weight and ratio of the actual liver weight to the body weight, and the actual lung and kidney weights to the body weight had significantly higher values, while the actual weights of the adrenal gland (the right only), heart and brain had significantly lower values. Among the males in the 1,000 ppm group, the ratio of the actual liver weight to the body weight had a significantly higher value.

Among the females in the 5,000 ppm group, compared with the control group, the actual weights of liver and lung (the left only) and ratios of the actual liver and lung (the left only) weights to the body weights, the actual weight of the adrenal gland (the left only), and the ratios of the actual weights of the ovary (the left only), spleen, heart, kidney and brain to the body weights had significantly higher values, while the actual weight of the brain had a significantly lower value.

In the 5,000 ppm groups of both sexes, compared with the control groups, the body weights at the time of necropsy had significantly lower values.

(3) Histopathologic Examination

The results for nonneoplastic lesions are listed in Appendices K 1 to 4 (the Summary Tables). The results for neoplastic lesions are listed in Appendices L 1 and 2 (the total number of animals with tumors and number of tumors), Appendices M 1 and 2 (the incidence rates and time a necropsy was performed), Appendices N 1 and 2 (the results of the statistical analysis: Peto, Cochran-Armitage, and Fisher's tests). Individual tables of histopathologic examinations are listed in Appendices X 1 and 2.

Nasal Cavity

In the 5,000 ppm groups of both sexes (among the non-surviving / moribund animals and those surviving at the time of the scheduled necropsy), significant increases in the following incidences were observed: adhesion of the nasal concha, squamous metaplasia of the respiratory epithelium, respiratory epithelial metaplasia of the olfactory epithelium, hydropic degeneration and hardening of the lamina propria, atrophy of the olfactory epithelium, and nuclear enlargement of the olfactory (the supporting cells) and respiratory epithelium. The 5,000 ppm female group did not show a significant increase in incidence of nuclear enlargement of the respiratory epithelium, though it showed the increased incidence. In addition to these findings, males surviving at the time of the scheduled necropsy showed significant increases in calcinosis, inflammation of the squamous epithelium, and acute rhinitis. Non-surviving / moribund females showed a significant increase in incidence of thrombus, and females surviving at the time of the scheduled necropsy showed significant increases in multiplication of the nasal glands, inflammation of the squamous epithelium, and acute rhinitis. Meanwhile, among the animals surviving at the time of the scheduled necropsy in the 5,000 ppm groups, decreased incidences of eosinophilic changes in the olfactory epithelium among the males and decreased incidences of eosinophilic changes of the respiratory epithelium among the females were observed. Furthermore, non-surviving / moribund females in the 5,000 ppm group showed a significant difference in incidences of eosinophilic changes in the olfactory epithelium in comparison with the control group. On the other hand, respiratory epithelial metaplasia of the olfactory epithelium, hydropic degeneration and hardening of the lamina propria, atrophy of the olfactory epithelium, nuclear enlargement of the olfactory epithelium (the supporting cells), calcinosis, and multiplication of the nasal glands were observed on the dorsal wall of the nasal cavity or on the olfactory epithelium in the posterior half of the nasal cavity. Adhesion was observed in both the nasal concha in the anterior half and the ethmoturbinals in the posterior half of the nasal cavity. (Table 12)

TABLE 12 NUMBER OF RAT WITH SELECTED NASAL LESIONS

	Male				Female			
Group	Control	200 ppm	1000 ppm	5000 ppm	Control	200 ppm	1000 ppm	5000 ppm
Number of examined animal	50	50	50	50	50	50	50	50
Adhesion	1	2	5	48	6	2	3	46
Thrombus	47	45	41	12	23	29	25	7
Deposit of calcium				48			1	30
Inflammation				13				15
Squamous cell metaplasia				31				35
Squamous cell hyperplasia				2				5
Rhinitis				15				12
Eosinophilic change:olfactry epithelium	38	36	37	22	42	41	43	35
Eosinophilic change:respiratory epithelium	9	6	8	2	19	18	20	3
Respiratory metaplasia	12	11	20	43	2		2	42
Hydropic change:lamina propria				46				46
Sclerosis:lamina propria			1	44				48
Atrophy:olfactory epithelium				36			1	40
Nuclear enlargement:olfactory epithelium			5	38			28	39
Nuclear enlargement:respiratory epithelium				26				13
Proliferation:nasal gland				3				11
Squamous cell carcinoma				3				7
Sarcoma:NOS				2				
Rhabdomyosarcoma				1				
Ethesioneuroepithcliona				1				1

Among neoplastic lesions, an increasing trend in incidence of squamous cell carcinoma in all of the dosed groups of both sexes was revealed by the Peto tests (the mortality method and the mortality method + the prevalence method) and the Cochrane–Armitage test. The increased incidence rate in the 5,000 ppm female group was revealed by the Fisher's exact test. In the 5,000 ppm groups, rhabdomyosarcoma, sarcoma NOS, and esthesioneuroepithelioma among the males and esthesioneuroepithelioma were observed among the females. After statistical processing of adding the numbers of squamous cell carcinoma and the above tumors, the Peto tests (the mortality method, the prevalence method, and the mortality method + the prevalence method) and the Cochrane–Armitage test revealed an increasing trend in both sexes, and the Fisher's exact test revealed the increased incidence rate in the 5,000 ppm groups. (Table 13 and 14)

TABLE 13 NEOPLASTIC LESIONS (NASAL CAVITY) INCIDENCE AND STATISTICAL ANALYSIS : RAT:MALE

Group Name	Control	200 ppm	1000 ppm	5000 ppm
SITE : nasal cavity TUMOUR : squamous cell carcinoma				
Overall Rates(a)	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)	3/50 (6.0)
Adjusted Rates(b)	0.0	0.0	0.0	3.23
Terminal Rates(c)	0/40 (0.0)	0/45 (0.0)	0/35 (0.0)	0/22 (0.0)
Standard Rates(d)	P=0.0037##?			
Prevalence Rates(d)	P=0.1287			
Combind analysis(d)	P=0.0002##?			
Cochran-Armitage Test(e)	P=0.0030##			
Fisher Exact Test(e)		P=0.5000	P=0.5000	P=0.1325
SITE : nasal cavity TUMOUR : rhabdomyosarcoma				
Overall Rates(a)	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)	1/50 (2.0)
Adjusted Rates(b)	0.0	0.0	0.0	4.35
Terminal Rates(c)	0/40 (0.0)	0/45 (0.0)	0/35 (0.0)	1/22 (4.5)
Standard Rates(d)	P=------			
Prevalence Rates(d)	P=0.1010			
Combind analysis(d)	P=------			
Cochran-Armitage Test(e)	P=0.0879			
Fisher Exact Test(e)		P=0.5000	P=0.5000	P=0.4950
SITE : nasal cavity TUMOUR : sarcoma:NOS				
Overall Rates(a)	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)	2/50 (4.0)
Adjusted Rates(b)	0.0	0.0	0.0	4.35
Terminal Rates(c)	0/40 (0.0)	0/45 (0.0)	0/35 (0.0)	2/22 (4.5)
Standard Rates(d)	P=0.1347			
Prevalence Rates(d)	P=0.1010			
Combind analysis(d)	P=0.0036##?			
Cochran-Armitage Test(e)	P=0.0155#			
Fisher Exact Test(e)		P=0.5000	P=0.5000	P=0.2574
SITE : nasal cavity TUMOUR : ethesioneuroepithelioma				
Overall Rates(a)	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)	1/50 (2.0)
Adjusted Rates(b)	0.0	0.0	0.0	0.0
Terminal Rates(c)	0/40 (0.0)	0/45 (0.0)	0/35 (0.0)	0/22 (0.0)
Standard Rates(d)	P=0.1602			
Prevalence Rates(d)	P=------			
Combind analysis(d)	P=0.1602			
Cochran-Armitage Test(e)	P=0.0879			
Fisher Exact Test(e)		P=0.5000	P=0.5000	P=0.4950
SITE : nasal cavity TUMOUR : ALL TUMOR				
Overall Rates(a)	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)	7/50 (14.0)
Adjusted Rates(b)	0.0	0.0	0.0	10.0
Terminal Rates(c)	0/40 (0.0)	0/45 (0.0)	0/35 (0.0)	2/22 (9.1)
Standard Rates(d)	P<0.0001##?			
Prevalence Rates(d)	P=0.0002##?			
Combind analysis(d)	P<0.0001##?			
Cochran-Armitage Test(e)	P<0.0001##			
Fisher Exact Test(e)		P=0.5000	P=0.5000	P=0.0101#

TABLE 14 NEOPLASTIC LESIONS (NASAL CAVITY) INCIDENCE AND STATISTICAL ANALYSIS : RAT:FEMALE

Group Name	Control	200 ppm	1000 ppm	5000 ppm
SITE : nasal cavity TUMOUR : squamous cell carcinoma				
Overall Rates(a)	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)	7/50 (14.0)
Adjusted Rates(b)	0.0	0.0	0.0	2.78
Terminal Rates(c)	0/38 (0.0)	0/37 (0.0)	2/38 (0.0)	0/24 (0.0)
Standard Rates(d)	P=0.0001‡‡			
Prevalence Rates(d)	P=0.0925			
Combind analysis(d)	P<0.0001‡‡			
Cochran-Armitage Test(e)	P<0.0001‡‡			
Fisher Exact Test(e)		P=0.5000	P=0.5000	P=0.0101‡
SITE : nasal cavity TUMOUR : ethesioncuroepithelioma				
Overall Rates(a)	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)	1/50 (2.0)
Adjusted Rates(b)	0.0	0.0	0.0	4.17
Terminal Rates(c)	0/38 (0.0)	0/37 (0.0)	0/38 (0.0)	1/24 (4.2)
Standard Rates(d)	P=------			
Prevalence Rates(d)	P=0.1082			
Combind analysis(d)	P=------			
Cochran-Armitage Test(e)	P=0.0879			
Fisher Exact Test(e)		P=0.5000	P=0.5000	P=0.4950
SITE : nasal cavity TUMOUR : ALL TUMOR				
Overall Rates(a)	0/50 (0.0)	0/50 (0.0)	0/50 (0.0)	8/50 (16.0)
Adjusted Rates(b)	0.0	0.0	0.0	5.56
Terminal Rates(c)	0/38 (0.0)	0/37 (0.0)	0/38 (0.0)	1/24 (4.2)
Standard Rates(d)	P<0.0001‡‡?			
Prevalence Rates(d)	P=0.0096‡‡?			
Combind analysis(d)	P<0.0001‡‡?			
Cochran-Armitage Test(e)	P<0.0001‡‡			
Fisher Exact Test(e)		P=0.5000	P=0.5000	P=0.0054‡‡

Liver

Non-surviving / moribund males in the 5,000 ppm group showed increased incidences of spongiosis hepatis. The males surviving at the time of the scheduled necropsy in the 5,000 and 1,000 ppm groups showed increased incidences of spongiosis hepatis, hyperplasia, and clear, acidophilic and mixed cell focus. The males surviving at the time of the scheduled necropsy in the 5,000 ppm group showed increased incidences of basophilic cell focus, and decreased incidences of in bile duct hyperplasia. Non-surviving / moribund females in the 5,000 ppm group showed increased incidences of hyperplasia and basophilic cell focus, and decreased incidences of leukemic cell infiltration. Non-surviving / moribund females in the 200 ppm group showed increased incidences of bile duct hyperplasia. The females surviving at the time of the scheduled necropsy showed increased incidences of spongiosis hepatis, cyst formation, hyperplasia, and mixed cell focus, and decreased incidences of in basophilic cell focus in the 5,000 ppm group. The females surviving at the time of the scheduled necropsy in the 1,000 ppm group showed a significant difference in incidences of vacuolar cell focus. (Table 15)

TABLE 15 NUMBER OF RAT WITH SELECTED LIVER LESIONS

	Male				Female			
Group	Control	200 ppm	1000 ppm	5000 ppm	Control	200 ppm	1000 ppm	5000 ppm
Number of examined animal	50	50	50	50	50	50	50	50
Hyperplasia	3	2	10	24	3	2	11	47
Clear cell focus	3	3	9	8	1	1	5	4
Acidophilic cell focus	12	8	6	4	1	1		1
Basophilic cell focus	7	11	6	16	22	27	29	8
Mixed cell focus	2	8	14	13	1	1	3	11
Spongiosis hepatis	12	20	25	40			1	20
Bile duct hyperplasia	50	49	47	48	19	25	17	5
Hepatocellular adenoma		2	4	24	1		5	38
Hepatocellular carcinoma				14				10
Hemangioendothelioma								1

Among neoplastic lesions, an increasing trend in incidence of hepatocellular adenoma in all of the dosed groups of both sexes was revealed by the Peto tests (the mortality method, and the mortality method + the prevalence method) and the Cochrane–Armitage test. The increased incidence rate in the 5,000 ppm groups of both sexes was revealed by the Fisher's exact test. An increasing trend in incidence of hepatoma in all of the dosed groups of both sexes was revealed by the Peto tests (the prevalence method for both sexes, the mortality method among the females, and the mortality method + the prevalence method) and the Cochrane–Armitage test. The increased incidence rate in the 5,000 ppm group of both sexes was revealed by the Fisher's exact test. After statistical processing of adding the numbers of hepatocellular adenoma and hepatoma, the Peto tests (the prevalence method for both sexes, the mortality method + the prevalence method, and the mortality method for females) and the Cochrane–Armitage test revealed the increasing trend. The Fisher's exact test revealed the increased incidence rate in the 5,000 ppm groups.
(Tables 16 and 17)

TABLE 16 NEOPLASTIC LESIONS (LIVER) INCIDENCE AND STATISTICAL ANALYSIS : RAT:MALE

Group Name	Control	200 ppm	1000 ppm	5000 ppm
SITE : liver TUMOUR : hepatocellular adenoma				
Overall Rates(a)	0/50 (0.0)	2/50 (4.0)	4/49 (8.2)	24/50 (48.0)
Adjusted Rates(b)	0.0	4.44	11.76	70.37
Terminal Rates(c)	0/40 (0.0)	2/45 (4.4)	4/34 (11.8)	15/22 (68.2)
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=0.2574	P=0.0662	P<0.0001**
SITE : liver TUMOUR : hepatocellular carcinoma				
Overall Rates(a)	0/50 (0.0)	0/50 (0.0)	0/49 (0.0)	14/50 (28.0)
Adjusted Rates(b)	0.0	0.0	0.0	37.50
Terminal Rates(c)	0/40 (0.0)	0/45 (0.0)	0/34 (0.0)	8/22 (36.4)
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=0.5000	P=0.5000	P=0.0002**
SITE : liver TUMOUR : hepatocellular adenoma, hepatocellular carcinoma				
Overall Rates(a)	0/50 (0.0)	2/50 (4.0)	4/49 (8.2)	33/50 (66.0)
Adjusted Rates(b)	0.0	4.44	11.76	85.19
Terminal Rates(c)	0/40 (0.0)	2/45 (4.4)	4/34 (11.8)	18/22 (81.8)
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=0.2574	P=0.0662	P<0.0001**

TABLE 17 NEOPLASTIC LESIONS (LIVER) INCIDENCE AND STATISTICAL ANALYSIS : RAT:FEMALE

Group Name	Control	200 ppm	1000 ppm	5000 ppm
SITE : liver TUMOUR : hepatocellular adenoma				
Overall Rates(a)	1/50 (2.0)	0/50 (0.0)	5/50 (10.0)	38/50 (76.0)
Adjusted Rates(b)	2.56	0.0	12.50	96.00
Terminal Rates(c)	1/38 (2.0)	0/37 (0.0)	4/38 (10.5)	23/24 (95.8)
Standard Rates(d)	P=0.1387			
Prevalence Rates(d)	P<0.0001‡‡?			
Combind analysis(d)	P<0.0001‡‡?			
Cochran-Armitage Test(e)	P<0.0001‡‡			
Fisher Exact Test(e)		P=0.4950	P=0.1210	P<0.0001‡‡
SITE : liver TUMOUR : hepatocellular carcinoma				
Overall Rates(a)	1/50 (2.0)	0/50 (0.0)	0/50 (0.0)	10/50 (20.0)
Adjusted Rates(b)	2.56	0.0	0.0	25.00
Terminal Rates(c)	1/38 (2.0)	0/37 (0.0)	0/38 (0.0)	6/24 (25.0)
Standard Rates(d)	P=0.0013‡‡?			
Prevalence Rates(d)	P<0.0001‡‡?			
Combind analysis(d)	P<0.0001‡‡?			
Cochran-Armitage Test(e)	P<0.0001‡‡			
Fisher Exact Test(e)		P=0.4950	P=0.5000	P=0.0016‡‡
SITE : liver TUMOUR : hepatocellular adenoma,hepatocellular carcinoma				
Overall Rates(a)	1/50 (2.0)	0/50 (0.0)	5/50 (10.0)	40/50 (80.0)
Adjusted Rates(b)	2.56	0.0	12.50	96.00
Terminal Rates(c)	1/38 (2.6)	0/37 (0.0)	4/38 (10.5)	23/24 (95.8)
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=0.4950	P=0.1210	P<0.0001‡‡

Subcutis

An increasing trend in incidence of fibroma in the male dosed groups was revealed by the Peto test (the prevalence method) and the Cochrane–Armitage test. (Table 18)

TABLE 18 NEOPLASTIC LESIONS (SUBCUTIS) INCIDENCE AND STATISTICAL ANALYSIS : RAT:MALE

Group Name	Control	200 ppm	1000 ppm	5000 ppm
SITE : subcutis TUMOUR : fibroma				
Overall Rates(a)	5/50 (10.0)	3/50 (6.0)	5/50 (10.0)	12/50 (24.0)
Adjusted Rates(b)	10.64	6.67	14.29	36.67
Terminal Rates(c)	3/40 (7.5)	3/45 (6.7)	5/35 (14.3)	8/22 (36.4)
Standard Rates(d)	P=------			
Prevalence Rates(d)	P=0.0005‡‡			
Combind analysis(d)	P=------			
Cochran-Armitage Test(e)	P=0.0046‡‡			
Fisher Exact Test(e)		P=0.3790	P=0.3710	P=0.0942

Mammary Gland

An increasing trend in incidence of fibroadenoma in the male dosed groups was revealed by the Peto test (the prevalence method) and the Cochrane–Armitage test. An increasing trend in incidence of adenoma in the female dosed groups was revealed by the Peto test (the prevalence method) and the Cochrane–Armitage test. The increased incidence rate in the 5,000 ppm female group was revealed by the Fisher's exact test. (Tables 19 and 20)

TABLE 19 NEOPLASTIC LESIONS (MAMMARY GLAND) INCIDENCE AND STATISTICAL ANALYSIS : RAT:MALE

Group Name	Control	200 ppm	1000 ppm	5000 ppm
SITE : mammary gland TUMOUR : fibroadenoma				
Overall Rates(a)	1/50 (2.0)	1/50 (2.0)	0/50 (0.0)	4/50 (8.0)
Adjusted Rates(b)	2.50	2.22	0.0	17.39
Terminal Rates(c)	1/40 (2.5)	1/45 (2.2)	0/35 (0.0)	4/22 (18.2)
Standard Rates(d)	P=------			
Prevalence Rates(d)	P=0.0027‡‡			
Combind analysis(d)	P=------			
Cochran-Armitage Test(e)	P=0.0258‡			
Fisher Exact Test(e)		P=0.2475	P=0.4950	P=0.1998

TABLE 20 NEOPLASTIC LESIONS (MAMMARY GLAND) INCIDENCE AND STATISTICAL ANALYSIS : RAT:FEMALE

Group Name	Control	200 ppm	1000 ppm	5000 ppm
SITE : mammary gland TUMOUR : adenoma				
Overall Rates(a)	6/50 (12.0)	7/50 (14.0)	10/50 (20.0)	16/50 (32.0)
Adjusted Rates(b)	12.24	16.67	23.68	34.29
Terminal Rates(c)	4/38 (10.5)	5/37 (13.5)	9/38 (23.7)	7/24 (29.2)
Standard Rates(d)	P=------			
Prevalence Rates(d)	P=0.0036‡‡			
Combind analysis(d)	P=------			
Cochran-Armitage Test(e)	P=0.0064‡‡			
Fisher Exact Test(e)		P=0.4863	P=0.2557	P=0.0430‡

Peritoneum

An increasing trend in incidence of mesothelioma in the male dosed groups was revealed by the Peto tests (the mortality method, the prevalence method, and the mortality method + the prevalence method) and the Cochrane–Armitage test. The increased incidence rate in the 5,000 ppm group was revealed by the Fisher's exact test. (Table 21)

TABLE 21 NEOPLASTIC LESIONS (PERITONEUM) INCIDENCE AND STATISTICAL ANALYSIS : RAT:MALE

Group Name	Control	200 ppm	1000 ppm	5000 ppm
SITE : peritoneum TUMOUR : mesothelioma				
Overall Rates(a)	2/50 (4.0)	2/50 (4.0)	5/50 (10.0)	28/50 (56.0)
Adjusted Rates(b)	2.50	2.22	11.43	56.52
Terminal Rates(c)	1/40 (2.5)	1/45 (4.4)	4/35 (11.4)	12/22 (54.5)
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=0.3088	P=0.2425	P<0.0001‡‡

Thyroid Gland

A decreasing trend in incidence of both C-cell adenoma and C-cell cancer tumor combined in the male dosed groups was revealed by the Cochrane–Armitage test.

Pancreas

A decreased incidence rate of pancreatic adenoma in the 5,000 ppm male group was revealed by the Fisher's exact test.

Kidney

A significant increase in incidence of nuclear enlargement of the proximal renal tubules was noted among the non-surviving / moribund animals and those surviving at the time of the scheduled necropsy in the 5,000 ppm groups of both sexes and the females surviving at the time of the scheduled necropsy in the 1,000 ppm group. A decrease in incidence of chronic nephropathy was noted among the non-surviving / moribund females in the 1,000 ppm group. (Table 22)

TABLE 22 NUMBER OF RAT WITH SELECTED KIDNEY LESIONS

	Male				Female			
Group	Control	200 ppm	1000 ppm	5000 ppm	Control	200 ppm	1000 ppm	5000 ppm
Number of examined animal	50	50	50	50	50	50	50	50
Nuclear enlargement:proximal tubule				50			6	39
Adenoma			2					
Lipoma		1		1	1		1	
Schwannoma				1				

Spleen

An increase in incidence of fibrosis was noted among the males surviving at the time of the scheduled necropsy in the 5,000 ppm group. A decrease in incidences of hemosiderosis and extramedullary hematopoiesis was noted among the males surviving at the time of the scheduled necropsy in all of the dosed groups. A decrease in incidence of extramedullary hematopoiesis was noted among the females surviving at the time of the scheduled necropsy in the 200 ppm group.

Lung

A decrease in incidence of leukemic cell infiltration was noted among the non-surviving / moribund females in the 5,000 ppm group.

Bone Marrow

A decrease in incidence of leukemic cell infiltration was noted among the non-surviving / moribund females in the 5,000 ppm group.

Heart

A decrease in incidence of myocardial fibrosis was noted among the non-surviving / moribund females in the 5,000 ppm group.

Arteries

A decrease in incidence of arteritis was noted among the males surviving at the time of the scheduled necropsy in the 1,000 ppm group.

(4) Causes of Deaths

Causes of deaths pathologically determined for the non-surviving / moribund animals are listed in Table 23.

More males in the 5,000 ppm group than those in the control group died from mesothelioma or nasal cavity tumor which was considered to be a cause of death. There were also two males died from nonneoplastic lesions in the nasal cavity. Many females in the 5,000 ppm group died from nasal cavity tumor or hepatic tumor which was considered to be a cause of death. There were a slightly high number of females died also from nonneoplastic lesions in the livers.

TABLE 23 CAUSE OF DEATH :RAT

Group		Male				Female			
		Control	200ppm	1000ppm	5000ppm	Control	200ppm	1000ppm	5000ppm
Number of dead/moribund animal		10	5	15	28	12	13	12	26
Hepatic lesion			1		1	1	1		4
Nasal lesion					2				2
Chronic nephropathy				1			1		
Pneumonia		1							
Tumor death	subcutis			2	1				
	nasal				4				6
	liver								4
	pituitary	2	1	4	1	2	5	1	3
	uterus							4	
	mammary gland						1	2	1
	brain		1		1				
	bone			2			1		
	mesothelioma	1	1	1	12	1			
	leukemia	2	1	3	4	8	2	5	5
	others	3		2	2		1		
No microscopical confirmation		1					1		1

III-2 Carcinogenicity Study in Mice

III-2-1 Observation of Animal Conditions

(1) Survival

Survival data in each group during the administration period is listed in Tables 24 and 25 and Figure 9 and 10.

Among the males, there was no significant difference in number of survivors in all of the dosed groups during the administration period, in comparison with the control group.

Among the females, there was a significant decrease in number of survivors in the 8,000 ppm group every week in the period of Week 62 and later, and in the 2,000 ppm group in Week 104, in comparison with the control group.

Among the males, the first death occurred in Week 38 in the 8,000 ppm group, Week 62 in the 2,000 ppm group, Week 48 in the 500 ppm group, and Week 62 in the control group. Among the females, the first death occurred in Week 32 in the 8,000 ppm group, Week 48 in the 2,000 ppm group, Week 72 in the 500 ppm, and Week 56 in the control group.

(2) General Symptoms

The observed symptoms of the non-survivors in each group from the start of administration to death, and those of the survivors in the late stage of administration (Weeks 92–104) are listed in Tables 26 and 27.

There was no difference in incidence of mass in the non-survivors between each dosed group of both sexes and the control group. However, hunchback, piloerection, wasting, and abnormal respiration were observed in many animals in the 8,000 ppm groups of both sexes. Abnormal nasal noises were observed only in the 8,000 ppm female group (2/45).

There was no difference in incidence of mass in survivors between each dosed group of both sexes and the control group. However, many animals in the 8,000 ppm groups of both sexes had hunchback, piloerection, and abnormal respiration, which were very similar conditions observed in dying animals as a sign of worsening health conditions.

TABLE 24 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN MALE MOUSE

Week-Day on Study	Control Av.Wt.	No.of Surviv.	500 ppm Av.Wt.	% of cont.	No.of Surviv.	2000 ppm Av.Wt.	% of cont.	No.of Surviv.	8000 ppm Av.Wt.	% of cont.	No.of Surviv.
0-0	23.9 (50)	50	23.9 (50)	100	50	23.9 (50)	100	50	23.9 (50)	100	50
1-7	25.7 (50)	50	25.7 (50)	100	50	25.5 (50)	99	50	25.4 (50)	99	50
2-7	27.0 (50)	50	26.9 (50)	100	50	26.7 (50)	99	50	26.5 (50)	98	50
3-7	27.9 (50)	50	27.7 (50)	99	50	27.7 (50)	99	50	27.3 (50)	98	50
4-7	29.3 (50)	50	29.1 (50)	99	50	28.8 (50)	98	50	28.4 (50)	97	50
5-7	30.0 (50)	50	30.1 (50)	100	50	29.9 (50)	100	50	29.4 (50)	98	50
6-7	31.5 (50)	50	31.1 (50)	99	50	31.0 (50)	98	50	30.0 (50)	95	50
7-7	32.2 (50)	50	32.2 (50)	100	50	32.0 (50)	99	50	30.8 (50)	96	50
8-7	33.0 (50)	50	32.7 (50)	99	50	32.6 (50)	99	50	31.1 (50)	94	50
9-7	34.0 (50)	50	33.8 (50)	99	50	33.5 (50)	99	50	32.8 (50)	96	50
10-7	34.8 (50)	50	34.6 (50)	99	50	34.6 (50)	99	50	33.5 (50)	96	50
11-7	35.4 (50)	50	35.4 (50)	100	50	35.5 (50)	100	50	33.9 (50)	96	50
12-7	36.2 (50)	50	36.2 (50)	100	50	36.3 (50)	100	50	33.9 (50)	94	50
13-7	37.3 (50)	50	37.2 (50)	100	50	36.9 (50)	99	50	35.3 (50)	95	50
14-7	38.4 (50)	50	38.0 (50)	99	50	37.9 (50)	99	50	36.2 (50)	94	50
16-7	38.9 (50)	50	38.9 (50)	100	50	38.7 (50)	99	50	36.9 (50)	95	50
18-7	40.5 (50)	50	40.4 (50)	100	50	40.2 (50)	99	50	38.1 (50)	94	50
20-7	41.4 (50)	50	41.3 (50)	100	50	41.2 (50)	100	50	38.8 (50)	94	50
22-7	42.5 (50)	50	42.4 (50)	100	50	42.1 (50)	99	50	39.9 (50)	94	50
24-7	43.5 (50)	50	43.6 (50)	100	50	43.4 (50)	100	50	40.7 (50)	94	50
26-7	45.0 (50)	50	44.5 (50)	99	50	44.0 (50)	98	50	41.6 (50)	92	50
28-7	45.6 (50)	50	44.7 (50)	98	50	44.6 (50)	98	50	41.8 (50)	92	50
30-7	46.4 (50)	50	45.9 (50)	99	50	45.5 (50)	98	50	43.0 (50)	93	50
32-7	46.6 (50)	50	46.5 (50)	100	50	46.2 (50)	99	50	43.3 (50)	93	50
34-7	47.1 (50)	50	47.0 (50)	100	50	46.6 (50)	99	50	43.8 (50)	93	50
36-7	47.3 (50)	50	47.2 (50)	100	50	46.9 (50)	99	50	44.2 (50)	93	50
38-7	48.1 (50)	50	47.9 (50)	100	50	47.8 (50)	99	50	44.7 (49)	93	49
40-7	48.6 (50)	50	48.1 (50)	99	50	47.9 (50)	99	50	45.3 (49)	93	49
42-7	49.5 (50)	50	49.4 (50)	100	50	49.5 (50)	100	50	46.6 (49)	94	49
44-7	50.0 (50)	50	50.5 (50)	101	50	50.1 (50)	100	50	47.3 (49)	95	49
46-7	50.5 (50)	50	50.5 (50)	100	50	50.2 (50)	99	50	47.3 (49)	94	49
48-7	50.6 (50)	50	50.5 (49)	100	49	50.5 (50)	100	50	47.5 (48)	94	48
50-7	50.8 (50)	50	51.5 (49)	101	49	50.6 (50)	100	50	47.6 (48)	94	48
52-7	51.7 (50)	50	51.8 (48)	100	48	51.4 (50)	99	50	47.8 (48)	92	48
54-7	51.8 (50)	50	52.3 (48)	101	48	51.6 (50)	100	50	47.9 (48)	92	48
56-7	52.0 (50)	50	52.4 (48)	101	48	51.8 (50)	100	50	48.3 (48)	93	48
58-7	52.1 (50)	50	52.2 (46)	100	46	52.1 (50)	100	50	48.0 (48)	92	48
60-7	52.3 (50)	50	52.4 (46)	100	46	52.2 (50)	100	50	48.2 (46)	92	46
62-7	52.3 (48)	48	52.4 (45)	100	45	52.8 (49)	101	49	48.1 (46)	92	46
64-7	52.1 (47)	47	53.0 (45)	102	45	52.8 (48)	101	48	48.0 (46)	92	46
66-7	52.3 (46)	46	53.0 (45)	101	45	53.0 (48)	101	48	47.2 (46)	90	46
68-7	52.5 (46)	46	53.0 (45)	101	45	53.0 (48)	101	48	46.7 (45)	89	44
70-7	52.9 (45)	45	53.5 (45)	101	45	53.2 (47)	101	47	46.9 (43)	89	43
72-7	53.2 (45)	45	53.4 (45)	100	45	53.5 (46)	101	46	46.5 (43)	87	43
74-7	53.7 (44)	44	53.5 (44)	100	44	53.5 (46)	100	45	45.9 (43)	85	43
76-7	53.6 (44)	44	53.8 (43)	100	43	53.7 (45)	100	45	45.6 (42)	85	42
78-7	54.0 (44)	44	54.0 (42)	100	42	53.3 (45)	99	45	43.8 (42)	81	42
80-7	53.9 (44)	44	53.9 (42)	100	42	53.1 (45)	99	45	42.6 (42)	79	42
82-7	53.7 (44)	44	53.5 (42)	100	42	52.9 (44)	99	44	40.9 (42)	76	42
84-7	53.6 (44)	44	53.2 (42)	99	42	52.7 (42)	98	42	39.6 (41)	74	41
86-7	53.3 (43)	43	52.4 (41)	98	40	51.5 (42)	97	42	37.9 (40)	71	40
88-7	53.1 (42)	42	53.5 (39)	101	39	50.7 (40)	95	40	37.0 (38)	70	38
90-7	53.1 (40)	40	53.6 (39)	101	39	49.6 (40)	93	40	36.4 (36)	69	36
92-7	53.8 (37)	37	53.0 (38)	99	38	48.7 (38)	91	37	35.7 (32)	66	32
94-7	53.1 (36)	36	52.6 (38)	99	38	49.0 (34)	92	34	34.9 (30)	66	30
96-7	52.4 (36)	36	52.4 (38)	100	38	48.8 (32)	93	32	33.3 (30)	64	30
98-7	51.9 (36)	35	51.6 (38)	99	38	48.6 (29)	94	29	32.9 (27)	63	27
100-7	52.6 (33)	33	51.7 (36)	98	36	48.1 (28)	91	28	31.9 (26)	61	26
102-7	52.1 (33)	33	51.2 (35)	98	34	48.2 (26)	93	26	31.2 (26)	60	26
104-7	52.9 (31)	31	51.5 (33)	97	33	48.3 (25)	91	25	30.3 (26)	57	26

No. of Survivars : Significant difference ; * : P≦0.05 ** : P≦0.05 Test of CHI SQUARE

TABLE 25 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN FEMALE MOUSE

Week-Day on Study	Control Av.Wt.	No.of Surviv.	500 ppm Av.Wt.	% of cont.	No.of Surviv.	2000 ppm Av.Wt.	% of cont.	No.of Surviv.	8000 ppm Av.Wt.	% of cont.	No.of Surviv.	
0-0	19.3 (50)	50	19.3 (50)	100	50	19.3 (50)	100	50	19.3 (50)	100	50	
1-7	20.3 (50)	50	20.8 (50)	102	50	20.4 (50)	100	50	20.3 (50)	100	50	
2-7	21.7 (50)	50	21.7 (50)	100	50	21.5 (50)	99	50	21.6 (50)	100	50	
3-7	22.4 (50)	50	22.5 (50)	100	50	22.4 (50)	100	50	21.9 (50)	98	50	
4-7	23.2 (50)	50	23.4 (50)	101	50	22.9 (50)	99	50	22.6 (50)	97	50	
5-7	23.8 (50)	50	24.1 (50)	101	50	24.0 (50)	101	50	23.3 (50)	98	50	
6-7	24.9 (50)	50	24.8 (50)	100	50	24.4 (50)	98	50	23.8 (50)	96	50	
7-7	24.8 (50)	50	25.0 (50)	101	50	24.8 (50)	100	50	23.9 (50)	96	50	
8-7	25.7 (50)	50	26.6 (50)	104	50	25.7 (50)	100	50	24.6 (50)	96	50	
9-7	25.7 (50)	50	26.6 (50)	104	50	26.2 (50)	102	50	25.2 (50)	98	50	
10-7	26.6 (50)	50	27.2 (50)	102	50	26.9 (50)	101	50	25.8 (50)	97	50	
11-7	27.2 (50)	50	27.3 (50)	100	50	27.3 (50)	100	50	26.0 (50)	96	50	
12-7	27.4 (50)	50	27.3 (50)	100	50	27.7 (50)	101	50	26.3 (50)	96	50	
13-7	28.2 (50)	50	28.1 (50)	100	50	28.2 (50)	100	50	26.4 (50)	94	50	
14-7	28.9 (50)	50	28.8 (50)	100	50	29.0 (50)	100	50	27.3 (50)	94	50	
16-7	28.7 (50)	50	29.2 (50)	102	50	29.1 (50)	101	50	27.2 (50)	95	50	
18-7	30.3 (50)	50	30.2 (50)	100	50	30.3 (50)	100	50	28.3 (50)	93	50	
20-7	30.9 (50)	50	30.1 (50)	97	50	30.1 (50)	97	50	28.2 (50)	91	50	
22-7	31.4 (50)	50	31.5 (50)	100	50	31.2 (50)	99	50	28.8 (50)	92	50	
24-7	31.6 (50)	50	31.8 (50)	101	50	31.5 (50)	100	50	29.2 (50)	92	50	
26-7	32.0 (50)	50	32.2 (50)	101	50	32.0 (50)	100	50	29.5 (50)	92	50	
28-7	32.5 (50)	50	32.7 (50)	101	50	32.4 (50)	100	50	29.7 (50)	91	50	
30-7	33.0 (50)	50	33.2 (50)	101	50	32.9 (50)	100	50	30.3 (50)	92	50	
32-7	33.7 (50)	50	34.1 (50)	101	50	33.2 (50)	99	50	30.7 (49)	91	49	
34-7	33.6 (50)	50	34.1 (50)	101	50	33.1 (50)	99	50	30.2 (49)	90	49	
36-7	33.8 (50)	50	34.1 (50)	101	50	33.4 (50)	99	50	30.2 (49)	89	49	
38-7	34.9 (50)	50	34.2 (50)	98	50	33.7 (50)	97	50	30.3 (49)	87	49	
40-7	34.6 (50)	50	34.4 (50)	99	50	33.7 (50)	97	50	30.0 (49)	87	49	
42-7	36.0 (50)	50	35.6 (50)	99	50	35.4 (50)	98	50	30.3 (49)	84	49	
44-7	36.0 (50)	50	35.9 (50)	100	50	36.0 (50)	100	50	30.5 (49)	85	49	
46-7	36.6 (50)	50	36.6 (50)	100	50	36.1 (50)	99	50	30.2 (49)	83	49	
48-7	37.1 (50)	50	36.8 (50)	99	50	36.8 (49)	99	49	30.0 (48)	81	48	
50-7	37.3 (50)	50	36.8 (50)	99	50	37.2 (48)	100	48	29.8 (48)	80	48	
52-7	38.9 (50)	50	38.6 (50)	99	50	38.8 (48)	100	48	30.3 (48)	78	48	
54-7	38.2 (50)	50	38.6 (50)	101	50	38.3 (48)	100	48	29.4 (48)	77	48	
56-7	38.5 (49)	49	38.7 (50)	101	50	38.6 (48)	100	47	29.0 (47)	75	47	
58-7	39.0 (49)	49	39.1 (50)	100	50	38.9 (47)	100	47	28.9 (47)	74	46	
60-7	39.3 (49)	49	39.5 (50)	101	50	39.1 (47)	99	47	28.7 (46)	73	46	
62-7	39.5 (49)	49	39.7 (50)	101	50	39.8 (47)	101	47	28.5 (43)	72	43	*
64-7	40.0 (49)	49	40.3 (50)	101	50	39.7 (46)	99	46	28.4 (43)	71	43	*
66-7	40.3 (49)	49	40.0 (50)	99	50	39.7 (46)	99	46	28.0 (43)	69	43	*
68-7	40.7 (49)	49	40.1 (50)	99	50	39.6 (46)	97	46	27.3 (42)	67	41	**
70-7	40.2 (48)	48	40.1 (50)	100	50	38.9 (46)	97	45	26.9 (39)	67	39	**
72-7	40.8 (48)	48	39.8 (49)	98	49	39.3 (43)	96	43	26.5 (39)	65	39	**
74-7	40.6 (47)	47	40.2 (49)	99	49	39.2 (42)	97	42	25.9 (38)	64	37	**
76-7	41.0 (46)	46	40.2 (47)	98	47	39.4 (41)	96	41	25.9 (36)	63	36	**
78-7	40.4 (45)	45	40.4 (45)	100	45	38.8 (41)	96	41	25.1 (36)	62	35	*
80-7	40.9 (45)	45	40.9 (44)	100	43	37.8 (41)	92	41	25.2 (31)	62	31	**
82-7	40.7 (44)	44	39.7 (43)	98	43	37.6 (41)	92	41	24.8 (30)	61	30	**
84-7	40.3 (43)	43	39.5 (42)	98	42	36.9 (40)	92	40	24.7 (25)	61	25	**
86-7	40.6 (42)	42	39.4 (40)	97	40	36.1 (39)	89	39	24.9 (22)	61	22	**
88-7	40.2 (41)	41	39.5 (40)	98	40	34.9 (38)	87	38	24.7 (21)	61	21	**
90-7	39.8 (39)	39	38.8 (40)	97	40	34.6 (37)	87	37	24.4 (18)	61	18	**
92-7	40.1 (37)	37	38.4 (39)	96	39	34.2 (34)	85	34	23.1 (16)	58	16	**
94-7	39.7 (36)	36	38.3 (39)	96	39	34.9 (31)	88	31	22.6 (16)	57	16	**
96-7	39.2 (35)	35	38.8 (37)	99	36	34.5 (26)	88	26	21.7 (11)	55	11	**
98-7	38.4 (34)	33	37.5 (33)	98	32	34.5 (24)	90	24	22.1 (8)	58	8	**
100-7	38.7 (30)	30	37.8 (31)	98	31	33.8 (22)	87	22	21.4 (7)	55	6	**
102-7	39.2 (29)	29	37.8 (30)	96	30	33.1 (20)	84	20	21.7 (5)	55	5	**
104-7	38.5 (29)	29	37.3 (29)	97	29	32.6 (18)	85	17 *	21.3 (5)	55	5	**

No. of Survivars : Significant difference ; * : P≦0.05 ** : P≦0.05 Test of CHI SQUARE

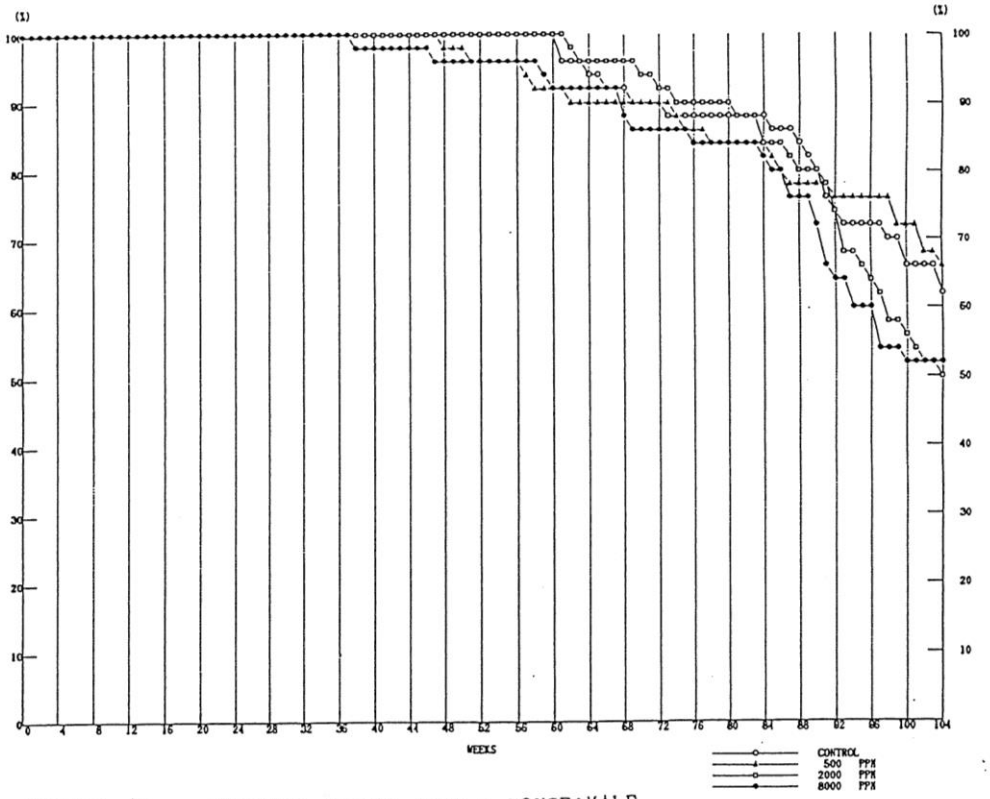


FIGURE 9 SURVIVAL ANIMAL RATE : MOUSE:MALE

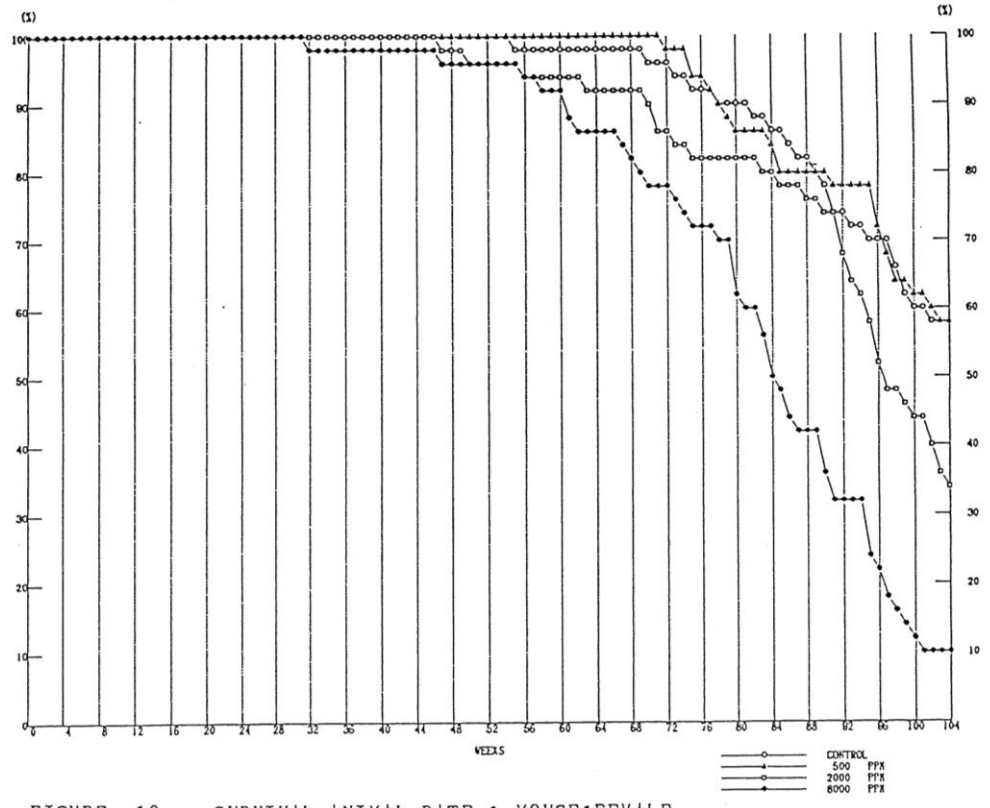


FIGURE 10 SURVIVAL ANIMAL RATE : MOUSE:FEMALE

TABLE 26 CLINICAL OBSERVATION OF DEAD AND MORIBUND MOUSE (0-104W SUMMARY)

Clinical sign	MALE				FEMALE			
	Control	500ppm	2000ppm	8000ppm	Control	500ppm	2000ppm	8000ppm
(DEAD AND MORIBUND ANIMAL NUMBERS)	(19)	(17)	(25)	(24)	(21)	(21)	(33)	(45)
LOCOMOTOR MOVEMENT DECR	1	4	2	2	1	1	5	3
HUNCHBACK POSITION	4	1	4	13	2	2	14	32
WASTING	0	0	1	5	0	0	7	18
PILOERECTION	10	12	18	22	12	15	26	41
FROG BELLY	4	2	2	1	9	4	5	0
EDEMA	0	0	1	1	2	1	1	0
ANEMIA	3	5	13	6	11	7	9	3
ASCITES	0	0	0	0	4	1	0	0
ABNORMAL RESPIRATION	6	4	11	19	9	15	17	35
NOISY	0	0	0	0	0	0	0	2
SUBNORMAL TEMP	2	4	1	3	2	2	3	3
INTERNAL MASS	4	3	4	2	5	3	7	3
EXTERNAL MASS								
M.NOSE	0	0	0	0	0	0	0	1
M.EYE	0	0	1	0	0	0	0	1
M.MANDIBULAR	0	0	0	0	0	0	0	1
M.EAR	0	0	0	1	0	0	0	1
M.PERI EAR	0	0	0	0	0	0	0	1
M.NECK	0	0	0	0	0	0	2	1
M.BREAST	0	0	0	1	0	0	2	0
M.ABDOMEN	0	0	0	0	1	0	2	0
M.ANTERIOR.DORSUM	0	0	0	0	0	1	1	1
M.INTERSCAPULUM	0	1	0	0	0	0	0	0
M.POSTERIOR DORSUM	0	0	1	0	0	0	0	0
M.HINDLIMB	0	0	0	1	0	0	0	0
M.GENITALIA	0	0	0	0	0	2	2	0
M.TAIL	0	1	0	0	0	0	0	0

TABLE 27 CLINICAL OBSERVATION OF SURVIVAL MOUSE (92-104W SUMMARY)

Clinical sign	MALE				FEMALE			
	Control	500ppm	2000ppm	8000ppm	Control	500ppm	2000ppm	8000ppm
(SURVIVAL ANIMAL NUMBERS)	(31)	(33)	(25)	(26)	(29)	(29)	(17)	(5)
LOCOMOTOR MOVEMENT DECR	0	1	1	0	1	1	0	2
HUNCHBACK POSITION	0	3	2	14	2	2	2	4
WASTING	0	2	0	6	0	1	1	1
PILOERECTION	5	6	8	26	5	5	10	5
FROG BELLY	0	1	0	0	2	0	1	0
EDEMA	0	0	0	0	1	0	0	0
ANEMIA	2	4	1	1	1	2	1	0
CYANOSIS	0	0	0	0	0	1	0	0
ABNORMAL RESPIRATION	3	2	3	24	5	6	7	5
NOISY	0	0	0	0	0	1	0	0
SUBNORMAL TEMP	0	0	1	0	1	0	0	0
INTERNAL MASS	1	5	4	5	2	2	3	0
EXTERNAL MASS								
M.EYE	0	0	1	0	0	0	0	0
M.ORAL CAVITY	0	0	0	1	0	0	0	0
M.EAR	0	0	0	0	0	1	0	0
M.NECK	0	0	1	1	0	0	0	0
M.BREAST	0	0	0	0	2	0	0	0
M.POSTERIOR DORSUM	0	1	0	0	0	1	0	0
M.GENITALIA	1	0	0	0	0	0	0	0
M.TAIL	0	1	1	0	1	1	0	0

(3) Body Weight

Changes in body weight in each group during the administration period are listed in Tables 24 and 25 and Figures 11 and 12.

Compared with the control group, males in the 8,000 ppm group showed 2–43% of suppression of body weight gain almost throughout the administration period and those in the 2,000 ppm group showed 6–9% of suppression of body weight gain in the late stage of administration (Weeks 90–104).

Compared with the control group, females in the 8,000 ppm group showed 2–45% of suppression of body weight gain almost throughout the administration period and those in the 2,000 ppm group showed 8–16% of suppression of body weight gain in the late stage of administration (Weeks 80–104). (Appendices B 3 and 4)

(4) Food Consumption

Food consumption (per animal per day) during the administration period is listed in Tables 28 and 29 and Figures 13 and 14.

Compared with the control group, males in the 8,000 ppm group had decreased food consumption of 2–22% almost throughout the administration period.

Compared with the control group, females in the 8,000 ppm group had decreased food consumption of 7–27% in the middle stage of administration and later (Weeks 38–102). (Appendices C 3 and 4)

(5) Water Consumption

Water consumption (per animal per day) during the administration period is listed in Tables 30 and 31 and Figures 15 and 16.

Compared with the control group, males in the 8,000 ppm group had decreased water consumption of 14–35% during the entire administration period.

Compared with the control group, females in the 8,000 ppm group had decreased food consumption of 15–48% during the entire administration period . (Appendices D 3 and 4)

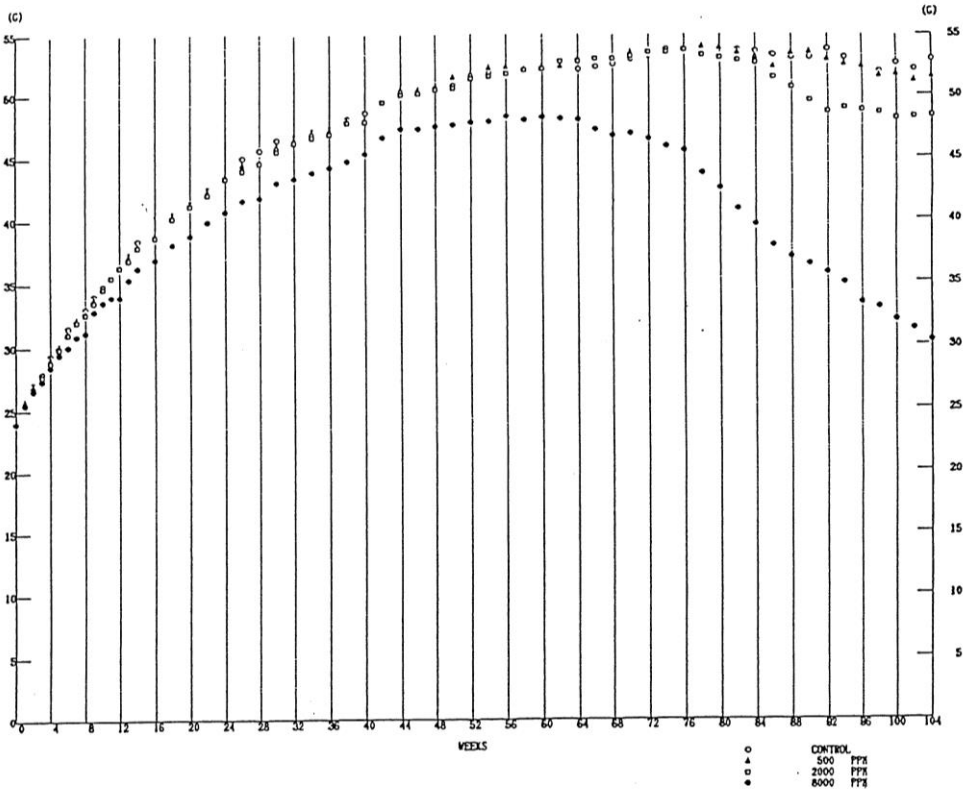


FIGURE 11 BODY WEIGHT CHANGES : MOUSE:MALE

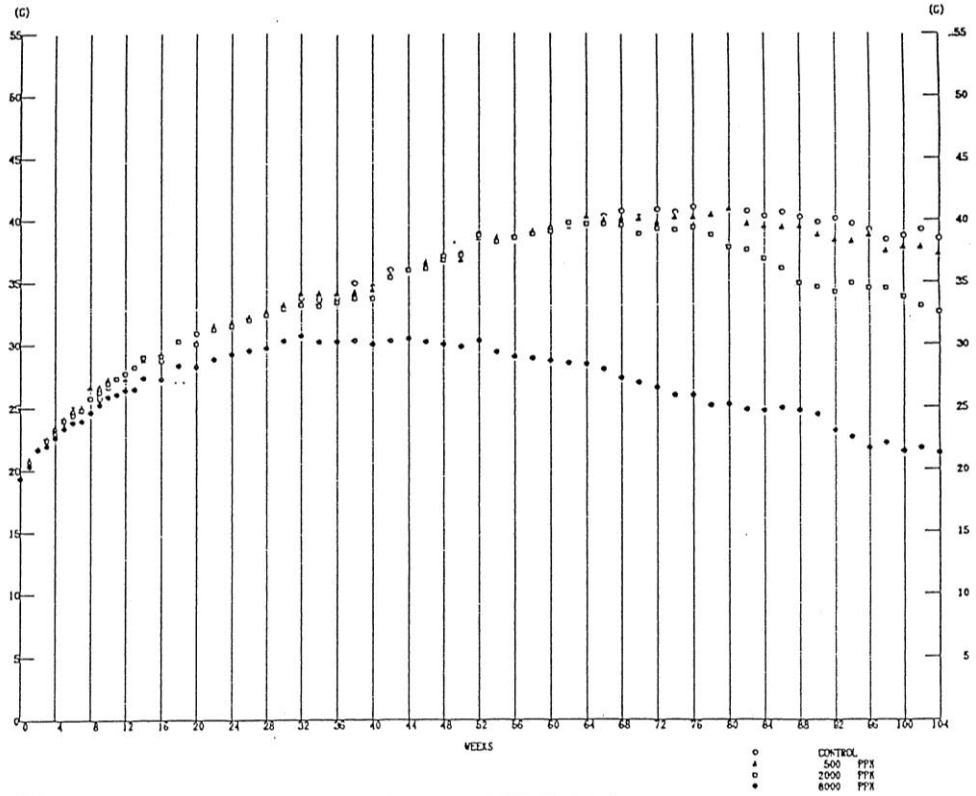


FIGURE 12 BODY WEIGHT CHANGES : MOUSE:FEMALE

TABLE 28 FOOD CONSUMPTION IN MALE MOUSE

Week-Day on Study	Control Av.FC.	No.of Surviv.	500 ppm Av.FC.	% of cont.	No.of Surviv.	2000 ppm Av.FC.	% of cont.	No.of Surviv.	8000 ppm Av.FC.	% of cont.	No.of Surviv.
1-7	4.2 (50)	50	4.2 (50)	100	50	4.1 (50)	98	50	4.0 (49)	95	50
2-7	4.2 (50)	50	4.1 (50)	98	50	4.1 (50)	98	50	4.0 (50)	95	50
3-7	4.0 (50)	50	4.0 (50)	100	50	3.9 (50)	98	50	3.9 (50)	98	50
4-7	4.1 (50)	50	4.1 (50)	100	50	4.0 (50)	98	50	4.0 (50)	98	50
5-7	4.0 (50)	50	4.1 (50)	103	50	4.0 (50)	100	50	3.9 (50)	98	50
6-7	4.3 (50)	50	4.2 (50)	98	50	4.2 (49)	98	50	4.1 (50)	95	50
7-7	4.2 (50)	50	4.0 (50)	95	50	4.1 (50)	98	50	4.0 (50)	95	50
8-7	4.3 (50)	50	4.3 (50)	100	50	4.2 (50)	98	50	4.1 (50)	95	50
9-7	4.1 (50)	50	4.2 (50)	102	50	4.2 (50)	102	50	4.1 (50)	100	50
10-7	4.3 (50)	50	4.2 (50)	98	50	4.3 (50)	100	50	4.2 (50)	98	50
11-7	4.1 (50)	50	4.2 (50)	102	50	4.2 (50)	102	50	4.0 (50)	98	50
12-7	4.3 (50)	50	4.3 (50)	100	50	4.3 (50)	100	50	4.1 (50)	95	50
13-7	4.2 (50)	50	4.2 (50)	100	50	4.1 (50)	98	50	4.1 (50)	98	50
14-7	4.4 (50)	50	4.4 (49)	100	50	4.3 (50)	98	50	4.2 (49)	95	50
18-7	4.1 (50)	50	4.2 (50)	102	50	4.2 (50)	102	50	4.0 (50)	98	50
22-7	4.3 (50)	50	4.4 (50)	102	50	4.3 (50)	100	50	4.3 (50)	100	50
26-7	4.6 (50)	50	4.6 (50)	100	50	4.5 (50)	98	50	4.4 (50)	96	50
30-7	4.5 (50)	50	4.5 (50)	100	50	4.4 (50)	98	50	4.4 (50)	98	50
34-7	4.5 (50)	50	4.5 (50)	100	50	4.3 (50)	96	50	4.2 (50)	93	50
38-7	4.5 (50)	50	4.4 (50)	98	50	4.4 (50)	98	50	4.3 (49)	96	49
42-7	4.4 (50)	50	4.4 (50)	100	50	4.4 (50)	100	50	4.3 (49)	98	49
46-7	4.3 (50)	50	4.4 (50)	102	50	4.2 (49)	98	50	4.1 (49)	95	49
50-7	4.5 (50)	50	4.5 (49)	100	49	4.3 (50)	96	50	4.3 (48)	96	48
52-7	4.5 (50)	50	4.6 (48)	102	48	4.5 (50)	100	50	4.3 (48)	96	48
54-7	4.5 (50)	50	4.4 (48)	98	48	4.3 (50)	96	50	4.2 (48)	93	48
58-7	4.4 (50)	50	4.4 (46)	100	46	4.4 (50)	100	50	4.2 (48)	95	48
62-7	4.5 (48)	48	4.5 (45)	100	45	4.5 (49)	100	49	4.3 (46)	96	46
66-7	4.9 (46)	46	4.9 (45)	100	45	4.8 (48)	98	48	4.4 (46)	90	46
70-7	4.9 (45)	45	4.8 (45)	98	45	4.8 (47)	98	47	4.5 (43)	92	43
74-7	4.7 (44)	44	4.7 (44)	100	44	4.8 (46)	102	45	4.3 (43)	91	43
78-7	4.9 (44)	44	4.9 (42)	100	42	4.8 (45)	98	45	4.2 (42)	86	42
82-7	4.9 (44)	44	4.9 (42)	100	42	4.9 (44)	100	44	4.2 (42)	86	42
86-7	4.8 (43)	43	4.7 (41)	98	40	4.7 (42)	98	42	4.0 (40)	83	40
90-7	4.7 (40)	40	4.9 (39)	104	39	4.6 (40)	98	40	3.8 (36)	81	36
94-7	4.9 (36)	36	4.8 (38)	98	38	4.7 (34)	96	34	3.8 (30)	78	30
98-7	4.8 (36)	35	4.6 (38)	96	38	4.8 (29)	100	29	3.9 (27)	81	27
102-7	4.9 (33)	33	4.9 (35)	100	34	4.9 (26)	100	26	4.1 (26)	84	26
104-7	4.6 (31)	31	4.6 (33)	100	33	4.6 (25)	100	25	3.8 (26)	83	26

TABLE 29 FOOD CONSUMPTION IN FEMALE MOUSE

Week-Day on Study	Control Av.FC.	No.of Surviv.	500 ppm Av.FC.	% of cont.	No.of Surviv.	2000 ppm Av.FC.	% of cont.	No.of Surviv.	8000 ppm Av.FC.	% of cont.	No.of Surviv.
1-7	3.7 (50)	50	3.8 (50)	103	50	3.8 (50)	103	50	3.7 (50)	100	50
2-7	3.7 (50)	50	3.7 (50)	100	50	3.7 (50)	100	50	3.8 (50)	103	50
3-7	3.7 (50)	50	3.7 (50)	100	50	3.7 (50)	100	50	3.7 (50)	100	50
4-7	3.9 (50)	50	3.8 (50)	97	50	3.9 (50)	100	50	3.8 (50)	97	50
5-7	3.8 (50)	50	3.8 (50)	100	50	3.9 (50)	103	50	3.8 (50)	100	50
6-7	4.0 (50)	50	4.0 (50)	100	50	4.1 (50)	103	50	4.0 (50)	100	50
7-7	3.9 (50)	50	3.8 (50)	97	50	4.0 (50)	103	50	3.9 (50)	100	50
8-7	4.0 (50)	50	4.0 (50)	100	50	4.1 (50)	103	50	4.0 (50)	100	50
9-7	4.0 (50)	50	4.0 (50)	100	50	4.1 (50)	103	50	4.0 (50)	100	50
10-7	4.1 (50)	50	4.1 (50)	100	50	4.2 (50)	102	50	4.1 (50)	100	50
11-7	4.2 (50)	50	4.1 (50)	98	50	4.2 (50)	100	50	4.0 (50)	95	50
12-7	4.2 (50)	50	4.2 (50)	100	50	4.3 (50)	102	50	4.2 (50)	100	50
13-7	4.1 (50)	50	4.0 (50)	98	50	4.0 (50)	98	50	3.8 (50)	93	50
14-7	4.3 (50)	50	4.2 (50)	98	50	4.4 (50)	102	50	4.2 (50)	98	50
18-7	4.1 (50)	50	4.0 (50)	98	50	4.1 (50)	100	50	4.0 (50)	98	50
22-7	4.1 (50)	50	4.2 (50)	102	50	4.3 (50)	105	50	4.1 (50)	100	50
26-7	4.3 (50)	50	4.1 (50)	95	50	4.3 (50)	100	50	4.1 (50)	95	50
30-7	4.3 (50)	50	4.2 (50)	98	50	4.4 (50)	102	50	4.3 (50)	100	50
34-7	4.1 (50)	50	4.0 (50)	98	50	4.0 (50)	98	50	3.9 (49)	95	49
38-7	4.2 (50)	50	3.9 (50)	93	50	4.1 (50)	98	50	3.8 (49)	90	49
42-7	4.1 (49)	50	4.2 (50)	102	50	4.2 (50)	102	50	3.8 (49)	93	49
46-7	4.1 (50)	50	4.2 (50)	102	50	4.2 (50)	102	50	3.7 (49)	90	49
50-7	4.1 (50)	50	4.0 (50)	98	50	4.2 (48)	102	48	3.6 (48)	88	48
52-7	4.3 (50)	50	4.2 (50)	98	50	4.3 (48)	100	48	3.7 (48)	86	48
54-7	4.0 (50)	50	4.2 (50)	105	50	4.1 (48)	103	48	3.4 (48)	85	48
58-7	4.1 (49)	49	4.2 (50)	102	50	4.2 (47)	102	47	3.5 (47)	85	46
62-7	4.1 (49)	49	4.2 (50)	102	50	4.4 (47)	107	47	3.5 (43)	85	43
66-7	4.4 (49)	49	4.2 (50)	95	50	4.4 (46)	100	46	3.6 (42)	82	43
70-7	4.4 (48)	48	4.6 (50)	105	50	4.3 (46)	98	45	3.7 (39)	84	39
74-7	4.4 (47)	47	4.4 (49)	100	49	4.4 (42)	100	42	3.5 (38)	80	37
78-7	4.3 (45)	45	4.3 (45)	100	45	4.5 (41)	105	41	3.6 (36)	84	35
82-7	4.6 (44)	44	4.5 (43)	98	43	4.5 (41)	98	41	3.6 (30)	78	30
86-7	4.3 (42)	42	4.5 (40)	105	40	4.3 (39)	100	39	3.6 (23)	84	22
90-7	4.3 (39)	39	4.3 (40)	100	40	4.2 (37)	98	37	3.5 (18)	81	18
94-7	4.5 (36)	36	4.3 (39)	96	39	4.2 (31)	93	31	3.3 (16)	73	16
98-7	4.2 (34)	33	4.5 (33)	107	32	4.3 (24)	102	24	3.4 (8)	81	8
102-7	4.7 (29)	29	4.7 (30)	100	30	4.7 (20)	100	20	3.8 (5)	81	5
104-7	4.3 (29)	29	4.4 (29)	102	29	4.4 (18)	102	17	3.6 (5)	84	5

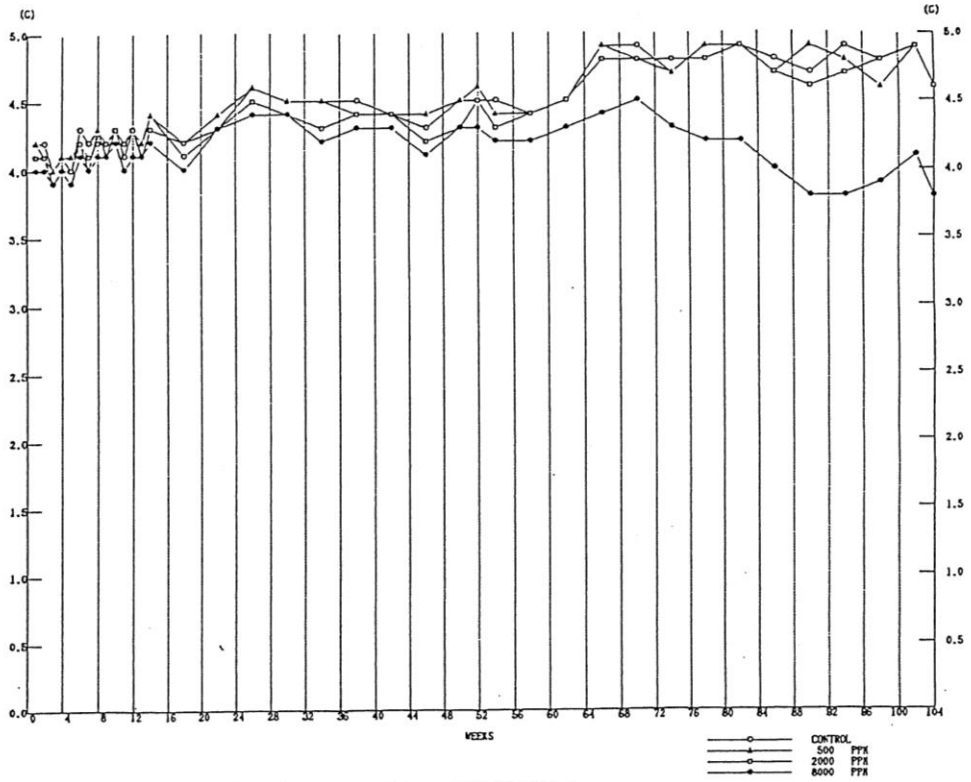


FIGURE 13 FOOD CONSUMPTION : MOUSE:MALE

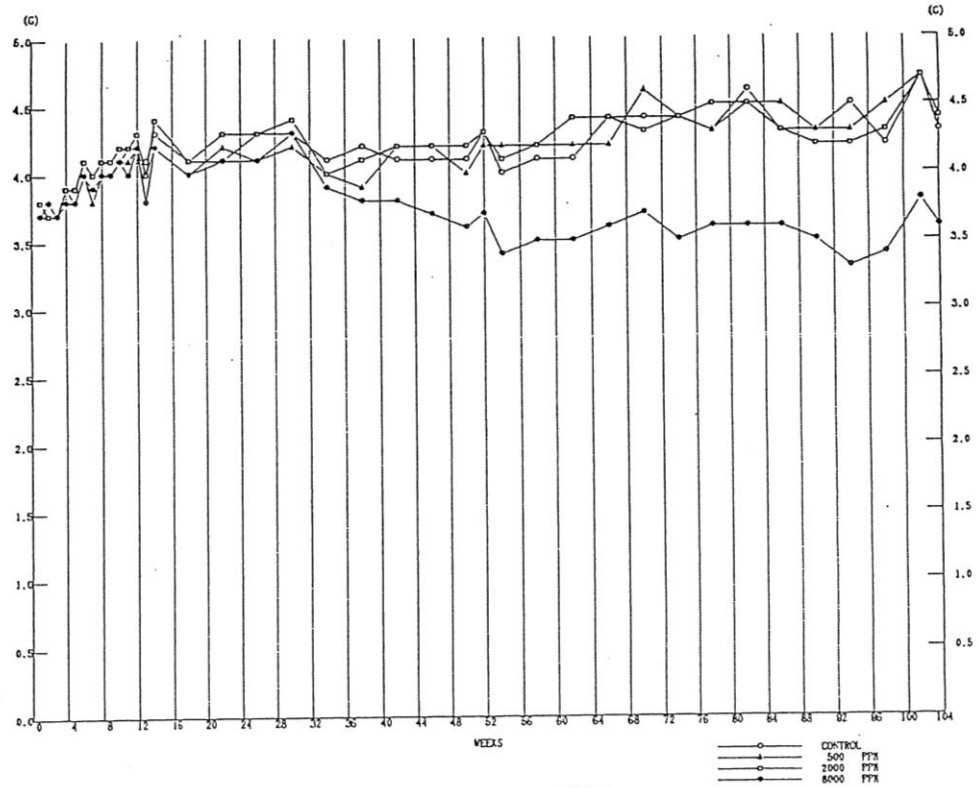


FIGURE 14 FOOD CONSUMPTION : MOUSE:FEMALE

TABLE 30 WATER CONSUMPTION IN MALE MOUSE

Week-Day on Study	Control Av.WC.	No.of Surviv.	500 ppm Av.WC.	% of cont.	No.of Surviv.	2000 ppm Av.WC.	% of cont.	No.of Surviv.	8000 ppm Av.WC.	% of cont.	No.of Surviv.
1-7	4.8 (50)	50	4.8 (50)	100	50	4.6 (50)	96	50	3.4 (50)	71	50
2-7	4.7 (50)	50	4.6 (50)	98	50	4.4 (50)	94	50	3.4 (50)	72	50
3-7	4.4 (50)	50	4.3 (49)	98	50	4.2 (50)	95	50	3.4 (50)	77	50
4-7	4.3 (50)	50	4.3 (49)	100	50	4.0 (50)	93	50	3.2 (50)	74	50
5-7	4.3 (50)	50	4.2 (50)	98	50	4.0 (50)	93	50	3.3 (50)	77	50
6-7	4.2 (50)	50	4.1 (50)	98	50	4.0 (50)	95	50	3.2 (50)	76	50
7-7	4.1 (49)	50	4.0 (49)	98	50	3.8 (50)	93	50	3.2 (50)	78	50
8-7	4.1 (50)	50	4.0 (50)	98	50	3.8 (50)	93	50	3.2 (50)	78	50
9-7	3.9 (50)	50	3.9 (50)	100	50	3.8 (50)	97	50	3.2 (50)	82	50
10-7	3.9 (50)	50	4.0 (50)	103	50	3.9 (50)	100	50	3.1 (50)	79	50
11-7	3.8 (50)	50	3.8 (50)	100	50	3.6 (50)	95	50	3.1 (50)	82	50
12-7	3.8 (50)	50	3.8 (50)	100	50	3.7 (50)	97	50	3.1 (50)	82	50
13-7	3.7 (50)	50	3.7 (50)	100	50	3.7 (50)	100	50	3.2 (50)	86	50
14-7	3.6 (50)	50	3.7 (50)	103	50	3.6 (50)	100	50	3.0 (50)	83	50
16-7	3.9 (50)	50	3.9 (50)	100	50	3.8 (50)	97	50	3.1 (50)	79	50
18-7	3.7 (50)	50	3.8 (50)	103	50	3.7 (50)	100	50	3.1 (50)	84	50
20-7	3.8 (50)	50	3.9 (50)	103	50	3.7 (50)	97	50	3.1 (50)	82	50
22-7	3.8 (50)	50	3.9 (50)	103	50	3.8 (50)	100	50	3.2 (50)	84	50
24-7	3.9 (50)	50	3.9 (50)	100	50	3.7 (50)	95	50	3.2 (50)	82	50
26-7	4.1 (50)	50	4.0 (50)	98	50	3.9 (50)	95	50	3.4 (50)	83	50
28-7	3.8 (49)	50	3.9 (50)	103	50	3.8 (50)	100	50	3.2 (50)	84	50
30-7	4.0 (50)	50	4.0 (50)	100	50	3.9 (50)	98	50	3.3 (50)	83	50
32-7	3.9 (50)	50	3.8 (50)	97	50	3.7 (50)	95	50	3.1 (50)	79	50
34-7	4.1 (50)	50	4.1 (50)	100	50	4.0 (50)	98	50	3.5 (50)	85	50
36-7	4.1 (50)	50	4.1 (50)	100	50	4.0 (50)	98	50	3.3 (50)	80	50
38-7	4.0 (50)	50	4.0 (50)	100	50	3.9 (50)	98	50	3.2 (49)	80	49
40-7	3.9 (50)	50	3.8 (50)	97	50	3.8 (50)	97	50	3.2 (49)	82	49
42-7	3.8 (50)	50	3.6 (50)	95	50	3.6 (50)	95	50	3.1 (49)	82	49
44-7	3.9 (50)	50	3.8 (50)	97	50	3.7 (50)	95	50	2.9 (49)	74	49
46-7	3.8 (50)	50	3.7 (50)	97	50	3.6 (50)	95	50	2.9 (49)	76	49
48-7	3.9 (50)	50	3.8 (49)	97	49	3.7 (50)	95	50	3.0 (48)	77	48
50-7	3.9 (50)	50	3.9 (49)	100	49	3.7 (50)	95	50	3.0 (48)	77	48
52-7	4.0 (50)	50	4.0 (48)	100	48	3.9 (50)	98	50	3.1 (48)	78	48
54-7	4.0 (50)	50	4.0 (48)	100	48	3.8 (50)	95	50	3.0 (48)	75	48
56-7	4.1 (50)	50	4.0 (48)	98	48	3.9 (50)	95	50	3.0 (48)	73	48
58-7	4.1 (50)	50	4.0 (46)	98	46	4.0 (50)	98	50	3.0 (48)	73	48
60-7	4.1 (50)	50	4.0 (45)	98	46	4.0 (50)	98	50	2.7 (46)	66	46
62-7	4.2 (48)	48	4.1 (45)	98	45	4.0 (49)	95	49	3.1 (46)	74	46
64-7	4.3 (46)	47	4.2 (45)	98	45	4.1 (48)	95	48	3.0 (46)	70	46
66-7	4.3 (46)	46	4.2 (45)	98	45	4.2 (48)	98	48	3.0 (46)	70	46
68-7	4.6 (46)	46	4.4 (45)	96	45	4.3 (47)	93	48	3.2 (45)	70	44
70-7	4.5 (45)	45	4.4 (45)	98	45	4.4 (46)	98	47	3.2 (43)	71	43
72-7	4.7 (45)	45	4.4 (45)	94	45	4.4 (45)	94	46	3.3 (43)	70	43
74-7	4.5 (44)	44	4.4 (44)	98	44	4.5 (45)	100	45	3.4 (43)	76	43
76-7	4.6 (44)	44	4.6 (43)	100	43	4.4 (43)	96	45	3.3 (42)	72	42
78-7	4.7 (44)	44	4.5 (42)	96	42	4.5 (43)	96	45	3.5 (42)	74	42
80-7	4.8 (44)	44	4.6 (42)	96	42	4.6 (43)	96	45	3.4 (42)	71	42
82-7	4.8 (43)	44	4.8 (42)	100	42	4.7 (44)	98	44	3.6 (42)	75	42
84-7	4.9 (43)	44	4.9 (42)	100	42	4.6 (40)	94	42	3.3 (40)	67	41
86-7	4.9 (42)	43	4.8 (41)	98	40	4.7 (40)	96	42	3.4 (39)	69	40
88-7	4.7 (40)	42	4.9 (38)	104	39	4.7 (37)	100	40	3.4 (38)	72	38
90-7	4.7 (37)	40	5.0 (39)	106	39	4.8 (36)	102	40	3.4 (36)	72	36
92-7	5.0 (36)	37	4.8 (37)	96	38	4.5 (33)	90	37	3.5 (32)	70	32
94-7	5.0 (35)	36	4.8 (37)	96	38	4.7 (30)	94	34	3.3 (30)	66	30
96-7	5.0 (35)	36	4.9 (37)	98	38	4.5 (28)	90	32	3.4 (30)	68	30
98-7	5.1 (35)	35	4.7 (36)	92	38	4.8 (26)	94	29	3.4 (27)	67	27
100-7	5.1 (31)	33	5.0 (35)	98	36	4.8 (27)	94	28	3.5 (26)	69	26
102-7	5.1 (32)	33	4.9 (33)	96	34	4.8 (25)	94	26	3.7 (26)	73	26
104-7	5.1 (30)	31	4.7 (30)	92	33	4.7 (24)	92	25	3.3 (26)	65	26

TABLE 31 WATER CONSUMPTION IN FEMALE MOUSE

Week-Day on Study	Control Av.WC.	No.of Surviv.	500 ppm Av.WC.	% of cont.	No.of Surviv.	2000 ppm Av.WC.	% of cont.	No.of Surviv.	8000 ppm Av.WC.	% of cont.	No.of Surviv.
1-7	4.5 (50)	50	4.5 (50)	100	50	4.6 (50)	102	50	3.5 (50)	78	50
2-7	4.4 (50)	50	4.4 (50)	100	50	4.5 (50)	102	50	3.5 (50)	80	50
3-7	4.3 (50)	50	4.4 (50)	102	50	4.4 (49)	102	50	3.6 (50)	84	50
4-7	4.2 (50)	50	4.3 (49)	102	50	4.6 (49)	110	50	3.5 (49)	83	50
5-7	4.4 (50)	50	4.5 (50)	102	50	4.9 (49)	111	50	3.5 (49)	80	50
6-7	4.3 (47)	50	4.4 (49)	102	50	4.7 (47)	109	50	3.6 (50)	84	50
7-7	4.3 (49)	50	4.3 (49)	100	50	4.6 (46)	107	50	3.5 (49)	81	50
8-7	4.3 (49)	50	4.3 (48)	100	50	4.8 (47)	112	50	3.5 (50)	81	50
9-7	4.4 (49)	50	4.3 (49)	98	50	4.6 (48)	105	50	3.5 (50)	80	50
10-7	4.4 (49)	50	4.4 (49)	100	50	4.7 (48)	107	50	3.6 (50)	82	50
11-7	4.5 (49)	50	4.3 (47)	96	50	4.4 (48)	98	50	3.5 (50)	78	50
12-7	4.5 (49)	50	4.3 (49)	96	50	4.4 (49)	98	50	3.5 (50)	78	50
13-7	4.3 (50)	50	4.1 (49)	95	50	4.1 (47)	95	50	3.4 (50)	79	50
14-7	4.3 (50)	50	4.2 (50)	98	50	4.2 (48)	98	50	3.4 (50)	79	50
16-7	4.3 (50)	50	4.1 (48)	95	50	4.3 (50)	100	50	3.6 (50)	84	50
18-7	4.3 (50)	50	4.3 (50)	100	50	4.4 (49)	102	50	3.4 (50)	79	50
20-7	4.0 (49)	50	4.0 (48)	100	50	4.2 (45)	105	50	3.4 (50)	85	50
22-7	4.4 (50)	50	4.5 (49)	102	50	4.7 (47)	107	50	3.5 (50)	80	50
24-7	4.4 (49)	50	4.3 (48)	98	50	4.4 (49)	100	50	3.5 (50)	80	50
26-7	4.5 (50)	50	4.4 (49)	98	50	4.4 (49)	98	50	3.6 (50)	80	50
28-7	4.3 (50)	50	4.4 (50)	102	50	4.4 (48)	102	50	3.5 (50)	81	50
30-7	4.3 (50)	50	4.1 (50)	95	50	4.4 (48)	102	50	3.6 (50)	84	50
32-7	4.2 (50)	50	4.1 (50)	98	50	4.0 (50)	95	50	3.4 (49)	81	49
34-7	4.4 (50)	50	4.3 (49)	98	50	4.3 (48)	98	50	3.5 (49)	80	49
36-7	4.2 (49)	50	4.3 (50)	102	50	4.2 (50)	100	50	3.5 (49)	83	49
38-7	4.3 (50)	50	4.1 (48)	95	50	4.3 (47)	100	50	3.4 (49)	79	49
40-7	4.1 (50)	50	4.2 (49)	102	50	4.1 (48)	100	50	3.3 (49)	80	49
42-7	3.9 (49)	50	4.0 (48)	103	50	3.9 (47)	100	50	3.1 (49)	79	49
44-7	4.0 (50)	50	4.0 (48)	100	50	4.1 (48)	103	50	3.3 (49)	83	49
46-7	4.0 (50)	50	4.1 (50)	103	50	4.2 (48)	105	50	3.1 (48)	78	49
48-7	3.9 (50)	50	4.0 (48)	103	50	4.2 (47)	108	49	3.0 (48)	77	48
50-7	3.9 (49)	50	4.1 (48)	105	50	4.1 (48)	105	48	3.1 (48)	79	48
52-7	3.9 (50)	50	3.9 (50)	100	50	3.7 (47)	95	48	2.9 (48)	74	48
54-7	4.0 (49)	50	4.0 (49)	100	50	3.9 (48)	98	48	2.9 (48)	73	48
56-7	3.9 (48)	49	3.9 (49)	100	50	3.8 (48)	97	47	2.8 (47)	72	47
58-7	4.0 (48)	49	4.0 (50)	100	50	3.9 (46)	98	47	2.8 (47)	70	46
60-7	4.0 (49)	49	4.0 (50)	100	50	4.1 (46)	103	47	2.7 (46)	68	46
62-7	3.7 (49)	49	3.5 (50)	95	50	3.8 (47)	103	47	2.7 (43)	73	43
64-7	4.3 (49)	49	4.1 (50)	95	50	4.1 (45)	95	46	2.9 (43)	67	43
66-7	4.1 (48)	49	4.0 (50)	98	50	3.9 (43)	95	46	2.8 (43)	68	43
68-7	4.3 (47)	49	4.0 (48)	93	50	4.1 (45)	95	46	2.7 (42)	63	41
70-7	4.2 (47)	48	4.2 (50)	100	50	4.1 (45)	98	45	2.8 (39)	67	39
72-7	4.3 (47)	48	4.1 (49)	95	49	4.2 (41)	98	43	2.7 (39)	63	39
74-7	4.3 (47)	47	4.3 (47)	100	49	4.5 (42)	105	42	2.8 (38)	65	37
76-7	4.5 (46)	46	4.2 (47)	93	47	4.6 (41)	102	41	2.7 (35)	60	36
78-7	4.3 (45)	45	4.4 (44)	102	45	4.2 (40)	98	41	2.5 (35)	58	35
80-7	4.3 (45)	45	4.3 (43)	100	43	4.4 (41)	102	41	2.6 (30)	60	31
82-7	4.5 (44)	44	4.6 (41)	102	43	4.5 (40)	100	41	2.7 (30)	60	30
84-7	4.5 (43)	43	4.5 (41)	100	42	4.3 (39)	96	40	2.5 (25)	56	25
86-7	4.5 (41)	42	4.7 (39)	104	40	4.5 (36)	100	39	2.7 (23)	60	22
88-7	4.6 (41)	41	4.5 (40)	98	40	4.3 (37)	93	38	2.6 (21)	57	21
90-7	4.5 (39)	39	4.2 (40)	93	40	4.3 (35)	96	37	2.5 (18)	56	18
92-7	4.6 (37)	37	4.3 (39)	93	39	4.2 (34)	91	34	2.4 (16)	52	16
94-7	4.4 (35)	36	4.2 (39)	95	39	4.0 (31)	91	31	2.5 (16)	57	16
96-7	4.6 (34)	35	4.5 (37)	98	36	4.3 (26)	93	26	2.5 (11)	54	11
98-7	4.7 (33)	33	4.3 (33)	91	32	4.2 (24)	89	24	2.6 (8)	55	8
100-7	4.6 (30)	30	4.5 (31)	98	31	4.4 (22)	96	22	2.6 (7)	57	6
102-7	4.5 (29)	29	4.2 (30)	93	30	4.0 (20)	89	20	2.6 (5)	58	5
104-7	4.6 (29)	29	4.1 (29)	89	29	3.8 (18)	83	17	2.6 (5)	57	5

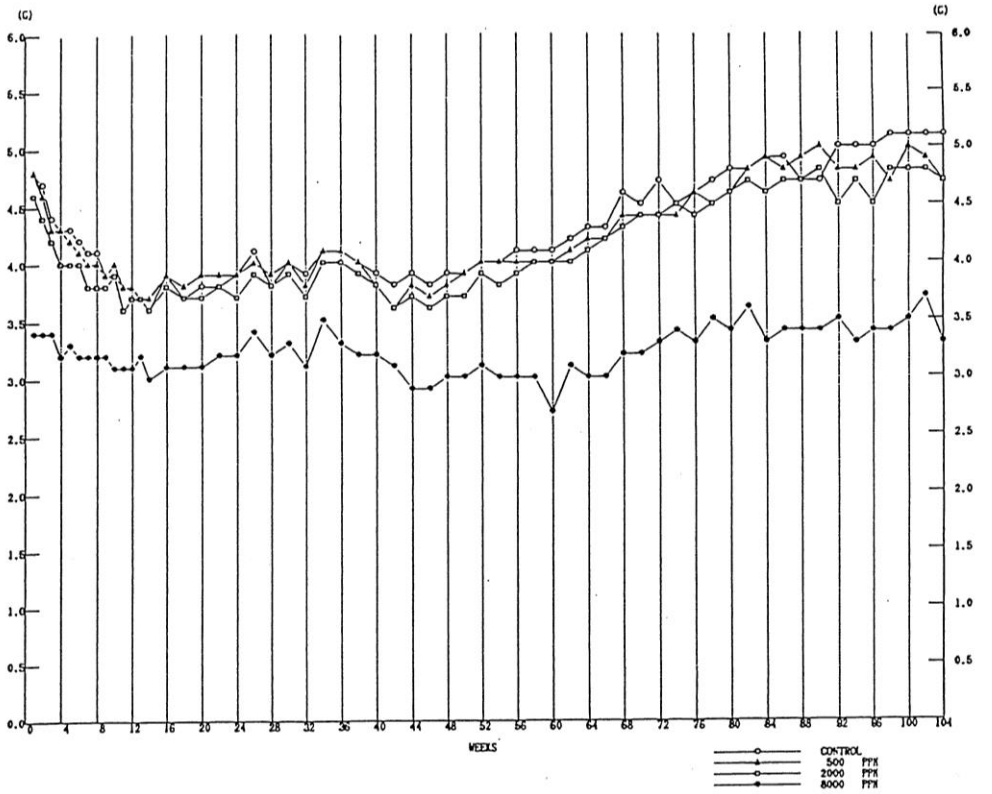


FIGURE 15 WATER CONSUMPTION : MOUSE:MALE

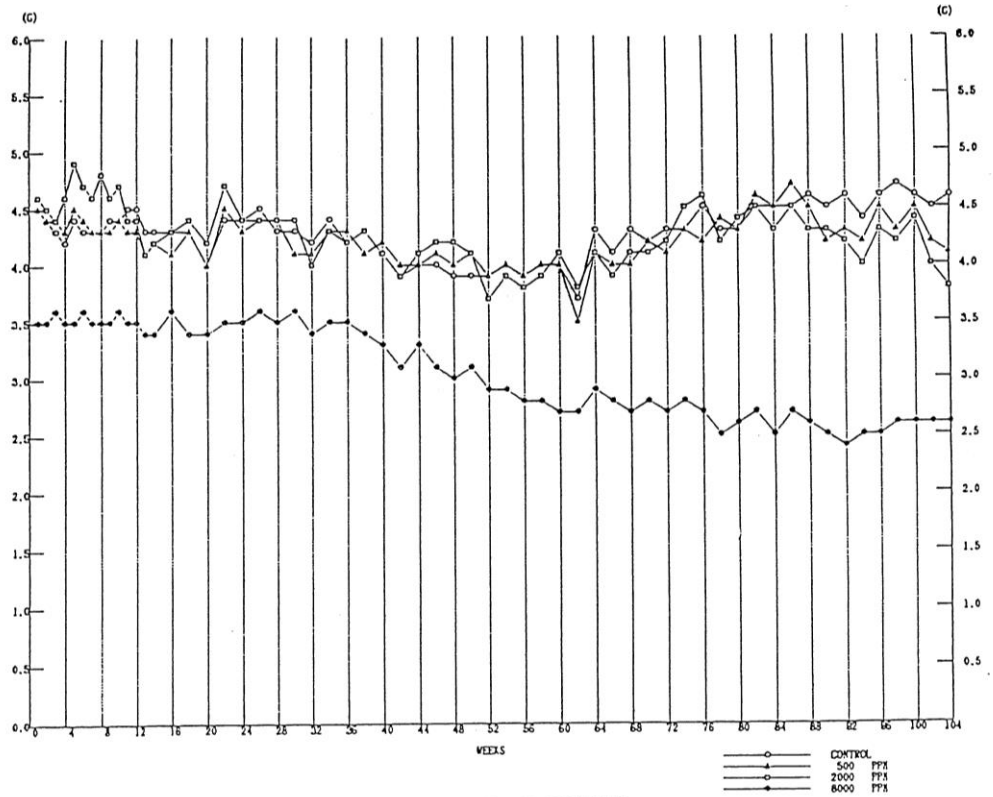


FIGURE 16 WATER CONSUMPTION : MOUSE:FEMALE

III-2-2 Hematology and Blood Biochemistry Examinations, and Urinalysis

(1) Hematology Examination

The results are listed in Appendices E 3 and 4 (the Summary Tables) and Appendices R 3 and 4 (the Individual Tables).

Among the males in the 8,000 ppm group, the following was observed: increases in the red blood cell counts, hemoglobin concentration, and hematocrit level. A decrease in eosinophil percentage was observed in the 8,000 ppm group.

Among the females, decreases in platelet counts in the 8,000 and 2,000 ppm groups were observed.

(2) Blood Biochemistry Examination

The results are listed in Appendices F 3 and 4 (the Summary Tables) and Appendices S 3 and 4 (the Individual Tables).

Among the males in the 8,000 ppm group, the following was observed: increases in GOT, GPT, LDH, ALP, LAP, and CPK and decreases in glucose and triglyceride. Also in the 2,000 ppm group, increases in GOT, GPT, LDH, and ALP were observed. Compared with the control group, the mean LDH value of the 2,000 ppm group was lower. This occurred because the control group contained an animal with a significant high LDH value (LDH: 16110 IU/l). This caused the mean value of the control group became higher than that of the 2,000 ppm group. The actual statistical significant difference occurred due to an increase in LDH. A decrease in urea nitrogen was observed in the 2,000 ppm group.

Among the females in the 8,000 ppm group, the following was observed: increases in GOT, GPT, LDH, ALP, and CPK and decreases in glucose, triglyceride, phospholipid, albumin, and calcium. Also in the 2,000 ppm group, increases in GOT, GPT, LDH, ALP, and LAP and decreases in glucose and triglyceride were observed.

(3) Urinalysis

The results are listed in Appendices G 3 and 4 (the Summary Tables) and Appendices T 3 and 4 (the Individual Tables).

Among the males, a decrease in the pH level was noted in the 8,000 ppm group.

Among the females, a decrease in the pH level, increases in protein and urobilinogen, and increased incidences of positive glucose and positive occult blood were noted in the 8,000 ppm group. Also in the 2,000 ppm group, an increase in protein and increased incidences of positive glucose and positive occult blood were noted.

III-2-3 Pathological Examination

(1) Necropsy

Findings observed at the time of necropsy are listed in Appendices H 5 to 8 (the Summary Tables) and Appendices U 3 and 4 (the Individual Tables). Among the findings, findings in the dosed groups with characteristics and a high incidence rate in comparison with the control groups are described below.

There were slightly more liver nodules observed in the non-surviving / moribund males of the 2,000 ppm group, and there were many liver nodules observed also in the dosed males surviving at the time of the scheduled necropsy. Many non-surviving / moribund females had red dots / macules on the lungs and livers, and liver nodules. Many females surviving at the time of the scheduled necropsy also had liver nodules.

(2) Organ Weight

The actual weights of the organs and their ratios to the body weights measured for all animals surviving until the time of the scheduled necropsy are listed in Appendices I 3 and 4 (the Summary Tables of the actual weights), Appendices J 3 and 4 (the Summary Tables of the actual weight to body weight ratio), Appendices V 3 and 4 (the Individual Tables of the actual weights), and Appendices W 3 and 4 (the Individual Tables of the actual weight to body weight ratio).

Compared with the control group, the ratios of all organs to the body weights had significantly higher values in the 8,000 ppm male group. However, only the actual weight of the lung was significantly heavier. The actual weights of the testis (the left only), heart, kidney, and brain had significant lower values. In the 2000 ppm group, the actual weights of the heart and kidney had significant lower values.

Compared with the control group, the actual weight of the lung and ratio to the body weights, and the ratios of the heart, kidney, liver, and brain to the body weights had significantly higher values in the 8,000 ppm female group. The actual weight of the ovary (the left only) and ratio to the body weight, and the actual weights of the adrenal gland and brain had significantly lower values. In the 2,000 ppm group, the actual weights of the lung, kidney, and heart and ratios to the body weights, and the ratios of the spleen and brain to the body weights had significantly higher values. The actual weight of the ovary (the right only) had a significantly lower value.

The body weights in the 8,000 ppm male group and the 8,000 and 2,000 ppm female groups at the time of necropsy had significantly lower values compared with those of the control groups.

(3) Histopathologic Examination

The results for nonneoplastic lesions are listed in Appendices K 5 to 8 (the Summary Tables). The results for neoplastic lesions are listed in Appendices L 3 and 4 (the total number of animals with tumors and number of tumors), Appendices M 3 and 4 (the incidence rates and time a necropsy was performed), Appendices N 3 and 4 (the results of the statistical analysis: Peto, Cochran-Armitage, and Fisher's tests). Individual tables of histopathologic examinations are listed in Appendices X 3 and 4.

Nasal cavity

In the 8,000 ppm groups of both sexes (among the non-surviving / moribund animals and those surviving at the time of the scheduled necropsy), significant increases in the following incidences were observed: rhinitis, atrophy of the olfactory epithelium, and nuclear enlargement of the respiratory epithelium. In the 8,000 ppm groups of both sexes (among the non-surviving / moribund animals and those surviving at the time of the scheduled necropsy), the 2,000 ppm female group (among the non-surviving / moribund animals), and the 2,000 ppm group of both sexes (among the animals surviving at the time of the scheduled necropsy), a significant increase in incidence of nuclear enlargement of the olfactory epithelium (the supporting cells) was observed. The non-surviving / moribund in the 8,000 ppm group showed an increased incidence of eosinophilic changes in the olfactory epithelium and a decreased incidence of respiratory epithelial metaplasia. The non-surviving / moribund in the 2,000 ppm group showed decreased incidences of eosinophilic changes in the olfactory epithelium and respiratory epithelial metaplasia. The males surviving at the time of the scheduled necropsy in the 8,000 ppm group showed decreased incidences of eosinophilic changes in the olfactory epithelium and respiratory epithelial metaplasia. The males surviving at the time of the scheduled necropsy in the 2,000 ppm group showed decreased incidences of eosinophilic changes in the respiratory epithelium and respiratory epithelial metaplasia. The males surviving at the time of the scheduled necropsy in the 500 ppm group showed a decreased incidence of respiratory epithelial metaplasia. The non-surviving / moribund females in the 8,000 ppm group showed an increased incidence of eosinophilic changes in the respiratory epithelium, decreased incidences of respiratory epithelial metaplasia, and atrophy of the respiratory epithelium. The non-surviving / moribund females in the 2,000 ppm group showed decreased incidences of respiratory epithelial metaplasia. The females surviving at the time of the scheduled necropsy in the 8,000 ppm group showed a decreased incidence of respiratory epithelial metaplasia. The females surviving at the time of the scheduled necropsy in the 2,000 ppm group showed an increased incidence of rhinitis, and decreased incidences of eosinophilic changes in the respiratory epithelium and respiratory epithelial metaplasia. (Table 32)

Among neoplastic lesions, an increasing trend in incidence of adenocarcinoma in the dosed female groups was revealed by the Peto tests (the mortality method, and the mortality method + the prevalence method). However, there was one incidence in the 8,000 ppm group. There was one incidence of esthesioneuroepithelioma in the 8,000 ppm male group and one incidence of papilloma in the 2,000 ppm female group.

TABLE 32 NUMBER OF MOUSE WITH SELECTED NASAL LESIONS

	Male				Female			
Group	Control	500 ppm	2000 ppm	8000 ppm	Control	500 ppm	2000 ppm	8000 ppm
Number of examined animal	50	50	50	50	50	50	50	50
Rhinitis	1	2	1	25	2		7	42
Respiratory metaplasia	40	40	15		30	27	1	
Nuclear enlargement:olfactory epithelium			9	49			41	33
Nuclear enlargement:respiratory epithelium				31				41
Atrophy:olfactory epithelium			1	48			1	42
Atrophy:respiratory epithelium								26
Adenocarcinoma								1
Papilloma							1	
Esthesioneuroepithelioma				1				

Liver

An increased incidence of vasodilatation was noted among the females surviving at the time of the scheduled necropsy in the 8,000 ppm group and an increased incidence of fatty degeneration was noted among the surviving 2,000 ppm group. A decreased incidence of uterine tumor metabasis was noted among the non-surviving / moribund females in the 8,000 ppm and 2,000 ppm groups. A decreased incidence of granulation formations was noted among animals surviving at the time of the scheduled necropsy in the 2,000 ppm and 500 ppm groups. (Table 33)

TABLE 33 NUMBER OF MOUSE WITH SELECTED LIVER LESIONS

	Male				Female			
Group	Control	500 ppm	2000 ppm	8000 ppm	Control	500 ppm	2000 ppm	8000 ppm
Number of examined animal	50	50	50	50	50	50	50	50
Angiectasis	2	3	4	16	6	3	5	1
Hepatocellular adenoma	7	16	22	8	4	30	20	2
Hepatocellular carcinoma	15	20	23	36		6	30	45
Hemangioendothelioma	3	8	8	4		3	1	2
Histiocyto sarcoma	2		3	3	1	3		2

Among neoplastic lesions, compared with the control groups, increased incidence rates of hepatocellular adenoma in the 2,000 ppm male, 2,000 and 500 ppm female groups were revealed by the Fisher's exact test. However, a decreasing trend in incidence of hepatocellular adenoma between the female dosed groups and the control group was revealed by the Cochrane–Armitage test. On the other hand, an increasing trend in incidence of hepatoma in the dosed groups of both sexes was revealed by the Peto tests (the mortality method, the prevalence method, and the mortality method + the prevalence method) and the Cochrane–Armitage test, and the increased incidence rates in the 8,000 ppm male group and all the female dosed groups were also revealed by the Fisher's exact test. After statistical processing of adding the numbers of hepatocellular adenoma and hepatoma, the Peto tests (the mortality method, the prevalence method, and the mortality method + the prevalence method) and the Cochrane–Armitage test revealed the increasing trend. The Fisher's exact test revealed the increased incidence rates in the 8,000 ppm male group and all of the female dosed groups.
(Tables 34 and 35)

TABLE 34 NEOPLASTIC LESIONS (LIVER) INCIDENCE AND STATISTICAL ANALYSIS : MOUSE:MALE

Group Name	Control	500 ppm	2000 ppm	8000 ppm
SITE : liver TUMOUR : hepatocellular adenoma				
Overall Rates(a)	7/50 (14.0)	16/50 (32.0)	22/50 (44.0)	8/50 (16.0)
Adjusted Rates(b)	19.35	45.45	56.00	26.92
Terminal Rates(c)	6/31 (19.4)	15/33 (45.5)	14/25 (56.0)	7/26 (26.9)
Standard Rates(d)	P=------			
Prevalence Rates(d)	P=0.7888			
Combind analysis(d)	P=------			
Cochran-Armitage Test(e)	P=0.2454			
Fisher Exact Test(e)		P=0.0704	P=0.0109*	P=0.4854
SITE : liver TUMOUR : hepatocellular carcinoma				
Overall Rates(a)	15/50 (30.0)	20/50 (40.0)	23/50 (46.0)	36/50 (72.0)
Adjusted Rates(b)	29.03	42.86	56.00	84.62
Terminal Rates(c)	9/31 (29.0)	13/33 (39.4)	14/25 (56.0)	22/26 (84.6)
Standard Rates(d)	P=0.0196*			
Prevalence Rates(d)	P<0.0001**			
Combind analysis(d)	P<0.0001**			
Cochran-Armitage Test(e)	P<0.0001**			
Fisher Exact Test(e)		P=0.2981	P=0.1801	P=0.0119*
SITE : liver TUMOUR : hepatocellular adenoma,hepatocellular carcinoma				
Overall Rates(a)	21/50 (42.0)	31/50 (62.0)	37/50 (74.0)	39/50 (78.0)
Adjusted Rates(b)	45.16	74.29	88.00	92.59
Terminal Rates(c)	14/31 (45.2)	24/33 (72.7)	22/25 (88.0)	24/26 (92.3)
Standard Rates(d)	P=0.0196*			
Prevalence Rates(d)	P<0.0001**			
Combind analysis(d)	P<0.0001**			
Cochran-Armitage Test(e)	P=0.0025**			
Fisher Exact Test(e)		P=0.1696	P=0.0646	P=0.0456*

TABLE 35 NEOPLASTIC LESIONS (LIVER) INCIDENCE AND STATISTICAL ANALYSIS : MOUSE:FEMALE

Group Name	Control	500 ppm	2000 ppm	8000 ppm
SITE : liver TUMOUR : hepatocellular adenoma				
Overall Rates(a)	4/50 (8.0)	30/50 (60.0)	20/50 (40.0)	2/50 (4.0)
Adjusted Rates(b)	11.11	83.33	52.00	20.00
Terminal Rates(c)	3/29 (10.3)	24/29 (82.8)	8/17 (47.1)	1/ 5 (20.0)
Standard Rates(d)	P=------			
Prevalence Rates(d)	P=0.9716			
Combind analysis(d)	P=------			
Cochran-Armitage Test(e)	P=0.0002‡‡			
Fisher Exact Test(e)		P<0.0001‡‡	P=0.0024‡‡	P=0.3574
SITE : liver TUMOUR : hepatocellular carcinoma				
Overall Rates(a)	0/50 (0.0)	6/50 (12.0)	30/50 (60.0)	45/50 (90.0)
Adjusted Rates(b)	0.0	11.63	81.82	100.00
Terminal Rates(c)	0/29 (0.0)	2/29 (6.9)	13/17 (76.5)	5/ 5(100.0)
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=0.0190‡	P<0.0001‡‡	P<0.0001‡‡
SITE : liver TUMOUR : hepatocellular adenoma,hepatocellular carcinoma				
Overall Rates(a)	4/50 (8.0)	34/50 (68.0)	41/50 (82.0)	46/50 (92.0)
Adjusted Rates(b)	11.11	86.67	86.55	100.00
Terminal Rates(c)	3/29 (10.3)	25/29 (86.2)	16/17 (94.1)	5/ 5(100.0)
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<0.0001‡‡	P<0.0001‡‡	P<0.0001‡‡

Mammary Gland

An increasing trend in incidence of adenocarcinoma in the female dosed groups was revealed by the Peto test (the prevalence method).

Heart

An increasing trend in incidence of hemangioendothelioma in the dosed male groups was revealed by the Peto test (the prevalence method) and the Cochrane–Armitage test.

Spleen

An increased incidence of follicular hyperplasia was noted among the males surviving at the time of the scheduled necropsy in the 2,000 ppm group and a decreased incidence of uterine tumor metabasis was noted among the non-surviving / moribund females in the 8,000 ppm group. An increasing trend in incidences of hemangioendothelioma and malignant lymphoma in the dosed male groups was revealed by the Peto test (the prevalence method). An increasing trend in incidence of malignant lymphoma in the dosed female groups was revealed by the Peto test (the prevalence method).

Lymph Node

A decreased incidence of uterine tumor metabasis was noted in the non-surviving / moribund females in the 8,000 ppm group. A decreasing trend in incidence of malignant lymphoma in the dosed female groups was revealed by the Cochrane–Armitage test.

Pituitary Gland

A decreasing trend in incidence of adenoma in the dosed female groups was revealed by the Cochrane–Armitage test, and the decreased incidence rate in the 8,000 ppm female group was revealed by the Fisher's exact test.

Harderian Gland

An increasing trend in incidence of adenoma in the dosed female groups was revealed by the Peto test (the prevalence method).

All Organs

After tallying and statistical processing of incidences of malignant lymphoma in all organs, the Peto test (the prevalence method) revealed an increasing trend among the males and the Cochrane-Armitage test revealed a decreasing trend among the females.

After tallying and statistical processing of incidences of hemangioendothelioma (benign) and hemangioendothelioma in all organs, an increasing trend in incidence of hemangioendothelioma (benign) among the males was revealed by the Peto test (the prevalence method), and a decreasing trend of hemangioendothelioma among the females was revealed by the Peto test (the mortality method).

Trachea

In the 8,000 ppm groups of both sexes (among the non-surviving / moribund animals and those surviving at the time of the scheduled necropsy), a significant increase in incidence of atrophy of the tracheal epithelium was observed. In the 8,000 ppm male group, an increase in incidence of nuclear enlargement of the tracheal epithelium was observed among the animals surviving at the time of the scheduled necropsy. (Table 36)

TABLE 36 NUMBER OF MOUSE WITH SELECTED TRACHEA LESIONS

	Male				Female			
Group	Control	500 ppm	2000 ppm	8000 ppm	Control	500 ppm	2000 ppm	8000 ppm
Number of examined animal	50	50	50	50	50	50	50	50
Nuclear enlargement:epithelium				17				
Atrophy: epithelium				42			2	49

Lung / Bronchial Tube

In the 8,000 ppm groups of both sexes (among the non-surviving / moribund animals and those surviving at the time of the scheduled necropsy), significant increases in incidence of atrophy of the epithelium (bronchial) and nuclear enlargement of the bronchial epithelium were observed. Also among the non-surviving / moribund females in the 2,000 ppm group, a significant increase in incidence of nuclear enlargement of the bronchial epithelium was observed. Among the females surviving at the time of the scheduled necropsy in the 2,000 ppm group, a significant increase in incidence of atrophy of the bronchial epithelium was observed.

In the 8,000 ppm groups, the non-surviving / moribund females and animals of both sexes surviving at the time of the scheduled had a significant increase in incidence of foamy cell formation. In 2,000 ppm groups, the non-surviving / moribund females had a decrease in incidence of leukemic cell infiltration.
(Table 37)

TABLE 37 NUMBER OF MOUSE WITH SELECTED LUNG/BRONCHUS LESIONS

	Male				Female			
Group	Control	500 ppm	2000 ppm	8000 ppm	Control	500 ppm	2000 ppm	8000 ppm
Number of examined animal	50	50	50	50	50	50	50	50
Nuclear enlargement:bronchial epithelium				31		1	22	48
Atrophy:epithelium				43			7	50
Accumulation of foamy cell	1			27		1	4	45
Bronchiolar-alveolar adenoma	3	2	5	1		1	3	1
Bronchiolar-alveolar carcinoma	6	8	8	4	1		3	

Kidney

In the 8,000 ppm male group (among the non-surviving / moribund animals and those surviving at the time of the scheduled necropsy), a significant increase in incidence of nuclear enlargement of the proximal tubules was observed. Incidences of vacuolation of the proximal tubular epithelium in males were increased among the non-surviving / moribund animals in the 8,000 ppm group. They were decreased among the survivors at the time of the scheduled necropsy in the 8,000 ppm group. They were increased among the survivors at the time of the scheduled necropsy in the 2,000 and 500 ppm groups. Among the non-surviving / moribund females in the 8,000 ppm groups, decreased incidences of uterine tumor metabasis and formation of hyaline droplets were observed.
(Table 38)

TABLE 38 NUMBER OF MOUSE WITH SELECTED KIDNEY LESIONS

	Male				Female			
Group	Control	500 ppm	2000 ppm	8000 ppm	Control	500 ppm	2000 ppm	8000 ppm
Number of examined animal	50	50	50	50	50	50	50	50
Nuclear enlargement:proximal tubule				39				8
Trasitional cell papilloma						1		

Teeth

Among the non-surviving / moribund animals and those surviving at the time of the scheduled necropsy in the 8,000 ppm male group, a decreased incidence of dysplasia was observed.

Testes

Among the non-surviving / moribund males in the 8,000 and 2,000 ppm groups, a decreased incidence of mineralization was observed.

Preputial Gland

Among the males surviving at the time of the scheduled necropsy in the 2,000 ppm group, an increased incidence of inflammation was observed.

Ovary

Among the non-surviving / moribund females in the 8,000 and 2,000 ppm groups, a decreased incidence of uterine tumor metabasis was observed. Among the non-surviving / moribund females in the 500 ppm group, an increased incidence of cysts was observed.

Uterus

Among the non-surviving / moribund females in the 2,000 ppm group, an increased incidence of cystic changes was observed.

Brain

Among the males surviving at the time of the scheduled necropsy in the 500 ppm group, an increased incidence of calcinosis was observed.

(4) Causes of Deaths

Causes of deaths pathologically determined for the non-surviving / moribund animals are listed in Table 39.

Slightly more males in the 8,000 ppm group than those in the control group died from hepatic tumor which was considered to be a cause of death (15 deaths in the 8,000 ppm group and 9 deaths in the control group). Many females in the 8,000 and 2,000 ppm groups died from hepatic tumor which was considered to be a cause of deaths (31 deaths in the 8,000 ppm group, 8 deaths in the 2,000 ppm group, and one death in the control group). There were a high number of deaths also from leukemia (primarily malignant lymphoma) in the 2,000 ppm female group (14 deaths in the 2,000 ppm group and 6 deaths in the control group).

TABLE 39 CAUSE OF DEATH :MOUSE

Group		Male				Female			
		Control	500ppm	2000ppm	8000ppm	Control	500ppm	2000ppm	8000ppm
Number of dead/moribund animal		19	17	25	24	21	21	33	45
Hepatic lesion		1				1			
Hemorrhage		1			3				
Urinary retention		1	1	1					
Tumor death	subcutis					1	1	2	1
	nasal				1				1
	spleen	2		3	1				1
	liver	9	8	11	15	1	2	8	31
	pituitary			2			1	1	1
	uterus					10	5	6	4
	leukemia	4	3	7	4	6	12	14	4
	others	1	2						1
Others						1		1	1
No microscopical confirmation			3	1		1		1	

VI Discussion

- Determining the Exposure Levels of the Carcinogenicity Study -

The exposure levels of the carcinogenicity study were determined based the results of the preliminary carcinogenicity studies (the thirteen-week studies) and the following criteria:

1) The carcinogenicity of the highest concentration of the substance can be proved. Types of cancers and the sites can be identified;
2) The median concentration must be a vicinity of a carcinogenic level;
3) The minimum concentration must be a non-carcinogenic level.

In the preliminary carcinogenicity studies, the drinking water which was prepared with 1,4-dioxane concentrations of 25,000, 10,000, 4,000, 1,600, 640 ppm, or 0 ppm was provided ad libitum to both rats and mice. As a result, there was one death each in the 25,000 ppm groups of both rats and mice. The final body weights of the survivors decreased by ≧ 20% compared with the control groups. Histopathological examinations revealed severe toxicity symptoms in the nasal cavity, trachea, liver, and brain. In the 10,000 mg/kg group of rats, females had a 12% decrease in the body weights and the animals of both sexes had a ≧ 35% decrease in water consumption in comparison with their control groups. They also had changes in the nasal cavity, trachea, and liver. In the 10,000 ppm group of mice, the body weights of both sexes stayed within a ≦ 10% decrease and water consumption showed a decrease of 48% among the males and of 30% among the females, compared with the control group. Histopathological examinations revealed changes in the nasal cavity, trachea, lungs, and liver. In the 4,000 ppm group of rats, the body weight decrease was extremely small, but water consumption decreased by 30% among the males and 20% among the females in comparison with their control groups. They also had changes in the nasal cavity, trachea, and liver. In the 4,000 ppm group of mice, the body weights hardly decreased, and water consumption stayed within a 14% decrease among the males. Histopathological examinations revealed changes in the nasal cavity, trachea, lungs, and liver. In the 1,600 ppm groups of both rats and mice, the body weights hardly decreased and water consumption decreased by 17% for male rats, 11% for female rats, and 14% for male mice. Histopathological examinations revealed changes in the nasal cavity and lungs for rats and the lungs for mice. In the 640 ppm groups of both rats and mice, there were no significant changes observed.

The rats in the ≧ 10,000 ppm groups had decreases in body weights and water consumption, and showed significant histopathological changes. The rats in the 4,000 ppm groups had decreases in water consumption, and changes in the nasal cavity, trachea, and liver. The rats in the 1,600 ppm groups showed changes in the nasal cavity and liver. The mice in the 25,000 ppm group indicated the same findings as those of the rats in the ≧ 10,000 ppm groups. The mice in the 10,000 and 4,000 ppm groups showed decreases in water consumption, and changes in the nasal cavity, trachea, lung, and liver. The mice in the 1,600 ppm group had changes only in the lung. These observations suggest rats has higher sensitivity to 1,4-dioxane than mice.

A past carcinogenicity study by administration of drinking water with 1,4-dioxane by NCI (1978, Reference 5) reported incidences of tumors in the nasal cavity (squamous cell cancer) in rats of both sexes, hepatocellular adenoma in female rats, and hepatocellular cancer in mice of both sexes at exposure levels of 5,000 and 10,000 ppm. According to the report, it was inferred that there could be little doubt that the concentrations ≧ 5,000 ppm of 1,4-dioxane caused carcinogenic effects on the nasal cavity and liver.

The dosage concentrations for carcinogenicity study were determined with a consideration of the above data. The median concentration was determined by looking at a concentration range that bordered carcinogenic levels yet did not cause severe general toxic symptoms. For rats, this range was determined to be 640–1,600 ppm, and the median concentration was determined to be 1,000 ppm. Considering the lower sensitivity seen in mice compared to rats, the median concentration for mice was determined to be 2,000 ppm, slightly higher than 1,600 ppm. As for the highest dosage concentration, a range of dosage concentrations was 4,000–5,000 ppm for rats. Based on the same common ratio as that of rats, a range of upper end dosage concentrations for mice was considered to be 8,000–10,000 ppm. Based on the study report by the NCI it was determined that the highest dosage concentration was 5,000 ppm for rats. The fact that mice avoid drinking water with 1,4-dioxanesuggested that the concentration level of 10,000 ppm was slightly high. Therefore, 8,000 ppm was chosen for mice. The common ratio was 5 for rats and 4 for mice. The lowest dosage concentration was determined to be 200 ppm for rats and 500 ppm for mice.

- Evaluation of the Results -

<Survivals>

For rats, compared with the control groups, number of survivors of both sexes at the ending of administration (Week 104) was significantly decreased in the 5,000 ppm groups. Among the males, a cause of death was primarily peritoneal mesothelioma, tumors or nonneoplastic lesions of the nasal cavity. Among the females, a cause of death was primarily tumors or nonneoplastic lesions of the nasal cavity or livers. Increased incidences of death were considered to be due to the administration of 1,4-dioxane which caused to increase these lesions. Among the non-survivors in the 5,000 ppm group which had histopathological findings of nasal changes, a few of them had an abnormal nasal noise and/or nasal mass during life. As for changes in body weight, suppression of body weight gain in the 5,000 ppm groups of both sexes was observed during the administration period, compared with the control groups.

Among the female mice, compared with the control groups, number of survivors at the ending of administration (Week 104) was significantly decreased in the 2,000 ppm group. A cause of death among the females was primarily the tumors of the livers in the 8,000 ppm group and the tumors of the livers or leukemia (primarily malignant lymphoma) in the 2,000 ppm group. Increased incidences of death among the females were considered to be due to the administration of 1,4-dioxane which caused to increase these lesions. Among the males, there was no significant difference in number of survivors in the dosed groups in comparison with the control group during the administration period.

As for changes in body weight, , compared with the control groups, suppression of body weight gain was observed in the 8,000 ppm groups of both sexes during the entire administration period and in the 2,000 ppm groups of both sexes at the end of administration.

<Neoplastic Lesions>

For rats, an increasing trend in incidence of nasal tumors was revealed by the Peto tests (the mortality method, the prevalence method, and the mortality method + the prevalence method) and the Cochrane–Armitage test, and the increased incidence rate in the 5,000 ppm groups of both sexes was also revealed by the Fisher's exact test. These results indicated 1,4-dioxane increased the incidences of nasal tumors. The most common type of nasal tumors was squamous cell carcinoma which showed a statistically significant increase. Though there were a few incidences, rhabdomyosarcoma, sarcoma NOS, and esthesioneuroepithelioma among the males and esthesioneuroepithelioma among the females were observed. The increased incidence of these tumors was not statistically significant. However, they were rare tumors and were considered to be caused by 1,4-dioxane.

Increasing trends in incidence of hepatocellular adenoma and hepatoma in rats were revealed by the Peto tests (the mortality method, the prevalence method, and the mortality method + the prevalence method) and the Cochrane–Armitage test, and the increased incidence rate in the 5,000 ppm groups of both sexes was revealed by the Fisher's exact test. These results indicated 1,4-dioxane increased the incidences of hepatocellular adenoma and hepatoma. The 1,000 ppm female group had many incidences of hepatocellular adenoma, but no statistically significant difference was observed.

An increasing trend in incidence of peritoneal mesothelioma only among male rats was revealed by the Peto tests (the mortality method, the prevalence method, and the mortality method + the prevalence method) and the Cochrane–Armitage test, and the increased incidence rate in the 5,000 ppm male group was revealed by the Fisher's exact test. The most common cause of death in the 5,000 ppm male group was mesothelioma. It is known that there is a gender difference in spontaneous occurrence of peritoneal mesothelioma in rats and that the tumor occurs more commonly in males. It was indicated that 1,4-dioxane further caused to increase incidences of this tumor in males.

As for other neoplastic lesions in rats, compared with the control groups, increasing trends in incidences of fibroma of the subcutis and fibroadenoma of the mammary glands in the male dosed groups and an increasing trend in incidence of adenoma of the mammary glands in the female dosed groups were revealed by the Peto test (the prevalence method) and the Cochrane–Armitage test. The increased incidence rate of adenoma of the mammary glands in the 5,000 ppm female group was revealed by the Fisher's exact test. The increased incidences of these tumors were also considered to be caused by 1,4-dioxane.

For mice, an increasing trend in incidence of adenocarcinoma in the nasal cavity in the dosed female groups was revealed by the Peto tests (the mortality method, and the mortality method + the prevalence method). However, there was one incidence in the 8,000 ppm group. There was one incidence of esthesioneuroepithelioma in the 8,000 ppm male group and only one incidence of papilloma in the 2,000 ppm female group. Spontaneuous occurrences of nasal cavity tumor in mice are extremely rare. Especially adenocarcinoma and esthesioneuroepithelioma which were observed in the 8,000 ppm groups of both sexes were considered to be caused by 1,4-dioxane, thought the number of the incidences was fewer than that of rats.

An increasing trend in incidence of hepatoma in mice in both sexes was revealed by the Peto tests (the mortality method, the prevalence method, and the mortality method + the prevalence method) and the Cochrane–Armitage test, and the increased incidence rates in the 8,000 ppm male group and all of the female dosed groups of mice was revealed by the Fisher's exact test. These results indicated 1,4-dioxane increased the incidences of hepatoma. The increased incidence of hepatoma was more significant among the females than the males, and the increased incidence was observed even in the lowest dosed group of 500 ppm among the females. As for hepatocellular adenoma, the Fisher's exact test revealed the increased incidence rate in the 2,000 ppm male group, and the 2,000 and 500 ppm female groups in comparison with the control groups. However, compared with the control group, a decreasing trend in incidence of hepatocellular adenoma in the female dosed groups was revealed by the Cochrane–Armitage test. Thus it was not clear that the increased trend was due to the administration of the substance. This phenomenon might have occurred due to the fact that there were very many incidences of hepatoma in the 8,000 ppm group of both sexes, resulting in decreased incidences of hepatocellular adenoma. After statistical processing of adding the numbers of hepatocellular adenoma and hepatoma, the Peto tests (the mortality method, the prevalence method, and the mortality method + the prevalence method) and the Cochrane–Armitage test revealed the increasing trend in the dosed groups of both sexes. The Fisher's exact test also revealed the increased incidence rates in the 8,000 ppm male group and all of the female dosed groups. Thus, even in consideration of the apparent decreasing trend of hepatocellular adenoma in the dosed groups, it was indicated that 1,4-dioxane increased the incidences of the tumors originated from a hepatocyte.

<Nonneoplastic Lesions>

In the nasal cavity of rats, many incidences of squamous metaplasia of the respiratory epithelium were observed in the 5,000 ppm groups of both sexes. A report on an inhalation study of formaldehyde by Swenberg et. al. (Reference 3) pointed out that squamous metaplasia of the respiratory epithelium was a precursor to squamous cell carcinoma. Thus the findings in the study should be considered as a precursor lesion to cancer. The following nonneoplastic lesions of the nasal cavity were observed in the 5,000 ppm groups in rats: adhesion of the nasal concha, respiratory epithelial metaplasia of the olfactory epithelium, hydropic degeneration and hardening of the lamina propria, atrophy of the olfactory epithelium, nuclear enlargement of the olfactory epithelium (the supporting cells) and respiratory epithelium, calcinosis, multiplication of the nasal glands, inflammation of the squamous epithelium, and acute rhinitis. These lesions were observed primarily in the respiratory epithelium located in the anterior half of the nasal cavity. Contrary to this, the following lesions were found the dorsal wall of the nasal cavity or on the olfactory epithelium in the posterior half of the nasal cavity: respiratory epithelial metaplasia of the olfactory epithelium, hydropic degeneration and hardening of the lamina propria, atrophy of the olfactory epithelium, nuclear enlargement of the olfactory epithelium (the supporting cells), calcinosis, and multiplication of the nasal glands. Adhesion was observed in both the nasal concha in the anterior half and the ethmoturbinals in the posterior half of the nasal cavity. Among these findings, hydropic degeneration and hardening of the lamina propria, and nuclear enlargement of the olfactory epithelium (the supporting cells) were specific lesions, which were rarely reported in any of the published literatures. A search for

a published literature reporting similar lesions located a report by Turk et. al. (Reference 4) which showed incidences of hydrops and fibrosis in the nasal mucosa after intraperitoneal injection of 3-methylindole. Their findings were morphologically very similar to hydropic degeneration and hardening of the lamina propria observed in the study. However, among the ≦ 1,000 ppm groups, nuclear enlargement of the olfactory epithelium (the supporting cells) was limited to some animals in the 1,000 ppm groups.

Among rats, an increased incidence of hyperplasia or cell focus in the livers was observed among the males in the ≧ 1,000 ppm groups and the females in the 5,000 ppm group. The types of cell focus increased were basophilic, acidophilic, clear, and mixed among males, and basophilic and mixed among females. The weights of the livers were significantly heavier in the 5,000 ppm group of both sexes, which was considered to be the weight increase caused by these lesions and tumors. Among other nonneoplastic lesions, there were increased incidences of spongiosis hepatis in the ≧ 1,000 ppm male groups and the 5,000 ppm female group. These changes were considered to be caused by 1,4-dioxane. Blood biochemistry examinations revealed that rats in the 5,000 ppm groups of both sexes had increases in GOT, GPT, LDH, ALP, and γ-GTP. These changes were related to the tumors and nonneoplastic lesions in the livers which were identified histopathologically after the administration of 1,4-dioxane.

In the nasal cavity of mice, rhinitis, atrophy of the olfactory epithelium, and nuclear enlargement of the respiratory epithelium and olfactory epithelium (the supporting cells) were observed in the 8,000 ppm groups of both sexes. Among the findings, nuclear enlargement of the olfactory epithelium (the supporting cells) was also observed in the 2,000 ppm groups of both sexes. These findings were considered to be changes caused by 1,4-dioxane, similar to those of rats.

In the livers of the 8,000 ppm male mice group, there were increased incidences of vasodilatation, which was considered to be changes caused by 1,4-dioxane. However, there was no increased incidence of hyperplasia or cell focus. Blood biochemistry examinations revealed that mice in the 8,000 and 2,000 ppm groups of both sexes had increases in GOT, GPT, LDH, and ALP. These changes were considered to be caused by the administration of 1,4-dioxane. Another nonneoplastic lesion caused by 1,4-dioxane was nuclear enlargement of the proximal renal tubules which was observed in rats in the 5,000 ppm groups of both sexes and mice in the 8,000 ppm male group. Nuclear enlargement of the trachea was observed among the mice in the 8,000 ppm male group and nuclear enlargement of the bronchi was observed in the 2,000 ppm groups of both sexes. These lesions accompanied atrophies of the tracheal and bronchial epithelium, and formation of foamy cells in alveoli. These changes of nuclear enlargement were also observed in the respiratory and olfactory epithelium in the nasal cavities of both rats and mice. It was inferred that 1,4-dioxane somehow affected nuclei of these tissues. Furthermore, rats in the 5,000 ppm male group showed an increased incidence of fibrosis in the spleen, which suggested a causal relationship to the administration of 1,4-dioxane.

- Study Result Comparison with Other Studies -

The results from the study were compared with the results from other published reports on incidences of tumors by R. J. Kociba (1974) (Reference 38) and NCI (1978) (Reference 5) (Tables 40 and 41).

According to the published reports, the types of tumors and the sites affected by 1,4-dioxane were primarily squamous cell carcinoma in the nasal cavity, hepatoma, and hepatocellular adenoma in rats, and hepatoma and hepatocellular adenoma in mice. The results of the study were the same as those of the published reports on mice. However, in addition to the results from the published reports on rats, increased incidences of peritoneal mesothelioma were observed in the study. As for the administration concentrations of 1,4-dioxane and tumor incidences, the published reports on rats indicated that tumors occurred at the concentrations of ≧ 5,000 ppm but no tumors occurred at the concentrations of ≦ 1,000 ppm. The significant increase in incidence of tumors observed in the published reports was observed only at 5,000 ppm in the study. The published reports on mice indicated that tumors occurred at ≧ 5,000 ppm, but there were no study reports on any concentrations below that level. The results of the study showed a significant increase in incidence of hepatic tumors at ≧ 2,000 ppm among the males and the lowest dosage group of 500 ppm among the females. The study observed that tumors occurred even at the low dosage level which was not tested in the other published reports.

The published reports noted only the following toxic lesions other than the tumors caused by 1,4-dioxane: inflammation in the nasal cavity and lesions in the livers and kidneys in rats, and inflammation in the nasal cavity and lungs in mice. The results of the study showed the following characteristic toxic lesions from 1,4-dioxane: nuclear enlargement of the proximal renal tubules, hydropic degeneration and hardening of the lamina propria in the nasal cavity, and nuclear enlargement of the olfactory epithelium (the supporting cells) and the respiratory epithelium (the supporting cells) for rats, and nuclear enlargement of the proximal renal tubules, the olfactory epithelium (the supporting cells), respiratory epithelium, trachea, and bronchi for mouse.

TABLE 40 TUMOR OBSERVED IN CARCINOGENESIS STUDIES OF 1,4-DIOXANE (DRINKING, RAT)

	Dose				
	100 ppm	200 ppm	1000 ppm	5000 ppm	10000 ppm
R. J. Kochiba et al. (1974) (Sherman Strain rat)	Negative (M, F)		Negative (M, F)		Hepatocellular Carcinoma (M, F) Nasal Carcinoma
NCI (1978) (Osborne-Mendel rat)				Nasal Tumor (M, F) Hepatocellular Adenoma (F)	Nasal Tumor (M, F) Hepatocellular Adenoma (F)
This Study (F344/DuCrj (Fischer) rat)		Negative (M, F)	Negative (M, F) (Hepatocellular Adenoma) (M:4/50, F:5/50)	Nasal Tumor (M, F). Hepatocellular Carcinoma (M, F) Hepatocellular Adenoma (M, F) Peritoneum Mesothelioma (M)	

TABLE 41 TUMOR OBSERVED IN CARCINOGENESIS STUDIES OF 1,4-DIOXANE (DRINKING, MOUSE)

	Dose				
	500 ppm	2000 ppm	5000 ppm	8000 ppm	10000 ppm
NCI (1978) ($B6C3F_1$ mouse)			Hepatocellular Carcinoma (M, F) Hepatocellular Adenoma (F)		Hepatocellular Carcinoma (M, F) Hepatocellular Adenoma (F)
This Study ($Crj:BDF_1$ mouse)	Negative (M) Hepatocellular Carcinoma (F) Hepatocellular Adenoma (F)	Hepatocellular Carcinoma (M, F) Hepatocellular Adenoma (F)		Hepatocellular Carcinoma (M, F)	

V <Conclusion>

To summarize two-year carcinogenicity study of 1,4-dioxane, it was found that F344/DuCrj (Fischer) rats showed increased incidences of primarily squamous cell carcinoma in the nasal cavity, hepatoma, and peritoneal mesothelioma in the 5,000 ppm groups, and that Crj: BDF_1 mice showed increased incidences of hepatoma in the ≧ 500 ppm groups. These indicated carcinogenicity of 1,4-dioxane in both rats and mice.

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