Report of Preliminary Carcinogenicity Studies (Acute, Two-Week, and Thirteen-Week Studies) 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 Preliminary Carcinogenicity Studies (Acute, Two-Week, and Thirteen-Week Studies) by Oral Administration of 1,4-Dioxane (Mixed with Water) to Rats and Mice

Study Objective

The studies were conducted to collect data from single oral administration, continuous two-week and thirteen-week oral administration of 1,4-dioxane to rats and mice, in order to determine concentration levels in a carcinogenicity study.

Name and Address of Study Facility

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

Study Schedules

Acute Studies

Animal Introduction	Rats	February 14, 1983
	Mice	February 15, 1983
Study Group Assignment	Rats	February 21, 1983
	Mice	February 22, 1983
Administration	Rats	February 21, 1983
	Mice	February 22, 1983
Scheduled Necropsy	Rats	March 7, 1983
	Mice	March 8, 1983

Two-Week Studies

Animal Introduction	Rats	July 5, 1983
	Mice	July 6, 1983
Study Group Assignment	Rats	July 19, 1983
	Mice	July 20, 1983
Beginning Administration	Rats	July 19, 1983
	Mice	July 20, 1983
Ending Administration	Rats	August 2, 1983
	Mice	August 3, 1983
Scheduled Necropsy	Rats	August 2, 1983
	Mice	August 3, 1983

Thirteen-Week Studies

Animal Introduction	Rats	July 2, 1984
	Mice	July 9, 1984
Study Group Assignment	Rats	July 16, 1984
	Mice	July 23, 1984
Beginning Administration	Rats	July 16, 1984
	Mice	July 23, 1984
Ending Administration	Rats	October 16–18, 1984
	Mice	October 23–25, 1984
Scheduled Necropsy	Rats	October 16–18, 1984
	Mice	October 23–25, 1984

Names of the Final Report Authors

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Statement by the Chief Investigator

Study Title : Preliminary Carcinogenicity Studies (Acute, Two-Week, and Thirteen-Week Studies) by Oral Administration of 1,4-Dioxane (Mixed with Water) to Rats and Mice

The studies were conducted in accordance with the Study Protocols. 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: 0003, 0004, 0018, 0019, 0053, 0054

Title: Preliminary Carcinogenicity Studies (Acute, Two-Week, and Thirteen-Week Studies) 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 studies were conducted accurately in accordance with the Study Protocols, 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 Preliminary Carcinogenicity Studies (Acute, Two-Week, and Thirteen-Week Studies) by Oral Administration of 1,4-Dioxane (Mixed with Water) to Rats and Mice

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Abstract

Acute, two-week, and thirteen-week studies were conducted as preliminary carcinogenicity studies of 1,4-dioxane in F344/DuCrj (Fischer) rats and Crj: BDF_1 mice.

In the acute studies, six study groups were created, with each group consisting of 10 males and 10 females of rats and 10 males and 10 females of mice, totaling 120 rats and 120 mice. The 1,4-dioxane solutions of 10,000, 7,143, 5,102, 3,644, or 2,603 mg/kg body-weight (with a common ratio of 1.4) for rats, and the 1,4-dioxane solutions of 9,000, 6,923, 5,325, 4,096, or 3,151 mg/kg body-weight (with a common ratio of 1.3) for mice were administered once with gavage using a stomach tube. A follow-up observation was conducted for two weeks after the gavage. Only a solvent of deionized water was administered to the control groups. The following was performed: observations of general symptoms, measurement of body weight, and pathological examinations. Up to two weeks after the administration, all male rats in the 10,000 mg/kg group, eight male rats in the 7,143 mg/kg group, and no male rats in the ≦ 5,102 mg/kg groups were found dead. All female rats in the ≧ 7,143 mg/kg groups, five female rats in the 5,102 mg/kg group, and no female rats in the ≦ 3,644 mg/kg groups were found dead. Up to two weeks after the administration, all mice of both sexes in the ≧ 6,923 groups, six mice of both sexes in the 5,325 mg/kg groups, and no mice of both sexes in the ≦ 4,096 mg/kg groups were found dead. Pathological examinations revealed effects of 1,4-dioxane in the kidney and liver for rats and the kidney for mice.

Based on the above results and taking into account water consumption by the animals, it was determined that the appropriate highest dosage concentration of 1,4-dioxane in drinking water for the two-week studies was 90,000 ppm in order to have a daily consumption of 1,4-dioxane of approximately 5,000 mg/kg.

In the two-week studies, six study groups were created, with each group consisting of 10 males and 10 females of rats and 10 males and 10 females of mice, totaling 120 rats and 120 mice. Drinking water with 1,4-dioxane was prepared for both rats and mice with concentrations of 90,000, 30,000, 10,000, 3,330, 1,110 ppm (with a common ratio of 3.0), or 0 ppm (for the control groups). The drinking water was provided ad libitum for two weeks. The following was performed: observations of general symptoms, measurement of body weight, food and water consumption, and pathological examinations. All rats of both sexes in the 90,000 ppm groups and two female rats in the 30,000 ppm group were found dead. Suppression of body weight gain was also found in rats of both sexes in the ≧ 10,000 ppm groups during the entire study period. Deaths of mice were found only in the 90,000 ppm groups; nine males and all females were found dead. Suppression of body weight gain was also found in mice of both sexes in the ≧ 10,000 ppm groups during the entire study period. Pathological examinations revealed effects of 1,4-dioxane to the liver, nasal cavity, brain, and kidney for rats and the kidney for mice. Based on these results, if the highest dosage concentration were to be set to ≧ 30,000 ppm for thirteen-week studies, it was expected that the animals would not tolerate the toxicity of 1,4-dioxane. Therefore, the lower concentration was considered desirable. In order to determine a No Observed Effect Concentration (NOEC), it was also considered desirable to set the lowest dosage concentration to a concentration lower than 1,100 ppm at which effects were observed during the necropsy in the two-week studies.

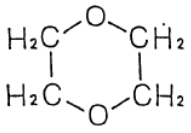
In the thirteen-week studies, six study groups were created, with each group consisting of 10 males and 10 females of rats and 10 males and 10 females of mice, totaling 120 rats and 120 mice. Drinking water with 1,4-dioxane for both rats and mice was prepared with concentrations of 25,000, 10,000, 4,000, 1,600, 640 ppm (with a common ratio of 2.5), or 0 ppm (for the control groups). The drinking water was provided ad libitum for thirteen 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. Rats in the ≧ 10,000 ppm groups had a decrease in body weight and water consumption and showed intense changes in the nasal cavity, trachea, liver, kidney, and brain during histopathological examinations. Rats in the 4,000 ppm groups had a decrease in water consumption and showed changes in the nasal cavity, trachea, and liver. Rats in the 1,600 ppm groups had changes in the nasal cavity and liver. These findings were considered to be effects of the 1,4-dioxane administration. There were no significant changes in rats in the 640 ppm groups. Mice in the 25,000 ppm group had the same findings as the rats in the ≧ 10,000 ppm groups. The mice in the 10,000 and 4,000 ppm groups had a decrease in water consumption and showed changes in the nasal cavity, trachea, lungs, and liver. These findings were considered to be effects of the 1,4-dioxane administration. The mice in the 1,600 ppm groups showed changes in the lungs only. There were no significant changes in mice in the 640 ppm groups.

Based on the above findings, rats had slightly higher sensitivity to 1,4-dioxane than mice.

The dosage concentrations for carcinogenicity studies were determined with a comprehensive consideration of the above results. 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 with rats, the median concentration for mice was determined to be 2,000 ppm, a level slightly higher than 1,600 ppm. The highest dosage concentrations were determined to be 5,000 ppm for rats and 8,000 ppm for mice, considering ≧ 5,000 ppm as the concentration at which no severe toxic changes appear but carcinogenicity could be reliably proved based on a published study by NCI (1978). The common ratio was 5 for rats and 4 for mice. Therefore the lowest dosage concentrations were determined to be 200 ppm for rats and 500 ppm for 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 1 and 4)

<Production Volume>

The production volumes of 1,4-dioxane 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 1, 5, 6, and 7)

<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 8 and 9)

<Effects on Humans>

The atmospheric concentration of 300 ppm causes irritation of the eyes, nose, and throat (Reference 10). 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 11)

I Study Materials

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

Lot Numbers		
Acute Studies	:	WKE1190
Two-Week Studies	:	PEN6246
Thirteen-week studies	:	CDP4224
Manufacturer	:	Wako Pure Chemical Industries, Ltd.
Grade	:	Special grade
Purity	:	≧ 99%
Moisture	:	≦ 0.3%
Nonvolatile Matter	:	≦ 0.01%
Stabilizer	:	Butyl hydroxytoluene, approximately 5 ppm

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. These results are provided in Appendix A 4-1 for the acute studies, Appendix B 5-1 for the two-week studies, and Appendix O 1 for the thirteen-week studies.

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. These results are provided in Appendix A 4-1 for the acute studies, Appendix B 5-1 for the two-week studies, and Appendix O 1 for the thirteen-week studies.

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 for the acute, two-week, and thirteen-week studies.

For the acute studies, 72 male and 72 female rats and 72 male and 72 female mice at 5 weeks old were introduced (the range of body weights at the time of acceptance: 85–100 g for male rats, 80–90 g for female rats, 20–25 g for male mice, and 15–19 g for female mice). After one week of quarantine and habituation, 60 male and 60 female rats and 60 male and 60 female mice with normal development, no abnormal general symptoms, and body weights close to the median weight were selected and used for the studies (the range of body weights at the beginning of administration: 104–119 g for male rats, 86–95 g for female rats, 19.9–22.0 g for male mice, and 15.9–18.1 g for female mice). Prior to the selection, no food was given to the animals on the day before the administration (≧ 18 hours) as a preparation for substance dosage.

For the two-week studies, 72 male and 72 female rats and 72 male and 72 female mice at 4 weeks old were introduced. (The range of the body weights at the time of acceptance: 55–65 g for male rats, 50–60 g for female rats, 15–20 g for male mice, and 13–17 g for female mice). After two weeks of quarantine and habituation, 60 male and 60 female rats and 60 male and 60 female mice with normal development, no abnormal general symptoms, and body weights close to the median weight were selected and used for the studies (the range of the body weights at the beginning of administration: 125–141 g for male rats, 100–109 g for female rats, 22.2–24.9 g for male mice, and 18.6–20.7 g for female mice).

For the thirteen-week studies, 72 male and 72 female rats and 72 male and 72 female mice at 4 weeks old were introduced. (The range of the 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 two weeks of quarantine and habituation, 60 male and 60 female rats and 60 male and 60 female mice with normal development, no abnormal general symptoms, and body weights close to the median weight were selected and used for the studies (the range of the body weights at the beginning of administration: 123–137 g for male rats, 97–107 g for female rats, 21.8–24.9 g for male mice, and 17.9–19.8 g for female mice).

The reason why F344/DuCrj (Fischer) rats (SPF) and Crj: BDF_1 mice (SPF) were used for the preliminary carcinogenicity studies was because these animals had the same characteristics as the animals used in the actual carcinogenicity study.

The reasons for selecting the laboratory animals used for the actual carcinogenicity study are as follows:

a) Genetic stability;
b) Fewer spontaneous tumor incidences; and
c) The data 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

In the acute studies, 1,4-dioxane solution was administered once with gavage using a stomach tube. The laboratory animals were fasted starting from the day before the administration. The dosage for each group was 0.1 ml of 1,4-dioxane solution per 10 g of body weight (measured on the day of the administration). Similarly, the control groups received 0.1 ml of deionized water per 10 g of body weight with gavage using a stomach tube.

Drinking water dissolved with 1,4-dioxane was easily accessible for the laboratory animals continuously for 14 days in the two-week studies and 92–94 days in the thirteen-week studies. Deionized water was easily accessible for the control groups during the periods.

II-1-2 Administration Dosage, Concentration, and Selection Reason

In the acute studies with rats, the highest dosage was set at 10,000 mg/kg (the dosage per 1 kg of body weight), and the lower dosages were set at 7,143, 5,102, 3,644, or 2,603 mg/kg (with a common ratio of 1.4). In the acute studies with mice, the highest dosage was set at 9,000 mg/kg, and the lower dosages were 6,923, 5,325, 4,096, or 3,151 mg/kg (with a common ratio of 1.3).

In the two-week studies for both rats and mice, the highest concentration was set at 90,000 ppm and the lower concentrations were set at 30,000, 10,000, 3,330, or 1,110 ppm (with a common ratio of 3.0).

In the thirteen-week studies for both rats and mice, the highest concentration was set at 25,000 ppm and the lower concentrations were set at 10,000, 4,000, 1,600, or 640 ppm (with a common ratio of 2.5).

The concentrations used in the acute studies were set based on the literature values (Reference 1). The concentrations in the two-week studies were set based on the results from the acute studies, and the concentrations in the thirteen-week studies were set based on the results from the two-week studies.

II-1-3 Study Solution Preparation

On the day of administration for the acute studies, the 1,4-dioxane solutions for the highest dosage groups were first prepared by dissolving the substance in deionized water (high pressure steam sterilized). Then the solutions for the remaining groups were prepared by a serial dilution method. The highest dosage concentration for rats was set at 1,000 g/l and the lower dosage concentrations were set at 714.3, 510.2, 364.4, or 260.3 g/l. The highest dosage concentration for mice was set at 900 g/l and the lower dosage concentrations were set at 692.3, 532.5, 409.6, or 315.1 g/l.

In the two-week and thirteen-week studies, once the solutions were prepared by mixing 1,4-dioxane with deionized water (ultraviolet ray and filter sterilized) to the pre-determined concentrations (10% (W/W) for the two-week studies and 16% for the thirteen-week studies), the prepared solutions were used to make the other solutions by adding deionized water in order to dilute them to the set concentrations. The concentrations in the studies were expressed as percentage and ppm values, which were both weight per weight concentrations. The solutions were prepared twice a week when the drinking water was changed.

II-1-4 Concentration Measurement at Time of Preparation

The concentrations of the 1,4-dioxane solutions administered in all three studies were measured using a gas chromatography method to ensure that the concentrations of the solutions would be measured in the range of the set concentrations of 97–101% for the acute studies, 92–100% for the two-week studies, and 95–103% for the thirteen-week studies. The results are listed in Appendices A 4-3, B 5-3, and O 3 for the acute, two-week, and thirteen week studies respectively.

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. The results are listed in Appendices B 5-4 and O 4 for the two-week and thirteen week studies respectively.

II-1-6 Amount of the Study Articles Consumed

The ingested amounts of 1,4-dioxane for the two-week and thirteen week studies were calculated from the measured body weights, the amounts of water consumed, and the set concentrations.

The results are listed in Appendices B 1-1 to 4 and Appendix C 1 to 4 for the two-week and thirteen week studies respectively.

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 for the acute, two-week, and thirteen-week studies (Reference 2).

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

The rats and mice for all of the studies 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 for all of the studies were reared in a barrier-system animal room with a temperature of 24 ± 1℃, 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 periods of quarantine and habituation for the acute studies and the periods of quarantine for the two-week and thirteen-week studies, 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 for the two-week and thirteen-week studies, 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 animals in the acute studies were fasted starting from the day before the administration up to the end of the administration (≧ 18 hours). The drinking water for the entire rearing period of the acute studies and for the quarantine periods of the two-week and thirteen-week studies was provided by Hadano City Waterworks Bureau. The water was filtered and sterilized by UV rays before it was placed into an automatic watering system from which the drinking water was provided ad libitum. The drinking water for the periods of habitation and administration of the two-week and thirteen-week studies 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.

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 after the administration, one, two, four and six hours after the administration on the administration day, and once daily after that for the acute studies, and once daily for the two-week and thirteen-week studies.

II-3-2 Measurement of Body Weight

In the acute studies, the body weight was measured on Days 1, 2, 3, 4 and 7 in Week 1, and Days 3 and 7 in Week 2 during the observation period. In the two-week studies, the body weight was measured on Days 1, 2, 4, and 7 in Week 1 and Days 3 and 7 in Week 2 after the start of the administration. In the thirteen-week studies, the body weight was measured once weekly after the start of the administration.

II-3-3 Measurement of Food Consumption

The food consumption was measured once weekly in the two-week and thirteen-week studies.

II-3-4 Measurement of Water Consumption

The water consumption was measured twice weekly in the two-week and thirteen-week studies.

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 in the thirteen-week studies and put under etherization immediately before necropsy. Hematological examinations in the thirteen-week studies 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 1 and Appendix P 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 in the thirteen-week studies and put under etherization immediately before necropsy. Heparin sodium 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 1 and Appendix P 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 in the thirteen-week studies.

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

II-3-8 Pathological Examination

All animals in each study were visually observed at the time of necropsy. Tissue sections of organs from two or more males and two or more females chosen arbitrarily in each group of the acute and two-week studies and those from all animals in the thirteen-week studies were studied histopathologically with an optical microscope. The tissues examined were from the organs listed in Table 1 and the ones only with visually observed changes. 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 tissue sections of the organs were prepared for pathological examinations by being embedded in paraffin, sliced thinly and stained with hematoxylin-eosin, after being fixed in 10% neutral phosphate buffered formalin solution.

The wet weight (actual weight) of organs listed in Table 1 was measured for all animals surviving up to the scheduled necropsy for the thirteen-week studies.

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

	Acute Studies	Two-week Studies	Thirteen-week Studies
Method of Administration	Gavage	Drinking water	Drinking water
Number of Groups	Male 6, Female 6	Male 6, Female 6	Male 6, Female 6
Size of Study Groups	10 males and 10 females of each groups	10 males and 10 females of each groups	10 males and 10 females of each groups
Animals			
Strain and Species	F344/DuCrj(Fischer)rat Crj:BDF_1mouse	F344/DuCrj(Fischer)rat Crj:BDF_1mouse	F344/DuCrj(Fischer)rat Crj:BDF_1mouse
Animal Source	Charles River Japan,Inc.	Charles River Japan,Inc.	Charles River Japan,Inc.
During of Time Held Before Study	1 wk	2 wk	2 wk
Age When Placed on Study	6 wk	6 wk	6 wk
Age When Killed	8 wk	8 wk	19 wk
Doses	0,2603,3644,5102,7143 or 10000 mg/kg;rat 0,3151,4096,5325,6923 or 9000 mg/kg;mouse	0,1110,3330,10000,30000 or 90000 ppm	0,640,1600,4000,10000 or 25000 ppm
Duration of Dosing	Single	7d/wk for 2 wk	7d/wk for 13 wk
Animal Maintenance			
Feed	CRF-1 (Oriental Yeast Co.,Ltd.) Sterilized by γ-ray Available ad libitum	Same as Acute Studies	Same as Acute Studies
Water	Sterilized by ultraviolet rays Automatic watering system Available ad libitum	Formulated water Deionized water sterilized by filter and ultraviolet ray Available ad libitum by water bottle	Same as Two-week Studies
Animals per Cage	Single	Single	Single
Animal Room Environment	Barrier system Temperature:24±2℃ Humidity :55±10% Fluorescent light 12h/d 15-17 room air changes /h	Same as Acute Studies	Same as Acute Studies
Type and Frequency of Observation			
Clinical Sign	Observed 1,2,4 and 6h after administration/ observed 1×d thereafter	Observed 1×d	Observed 1×d
Body Weight	Weighed 0-0,1-1,1-2,1-3,1-4, 1-7,2-3 and 2-7(wk-d)	Weighed 0-0,1-1,1-2,1-4,1-7, 2-3 and 2-7(wk-d)	Weighed 1×wk for 13wk
Food Consumption	None	Weighed 1×wk for 2wk	Weighed 1×wk for 13wk
Water Consumption	None	Weighed 2×wk for 2wk	Weighed 2×wk for 13wk
Hematology	None	None	Red blood cell(RBC) Hemoglobin Hematocrit Mean corpuscular volume(MCV) Platelet White blood cell(WBC) Differential WBC

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

	Acute Studies	Two-week Studies	Thirteen-week Studies
Blood Biochemistry	None	None	Total protein Albumin A/G ratio T-bilirubin Glucose T-cholesterol Triglyceride <rat only> Glutamic oxaloacetic transaminase(GOT) Glutamic pyruvic transaminase(GPT) Lactate dehydrogenase(LDH) Alkaline phosphatase(ALP) Leucine aminopeptidase(LAP) Creatine phosphokinase(CPK) <rat only> Urea nitrogen Creatinine <rat only> Sodium Potassium Chloride Calcium <rat only> Inorganic phosphorus <rat only>
Urinalysis	None	None	pH Protein Glucose Ketone body Bilirubin <rat only> Occult blood Urobilinogen
Necropsy	Necropsy performed on all animals.	Same as acute studies	Same as acute studies
Organ Weight	None	None	Organ weight measurement performed on schedule sacrificed animals. The following organs were weighed: brain,lung,liver,spleen,heart, kidney,adrenal,testis,ovary,thymus.
Histopathologic Examination	Histopathologic examination performed on at least two animals per sex per group. The following organs were examined :brain,lung,liver,spleen,heart, kidney,adrenal,testis,ovary, pancreas,stomach, small intestine,large intestine, thymus,lymph nodes(axilla,inguinal), pituitary,bone marrow.	Same as acute studies	Histopathologic examination performed on all animals. The following organs were examined :brain,lung,liver,spleen,heart, kidney,adrenal,testis,ovary, thyroid,parathyroid,pancreas,stomach, small intestine,large intestine, thymus,lymph nodes(axilla,inguinal), pituitary,urinary bladder,eye, tongue,spinal cord, peripheral nerve(sciatic), esophargus,bone marrow,epididimys, seminal vesicle,prostate, salivary gland,skin,uterus,vagina, mammary gland,muscle,trachea, Harder gland,nasal cavity,bone.

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 three to 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 P 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 and water consumption 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 number of animals examined was used as the population size for the histopathological data for the acute and two-week studies.

(3) Statistical Methodology

With the control groups used as the standard groups, all measurements obtained in the studies 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 thirteen-week study 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 urinalysis.

II-5 Archive of Study Records and Materials

The Study Protocols, samples, raw data, records, final report, Proof of Quality Assurance, study materials, and any other materials related to the studies 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 Preliminary Carcinogenicity Studies in Rats

III-1-1 Acute Studies

(1) Observation of Animal Conditions

Survival

Deaths were observed in the ≧ 7,143 mg/kg male groups and the ≧ 5,102 mg/kg female groups. The following deaths were observed in the 10,000 mg/kg group of 10 males: five on the date of administration (four in 2–4 hours and one in 4–6 hours after the administration) and five on Day 1 in Week 1, totaling 10 deaths. The following deaths were observed in the 7,143 mg/kg group of 10 males: one on the date of administration (2–4 hours after the administration), six on Day 1 in Week 1, and one on Day 2 in Week 1, totaling eight deaths. The following deaths were observed in the 10,000 mg/kg group of 10 females: four on the date of administration (two in 1–2 hours and two in 2–4 hours after the administration) and six on Day 1 in Week 1, totaling 10 deaths. In the 7,143 mg/kg group of 10 females, 10 died on Day 1 in Week 1. The following deaths were observed in the 5,102 mg/kg group of 10 females: three on Day 2 in Week 1, one on Day 3 in Week 1, and one on Day 4 in Week 1, totaling five deaths. (Tables 2 and 3)

General Symptoms

The observed symptoms of the non-survivors in each group of both sexes included decreased locomotor movement, piloerection, maintenance of the abdominal and lying positions, closed eyelids, watery eyes, weakening, loss of righting reflex, cyanosis, abnormal respiration (irregular, depressed and difficult respiration), and decreased body temperature. The observed symptoms of the survivors in each group of both sexes included decreased locomotor movement, piloerection, abnormal body position (hunchback, abdominal and lying position), watery eyes, coloration, bloody nasal discharge, ataxic gait, abnormal respiration, and closed eyelids, all of which improved over time. The symptoms such as piloerection and hunchback position were observed up to the middle of the observation period, but they were barely observed after that. (Appendices A 1-1 and 2)

Body Weight

Survivors of both sexes in all groups showed suppression of body weight gain corresponding to the dosages received in comparison with the control groups. The final body weights in percentage in comparison with the control groups (Day 7 in Week 2) were 92% in the 7,143 mg/kg group, 86% in the 5,102 mg/kg group, 91% in the 3,644 mg/kg group, and 93% in the 2,603 mg/kg group of males and 93% in the 5,102 mg/kg group, 94% in the 3,644 mg/kg group, and 96% in the 2,603 mg/kg group of females. (Tables 2 and 3, Figures 1 and 2)

TABLE 2 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN MALE RAT (ACUTE STUDIES)

Week-Day on Study	Control Av.Wt.	No.of Surviv.	2603 Av.Wt.	mg/kg % of cont.	No.of Surviv.	3644 Av.Wt.	mg/kg % of cont.	No.of Surviv.	5102 Av.Wt.	mg/kg % of cont.	No.of Surviv.	7143 Av.Wt.	mg/kg % of cont.	No.of Surviv.	10000 Av.Wt.	mg/kg % of cont.	No.of Surviv.
0-0	113 (10)	10	113 (10)	100	10	113 (10)	100	10	113 (10)	100	10	113 (10)	100	9	113 (10)	100	5
1-1	126 (10)	10	118 (10)	94	10	110 (10)	87	10	103 (10)	82	10	110 (9)	87	3	111 (5)	88	0
1-2	133 (10)	10	125 (10)	94	10	117 (10)	88	10	103 (10)	77	10	108 (3)	81	2			
1-3	139 (10)	10	130 (10)	94	10	125 (10)	90	10	113 (10)	81	10	113 (2)	81	2			
1-4	146 (10)	10	136 (10)	93	10	131 (10)	90	10	118 (9)	81	10	118 (2)	81	2			
1-7	162 (10)	10	152 (10)	94	10	147 (10)	91	10	137 (9)	85	10	141 (2)	87	2			
2-3	180 (10)	10	167 (10)	93	10	162 (10)	90	10	153 (9)	85	10	160 (2)	89	2			
2-7	200 (9)	10	186 (10)	93	10	181 (10)	91	10	172 (9)	86	10	183 (2)	92	2			

TABLE 3 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN FEMALE RAT (ACUTE STUDIES)

Week-Day on Study	Control Av.Wt.	No.of Surviv.	2603 Av.Wt.	mg/kg % of cont.	No.of Surviv.	3644 Av.Wt.	mg/kg % of cont.	No.of Surviv.	5102 Av.Wt.	mg/kg % of cont.	No.of Surviv.	7143 Av.Wt.	mg/kg % of cont.	No.of Surviv.	10000 Av.Wt.	mg/kg % of cont.	No.of Surviv.
0-0	90 (10)	10	90 (10)	100	10	90 (10)	100	10	90 (10)	100	10	90 (10)	100	10	90 (10)	100	6
1-1	99 (10)	10	91 (10)	92	10	87 (10)	88	10	85 (10)	86	10	87 (10)	88	0	87 (6)	88	0
1-2	103 (10)	10	97 (10)	94	10	91 (10)	88	10	80 (10)	78	7						
1-3	107 (10)	10	100 (10)	93	10	96 (10)	90	10	82 (7)	77	6						
1-4	109 (10)	10	104 (10)	95	10	100 (10)	92	10	91 (5)	83	5						
1-7	117 (10)	10	113 (10)	97	10	110 (9)	94	10	103 (5)	88	5						
2-3	125 (10)	10	118 (10)	94	10	118 (10)	94	10	114 (5)	91	5						
2-7	134 (10)	10	129 (10)	96	10	126 (10)	94	10	125 (5)	93	5						

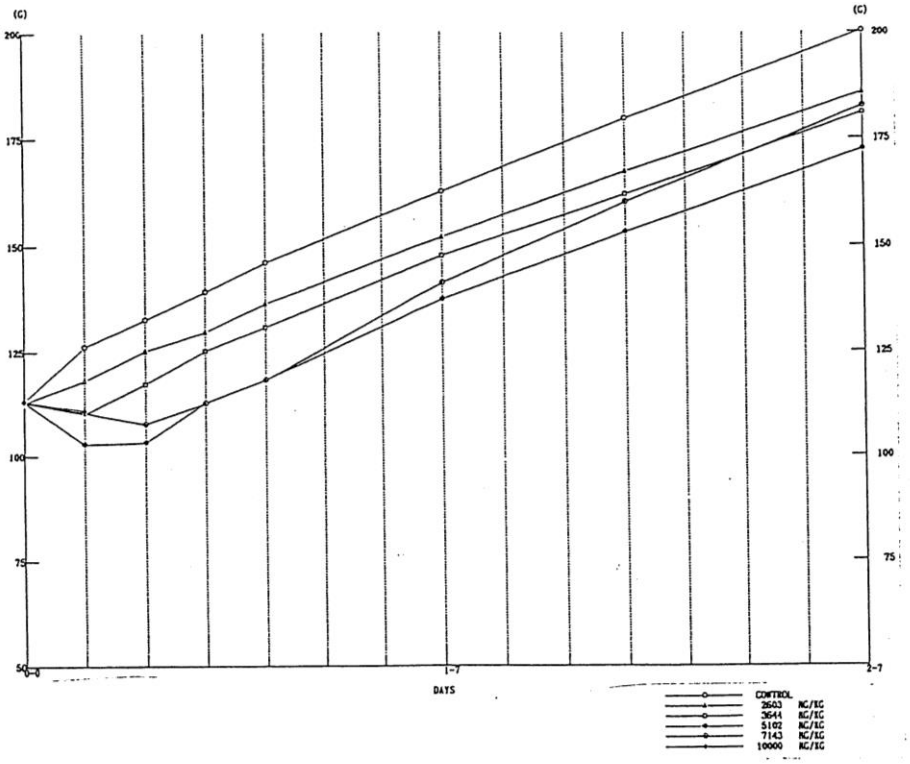


FIGURE 1 BODY WEIGHT CHANGES : RAT:MALE (ACUTE STUDIES)

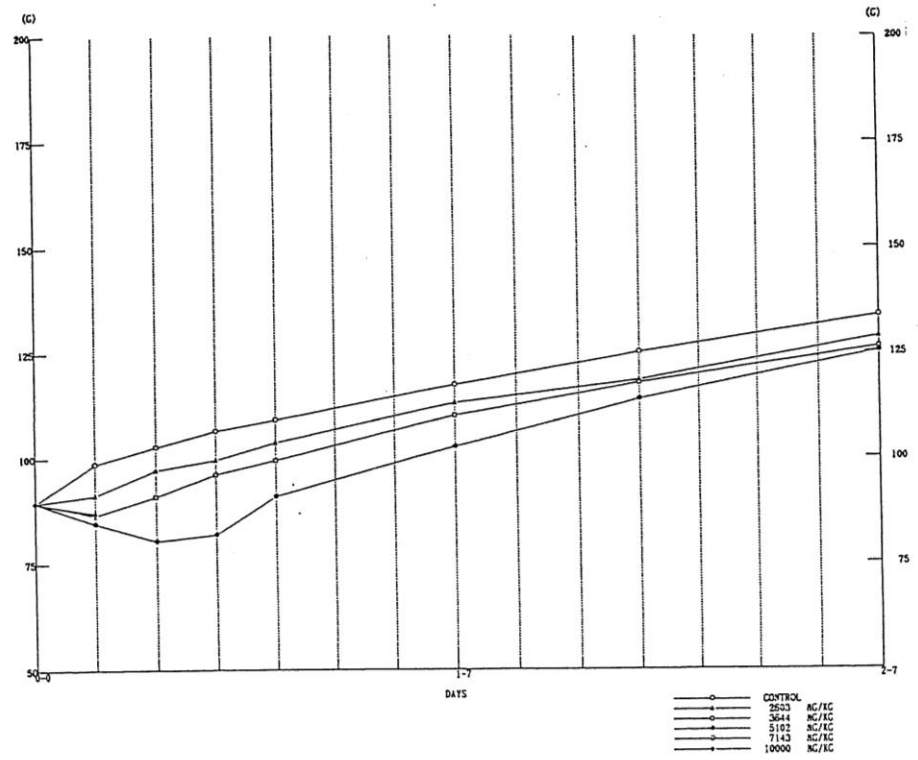


FIGURE 2 BODY WEIGHT CHANGES : RAT:FEMALE (ACUTE STUDIES)

(2) Pathological Examination

Necropsy

Findings observed at the time of necropsy are listed in Appendices A 2-1 to 4 (the Summary Tables) and Appendices A 5-1 and 2 (the Individual Tables).

Red dots / macules on the thymic and stomach glands and black, brown, red or clear fluid accumulation in the stomach or small intestines were observed in many non-survivors of both sexes. The following was observed: kidney enlargement in not less than half of the animals in the 7,143 mg/kg female group and the enlargement and anemic-like coloration of the kidneys in not less than half of the animals in the 5,102 mg/kg female group.

There were no characteristic findings in the study groups of both sexes surviving at the time of the scheduled necropsy.

Histopathological Examination

Histopathological examinations on the non-survivors were conducted on three males and three females in the 10,000 mg/kg groups, three males and two females in the 7,143 mg/kg groups, and three females in the 5,102 mg/kg. Histopathological examinations on the survivors at the time of the scheduled necropsy were conducted on two males and two females in the 7,143 mg/kg groups. The results are shown in Appendices A 3-1 to 4 (the Summary Tables, nonneoplastic lesions) and Appendices A 6-1 and 2 (the Individual Tables).

Non-survivors showed congested lungs, edema and hemorrhage near the pulmonary vessels, congested spleens, erosion of stomach glands, renal tubular necrosis and hydropic degeneration of the proximal renal tubules, hemorrhage and karyorrhexis in the thymic gland, hemorrhage in the heart, centrilobular hepatic vacuolar degeneration, adrenal hemorrhages, and corneal inflammation.

Animals surviving at the time of the scheduled necropsy showed pulmonary hemorrhages, reproduction images of the proximal renal tubules, and splenic extramedullary hemopoiesis. The control groups showed pulmonary hemorrhages and mineralization of the kidneys.

III-1-2 Two-Week Studies

(1) Observation of Animal Conditions

Survival

Deaths were observed in the 90,000 mg/kg male group and the ≧ 30,000 mg/kg female groups. The following deaths were observed in the 90,000 mg/kg group of 10 males in Week 2: one on Day 1, four on Day 3, two on Day 4, two on Day 5, and one on Day 6, totaling 10 deaths. The following deaths were observed in the 90,000 mg/kg group of 10 females in Week 2: one on Day 1, three on Day 2, three on Day 3, three on Day 4, totaling 10 deaths. The following deaths were observed in the 30,000 mg/kg group of 10 females in Week 2: one on Day 3 and one on Day 5, totaling two. (Tables 4 and 5)

General Symptoms

The symptoms of the non-survivors in each group of both sexes were observed starting from Day 2 to Day 4 in Week 1. These symptoms included decreased locomotor movement, piloerection, hunchback position, bloody nasal discharge, or bloody ocular discharge, contamination, and ataxic gait. The animals became weak as time passed. Body wasting, abnormal respiration and decreased body temperature were observed before the eventual deaths. Many survivors of both sexes in the 30,000 ppm groups had symptoms such as piloerection, hunchback position, and bloody nasal discharge starting from Day 2 in Week 1 during the administration period. (Appendices B 1-1 and 2)

Body Weight

Survivors of both sexes in the ≧ 10,000 ppm groups showed suppression of body weight gain corresponding to the dosages received in comparison with the control groups. The final body weights in percentage in comparison with the control groups (Day 7 in Week 2) were 73% in the 30,000 ppm male group and 94% in the 10,000 ppm male group and 76% in the 30,000 ppm female group and 97% in the 10,000 female group. (Tables 4 and 5, Figures 3 and 4)

Food Consumption

Decreased food consumption was observed in the ≧ 10,000 ppm groups of both sexes, closely corresponding to the suppression of body weight gain. (Tables 6 and 7)

Water Consumption

Decreased water consumption was observed during the entire administration period in the ≧ 3,300 ppm groups of both sexes, corresponding to the decrease in the administration concentration received in comparison with the control groups. The water consumption percentage in the ≧ 3,300 ppm groups of both sexes in comparison with the control groups were ± 10% in the 90,000 ppm groups, approximately 50–60% in the 30,000 ppm groups, approximately 80% in the 10,000 ppm groups, and 90% in the 3,330 groups. (Tables 8 and 9)

TABLE 4 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN MALE RAT (TWO-WEEK STUDIES)

Week-Day on Study	Control Av.Wt.	No.of Surviv.	1110 ppm Av.Wt.	% of cont.	No.of Surviv.	3330 ppm Av.Wt.	% of cont.	No.of Surviv.	10000 ppm Av.Wt.	% of cont.	No.of Surviv.	30000 ppm Av.Wt.	% of cont.	No.of Surviv.	90000 ppm Av.Wt.	% of cont.	No.of Surviv.
0-0	132 (10)	10	132 (10)	100	10	132 (10)	100	10	132 (10)	100	10	132 (10)	100	10	132 (10)	100	10
1-1	139 (10)	10	137 (10)	99	10	137 (10)	99	10	134 (10)	96	10	126 (10)	91	10	122 (10)	88	10
1-2	144 (10)	10	142 (10)	99	10	142 (10)	99	10	138 (10)	96	10	123 (10)	85	10	113 (10)	78	10
1-4	155 (10)	10	153 (10)	99	10	152 (10)	98	10	148 (10)	95	10	122 (10)	79	10	98 (10)	63	10
1-7	172 (10)	10	168 (10)	98	10	168 (10)	98	10	164 (10)	95	10	128 (10)	74	10	80 (10)	47	10
2-3	188 (10)	10	185 (10)	98	10	184 (10)	98	10	179 (10)	95	10	138 (10)	73	10	68 (9)	36	5
2-7	209 (10)	10	206 (10)	99	10	205 (10)	98	10	197 (10)	94	10	153 (10)	73	10			

TABLE 5 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN FEMALE RAT (TWO-WEEK STUDIES)

Week-Day on Study	Control Av.Wt.	No.of Surviv.	1110 ppm Av.Wt.	% of cont.	No.of Surviv.	3330 ppm Av.Wt.	% of cont.	No.of Surviv.	10000 ppm Av.Wt.	% of cont.	No.of Surviv.	30000 ppm Av.Wt.	% of cont.	No.of Surviv.	90000 ppm Av.Wt.	% of cont.	No.of Surviv.
0-0	104 (10)	10	104 (10)	100	10	104 (10)	100	10	104 (10)	100	10	105 (10)	101	10	105 (10)	101	10
1-1	107 (10)	10	108 (10)	101	10	107 (10)	100	10	104 (10)	97	10	99 (10)	93	10	96 (10)	90	10
1-2	110 (10)	10	110 (10)	100	10	109 (10)	99	10	107 (10)	97	10	98 (10)	89	10	89 (10)	81	10
1-4	116 (10)	10	115 (10)	99	10	115 (10)	99	10	112 (10)	97	10	98 (10)	84	10	79 (10)	68	10
1-7	124 (10)	10	123 (10)	99	10	123 (10)	99	10	120 (10)	97	10	96 (10)	77	10	64 (10)	52	10
2-3	133 (10)	10	132 (10)	99	10	132 (10)	99	10	128 (10)	96	10	97 (10)	73	9	55 (6)	41	3
2-7	141 (10)	10	141 (10)	100	10	142 (10)	101	10	137 (10)	97	10	107 (8)	76	8			

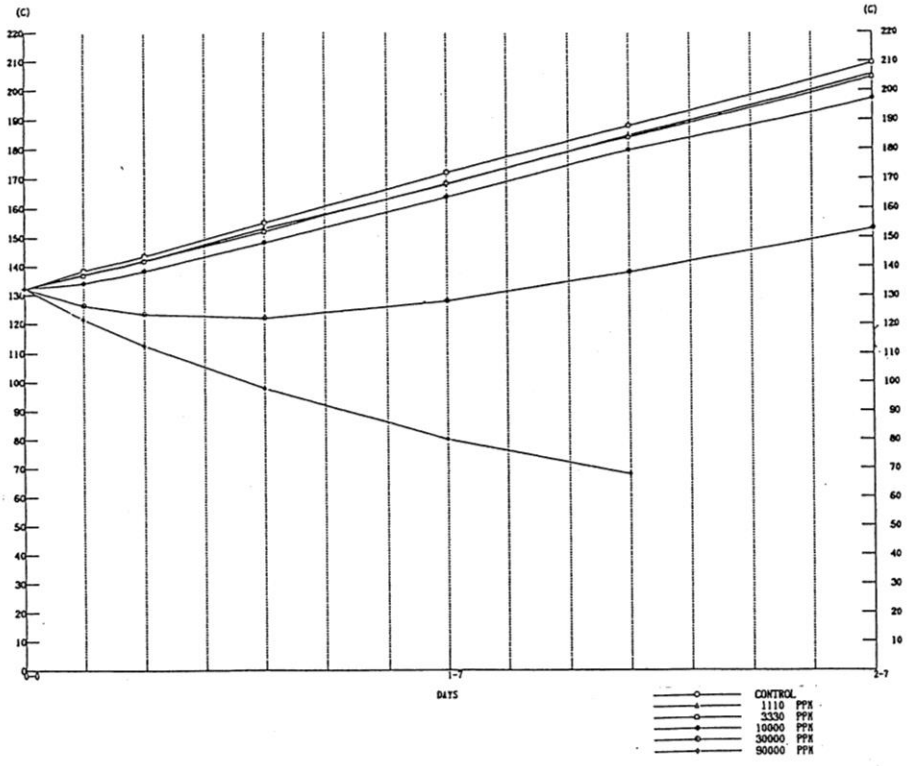


FIGURE 3 BODY WEIGHT CHANGES : RAT:MALE (TWO-WEEK STUDIES)

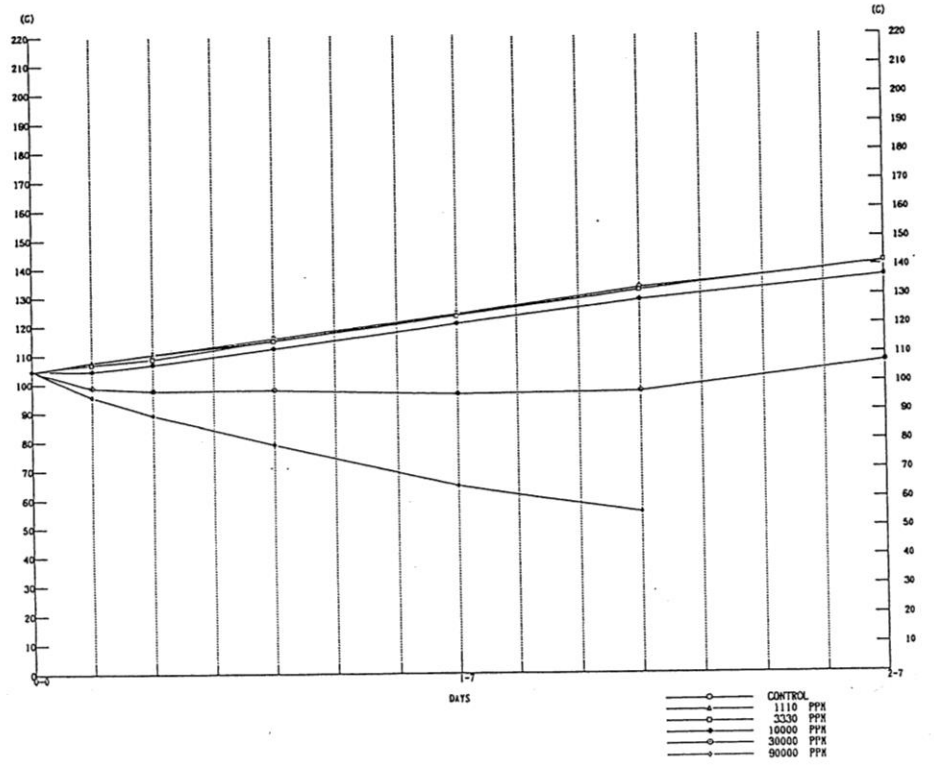


FIGURE 4 BODY WEIGHT CHANGES : RAT:FEMALE (TWO-WEEK STUDIES)

TABLE 6 FOOD CONSUMPTION IN MALE RAT (TWO-WEEK STUDIES)

Week-Day on Study	Control Av.FC.	No.of Surviv.	1110 ppm Av.FC.	% of cont.	No.of Surviv.	3330 ppm Av.FC.	% of cont.	No.of Surviv.	10000 ppm Av.FC.	% of cont.	No.of Surviv.	30000 ppm Av.FC.	% of cont.	No.of Surviv.	90000 ppm Av.FC.	% of cont.	No.of Surviv.
1-7	16.5 (10)	10	16.0 (10)	97	10	16.0 (10)	97	10	15.3 (10)	93	10	10.6 (10)	64	10	3.0 (10)	18	10
2-7	17.4 (10)	10	17.1 (10)	98	10	17.1 (10)	98	10	16.3 (10)	94	10	13.0 (10)	75	10			

TABLE 7 FOOD CONSUMPTION IN FEMALE RAT (TWO-WEEK STUDIES)

Week-Day on Study	Control Av.FC.	No.of Surviv.	1110 ppm Av.FC.	% of cont.	No.of Surviv.	3330 ppm Av.FC.	% of cont.	No.of Surviv.	10000 ppm Av.FC.	% of cont.	No.of Surviv.	30000 ppm Av.FC.	% of cont.	No.of Surviv.	90000 ppm Av.FC.	% of cont.	No.of Surviv.
1-7	11.9 (10)	10	12.0 (10)	101	10	11.9 (10)	100	10	11.5 (10)	97	10	8.5 (10)	71	10	2.7 (10)	23	10
2-7	11.7 (10)	10	11.9 (10)	102	10	12.0 (10)	103	10	11.8 (10)	101	10	8.3 (8)	71	8			

TABLE 8 WATER CONSUMPTION IN MALE RAT (TWO-WEEK STUDIES)

Week-Day on Study	Control Av.WC.	No.of Surviv.	1110 ppm Av.WC.	% of cont.	No.of Surviv.	3330 ppm Av.WC.	% of cont.	No.of Surviv.	10000 ppm Av.WC.	% of cont.	No.of Surviv.	30000 ppm Av.WC.	% of cont.	No.of Surviv.	90000 ppm Av.WC.	% of cont.	No.of Surviv.
1-4	22.0 (10)	10	20.6 (10)	94	10	19.9 (10)	90	10	17.6 (10)	80	10	9.8 (10)	45	10	2.6 (10)	12	10
1-7	22.1 (10)	10	20.4 (10)	92	10	20.1 (10)	91	10	18.0 (10)	81	10	12.7 (10)	57	10	0.8 (10)	4	10
2-3	22.5 (10)	10	21.0 (10)	93	10	20.3 (10)	90	10	18.0 (10)	80	10	14.2 (10)	63	10	1.1 (6)	5	5
2-7	23.6 (10)	10	22.0 (10)	93	10	20.9 (10)	89	10	18.2 (10)	77	10	15.0 (10)	64	10			

TABLE 9 WATER CONSUMPTION IN FEMALE RAT (TWO-WEEK STUDIES)

Week-Day on Study	Control Av.WC.	No.of Surviv.	1110 ppm Av.WC.	% of cont.	No.of Surviv.	3330 ppm Av.WC.	% of cont.	No.of Surviv.	10000 ppm Av.WC.	% of cont.	No.of Surviv.	30000 ppm Av.WC.	% of cont.	No.of Surviv.	90000 ppm Av.WC.	% of cont.	No.of Surviv.
1-4	17.1 (10)	10	17.4 (10)	102	10	15.3 (10)	89	10	12.8 (10)	75	10	7.4 (10)	43	10	2.0 (10)	12	10
1-7	16.9 (10)	10	17.5 (10)	104	10	15.2 (10)	90	10	13.4 (10)	79	10	9.1 (10)	54	10	0.9 (10)	5	10
2-3	17.6 (10)	10	17.8 (10)	101	10	16.1 (10)	91	10	13.7 (10)	78	10	10.0 (9)	57	9	1.0 (3)	6	3
2-7	17.7 (10)	10	18.8 (10)	106	10	16.0 (10)	90	10	13.1 (10)	74	10	9.6 (8)	54	8			

(2) Pathological Examination

Necropsy

Findings observed at the time of necropsy are listed in Appendices B 3-1 to 4 (the Summary Tables) and Appendices B 6-1 and 2 (the Individual Tables).

Many non-surviving / moribund animals in the 90,000 ppm groups of both sexes had thymic gland atrophy and red dots / macules on the stomach glands; a few males had black fluid accumulation in the stomach and a few females had reddening of the liver. Females in the 30,000 ppm group had thymic gland atrophy and reddening, red dots / macules on the stomach glands, cloudy livers, and enlargement, anemic-like coloration and coarse surface of the kidneys.

Males surviving at the time of the scheduled necropsy had anemic-like coloration of the liver and kidneys in all groups and many males in the 30,000 ppm group had yellowing of the liver and a well defined hepatic lobule structure. Many females had anemic-like coloration of the kidneys in the ≧ 3,330 ppm groups and thymic gland atrophy in the 30,000 ppm group.

Histopathological Examination

Histopathological examinations on the non-surviving / moribund animals were conducted on three males and three females in the 90,000 ppm groups and two females in the 30,000 ppm group. Histopathological examinations were conducted on one male and one female in the ≤ 30,000 ppm groups which were surviving at the time of the scheduled necropsy. The results are shown in Appendices B 4-1 to 4 (the Summary Tables) and Appendices B 7-1 and 2 (the Individual Tables).

Non-survivors showed hemorrhaging in the lungs and heart, erosion and bleeding in stomach glands, hydropic degeneration of the proximal renal tubules, thymic gland hemorrhages, adrenal hemorrhages, and centrilobular hepatic and cerebral vacuolar degeneration.

Animals surviving at the time of the scheduled necropsy showed nuclear enlargement of the olfactory epithelium (the supporting cells) in the nasal cavity, edema near the pulmonary vessels, centrilobular hepatic cellular swelling and vacuolar degeneration, appearance of acidophilic bodies in the kidneys and hydropic degeneration of the proximal renal tubules, reproduction images of the proximal renal tubules, cerebral vacuolar degeneration, hemorrhaging in the heart, and adrenal hemorrhages. The control groups showed hemorrhaging in the heart and appearance of acidophilic bodies in the kidneys.

III-1-3 Thirteen-Week Studies

(1) Observation of Animal Conditions

Survival

One death occurred only in the 25,000 ppm female group in Week 2 after the start of the administration. (Tables 10 and 11)

General Symptoms

The observed symptoms of the non-survivors included piloerection, hunchback position, bloody nasal discharge, contamination, coloration of fur, and contamination of surrounding areas of the external genitalia from urine. Many survivors in the ≧ 25,000 ppm groups of both sexes had piloerection, bloody nasal discharge, bloody ocular discharge, and coloration of fur. (Appendices D 1 and 2)

Body Weight

Animals of both sexes in the ≧ 4,000 ppm groups showed suppression of body weight gain corresponding to the dosage received in comparison with the control groups. The final body weights in percentage comparison with the control groups (Week 13) were 79% in the 25,000 ppm group, 93% in the 10,000 ppm group, 97% in the 4,000 ppm group, 101% in the 1,600 ppm group, 101% in the 640 group of males and 79% in the 25,000 ppm group, 88% in the 10,000 ppm group, 94% in the 4,000 ppm group, 97% in the 1,600 ppm group, and 101% in the 640 group of females. (Tables 10 and 11, Figures 5 and 6, Appendices E 1 and 2 (the Summary Tables), Appendices Q 1 and 2 (the Individual Tables))

Food Consumption

Decreased food consumption in the 25,000 ppm male group and the ≧ 10,000 ppm female groups was observed. Food consumption in percentage comparison with the control groups were 84–97% in the 25,000 ppm male group and 82–90% in the 25,000 female group and 87–97% in the 10,000 ppm female group. (Tables 12 and 13, Figures 7 and 8, Appendices F 1 and 2 (the Summary Tables), Appendices R 1 and 2 (the Individual Tables))

Water Consumption

Apparent decrease in water consumption in the ≧ 4,000 ppm groups of both sexes was observed corresponding to i the administration concentration received in comparison with the control groups. Water consumption in percentage comparison with the control groups were 43–74% in the 25,000 ppm group, 56–83% in the 10,000 ppm group, 62–91% in the 4,000 ppm group of males and 32–58% in the 25,000 ppm group, 41–75% in the 10,000 ppm group, 57–97% in the 4,000 ppm group of females. (Tables 14 and 15, Figures 9 and 10, Appendices G 1 and 2 (the Summary Tables), Appendices S 1 and 2 (the Individual Tables))

TABLE 10 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN MALE RAT (THIRTEEN-WEEK STUDIES)

Week-Day on Study	Control Av.Wt.	No.of Surviv.	640 ppm Av.Wt.	% of cont.	No.of Surviv.	1600 ppm Av.Wt.	% of cont.	No.of Surviv.	4000 ppm Av.Wt.	% of cont.	No.of Surviv.	10000 ppm Av.Wt.	% of cont.	No.of Surviv.	25000 ppm Av.Wt.	% of cont.	No.of Surviv.
0-0	130 (10)	10	130 (10)	100	10	130 (10)	100	10	130 (10)	100	10	130 (10)	100	10	130 (10)	100	10
1-7	162 (10)	10	158 (10)	98	10	165 (10)	102	10	162 (10)	100	10	158 (10)	98	10	137 (10)	85	10
2-7	195 (10)	10	194 (10)	99	10	201 (10)	103	10	197 (10)	101	10	193 (10)	99	10	165 (10)	85	10
3-7	224 (10)	10	225 (10)	100	10	231 (10)	103	10	224 (10)	100	10	219 (10)	98	10	191 (10)	85	10
4-7	246 (10)	10	250 (10)	102	10	255 (10)	104	10	247 (10)	100	10	240 (10)	98	10	210 (10)	85	10
5-7	265 (10)	10	268 (10)	101	10	272 (10)	103	10	264 (10)	100	10	255 (10)	96	10	222 (10)	84	10
6-7	280 (10)	10	281 (10)	100	10	286 (10)	102	10	277 (10)	99	10	267 (10)	95	10	231 (10)	83	10
7-7	293 (10)	10	296 (10)	101	10	301 (10)	103	10	289 (10)	99	10	280 (10)	96	10	238 (10)	81	10
8-7	308 (10)	10	310 (10)	101	10	315 (10)	102	10	300 (10)	97	10	290 (10)	94	10	246 (10)	80	10
9-7	319 (10)	10	322 (10)	101	10	327 (10)	103	10	313 (10)	98	10	302 (10)	95	10	255 (10)	80	10
10-7	328 (10)	10	331 (10)	101	10	334 (10)	102	10	319 (10)	97	10	309 (10)	94	10	264 (10)	80	10
11-7	336 (10)	10	340 (10)	101	10	343 (10)	102	10	326 (10)	97	10	315 (10)	94	10	269 (10)	80	10
12-7	345 (10)	10	351 (10)	102	10	352 (10)	102	10	334 (10)	97	10	322 (10)	93	10	273 (10)	79	10
13-7	355 (10)	10	359 (10)	101	10	360 (10)	101	10	343 (10)	97	10	329 (10)	93	10	279 (10)	79	10

TABLE 11 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN FEMALE RAT (THIRTEEN-WEEK STUDIES)

Week-Day on Study	Control Av.Wt.	No.of Surviv.	640 ppm Av.Wt.	% of cont.	No.of Surviv.	1600 ppm Av.Wt.	% of cont.	No.of Surviv.	4000 ppm Av.Wt.	% of cont.	No.of Surviv.	10000 ppm Av.Wt.	% of cont.	No.of Surviv.	25000 ppm Av.Wt.	% of cont.	No.of Surviv.
0-0	102 (10)	10	102 (10)	100	10	102 (10)	100	10	102 (10)	100	10	102 (10)	100	10	102 (10)	100	10
1-7	119 (10)	10	121 (10)	102	10	119 (10)	100	10	118 (10)	99	10	116 (10)	97	10	104 (10)	87	10
2-7	135 (10)	10	138 (10)	102	10	136 (10)	101	10	134 (10)	99	10	131 (10)	97	10	121 (9)	90	9
3-7	147 (10)	10	150 (10)	102	10	147 (10)	100	10	146 (10)	99	10	141 (10)	96	10	131 (9)	89	9
4-7	158 (10)	10	161 (10)	102	10	158 (10)	100	10	155 (10)	98	10	149 (10)	94	10	138 (9)	87	9
5-7	167 (10)	10	170 (10)	102	10	166 (10)	99	10	165 (10)	99	10	156 (10)	93	10	142 (9)	85	9
6-7	175 (10)	10	178 (10)	102	10	173 (10)	99	10	171 (10)	98	10	161 (10)	92	10	148 (9)	85	9
7-7	184 (10)	10	186 (10)	101	10	179 (10)	97	10	177 (10)	96	10	166 (10)	90	10	152 (9)	83	9
8-7	188 (10)	10	190 (10)	101	10	185 (10)	98	10	181 (10)	96	10	169 (10)	90	10	154 (9)	82	9
9-7	196 (10)	10	198 (10)	101	10	190 (10)	97	10	186 (10)	95	10	173 (10)	88	10	157 (9)	80	9
10-7	198 (10)	10	200 (10)	101	10	193 (10)	97	10	189 (10)	95	10	176 (10)	89	10	160 (9)	81	9
11-7	203 (10)	10	206 (10)	101	10	198 (10)	98	10	193 (10)	95	10	180 (10)	89	10	162 (9)	80	9
12-7	206 (10)	10	209 (10)	101	10	201 (10)	98	10	196 (10)	95	10	182 (10)	88	10	163 (9)	79	9
13-7	210 (10)	10	213 (10)	101	10	204 (10)	97	10	198 (10)	94	10	184 (10)	88	10	165 (9)	79	9

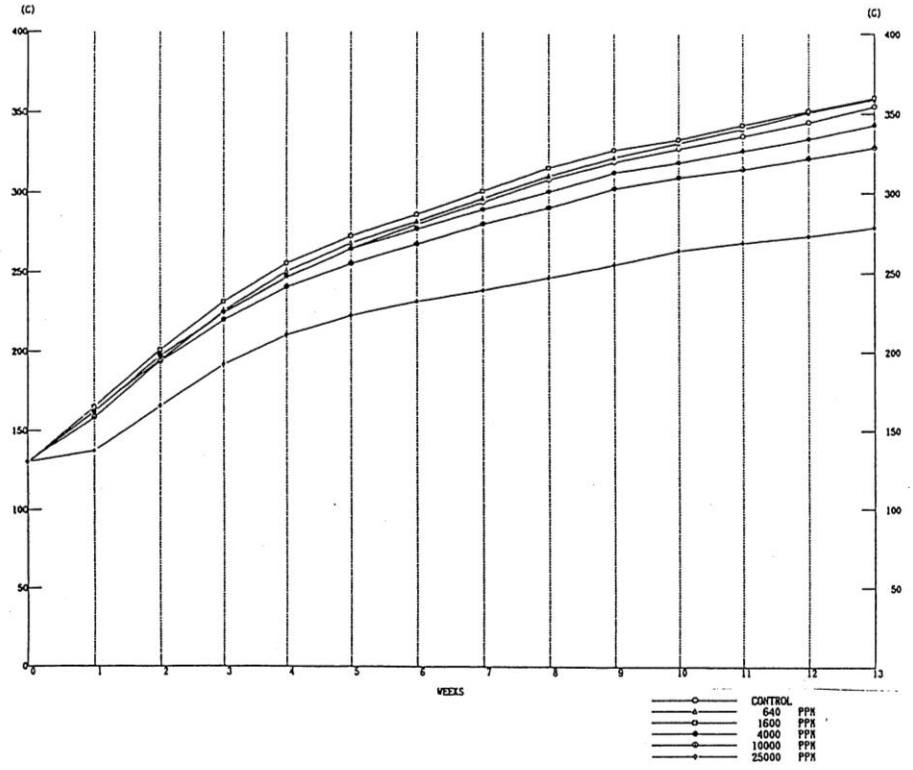


FIGURE 5 BODY WEIGHT CHANGES : RAT:MALE (THIRTEEN-WEEK STUDIES)

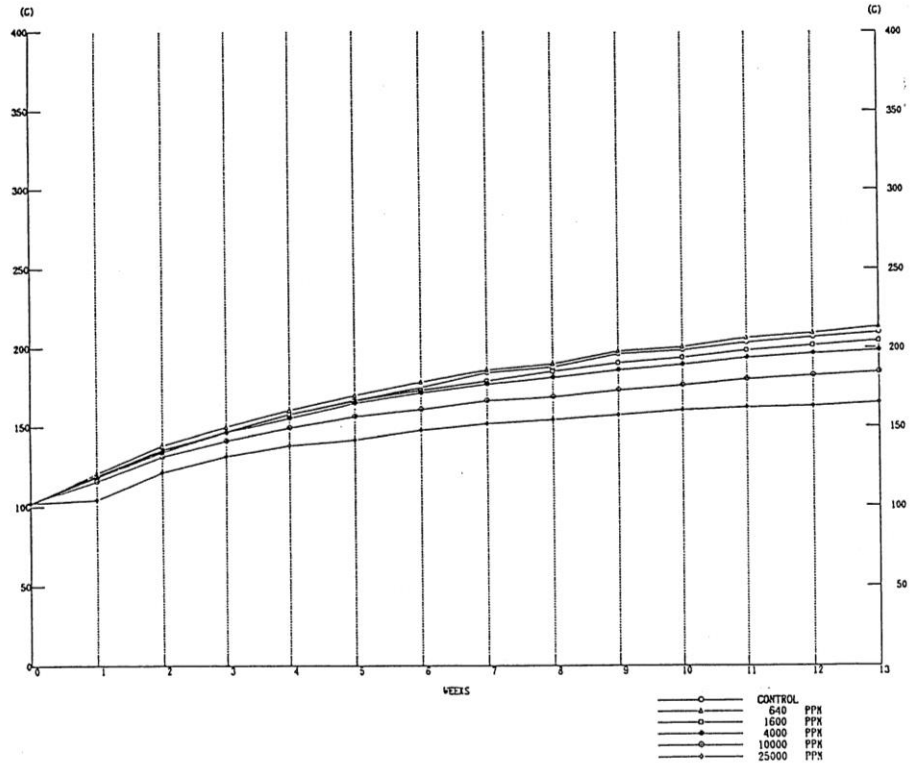


FIGURE 6 BODY WEIGHT CHANGES : RAT:FEMALE (THIRTEEN-WEEK STUDIES)

TABLE 12 FOOD CONSUMPTION IN MALE RAT (THIRTEEN-WEEK STUDIES)

Week-Day on Study	Control Av.FC.	No.of Surviv.	640 ppm Av.FC.	% of cont.	No.of Surviv.	1600 ppm Av.FC.	% of cont.	No.of Surviv.	4000 ppm Av.FC.	% of cont.	No.of Surviv.	10000 ppm Av.FC.	% of cont.	No.of Surviv.	25000 ppm Av.FC.	% of cont.	No.of Surviv.
1-7	15.0 (10)	10	14.3 (10)	95	10	15.0 (10)	100	10	14.8 (10)	99	10	14.1 (10)	94	10	12.9 (10)	86	10
2-7	15.7 (10)	10	16.5 (10)	105	10	17.0 (10)	108	10	16.3 (10)	104	10	15.7 (10)	100	10	13.8 (10)	88	10
3-7	17.0 (10)	10	17.3 (10)	102	10	17.8 (10)	105	10	16.7 (10)	98	10	16.1 (10)	95	10	14.3 (10)	84	10
4-7	16.8 (10)	10	17.9 (10)	107	10	18.1 (10)	108	10	17.3 (10)	103	10	16.7 (10)	99	10	15.3 (10)	91	10
5-7	17.4 (10)	10	18.3 (10)	105	10	18.1 (10)	104	10	17.5 (9)	101	10	17.2 (10)	99	10	16.2 (10)	93	10
6-7	17.5 (10)	10	17.9 (10)	102	10	18.0 (10)	103	10	17.2 (10)	98	10	17.4 (10)	99	10	16.3 (10)	93	10
7-7	18.4 (10)	10	18.7 (10)	102	10	18.7 (10)	102	10	17.6 (10)	96	10	18.2 (10)	99	10	16.6 (10)	90	10
8-7	17.7 (10)	10	18.0 (10)	102	10	17.9 (10)	101	10	17.3 (10)	98	10	17.2 (10)	97	10	16.5 (10)	93	10
9-7	17.5 (10)	10	18.2 (10)	104	10	18.2 (10)	104	10	17.6 (10)	101	10	17.8 (10)	102	10	16.7 (10)	95	10
10-7	17.6 (10)	10	18.4 (10)	105	10	18.3 (10)	104	10	17.6 (10)	100	10	17.8 (10)	101	10	17.0 (10)	97	10
11-7	18.1 (10)	10	18.6 (10)	103	10	18.8 (10)	104	10	17.9 (10)	99	10	18.2 (10)	101	10	17.2 (10)	95	10
12-7	17.6 (10)	10	18.1 (10)	103	10	18.4 (10)	105	10	17.3 (10)	98	10	17.6 (10)	100	10	16.2 (10)	92	10
13-7	17.8 (10)	10	18.4 (10)	103	10	18.3 (10)	103	10	17.5 (10)	98	10	17.4 (10)	98	10	16.1 (10)	90	10

TABLE 13 FOOD CONSUMPTION IN FEMALE RAT (THIRTEEN-WEEK STUDIES)

Week-Day on Study	Control Av.FC.	No.of Surviv.	640 ppm Av.FC.	% of cont.	No.of Surviv.	1600 ppm Av.FC.	% of cont.	No.of Surviv.	4000 ppm Av.FC.	% of cont.	No.of Surviv.	10000 ppm Av.FC.	% of cont.	No.of Surviv.	25000 ppm Av.FC.	% of cont.	No.of Surviv.
1-7	11.4 (10)	10	11.8 (10)	104	10	11.5 (10)	101	10	11.6 (10)	102	10	10.9 (10)	96	10	9.4 (10)	82	10
2-7	11.8 (10)	10	12.5 (10)	106	10	12.3 (10)	104	10	12.0 (10)	102	10	11.5 (10)	97	10	10.3 (9)	87	9
3-7	11.8 (10)	10	12.5 (10)	106	10	12.0 (10)	102	10	11.7 (10)	99	10	11.0 (10)	93	10	10.3 (9)	87	9
4-7	12.0 (10)	10	12.7 (10)	106	10	12.3 (10)	103	10	11.9 (10)	99	10	11.2 (10)	93	10	10.5 (9)	88	9
5-7	12.9 (10)	10	13.1 (10)	102	10	12.6 (10)	98	10	12.2 (10)	95	10	11.3 (10)	88	10	10.8 (9)	84	9
6-7	12.9 (10)	10	13.2 (10)	102	10	12.9 (10)	100	10	12.3 (10)	95	10	11.3 (10)	88	10	10.8 (9)	84	9
7-7	13.4 (10)	10	13.4 (10)	100	10	12.9 (10)	96	10	12.3 (10)	92	10	11.7 (10)	87	10	11.2 (9)	84	9
8-7	12.3 (10)	10	12.7 (10)	103	10	12.6 (10)	102	10	11.7 (10)	95	10	11.0 (10)	89	10	11.0 (9)	89	9
9-7	12.9 (10)	10	13.0 (10)	101	10	12.5 (10)	97	10	11.7 (10)	91	10	11.2 (10)	87	10	10.9 (9)	84	9
10-7	12.8 (10)	10	12.9 (10)	101	10	12.5 (10)	98	10	12.2 (10)	95	10	11.4 (10)	89	10	11.5 (9)	90	9
11-7	12.5 (10)	10	13.1 (10)	105	10	12.8 (10)	102	10	12.1 (10)	97	10	11.7 (10)	94	10	11.3 (9)	90	9
12-7	12.7 (10)	10	12.7 (10)	100	10	12.7 (10)	100	10	12.0 (10)	94	10	11.4 (10)	90	10	11.1 (9)	87	9
13-7	12.2 (10)	10	12.7 (10)	104	10	12.0 (10)	98	10	11.6 (10)	95	10	11.2 (10)	92	10	11.0 (9)	90	9
	12.4		12.8			12.4			11.9			11.3			10.8		

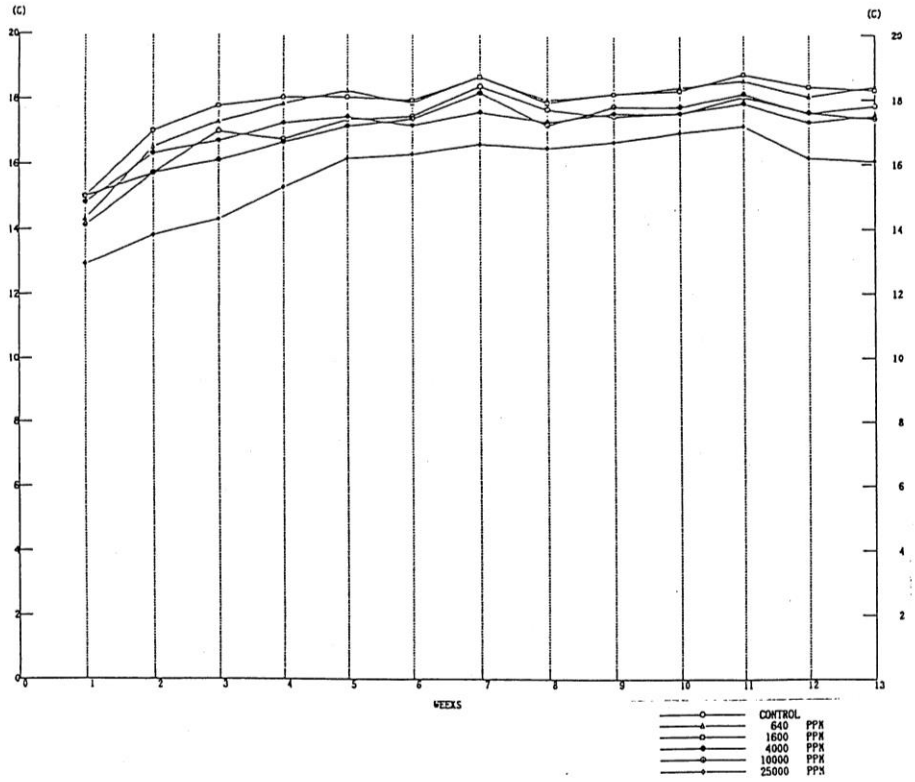


FIGURE 7 FOOD CONSUMPTION : RAT:MALE (THIRTEEN-WEEK STUDIES)

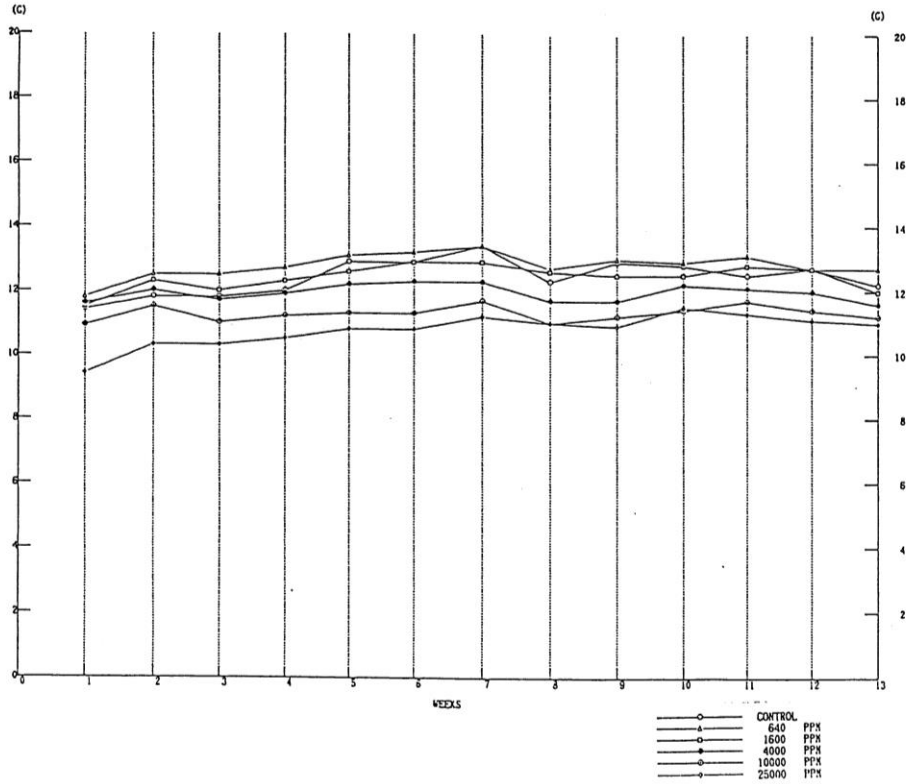


FIGURE 8 FOOD CONSUMPTION : RAT:FEMALE (THIRTEEN-WEEK STUDIES)

TABLE 14 WATER CONSUMPTION IN MALE RAT (THIRTEEN-WEEK STUDIES)

Week-Day on Study	Control Av.WC.	No.of Surviv.	640 ppm Av.WC.	% of cont.	No.of Surviv.	1600 ppm Av.WC.	% of cont.	No.of Surviv.	4000 ppm Av.WC.	% of cont.	No.of Surviv.	10000 ppm Av.WC.	% of cont.	No.of Surviv.	25000 ppm Av.WC.	% of cont.	No.of Surviv.
1-3	19.8 (10)	10	18.1 (10)	91	10	18.2 (10)	92	10	17.2 (10)	87	10	16.1 (10)	81	10	10.8 (10)	55	10
1-7	20.3 (10)	10	16.8 (10)	83	10	19.1 (10)	94	10	17.8 (10)	88	10	15.9 (10)	78	10	14.5 (10)	71	10
2-3	20.5 (10)	10	20.4 (10)	100	10	20.5 (10)	100	10	18.7 (10)	91	10	17.0 (10)	83	10	15.2 (10)	74	10
2-7	21.4 (10)	10	20.7 (10)	97	10	21.8 (10)	102	10	19.3 (10)	90	10	17.6 (10)	82	10	15.1 (10)	71	10
3-3	28.7 (10)	10	21.3 (10)	74	10	22.7 (10)	79	10	19.0 (10)	66	10	17.2 (10)	60	10	14.6 (10)	51	10
3-7	29.8 (10)	10	22.2 (10)	74	10	24.0 (10)	81	10	19.9 (10)	67	10	18.2 (10)	61	10	15.3 (10)	51	10
4-3	25.6 (10)	10	22.4 (10)	88	10	23.5 (10)	92	10	19.7 (10)	77	10	18.1 (10)	71	10	15.1 (10)	59	10
4-7	27.9 (10)	10	22.2 (10)	80	10	23.9 (10)	86	10	19.9 (10)	71	10	18.2 (10)	65	10	14.6 (10)	52	10
5-3	27.3 (10)	10	22.8 (10)	84	10	25.4 (10)	93	10	19.6 (10)	72	10	18.3 (10)	67	10	14.8 (10)	54	10
5-7	28.8 (10)	10	24.0 (10)	83	10	25.3 (10)	88	10	19.7 (10)	68	10	18.3 (10)	64	10	15.2 (10)	53	10
6-3	27.6 (10)	10	24.0 (10)	87	10	23.3 (10)	84	10	19.2 (10)	70	10	17.6 (10)	64	10	14.5 (10)	53	10
6-7	28.2 (10)	10	23.1 (10)	82	10	23.1 (10)	82	10	19.7 (10)	70	10	18.2 (10)	65	10	14.8 (10)	52	10
7-3	29.6 (10)	10	23.6 (10)	80	10	22.4 (10)	76	10	18.4 (10)	62	10	21.9 (10)	74	10	12.9 (10)	44	10
7-7	30.7 (10)	10	23.5 (10)	77	10	22.6 (10)	74	10	19.1 (10)	62	10	21.6 (10)	70	10	14.9 (10)	49	10
8-3	28.7 (10)	10	23.6 (10)	82	10	21.7 (10)	76	10	18.5 (10)	64	10	16.7 (10)	58	10	12.9 (10)	45	10
8-7	29.0 (10)	10	23.7 (10)	82	10	22.7 (10)	78	10	19.1 (10)	66	10	17.3 (10)	60	10	15.2 (10)	52	10
9-3	26.4 (10)	10	24.9 (10)	94	10	21.7 (10)	82	10	19.4 (10)	73	10	16.7 (10)	63	10	13.3 (10)	50	10
9-7	28.6 (10)	10	24.7 (10)	86	10	21.9 (10)	77	10	19.2 (10)	67	10	17.2 (10)	60	10	14.3 (10)	50	10
10-3	26.6 (10)	10	24.6 (10)	92	10	21.9 (10)	82	10	18.0 (10)	68	10	15.7 (10)	59	10	13.6 (10)	51	10
10-7	30.4 (10)	10	25.6 (10)	84	10	23.3 (10)	77	10	19.5 (10)	64	10	18.0 (10)	59	10	15.4 (10)	51	10
11-3	27.5 (10)	10	25.5 (10)	93	10	23.5 (10)	85	10	18.5 (10)	67	10	17.0 (10)	62	10	14.0 (10)	51	10
11-7	28.6 (10)	10	24.8 (10)	87	10	22.9 (10)	80	10	18.7 (10)	65	10	16.8 (10)	59	10	14.2 (10)	50	10
12-3	27.6 (10)	10	24.8 (10)	90	10	22.7 (10)	82	10	18.7 (10)	68	10	17.5 (10)	63	10	14.2 (10)	51	10
12-7	29.6 (10)	10	25.4 (10)	86	10	23.3 (10)	79	10	18.3 (10)	62	10	16.5 (10)	56	10	12.6 (10)	43	10
13-3	29.0 (10)	10	24.6 (10)	85	10	24.2 (10)	83	10	18.4 (10)	63	10	16.4 (10)	57	10	13.2 (10)	46	10
13-7	27.7 (10)	10	24.5 (10)	88	10	22.9 (10)	83	10	18.4 (10)	66	10	16.9 (10)	61	10	13.5 (10)	49	10
	27.2		23.1			22.6			18.9			17.6			14.2		

TABLE 15 WATER CONSUMPTION IN FEMALE RAT (THIRTEEN-WEEK STUDIES)

Week-Day on Study	Control Av.WC.	No.of Surviv.	640 ppm Av.WC.	% of cont.	No.of Surviv.	1600 ppm Av.WC.	% of cont.	No.of Surviv.	4000 ppm Av.WC.	% of cont.	No.of Surviv.	10000 ppm Av.WC.	% of cont.	No.of Surviv.	25000 ppm Av.WC.	% of cont.	No.of Surviv.
1-3	16.6 (10)	10	16.8 (10)	101	10	15.8 (10)	95	10	14.8 (10)	89	10	12.5 (10)	75	10	7.9 (10)	48	10
1-7	19.2 (10)	10	18.9 (10)	98	10	16.8 (10)	88	10	15.3 (10)	80	10	12.9 (10)	67	10	11.2 (10)	58	10
2-3	17.8 (10)	10	19.7 (10)	111	10	17.3 (10)	97	10	15.2 (10)	85	10	12.7 (10)	71	10	10.3 (10)	58	9
2-7	18.7 (10)	10	25.1 (10)	134	10	19.2 (10)	103	10	15.9 (10)	85	10	13.0 (10)	70	10	10.7 (9)	57	9
3-3	18.7 (10)	10	24.5 (10)	131	10	18.5 (10)	99	10	15.7 (10)	84	10	12.2 (10)	65	10	10.3 (9)	55	9
3-7	20.2 (10)	10	23.0 (10)	114	10	19.2 (10)	95	10	16.2 (10)	80	10	12.7 (10)	63	10	10.0 (9)	50	9
4-3	19.6 (10)	10	23.8 (10)	121	10	19.5 (10)	99	10	16.2 (10)	83	10	12.6 (10)	64	10	8.7 (9)	44	9
4-7	20.4 (10)	10	24.9 (10)	122	10	20.2 (10)	99	10	19.6 (10)	96	10	12.6 (10)	62	10	10.1 (9)	50	9
5-3	21.4 (10)	10	23.6 (10)	110	10	19.1 (10)	89	10	20.6 (10)	96	10	12.1 (10)	57	10	8.9 (9)	42	9
5-7	21.9 (10)	10	23.5 (10)	107	10	20.6 (10)	94	10	21.0 (10)	96	10	12.5 (10)	57	10	10.0 (9)	46	9
6-3	21.1 (10)	10	24.2 (10)	115	10	21.2 (10)	100	10	17.9 (10)	85	10	12.2 (10)	58	10	9.2 (9)	44	9
6-7	24.9 (10)	10	24.4 (10)	98	10	23.4 (10)	94	10	21.2 (10)	85	10	11.9 (10)	48	10	9.3 (9)	37	9
7-3	23.5 (10)	10	23.0 (10)	98	10	19.1 (10)	81	10	22.5 (10)	96	10	11.8 (10)	50	10	8.6 (9)	37	9
7-7	24.3 (10)	10	22.7 (10)	93	10	20.6 (10)	85	10	21.6 (10)	89	10	12.1 (10)	50	10	9.5 (9)	39	9
8-3	22.5 (10)	10	21.2 (10)	94	10	19.7 (10)	88	10	20.2 (10)	90	10	11.5 (10)	51	10	8.2 (9)	36	9
8-7	23.1 (10)	10	22.4 (10)	97	10	19.7 (10)	85	10	18.1 (10)	78	10	11.9 (10)	52	10	9.4 (9)	41	9
9-3	23.1 (10)	10	22.0 (10)	95	10	23.9 (10)	103	10	22.3 (10)	97	10	11.5 (10)	50	10	9.1 (9)	39	9
9-7	27.3 (10)	10	26.2 (10)	96	10	22.1 (10)	81	10	20.9 (10)	77	10	11.6 (10)	42	10	8.7 (9)	32	9
10-3	21.8 (10)	10	22.5 (10)	103	10	18.8 (10)	86	10	17.7 (10)	81	10	11.6 (10)	53	10	8.7 (9)	40	9
10-7	30.0 (10)	10	25.9 (10)	86	10	22.5 (10)	75	10	17.2 (10)	57	10	12.3 (10)	41	10	9.9 (9)	33	9
11-3	23.5 (10)	10	22.9 (10)	97	10	19.0 (10)	81	10	17.5 (10)	74	10	12.2 (10)	52	10	8.9 (9)	38	9
11-7	25.9 (10)	10	26.9 (10)	104	10	20.8 (10)	80	10	18.1 (10)	70	10	12.0 (10)	46	10	9.1 (9)	35	9
12-3	25.9 (10)	10	21.8 (10)	84	10	20.1 (10)	78	10	17.3 (10)	67	10	12.1 (10)	47	10	9.4 (9)	36	9
12-7	25.2 (10)	10	22.4 (10)	89	10	22.1 (10)	88	10	16.4 (10)	65	10	11.9 (10)	47	10	8.7 (9)	35	9
13-3	26.3 (10)	10	21.9 (10)	83	10	19.4 (10)	74	10	16.4 (10)	62	10	11.6 (10)	44	10	8.6 (9)	33	9
13-7	25.1 (9)	10	22.9 (10)	91	10	21.5 (10)	86	10	16.1 (10)	64	10	11.4 (10)	45	10	9.2 (9)	37	9
	22.6		23.0			20.0			18.2			12.1			9.3		

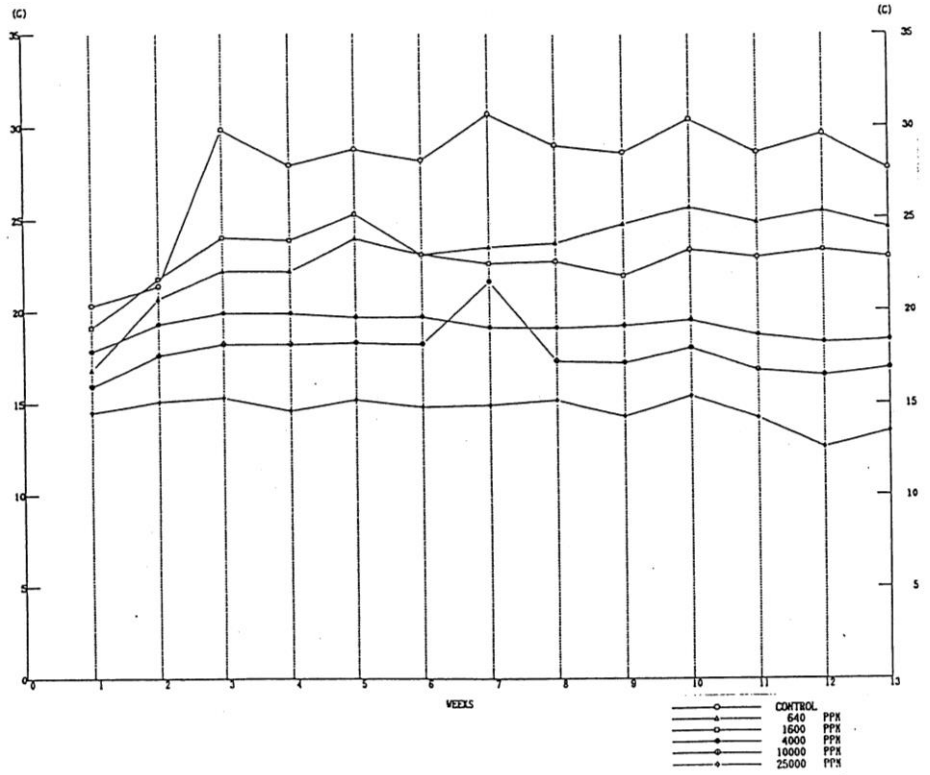


FIGURE 9 WATER CONSUMPTION : RAT:MALE (THIRTEEN-WEEK STUDIES)

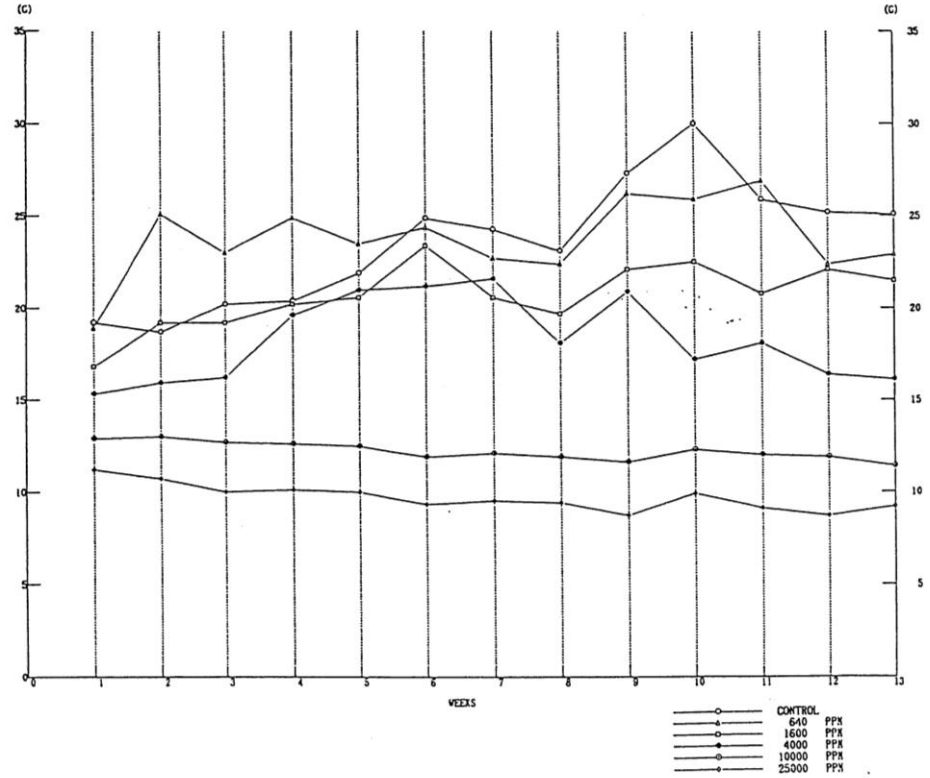


FIGURE 10 WATER CONSUMPTION : RAT:FEMALE (THIRTEEN-WEEK STUDIES)

(2) Hematology and Blood Biochemistry Examinations, and Urinalysis

Hematology Examination

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

For males in the 25,000 ppm group, the following was observed: increases in the red blood cell counts, hemoglobin concentration, hematocrit level, and segmented neutrophil ratio, and decrease in lymphocyte ratio.

For females, the following was observed: decreases in the mean corpuscular volume and platelet counts in the 25,000 ppm group, and decreases in the mean corpuscular volume in the 10,000 ppm group.

Blood Biochemistry Examination

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

For males in the 25,000 ppm group, the following was observed: increases in GOT, GPT, ALP, LAP, A/G ratio and decreases in total protein, albumin, glucose, total cholesterol, triglyceride, and sodium. In the 10,000 ppm group, the following was observed: increases in ALP and LAP and decreases in total protein and albumin. In the 4,000 ppm group, decreases in total protein and albumin were observed.

For females in the 25,000 ppm group, the following was observed: increases in GOT, ALP, LAP, A/G ratio and decreases in total protein, albumin, glucose, total cholesterol, sodium, and calcium. In the 10,000 ppm group, the following was observed: increases in LAP and decreases in total protein and albumin. In the 4,000 ppm group, decreases in total protein and albumin were observed.

Urinalysis

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

For males, a decrease in pH level was noted in the 25,000, 10,000, and 4,000 ppm groups.

For females, a decrease in pH level was noted in the 25,000 and 10,000 ppm groups.

(3) Pathological Examination

Necropsy

Findings observed at the time of necropsy are listed in Appendices K 1 to 3 (the Summary Tables) and Appendices W 1 and 2 (the Individual Tables).

Thymic and splenic atrophy, a well defined hepatic lobule structure, and an enlarged kidney were observed in one non-surviving male in the 25,000 ppm group. There were no characteristic findings in the study groups of both sexes surviving at the time of scheduled necropsy.

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 L 1 and 2 (the Summary Tables of the actual weights), Appendices M 1 and 2 (the Summary Tables of the actual weight to body weight ratio), Appendices X 1 and 2 (the Individual Tables of the actual weights), and Appendices Y 1 and 2 (the Individual Tables of the actual weight to body weight ratio).

For males, compared with the control group, the actual weights of the thymic gland, liver, spleen, lung (the right only), kidney (the right only) and heart in the 25,000 ppm group had significantly lower values, and the ratio of the brain, adrenal gland, testes, lung, kidney, and liver weights to the body weights had significantly higher values. In the 10,000 ppm group, the actual weight of the heart was significantly lower; and the ratios of the actual weight of the adrenal gland (the left only), testis (the left only), kidney (the right only), and liver to the body weights had significantly higher values. In the 4,000 ppm group, the ratio of the actual weight of the lung (the left only) to the body weights was significantly higher.

For females, compared with the control group, the actual weights of the thymic gland, adrenal gland (the left only), lungs, spleen, brain and heart in the 25,000 ppm group had significantly lower values. The actual weight of the kidneys and ratio to the body weight, and the ratio of the actual weights of the lung, liver, and brain to the body weights had significantly higher values. In the 10,000 ppm group, the actual weight of the heart was significantly lower. The actual weight of the kidneys and ratio to the body weight, and the ratios of the actual weight of the lung (the right only), spleen, liver and brain to the body weights had significantly higher values. In the 4,000 ppm group, the ratios of the actual weights of the kidney, liver, and brain to the body weights had significantly higher values. In the 1,600 ppm group, the actual weights of the kidneys and liver and their ratios to the body weights had significantly higher values.

The body weights of males in the ≧ 10,000 ppm groups and the body weights of females in the ≧ 4,000 ppm groups at the time of necropsy had significantly lower values compared with those of the control groups.

Histopathologic Examination

The results of the histopathologic examination are listed in Appendices N 1 to 3 (the Summary Tables) and Appendices Z 1 and 2 (the Individual Tables). The main findings are summarized in Tables 16 and 17.

One female that died in the 25,000 ppm group had nuclear enlargement in the olfactory epithelium (the supporting cells) in the nasal cavity, respiratory and tracheal epithelium, centrilobular hepatic vacuolar degeneration, hydropic degeneration of the proximal renal tubules, mineralization of the kidney cortex, and cerebral vacuolar degeneration. The cause of death was diagnosed as renal lesions (the hydropic degeneration of the proximal renal tubules).

Among the findings which were observed at the time of the scheduled necropsy, significant differences in comparison with the control groups are described below.

In the ≧ 4,000 ppm groups of both sexes, increased incidences of nuclear enlargement of the olfactory epithelium (the supporting cells) in the nasal cavity were noted. In the ≧ 1,600 ppm groups for both sexes, nuclear enlargement of the respiratory epithelum were noted.

In the ≧ 4,000 ppm groups of both sexes, increased incidences of nuclear enlargement of the tracheal epithelium were noted.

In the 25,000 ppm male group, increased incidences of nuclear enlargement of the bronchial epithelium in the lungs were noted.

In the 25,000 ppm groups of both sexes and in the 4,000 ppm male group, increased incidences of granulation formation in liver were noted. In the ≧ 1,600 ppm male groups and the ≧ 10,000 ppm female groups, increased incidences of centrilobular hepatic cellular swelling were noted. In the ≧ 10,000 ppm male groups and the 25,000 ppm female group, increased incidences of centrilobular hepatic vacuolar degeneration were noted.

In the 25,000 ppm groups of both sexes, increased incidences of hydropic degeneration of the proximal renal tubules were noted. In the ≧ 10,000 ppm groups of both sexes, increased incidences of nuclear enlargement of the proximal renal tubule epithelium were noted. The degree of acidophilic body appearance was reduced in the ≧ 4,000 ppm male groups. There was one male in the 4,000 ppm group which had nephroblastoma as a neoplastic lesion.

Increased incidences of cerebral vacuolar degeneration in the 25,000 ppm groups of both sexes were observed.

Decreased incidences of inflammation with degeneration in the harderian gland were noted in the 25,000 ppm female group.

TABLE 16 NUMBER OF MALE RAT WITH SELECTED LESIONS

Group (ppm)	Control	640	1600	4000	10000	25000
Number of Examined Animals	10	10	10	10	10	10
NASAL CAVITY						
Nuclear enlargement:olfactory epithelium				10	9	10
Nuclear enlargement:respiratory epithelium			9	10	9	10
TRACHEA						
Nuclear enlargement:epithelium				10	10	10
LUNG/BRONCH						
Nuclear enlargement:bronchial epithelium					1	2
LIVER						
Granulation				5	2	10
Swelling:central			9	10	10	10
Vacuolic change:central			1		10	10
KIDNEY						
Nuclear enlargement:proximal tubule				1	5	9
Hydropic change:proximal tubule						7
BRAIN						
Vacuolic change						10
HARDERIAN GLAND						
Inflammation	2	2	1	2		

TABLE 17 NUMBER OF FEMALE RAT WITH SELECTED LESIONS

Group (ppm)	Control	640	1600	4000	10000	25000
Number of Examined Animals	10	10	10	10	10	10
NASAL CAVITY						
Nuclear enlargement:olfactory epithelium				9	10	9
Nuclear enlargement:respiratory epithelium			5	10	10	9
TRACHEA						
Nuclear enlargement:epithelium				9	10	10
LUNG/BRONCH						
Nuclear enlargement:bronchial epithelium				1	1	6
LIVER						
Granulation	2		1	5	5	8
Swelling:central			1		9	9
Vacuolic change:central						10
KIDNEY						
Nuclear enlargement:proximal tubule					8	9
Hydropic change:proximal tubule						6
BRAIN						
Vacuolic change						9
HARDERIAN GLAND						
Inflammation	6	5	6	4	4	

III-2 Preliminary Carcinogenicity Studies in Mice

III-2-1 Acute Studies

(1) Observation of Animal Conditions

Survival

Deaths were observed in the ≧ 5,325 mg/kg groups of both sexes. For males the following deaths were observed: five in the 9,000 mg/kg group of 10 animals on the day of administration (four in 2–4 hours and one in 4–6 hours after the administration) and five on Day 1 in Week 1, totaling 10 deaths. In the 6,923 mg/kg group of 10 animals the following deaths were observed: eight on Day 1 in Week 1, two on Day 2 in Week 1, totaling 10 deaths. In the 5,325 mg/kg group of 10 animals the following deaths were observed: three on Day 1 in Week 1, three on Day 2 in Week 1, totaling 6 deaths. For females in the 9,000 mg/kg group of 10 animals, the following deaths were observed: one on the date of administration (4–6 hours after the administration) and nine on Day 1 in Week 1, totaling 10 deaths. In the 6,923 mg/kg group, the following deaths were observed: nine on Day 1 in Week 1 and one on Day 2 in Week 1, totaling 10 deaths. In the 5,325 mg/kg group, the following deaths were observed: one on Day 1 in Week 1 and five on Day 2 in Week 1, totaling six deaths. (Tables 18 and 19)

General Symptoms

The observed symptoms of the non-survivors in each group of both sexes included decreased locomotor movement, piloerection, abnormal body position (hunchback, abdominal and lying position), ataxic gait, watery eyes, weakening, loss of righting reflex, abnormal respiration (irregular, depressed and difficult respiration), and a decrease in body temperature. The observed symptoms of the survivors in each group of both sexes after the administration included decreased locomotor movement, piloerection, hunchback position, ataxic gait, and abnormal respiration. These symptoms improved over time, and they were barely observed starting from Day 3 in Week 3. (Appendices A 1-3 and 4)

Body Weight

Among survivors of both sexes in the ≦ 5,325 mg/kg groups, the 5,325 mg/kg group showed suppression of body weight gain in comparison with the control groups on Days 1 and 2 in Week 1 only. However, suppression disappeared starting from Day 3 in Week 1. The final body weights in percentage comparison with the control groups (Day 7 in Week 2) were 100% in the 5,325 mg/kg group, 100% in the 4,096 mg/kg group, 102% in the 3,151 mg/kg group of males and 100% in the 5,325 mg/kg group, 101% in the 4,096 mg/kg group, and 99% in the 3,151 mg/kg group of females. (Tables 18 and 19, Figures 11 and 12)

TABLE 18 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN MALE MOUSE (ACUTE STUDIES)

Week-Day on Study	Control Av.Wt.	No.of Surviv.	3151 Av.Wt.	mg/kg % of cont.	No.of Surviv.	4096 Av.Wt.	mg/kg % of cont.	No.of Surviv.	5325 Av.Wt.	mg/kg % of cont.	No.of Surviv.	6923 Av.Wt.	mg/kg % of cont.	No.of Surviv.	9000 Av.Wt.	mg/kg % of cont.	No.of Surviv.
0-0	20.9 (10)	10	20.9 (10)	100	10	20.9 (10)	100	10	20.9 (10)	100	10	20.9 (10)	100	10	20.9 (10)	100	5
1-1	23.5 (10)	10	21.6 (10)	92	10	22.0 (10)	94	10	20.7 (10)	88	7	19.9 (10)	85	2	19.9 (5)	85	0
1-2	23.8 (10)	10	23.0 (10)	97	10	22.8 (10)	96	10	21.1 (7)	89	4	20.4 (2)	86	0			
1-3	24.3 (10)	10	23.6 (10)	97	10	23.4 (10)	96	10	23.4 (4)	96	4						
1-4	24.5 (10)	10	23.9 (10)	98	10	23.7 (10)	97	10	23.9 (4)	98	4						
1-7	25.3 (10)	10	24.9 (10)	98	10	24.4 (10)	96	10	24.4 (4)	96	4						
2-3	25.6 (10)	10	25.3 (10)	99	10	25.1 (10)	98	10	25.1 (4)	98	4						
2-7	26.2 (10)	10	26.6 (10)	102	10	26.2 (10)	100	10	26.3 (4)	100	4						

TABLE 19 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN FEMALE MOUSE (ACUTE STUDIES)

Week-Day on Study	Control Av.Wt.	No.of Surviv.	3151 Av.Wt.	mg/kg % of cont.	No.of Surviv.	4096 Av.Wt.	mg/kg % of cont.	No.of Surviv.	5325 Av.Wt.	mg/kg % of cont.	No.of Surviv.	6923 Av.Wt.	mg/kg % of cont.	No.of Surviv.	9000 Av.Wt.	mg/kg % of cont.	No.of Surviv.
0-0	17.1 (10)	10	17.1 (10)	100	10	17.1 (10)	100	10	17.1 (10)	100	10	17.1 (10)	100	10	17.1 (10)	100	9
1-1	18.7 (10)	10	17.9 (10)	96	10	17.9 (10)	96	10	16.2 (10)	87	9	16.3 (10)	87	1	16.5 (9)	88	0
1-2	19.2 (10)	10	18.7 (10)	97	10	18.6 (10)	97	10	16.2 (9)	84	4	15.9 (1)	83	0			
1-3	19.4 (10)	10	19.0 (10)	98	10	19.2 (10)	99	10	18.6 (4)	96	4						
1-4	19.2 (10)	10	18.9 (10)	98	10	19.4 (10)	101	10	18.7 (4)	97	4						
1-7	19.6 (10)	10	19.3 (10)	98	10	19.4 (10)	99	10	18.8 (4)	96	4						
2-3	19.5 (10)	10	19.3 (10)	99	10	19.7 (10)	101	10	19.6 (4)	101	4						
2-7	20.2 (10)	10	19.9 (10)	99	10	20.5 (10)	101	10	20.2 (4)	100	4						

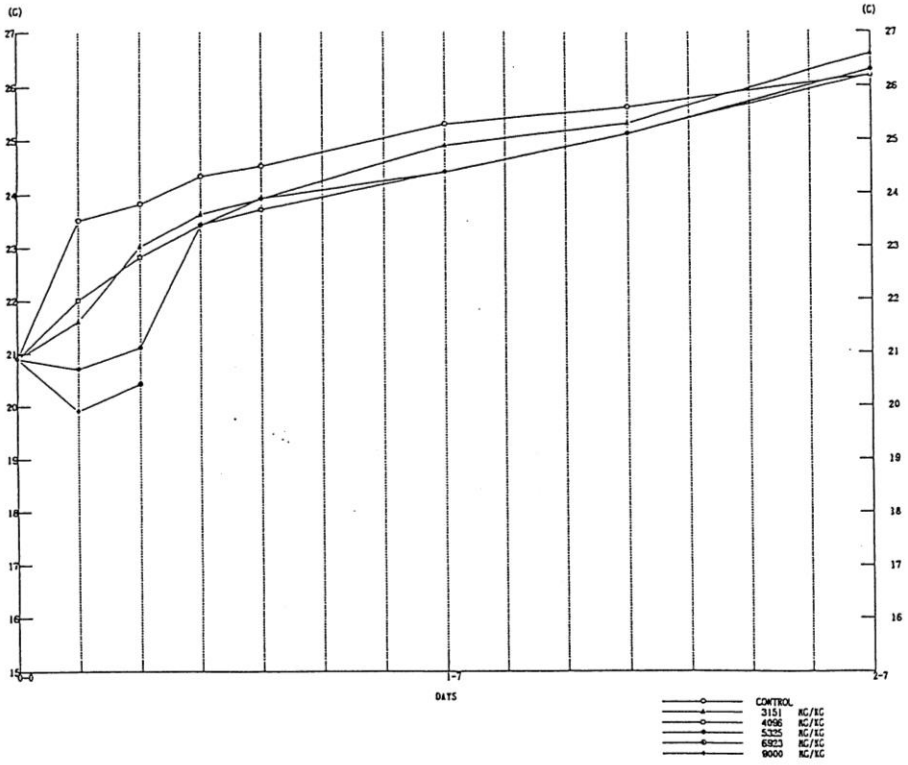


FIGURE 11 BODY WEIGHT CHANGES : MOUSE:MALE (ACUTE STUDIES)

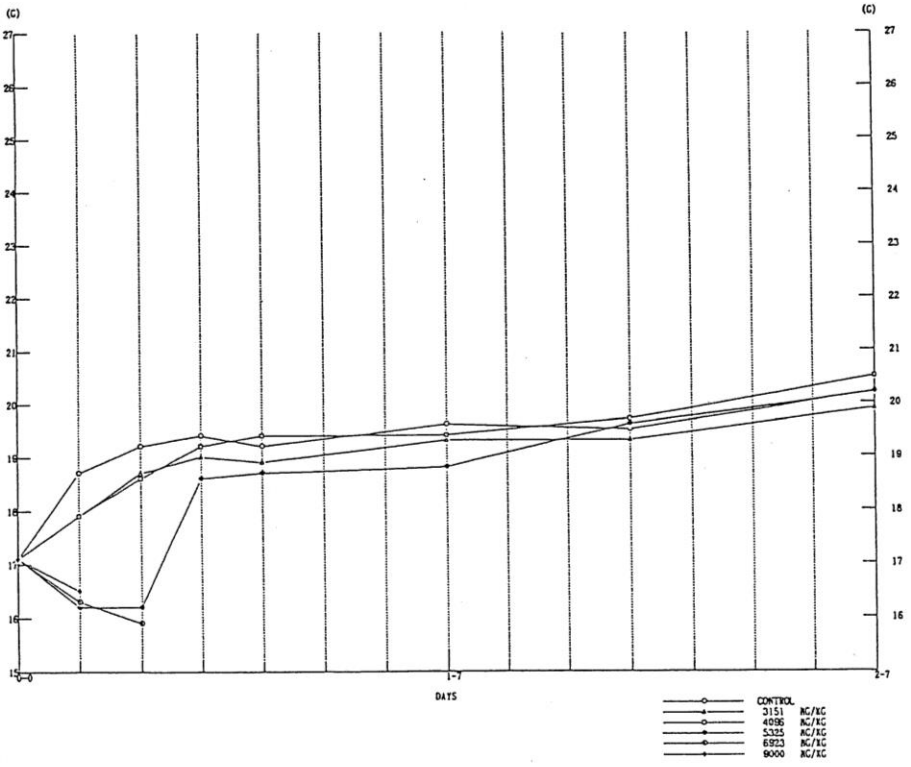


FIGURE 12 BODY WEIGHT CHANGES : MOUSE:FEMALE (ACUTE STUDIES)

(2) Pathological Examination

Necropsy

Findings observed at the time of necropsy are listed in Appendices A 2-5 to 8 (the Summary Tables) and Appendices A 5-1 and 2 (the Individual Tables).

Reddening of the lung and red dots / macules on the glandular stomach were observed in many non-survivors of both sexes in the ≧ 6,923 mg/kg groups. Black, brown, red or clear fluid accumulation in the stomach or small intestines was observed in many non-survivors in the 6,923 mg/kg male group and the ≧ 6,923 mg/kg female groups.

The same findings as those in the 6,923 mg/kg male group were observed in a small number of animals in the 5,325 mg/kg male group. Reddening of the lungs and black, brown, or red fluid accumulation in the stomach or small intestines were observed in a large number of females in the 5,325 mg/kg group. Red dots / macules on the glandular stomach were found in a small number of females in the same group. Anemic-like coloration of the liver and kidney was found in not less than half the animals of the 5,325 mg/kg groups of both sexes.

There were no characteristic findings in the study groups of both sexes surviving at the time of scheduled necropsy.

Histopathological Examination

Histopathological examinations on the non-survivors were conducted on two males and two females in the 9,000 mg/kg groups, two males and two females in the 6,923 mg/kg groups, and two males and one female in the 5,325 mg/kg groups. Histopathological examinations on the survivors at the time of the scheduled necropsy were conducted on two males and two females in the ≦ 5,325 mg/kg groups of both sexes. The results are shown in Appendices A 3-5 to 8 (the Summary Tables) and Appendices A 6-1 and 2 (the Individual Tables).

The following symptoms were observed in the non-survivors: congestion of the lungs and bone marrow, karyorrhexis images of the lymph node, congestion of the spleen, karyorrhexis images of the spleen, hydronephrosis, congestion of the pituitary gland, adrenal gland and brain, karyorrhexis images of the thymic gland, necrosis of the proximal renal tubules (cortico-medullary junction), and hemorrhaging of the glandular stomach.

Animals surviving at the time of the scheduled necropsy showed forestomach ulcers and erosions, thymic gland hemorrhages, testicular atrophies, congested fatty tissue, and melanin deposition on the spleen.

III-2-2 Two-Week Studies

Survival

Deaths were observed only in the 90,000 ppm groups of 10 animals of both sexes. For males, the following deaths were observed: two on Day 1, two on Day 2, two on Day 3, two on Day 4, and one on Day 5 in Week 2, totaling nine deaths. For females, the following deaths were observed: one on Day 7 in Week 1, five on Day 1, one on Day 2, three on Day 3 in Week 2, totaling 10 deaths. (Tables 20 and 21)

General Symptoms

The symptoms of the non-survivors in each group of both sexes were observed starting from Day 3 in Week 1. The symptoms were piloerection, hunchback position, contamination, and ataxic gait. The animals became weak as time passed. Decreased locomotor movement and wasting were observed before the eventual deaths of the animals. The symptoms of the survivors were observed starting from Day 3 in Week 1 in the ≧ 10,000 ppm male groups and the 30,000 ppm female group. One surviving male in the 90,000 ppm group continued to show decreased locomotor movement, piloerection, hunchback position, contamination, and weakening on the final day of administration (Day 7 in Week 2). Males in the 30,000 ppm group showed decreased locomotor movement, piloerection, and hunchback position. Many males in the 10,000 ppm group showed piloerection. Many females in the 30,000 ppm group showed piloerection and hunchback position. (Appendices B 1-3 and 4)

Body Weight

Throughout almost the entire administration period, survivors of both sexes in the ≧ 30,000 ppm groups showed suppression of body weight gain corresponding to the dosages received in comparison with the control groups. The final body weights in percentage comparison with the control groups (Day 7 in Week 2) were 52% in the 90,000 ppm male group, 85% in the 30,000 ppm male group, and 91% in the 30,000 ppm female group. (Tables 20 and 21, Figures 13 and 14)

Food Consumption

Decreased food consumption in the ≧ 30,000 ppm groups of both sexes was observed closely corresponding to the administration concentrations received in comparison with the control groups. (Tables 22 and 23)

Water Consumption

Decreased water consumption in the ≧ 10,000 ppm groups of both sexes was observed corresponding to a decrease in the administration concentrations received in comparison with the control groups. The water consumption percentages of both sexes in comparison with the control groups were ± 10% in the 90,000 ppm groups, approximately 20–50% in the 30,000 ppm groups, and approximately 70–80% in the 10,000 ppm groups. (Tables 24 and 25)

TABLE 20 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN MALE MOUSE (TWO-WEEK STUDIES)

Week-Day on Study	Control Av.Wt.	No.of Surviv.	1110 ppm Av.Wt.	% of cont.	No.of Surviv.	3330 ppm Av.Wt.	% of cont.	No.of Surviv.	10000 ppm Av.Wt.	% of cont.	No.of Surviv.	30000 ppm Av.Wt.	% of cont.	No.of Surviv.	90000 ppm Av.Wt.	% of cont.	No.of Surviv.
0-0	23.7 (10)	10	23.7 (10)	100	10	23.8 (10)	100	10	23.8 (10)	100	10	23.8 (10)	100	10	23.8 (10)	100	10
1-1	23.4 (10)	10	23.5 (10)	100	10	23.4 (10)	100	10	22.2 (10)	95	10	21.5 (10)	92	10	21.2 (10)	91	10
1-2	24.4 (10)	10	24.2 (10)	99	10	24.2 (10)	99	10	22.5 (10)	92	10	20.2 (10)	83	10	19.9 (10)	82	10
1-4	24.7 (10)	10	24.8 (10)	100	10	24.5 (10)	99	10	23.8 (10)	96	10	18.8 (10)	76	10	17.8 (10)	72	10
1-7	25.1 (10)	10	25.0 (10)	100	10	25.4 (10)	101	10	24.5 (10)	98	10	17.5 (10)	70	10	15.0 (10)	60	10
2-3	25.2 (10)	10	25.5 (10)	101	10	25.7 (10)	102	10	24.9 (10)	99	10	19.7 (10)	78	10	13.7 (6)	54	4
2-7	26.0 (10)	10	26.2 (10)	101	10	26.4 (10)	102	10	25.8 (10)	99	10	22.0 (10)	85	10	13.5 (1)	52	1

TABLE 21 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN FEMALE MOUSE (TWO-WEEK STUDIES)

Week-Day on Study	Control Av.Wt.	No.of Surviv.	1110 ppm Av.Wt.	% of cont.	No.of Surviv.	3330 ppm Av.Wt.	% of cont.	No.of Surviv.	10000 ppm Av.Wt.	% of cont.	No.of Surviv.	30000 ppm Av.Wt.	% of cont.	No.of Surviv.	90000 ppm Av.Wt.	% of cont.	No.of Surviv.
0-0	19.6 (10)	10	19.6 (10)	100	10	19.6 (10)	100	10	19.6 (10)	100	10	19.6 (10)	100	10	19.6 (10)	100	10
1-1	19.3 (10)	10	18.6 (10)	96	10	19.0 (10)	98	10	18.8 (10)	97	10	17.4 (10)	90	10	16.8 (10)	87	10
1-2	19.2 (10)	10	18.9 (10)	98	10	18.8 (10)	98	10	19.2 (10)	100	10	16.5 (10)	86	10	15.5 (10)	81	10
1-4	19.9 (10)	10	19.3 (10)	97	10	19.3 (10)	97	10	19.7 (10)	99	10	16.9 (10)	85	10	13.5 (10)	68	10
1-7	19.9 (10)	10	19.6 (10)	98	10	19.1 (10)	96	10	19.8 (10)	99	10	17.5 (10)	88	10	11.1 (10)	56	9
2-3	20.0 (10)	10	20.2 (10)	101	10	19.8 (10)	99	10	20.0 (10)	100	10	18.8 (10)	94	10	10.4 (3)	52	0
2-7	20.8 (10)	10	20.9 (10)	100	10	20.4 (10)	98	10	20.7 (10)	100	10	19.0 (3)	91	10			

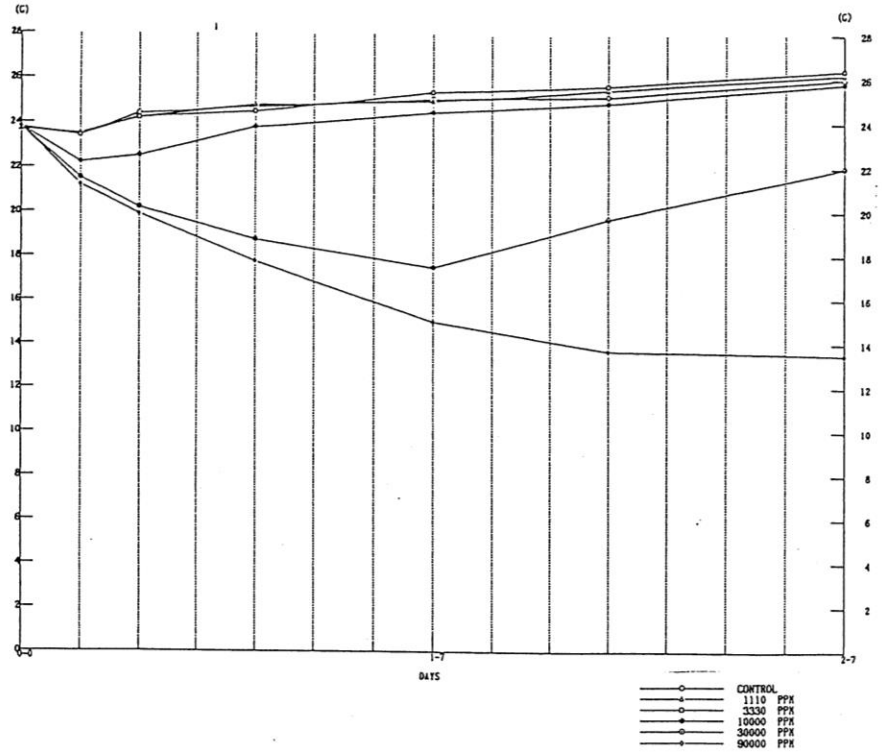


FIGURE 13 BODY WEIGHT CHANGES : MOUSE:MALE (TWO-WEEK STUDIES)

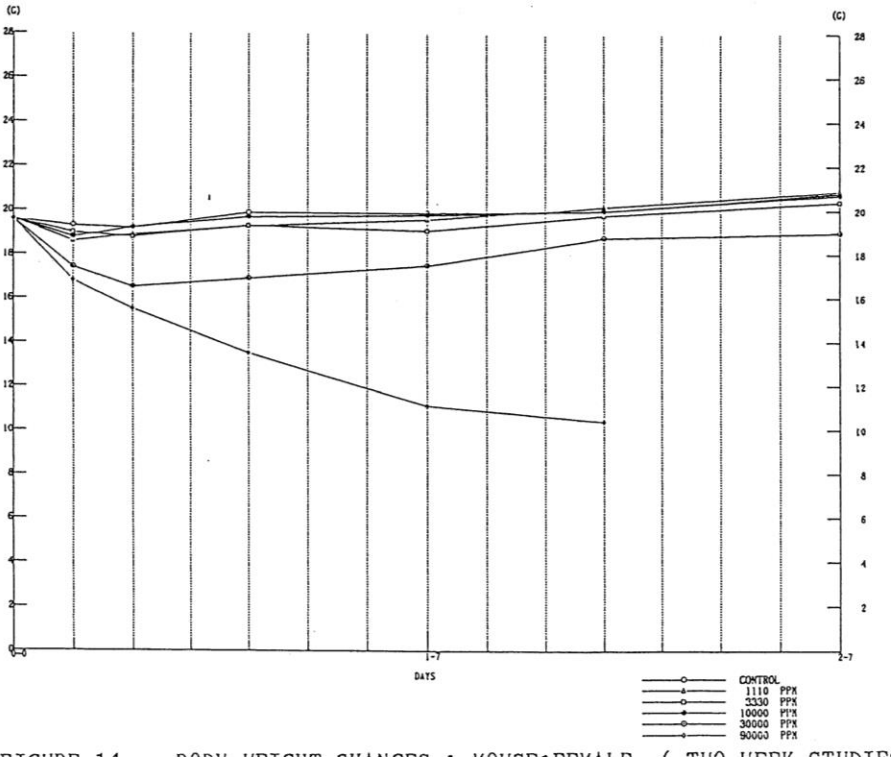


FIGURE 14 BODY WEIGHT CHANGES : MOUSE:FEMALE (TWO-WEEK STUDIES)

TABLE 22 FOOD CONSUMPTION IN MALE MOUSE (TWO-WEEK STUDIES)

Week-Day on Study	Control Av.FC.	No.of Surviv.	1110 ppm Av.FC.	% of cont.	No.of Surviv.	3330 ppm Av.FC.	% of cont.	No.of Surviv.	10000 ppm Av.FC.	% of cont.	No.of Surviv.	30000 ppm Av.FC.	% of cont.	No.of Surviv.	90000 ppm Av.FC.	% of cont.	No.of Surviv.
1-7	3.8 (10)	10	3.9 (10)	103	10	3.9 (10)	103	10	3.6 (10)	95	10	1.8 (10)	47	10	1.4 (10)	37	10
2-7	3.6 (10)	10	3.6 (10)	100	10	3.7 (10)	103	10	3.7 (10)	103	10	3.6 (10)	100	10	1.0 (1)	28	1

TABLE 23 FOOD CONSUMPTION IN FEMALE MOUSE (TWO-WEEK STUDIES)

Week-Day on Study	Control Av.FC.	No.of Surviv.	1110 ppm Av.FC.	% of cont.	No.of Surviv.	3330 ppm Av.FC.	% of cont.	No.of Surviv.	10000 ppm Av.FC.	% of cont.	No.of Surviv.	30000 ppm Av.FC.	% of cont.	No.of Surviv.	90000 ppm Av.FC.	% of cont.	No.of Surviv.
1-7	3.2 (10)	10	3.1 (10)	97	10	3.1 (10)	97	10	3.3 (10)	103	10	2.5 (10)	78	10	1.3 (10)	41	9
2-7	3.3 (10)	10	3.3 (10)	100	10	3.2 (10)	97	10	3.3 (10)	100	10	3.1 (3)	94	10			

TABLE 24 WATER CONSUMPTION IN MALE MOUSE (TWO-WEEK STUDIES)

Week-Day on Study	Control Av.WC.	No.of Surviv.	1110 ppm Av.WC.	% of cont.	No.of Surviv.	3330 ppm Av.WC.	% of cont.	No.of Surviv.	10000 ppm Av.WC.	% of cont.	No.of Surviv.	30000 ppm Av.WC.	% of cont.	No.of Surviv.	90000 ppm Av.WC.	% of cont.	No.of Surviv.
1-4	4.7 (10)	10	4.5 (10)	96	10	4.8 (10)	102	10	3.1 (10)	66	10	0.9 (10)	19	10	0.5 (10)	11	10
1-7	4.8 (10)	10	4.5 (10)	94	10	5.1 (10)	106	10	3.3 (10)	69	10	1.0 (10)	21	10	0.3 (10)	6	10
2-3	5.0 (10)	10	4.7 (10)	94	10	4.9 (10)	98	10	3.4 (10)	68	10	2.2 (10)	44	10	0.5 (4)	10	4
2-7	5.2 (10)	10	5.0 (10)	96	10	5.2 (10)	100	10	3.6 (10)	69	10	2.5 (10)	48	10	0.8 (1)	15	1

TABLE 25 WATER CONSUMPTION IN FEMALE MOUSE (TWO-WEEK STUDIES)

Week-Day on Study	Control Av.WC.	No.of Surviv.	1110 ppm Av.WC.	% of cont.	No.of Surviv.	3330 ppm Av.WC.	% of cont.	No.of Surviv.	10000 ppm Av.WC.	% of cont.	No.of Surviv.	30000 ppm Av.WC.	% of cont.	No.of Surviv.	90000 ppm Av.WC.	% of cont.	No.of Surviv.
1-4	4.6 (10)	10	4.4 (10)	96	10	4.3 (10)	93	10	3.5 (10)	76	10	1.6 (10)	35	10	0.5 (10)	11	10
1-7	4.6 (10)	10	3.8 (10)	83	10	4.4 (10)	96	10	3.6 (10)	78	10	2.1 (10)	46	10	0.4 (10)	9	9
2-3	4.8 (10)	10	4.8 (10)	100	10	4.3 (10)	90	10	3.4 (10)	71	10	2.4 (10)	50	10			
2-7	4.8 (10)	10	4.9 (10)	102	10	4.5 (10)	94	10	3.6 (10)	75	10	2.2 (10)	46	10			

(2) Pathological Examination

Necropsy

Findings observed at the time of necropsy are listed in Appendices B 3-5 to 8 (the Summary Tables) and Appendices B 6-3 and 4 (the Individual Tables).

Many non-surviving / moribund animals in the 90,000 ppm groups of both sexes had thymic gland atrophy and red dots / macules on the glandular stomach, and a few animals had red dots / macules on the lungs and brown fluid accumulation in the small intestines.

For survivors, almost the same findings as those for the non-survivors were observed in one animal in the 90,000 ppm male group. In the 30,000 ppm male group, many showed a well defined hepatic lobule structure and anemic-like coloration of the liver, and not less than half showed thymic gland atrophy. In the 10,000 ppm male group, approximately half showed anemic-like coloration of the liver and a small number showed anemic-like coloration of the kidney. In the 30,000 ppm female group, many showed a well defined hepatic lobule structure and anemic-like coloration of the liver, and a small number showed thymic gland atrophy. In the 10,000 and 3,330 ppm female groups, a small number showed a well defined hepatic lobule structure.

Histopathological Examination

Histopathological examinations on the non-survivors were conducted on three males and two females in the 90,000 ppm groups. Histopathological examinations on the survivors were conducted on one male in the 90,000 ppm group, and two males and two females each in the ≦ 30,000 ppm groups. The results are shown in Appendices B 4-5 to 8 (the Summary Tables) and Appendices B 7-3 and 4 (the Individual Tables).

Non-surviving / moribund males showed congestion of the bone marrow, thymic gland atrophy, karyorrhexis images of the thymic gland, spleen atrophy, and hepatic single cell necrosis.

Animals surviving at the time of the scheduled necropsy showed congestion of the bone marrow, thymic gland atrophy, and centrilobular hepatic cellular swelling.

The control groups showed melanin deposition on the spleen.

III-2-3 Thirteen-Week Studies

(1) Observation of Animal Conditions

Survival

One death occurred only in the 25,000 ppm male group during Week 2 after the start of the administration. (Tables 26 and 27)

General Symptoms

The observed symptoms of the non-survivors included piloerection, hunchback position, contamination, contamination of surrounding areas of the external genitalia from urine and wasting. Many animals surviving in the ≧ 25,000 ppm male groups had piloerection. (Appendices D 3 and 4)

Body Weight

Only males in the 25,000 ppm group showed suppression of body weight gain in comparison with the control groups. The final body weights in percentage comparison with the control groups (Week 13) were 71% in the 25,000 ppm group, 94% in the 10,000 ppm group, 100% in the 4,000 ppm group, 102% in the 1,600 ppm group, and 105% in the 640 ppm group for males and 96% in the 25,000 ppm group, 103% in the 10,000 ppm group, 103% in the 4,000 ppm group, 102% in the 1,600 ppm group, and 102% in the 640 ppm group for females. (Tables 26 and 27, Figures 15 and 16, Appendices E 3 and 4 (the Summary Tables), Appendices Q 3 and 4 (the Individual Tables))

Food Consumption

Decreased food consumption was observed only in the 25,000 ppm male group in comparison with the control group and corresponded to the suppression of body weight gain. (Tables 28 and 29, Figures 17 and 18, Appendices F 3 and 4 (the Summary Tables), Appendices R 3 and 4 (the Individual Tables))

Water Consumption

Apparent decrease in water consumption in the ≧ 10,000 ppm groups of both sexes was observed corresponding to a decrease in the administration concentrations received in comparison with the control groups. The water consumption percentages in comparison with the control groups were 7–48% in the 25,000 ppm group and 43–63% in the 10,000 ppm group for males and 24–50% in the 25,000 ppm group and 61–74% in the 10,000 ppm group for females. (Tables 30 and 31, Figures 19 and 20, Appendices G 3 and 4 (the Summary Tables), Appendices S 3 and 4 (the Individual Tables))

TABLE 26 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN MALE MOUSE (THIRTEEN-WEEK STUDIES)

Week-Day on Study	Control Av.Wt.	No.of Surviv.	640 ppm Av.Wt.	% of cont.	No.of Surviv.	1600 ppm Av.Wt.	% of cont.	No.of Surviv.	4000 ppm Av.Wt.	% of cont.	No.of Surviv.	10000 ppm Av.Wt.	% of cont.	No.of Surviv.	25000 ppm Av.Wt.	% of cont.	No.of Surviv.
0-0	23.5 (10)	10	23.5 (10)	100	10	23.5 (10)	100	10	23.5 (10)	100	10	23.5 (10)	100	10	23.5 (10)	100	10
1-7	24.7 (10)	10	24.9 (10)	101	10	24.8 (10)	100	10	24.6 (10)	100	10	24.1 (10)	98	10	19.3 (10)	78	10
2-7	25.6 (10)	10	26.1 (10)	102	10	26.2 (10)	102	10	25.7 (10)	100	10	25.4 (10)	99	10	22.9 (9)	89	9
3-7	26.6 (10)	10	27.4 (10)	103	10	27.4 (10)	103	10	26.6 (10)	100	10	26.5 (10)	100	10	23.9 (9)	90	9
4-7	27.3 (10)	10	28.3 (10)	104	10	28.1 (10)	103	10	27.8 (10)	102	10	27.7 (10)	101	10	24.2 (9)	89	9
5-7	28.3 (10)	10	29.1 (10)	103	10	28.9 (10)	102	10	28.4 (10)	100	10	28.0 (10)	99	10	24.4 (9)	86	9
6-7	29.5 (10)	10	30.2 (10)	102	10	29.9 (10)	101	10	29.2 (10)	99	10	28.9 (10)	98	10	24.7 (9)	84	9
7-7	29.9 (10)	10	31.3 (10)	105	10	30.9 (10)	103	10	30.0 (10)	100	10	29.9 (10)	100	10	25.2 (9)	84	9
8-7	30.9 (10)	10	32.0 (10)	104	10	31.7 (10)	103	10	31.1 (10)	101	10	30.7 (10)	99	10	25.7 (9)	83	9
9-7	31.6 (10)	10	32.9 (10)	104	10	32.2 (10)	102	10	31.6 (10)	100	10	31.4 (10)	99	10	25.8 (9)	82	9
10-7	32.8 (10)	10	34.0 (10)	104	10	33.1 (10)	101	10	32.6 (10)	99	10	32.1 (10)	98	10	25.8 (9)	79	9
11-7	33.5 (10)	10	34.7 (10)	104	10	33.9 (10)	101	10	33.7 (10)	101	10	32.4 (10)	97	10	25.9 (9)	77	9
12-7	34.4 (10)	10	35.7 (10)	104	10	34.7 (10)	101	10	33.1 (10)	96	10	32.7 (10)	95	10	25.3 (9)	74	9
13-7	34.5 (10)	10	36.1 (10)	105	10	35.2 (10)	102	10	34.6 (10)	100	10	32.5 (10)	94	10	24.6 (9)	71	9

TABLE 27 SURVIVAL ANIMAL NUMBERS AND BODY WEIGHT CHANGES IN FEMALE MOUSE (THIRTEEN-WEEK STUDIES)

Week-Day on Study	Control Av.Wt.	No.of Surviv.	640 ppm Av.Wt.	% of cont.	No.of Surviv.	1600 ppm Av.Wt.	% of cont.	No.of Surviv.	4000 ppm Av.Wt.	% of cont.	No.of Surviv.	10000 ppm Av.Wt.	% of cont.	No.of Surviv.	25000 ppm Av.Wt.	% of cont.	No.of Surviv.
0-0	18.9 (10)	10	18.9 (10)	100	10	18.9 (10)	100	10	18.9 (10)	100	10	18.9 (10)	100	10	18.9 (10)	100	10
1-7	19.3 (10)	10	19.4 (10)	101	10	19.2 (10)	99	10	19.3 (10)	100	10	19.4 (10)	101	10	18.7 (10)	97	10
2-7	19.4 (10)	10	20.3 (10)	105	10	19.7 (10)	102	10	20.4 (10)	105	10	20.4 (10)	105	10	19.7 (10)	102	10
3-7	20.9 (10)	10	20.7 (10)	99	10	20.9 (10)	100	10	21.0 (10)	100	10	21.1 (10)	101	10	20.3 (10)	97	10
4-7	20.8 (10)	10	20.9 (10)	100	10	20.9 (10)	100	10	21.4 (10)	103	10	21.3 (10)	102	10	20.9 (10)	100	10
5-7	21.2 (10)	10	21.3 (10)	100	10	21.7 (10)	102	10	21.9 (10)	103	10	21.9 (10)	103	10	21.2 (10)	100	10
6-7	21.4 (10)	10	21.8 (10)	102	10	21.9 (10)	102	10	22.0 (10)	103	10	22.2 (10)	104	10	21.0 (10)	98	10
7-7	22.7 (10)	10	22.3 (10)	98	10	22.3 (10)	98	10	22.7 (10)	100	10	22.4 (10)	99	10	22.1 (10)	97	10
8-7	22.4 (10)	10	22.5 (10)	100	10	22.7 (10)	101	10	23.2 (10)	104	10	23.2 (10)	104	10	21.7 (10)	97	10
9-7	22.8 (10)	10	23.1 (10)	101	10	23.7 (10)	104	10	24.3 (10)	107	10	24.1 (10)	106	10	22.3 (10)	98	10
10-7	23.3 (10)	10	23.3 (10)	100	10	24.4 (10)	105	10	24.1 (10)	103	10	23.7 (10)	102	10	22.0 (10)	94	10
11-7	24.2 (10)	10	24.2 (10)	100	10	24.6 (10)	102	10	24.8 (10)	102	10	24.5 (10)	101	10	22.1 (10)	91	10
12-7	23.8 (10)	10	24.4 (10)	103	10	24.5 (10)	103	10	24.2 (10)	102	10	24.3 (10)	102	10	22.4 (10)	94	10
13-7	23.8 (10)	10	24.2 (10)	102	10	24.2 (10)	102	10	24.5 (10)	103	10	24.5 (10)	103	10	22.9 (10)	96	10

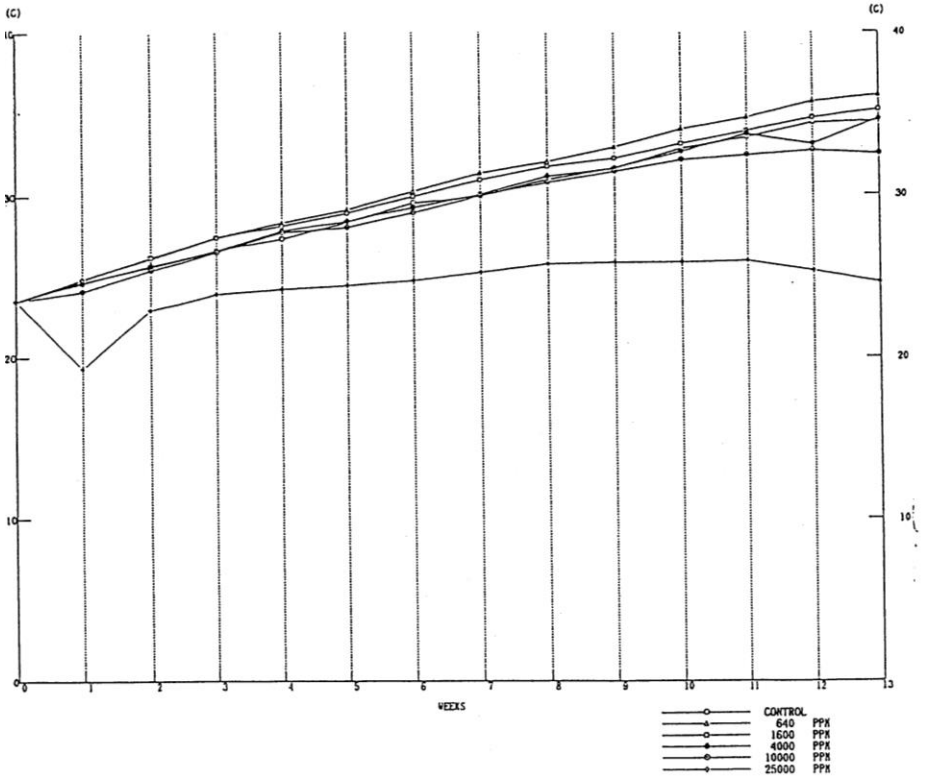


FIGURE 15 BODY WEIGHT CHANGES : MOUSE:MALE (THIRTEEN-WEEK STUDIES)

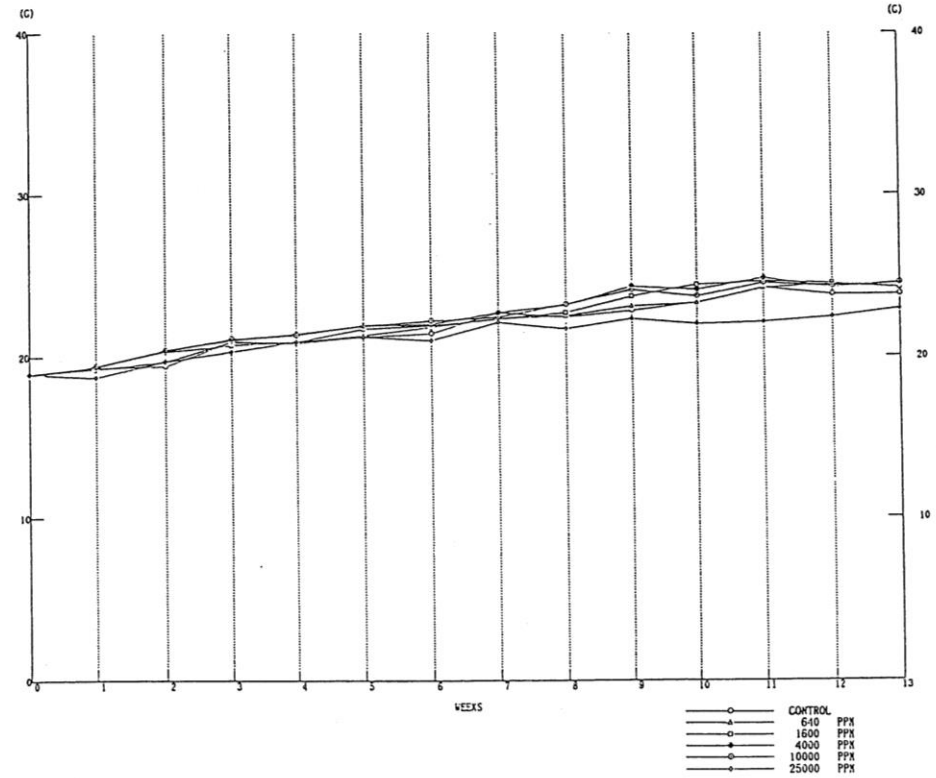


FIGURE 16 BODY WEIGHT CHANGES : MOUSE:FEMALE (THIRTEEN-WEEK STUDIES)

TABLE 28 FOOD CONSUMPTION IN MALE MOUSE (THIRTEEN-WEEK STUDIES)

Week-Day on Study	Control Av.FC.	No.of Surviv.	640 ppm Av.FC.	% of cont.	No.of Surviv.	1600 ppm Av.FC.	% of cont.	No.of Surviv.	4000 ppm Av.FC.	% of cont.	No.of Surviv.	10000 ppm Av.FC.	% of cont.	No.of Surviv.	25000 ppm Av.FC.	% of cont.	No.of Surviv.
1-7	3.8 (10)	10	3.8 (10)	100	10	3.8 (10)	100	10	3.8 (10)	100	10	3.5 (10)	92	10	2.5 (10)	66	10
2-7	3.7 (10)	10	3.8 (10)	103	10	3.9 (10)	105	10	3.8 (10)	103	10	3.7 (10)	100	10	4.0 (9)	108	9
3-7	3.8 (10)	10	3.9 (10)	103	10	4.0 (10)	105	10	3.9 (10)	103	10	3.8 (10)	100	10	3.5 (9)	92	9
4-7	4.0 (10)	10	4.1 (10)	103	10	4.1 (10)	103	10	4.0 (10)	100	10	4.0 (10)	100	10	3.7 (9)	93	9
5-7	4.1 (10)	10	4.1 (10)	100	10	4.2 (10)	102	10	4.1 (10)	100	10	4.0 (10)	98	10	3.8 (9)	93	9
6-7	4.4 (10)	10	4.3 (10)	98	10	4.4 (10)	100	10	4.3 (10)	98	10	4.2 (10)	95	10	3.9 (9)	89	9
7-7	4.1 (10)	10	4.1 (10)	100	10	4.2 (10)	102	10	4.1 (10)	100	10	4.1 (10)	100	10	3.8 (9)	93	9
8-7	4.2 (10)	10	4.1 (10)	98	10	4.2 (10)	100	10	4.1 (10)	98	10	4.0 (10)	95	10	3.9 (9)	93	9
9-7	4.3 (10)	10	4.3 (10)	100	10	4.3 (10)	100	10	4.3 (10)	100	10	4.2 (10)	98	10	3.9 (9)	91	9
10-7	4.6 (10)	10	4.6 (10)	100	10	4.4 (10)	96	10	4.5 (10)	98	10	4.3 (10)	93	10	4.0 (9)	87	9
11-7	4.2 (10)	10	4.2 (10)	100	10	4.2 (10)	100	10	4.3 (10)	102	10	4.0 (10)	95	10	3.7 (9)	88	9
12-7	4.3 (10)	10	4.3 (10)	100	10	4.3 (10)	100	10	4.0 (10)	93	10	3.9 (10)	91	10	3.5 (9)	81	9
13-7	3.8 (10)	10	3.8 (10)	100	10	3.9 (10)	103	10	3.9 (10)	103	10	3.6 (10)	95	10	3.3 (9)	87	9

TABLE 29 FOOD CONSUMPTION IN FEMALE MOUSE (THIRTEEN-WEEK STUDIES)

Week-Day on Study	Control Av.FC.	No.of Surviv.	640 ppm Av.FC.	% of cont.	No.of Surviv.	1600 ppm Av.FC.	% of cont.	No.of Surviv.	4000 ppm Av.FC.	% of cont.	No.of Surviv.	10000 ppm Av.FC.	% of cont.	No.of Surviv.	25000 ppm Av.FC.	% of cont.	No.of Surviv.
1-7	3.2 (10)	10	3.2 (10)	100	10	3.2 (10)	100	10	3.3 (10)	103	10	3.2 (10)	100	10	3.0 (10)	94	10
2-7	3.3 (10)	10	3.4 (10)	103	10	3.5 (10)	106	10	3.5 (10)	106	10	3.4 (10)	103	10	3.3 (10)	100	10
3-7	3.6 (10)	10	3.5 (10)	97	10	3.6 (10)	100	10	3.6 (10)	100	10	3.6 (10)	100	10	3.5 (10)	97	10
4-7	4.0 (10)	10	3.9 (10)	98	10	4.0 (10)	100	10	4.1 (10)	103	10	4.0 (10)	100	10	3.9 (10)	98	10
5-7	4.0 (10)	10	4.0 (10)	100	10	4.0 (10)	100	10	4.0 (10)	100	10	4.0 (10)	100	10	3.9 (10)	98	10
6-7	4.3 (10)	10	4.2 (10)	98	10	4.2 (10)	98	10	4.2 (10)	98	10	4.2 (10)	98	10	4.0 (10)	93	10
7-7	4.3 (10)	10	4.1 (10)	95	10	4.1 (10)	95	10	4.3 (10)	100	10	4.1 (10)	95	10	4.1 (10)	95	10
8-7	4.2 (10)	10	4.1 (10)	98	10	4.1 (10)	98	10	4.2 (10)	100	10	4.1 (10)	98	10	3.8 (10)	90	10
9-7	4.5 (10)	10	4.5 (10)	100	10	4.4 (10)	98	10	4.5 (10)	100	10	4.4 (10)	98	10	4.2 (10)	93	10
10-7	4.7 (10)	10	4.4 (10)	94	10	4.6 (10)	98	10	4.6 (10)	98	10	4.4 (10)	94	10	4.2 (10)	89	10
11-7	4.5 (10)	10	4.3 (10)	96	10	4.4 (10)	98	10	4.4 (10)	98	10	4.2 (10)	93	10	4.0 (10)	89	10
12-7	4.3 (10)	10	4.4 (10)	102	10	4.6 (10)	107	10	4.2 (10)	98	10	4.2 (10)	98	10	4.1 (10)	95	10
13-7	3.9 (10)	10	3.8 (10)	97	10	3.8 (10)	97	10	3.8 (10)	97	10	3.8 (10)	97	10	3.8 (10)	97	10

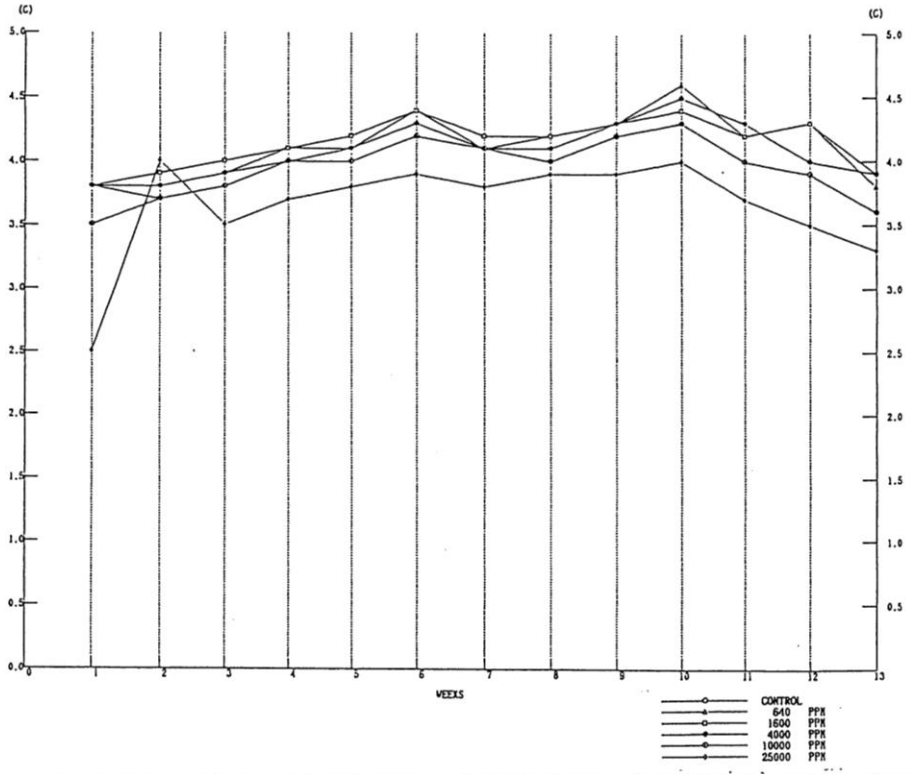


FIGURE 17 FOOD CONSUMPTION : MOUSE:MALE (THIRTEEN-WEEK STUDIES)

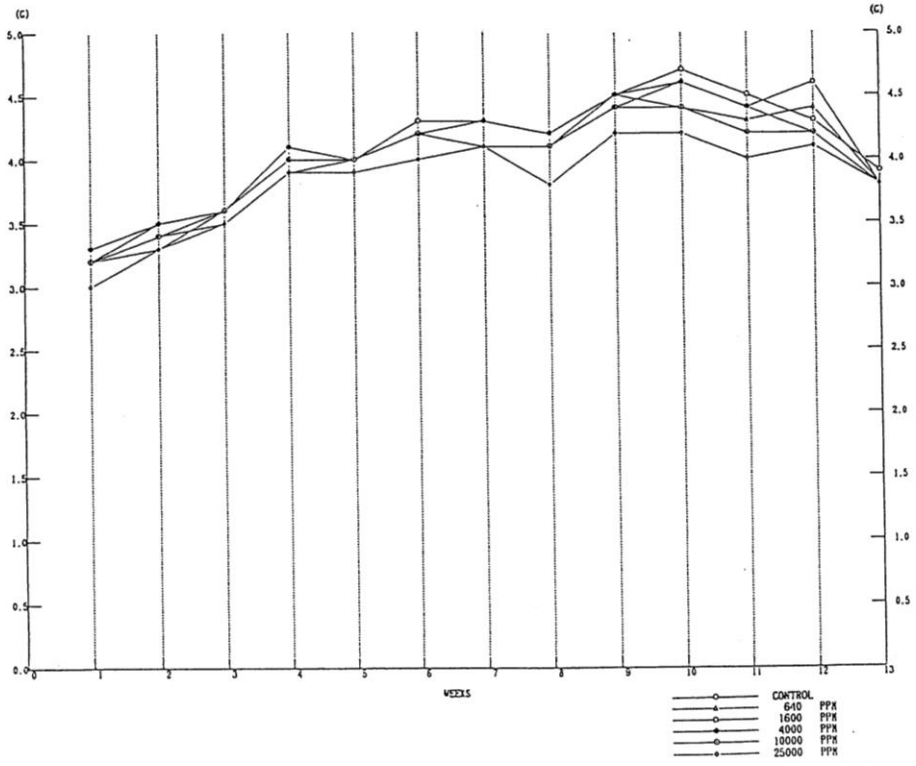


FIGURE 18 FOOD CONSUMPTION : MOUSE:FEMALE (THIRTEEN-WEEK STUDIES)

TABLE 30 WATER CONSUMPTION IN MALE MOUSE (THIRTEEN-WEEK STUDIES)

Week-Day on Study	Control Av.WC.	No.of Surviv.	640 ppm Av.WC.	% of cont.	No.of Surviv.	1600 ppm Av.WC.	% of cont.	No.of Surviv.	4000 ppm Av.WC.	% of cont.	No.of Surviv.	10000 ppm Av.WC.	% of cont.	No.of Surviv.	25000 ppm Av.WC.	% of cont.	No.of Surviv.
1-3	4.6 (10)	10	4.4 (10)	96	10	4.6 (10)	100	10	4.2 (10)	91	10	2.3 (10)	50	10	0.3 (10)	7	10
1-7	5.0 (10)	10	4.8 (10)	96	10	4.9 (10)	98	10	4.5 (10)	90	10	2.8 (10)	56	10	1.4 (10)	28	10
2-3	4.8 (10)	10	4.3 (10)	90	10	4.8 (10)	100	10	4.3 (10)	90	10	2.5 (10)	52	10	2.3 (9)	48	9
2-7	5.1 (10)	10	4.4 (10)	86	10	4.7 (10)	92	10	5.0 (10)	98	10	2.7 (10)	53	10	2.1 (9)	41	9
3-3	6.1 (10)	10	4.6 (10)	75	10	4.4 (10)	72	10	4.7 (10)	77	10	2.6 (10)	43	10	1.5 (9)	25	9
3-7	5.8 (10)	10	4.4 (10)	76	10	4.4 (10)	76	10	5.0 (10)	86	10	2.8 (10)	48	10	1.9 (9)	33	9
4-3	6.6 (10)	10	4.5 (10)	68	10	4.5 (10)	68	10	5.3 (10)	80	10	2.9 (10)	44	10	1.7 (9)	26	9
4-7	5.8 (10)	10	4.3 (10)	74	10	4.8 (10)	83	10	4.6 (10)	79	10	2.8 (10)	48	10	1.7 (9)	29	9
5-3	5.6 (10)	10	4.0 (10)	71	10	4.1 (10)	73	10	5.1 (10)	91	10	2.6 (10)	46	10	1.6 (9)	29	9
5-7	4.8 (10)	10	4.1 (10)	85	10	4.3 (10)	90	10	4.1 (10)	85	10	2.7 (10)	56	10	1.6 (9)	33	9
6-3	5.2 (10)	10	4.0 (10)	77	10	4.2 (10)	81	10	4.4 (10)	85	10	2.8 (10)	54	10	1.3 (9)	25	9
6-7	5.8 (10)	10	4.2 (10)	72	10	4.5 (10)	78	10	4.1 (10)	71	10	2.7 (10)	47	10	1.5 (9)	26	9
7-3	5.4 (10)	10	4.2 (10)	78	10	4.2 (10)	78	10	4.5 (10)	83	10	2.7 (10)	50	10	1.6 (9)	30	9
7-7	5.4 (10)	10	4.4 (10)	81	10	4.3 (10)	80	10	4.7 (10)	87	10	2.7 (10)	50	10	1.7 (9)	31	9
8-3	5.4 (10)	10	4.0 (10)	74	10	4.5 (10)	83	10	4.3 (10)	80	10	2.5 (10)	46	10	1.5 (9)	28	9
8-7	5.1 (10)	10	4.1 (10)	80	10	4.6 (10)	90	10	4.0 (10)	78	10	2.6 (10)	51	10	1.7 (9)	33	9
9-3	5.0 (10)	10	4.2 (10)	84	10	4.3 (10)	86	10	4.0 (10)	80	10	2.6 (10)	52	10	1.4 (9)	28	9
9-7	4.6 (10)	10	4.0 (10)	87	10	4.2 (10)	91	10	4.5 (10)	98	10	2.5 (10)	54	10	1.5 (9)	33	9
10-3	4.5 (10)	10	4.1 (10)	91	10	4.3 (10)	96	10	4.1 (10)	91	10	2.7 (10)	60	10	1.7 (9)	38	9
10-7	4.3 (10)	10	3.9 (10)	91	10	3.9 (10)	91	10	3.8 (10)	88	10	2.4 (10)	56	10	1.4 (9)	33	9
11-3	4.4 (10)	10	3.7 (10)	84	10	4.0 (10)	91	10	3.8 (10)	86	10	2.5 (10)	57	10	1.0 (9)	23	9
11-7	4.1 (10)	10	3.6 (10)	88	10	4.0 (10)	98	10	3.6 (10)	88	10	2.4 (10)	59	10	1.6 (9)	39	9
12-3	4.4 (10)	10	3.8 (10)	86	10	4.2 (10)	95	10	3.6 (10)	82	10	2.5 (10)	57	10	1.0 (9)	23	9
12-7	4.2 (10)	10	3.8 (10)	90	10	4.0 (10)	95	10	3.0 (10)	71	10	2.2 (10)	52	10	1.4 (9)	33	9
13-3	4.1 (10)	10	3.8 (10)	93	10	4.2 (10)	102	10	4.0 (10)	98	10	2.6 (10)	63	10	1.3 (9)	32	9
13-7	4.1 (10)	10	3.8 (10)	93	10	4.1 (10)	100	10	4.2 (10)	102	10	2.4 (10)	59	10	1.5 (9)	37	9

TABLE 31 WATER CONSUMPTION IN FEMALE MOUSE (THIRTEEN-WEEK STUDIES)

Week-Day on Study	Control Av.WC.	No.of Surviv.	640 ppm Av.WC.	% of cont.	No.of Surviv.	1600 ppm Av.WC.	% of cont.	No.of Surviv.	4000 ppm Av.WC.	% of cont.	No.of Surviv.	10000 ppm Av.WC.	% of cont.	No.of Surviv.	25000 ppm Av.WC.	% of cont.	No.of Surviv.
1-3	4.1 (10)	10	4.0 (10)	98	10	4.8 (10)	117	10	3.8 (10)	93	10	2.9 (10)	71	10	1.0 (10)	24	10
1-7	5.0 (10)	10	4.6 (10)	92	10	5.0 (10)	100	10	4.4 (10)	88	10	3.2 (10)	64	10	2.2 (10)	44	10
2-3	4.7 (10)	10	4.6 (10)	98	10	5.3 (10)	113	10	4.6 (10)	98	10	3.3 (10)	70	10	2.2 (10)	47	10
2-7	4.8 (10)	10	4.8 (10)	100	10	6.2 (10)	129	10	5.0 (10)	104	10	3.5 (10)	73	10	2.2 (10)	46	10
3-3	5.3 (10)	10	4.7 (10)	89	10	5.6 (10)	106	10	4.7 (10)	89	10	3.5 (10)	66	10	2.2 (10)	42	10
3-7	5.6 (10)	10	5.7 (10)	102	10	6.0 (10)	107	10	5.3 (10)	95	10	3.6 (10)	64	10	2.4 (10)	43	10
4-3	5.8 (10)	10	6.0 (10)	103	10	5.9 (10)	102	10	5.2 (10)	90	10	3.9 (10)	67	10	2.5 (10)	43	10
4-7	6.1 (10)	10	6.3 (10)	103	10	6.2 (10)	102	10	5.5 (10)	90	10	4.2 (10)	69	10	2.5 (10)	41	10
5-3	5.5 (10)	10	6.0 (10)	109	10	5.4 (10)	98	10	5.3 (10)	96	10	3.9 (10)	71	10	2.1 (10)	38	10
5-7	5.5 (10)	10	6.0 (10)	109	10	5.0 (10)	91	10	5.1 (10)	93	10	3.9 (10)	71	10	2.4 (10)	44	10
6-3	5.6 (10)	10	6.1 (10)	109	10	5.3 (10)	95	10	4.6 (10)	82	10	3.7 (10)	66	10	2.1 (10)	38	10
6-7	5.5 (10)	10	6.5 (10)	118	10	5.8 (10)	105	10	5.3 (10)	96	10	3.8 (10)	69	10	2.3 (10)	42	10
7-3	5.7 (10)	10	6.1 (10)	107	10	6.0 (10)	105	10	5.7 (10)	100	10	3.9 (10)	68	10	2.4 (10)	42	10
7-7	5.5 (10)	10	6.3 (10)	115	10	5.7 (10)	104	10	4.9 (10)	89	10	3.9 (10)	71	10	2.5 (10)	45	10
8-3	5.3 (10)	10	6.1 (10)	115	10	5.9 (10)	111	10	5.1 (10)	96	10	3.8 (10)	72	10	2.2 (10)	42	10
8-7	5.0 (10)	10	5.5 (10)	110	10	5.1 (10)	102	10	4.9 (10)	98	10	3.7 (10)	74	10	2.5 (10)	50	10
9-3	6.2 (10)	10	6.7 (10)	108	10	5.1 (10)	82	10	5.4 (10)	87	10	3.8 (10)	61	10	2.5 (10)	40	10
9-7	5.8 (10)	10	5.9 (10)	102	10	5.0 (10)	86	10	5.7 (10)	98	10	3.6 (10)	62	10	2.4 (10)	41	10
10-3	5.3 (10)	10	6.7 (10)	126	10	5.7 (10)	108	10	5.1 (10)	96	10	3.7 (10)	70	10	2.4 (10)	45	10
10-7	5.4 (10)	10	6.4 (10)	119	10	4.8 (10)	89	10	5.1 (10)	94	10	3.5 (10)	65	10	2.4 (10)	44	10
11-3	5.6 (10)	10	6.0 (10)	107	10	4.8 (10)	86	10	5.3 (10)	95	10	3.7 (10)	66	10	2.3 (10)	41	10
11-7	5.6 (10)	10	6.6 (10)	118	10	5.1 (10)	91	10	5.3 (10)	95	10	3.6 (10)	64	10	2.3 (10)	41	10
12-3	5.5 (10)	10	7.3 (10)	133	10	5.2 (10)	95	10	5.1 (10)	93	10	3.7 (10)	67	10	2.3 (10)	42	10
12-7	4.9 (10)	10	5.9 (10)	120	10	4.6 (10)	94	10	4.8 (10)	98	10	3.5 (10)	71	10	2.2 (10)	45	10
13-3	5.7 (10)	10	6.4 (10)	112	10	5.2 (10)	91	10	4.7 (10)	82	10	3.6 (10)	63	10	2.2 (10)	39	10
13-7	5.7 (10)	10	5.8 (10)	102	10	5.1 (10)	89	10	4.8 (10)	84	10	3.6 (10)	63	10	2.4 (10)	42	10

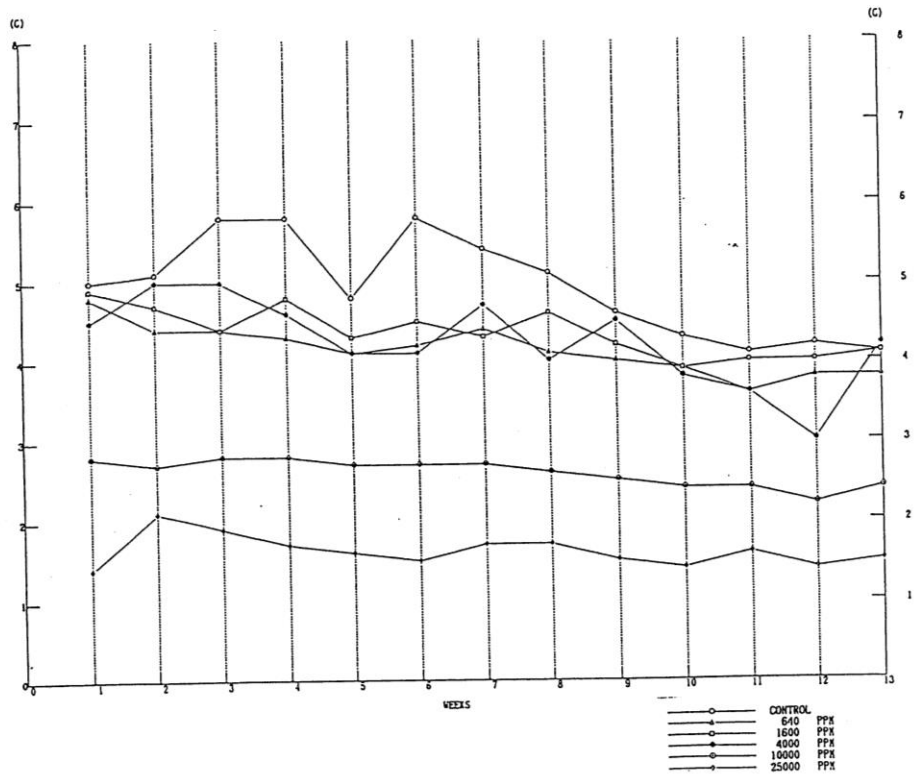


FIGURE 19 WATER CONSUMPTION : MOUSE:MALE (THIRTEEN-WEEK STUDIES)

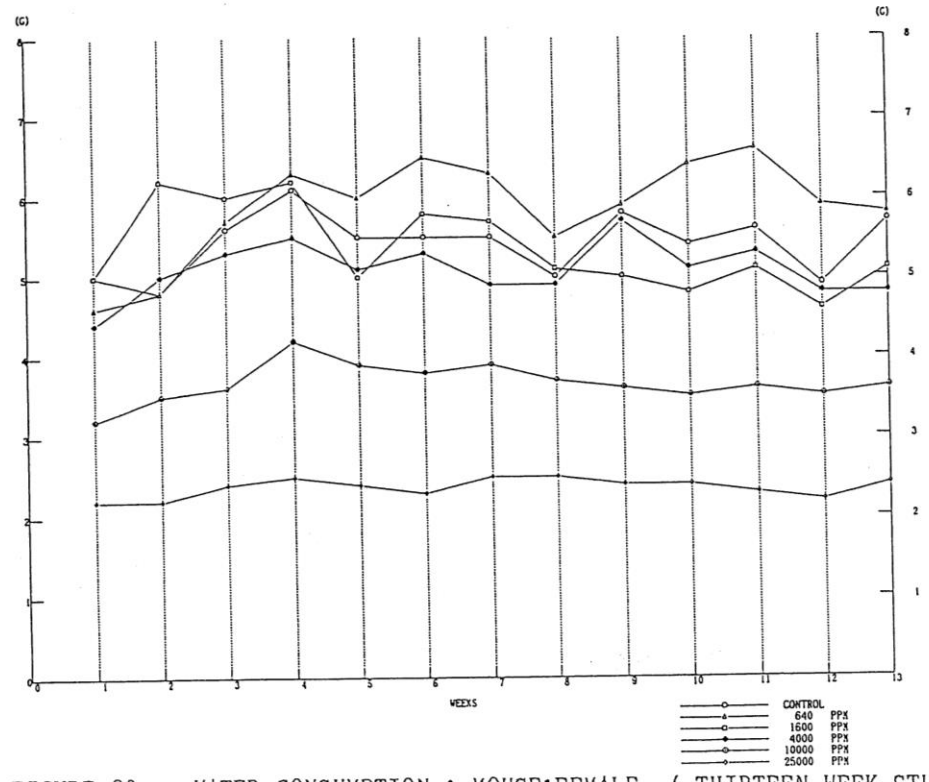


FIGURE 20 WATER CONSUMPTION : MOUSE:FEMALE (THIRTEEN-WEEK STUDIES)

(2) Hematology and Blood Biochemistry Examinations, and Urinalysis

Hematology Examination

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

For males in the 25,000 ppm group, the following was observed: increases in the red blood cell counts, hemoglobin concentration, hematocrit level, and the mean corpuscular volume. An increase in the mean corpuscular volume was also observed in the 10,000 ppm group. An increase in the platelet counts was found in the 4,000 ppm group.

For females, increases in the mean corpuscular volume in the 25,000 and 10,000 ppm groups were observed.

Blood Biochemistry Examination

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

For males in the 25,000 ppm group, the following was observed: increases in GOT, GPT and ALP, and decreases in total protein, albumin, glucose, and total cholesterol. In the 10,000 and 4,000 ppm groups, an increase in ALP and a decrease in total cholesterol were observed. In the 10,000 and 1,600 ppm groups a decrease in LAP was observed. In the 1,600 and 640 ppm groups an increase in potassium was observed.

For females in the 25,000 ppm group, the following was observed: increases in GOT, GPT, LDH, and ALP, and decreases in total protein, albumin, glucose, and total cholesterol. In the 10,000 ppm group, increases in GPT and LDH and decreases in total protein, glucose, and total cholesterol were observed.

Urinalysis

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

For males, a decrease in the pH level and an increase in protein were noted in the 25,000 ppm. The pH level decreased in the 10,000 ppm group. The protein level increased in the 1,600 ppm group.

For females, a decrease in the pH level was noted in the 25,000 and 10,000 ppm groups.

(3) Pathological Examination

Necropsy

Findings observed at the time of necropsy are listed in Appendices K 4 to 6 (the Summary Tables) and Appendices W 3 and 4 (the Individual Tables).

Thymic atrophy, black fluid accumulation in the glandular stomach and duodenum were observed in one non-surviving male in the 25,000 ppm group. There were no characteristic findings in the study groups of both sexes surviving at the time of scheduled necropsy.

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 L 3 and 4 (the Summary Tables of the actual weights), Appendices M 3 and 4 (the Summary Tables of the actual weight to body weight ratio), Appendices X 3 and 4 (the Individual Tables of the actual weights), and Appendices Y 3 and 4 (the Individual Tables of the actual weight to body weight ratio).

Compared with the control group, the actual weights of the thymic gland, heart, kidney (the right only), spleen, liver, and brain for males in the 25,000 ppm group had significantly lower values. The actual weights of the lungs and their ratio to the body weights, and the ratios of the actual weights of the adrenal gland (the left only), testis, kidney, and brain to the body weights had significantly higher values.

Compared with the control group, the actual weights of the lungs and kidney, and their ratios to the body weights for females in the 25,000 ppm group had significantly higher values. The actual weights of the livers and their ratios to the body weights and the actual weights of the brain had significantly lower values. In the 10,000 ppm group, the actual weights of the lungs and their ratios to the body weights had significantly higher values. The actual weights of the kidneys had significantly higher values.

The body weights in the 25,000 ppm male group at the time of necropsy had significantly lower values compared with those of the control group.

Histopathologic Examination

The findings observed at the time of necropsy are listed in Appendices N 4 to 6 (the Summary Tables) and Appendices Z 3 and 4 (the Individual Tables). The main findings are summarized in Tables 32 and 33.

One death in the 25,000 ppm male group showed nuclear enlargement in the olfactory epithelium in the nasal cavity, pulmonary / bronchial emphysema, erosion in glandular stomach, congestion of the bone marrow, and atrophy of the thymic gland and spleen. However, the cause of death could not be determined pathologically.

Among the findings which were observed at the time of the scheduled necropsy, significant differences in comparison with the control groups are described below.

Increased incidences of eosinophilic changes in the olfactory and respiratory epithelium in the nasal cavity in the 25,000 ppm male group and in the ≧ 10,000 ppm female group were noted. Increased incidences of nuclear enlargement of the olfactory epithelum (the supporting cells) in the ≧ 4,000 ppm groups of both sexes and increased incidences of nuclear enlargement of the respiratory epithelium in the 10,000 ppm male group and the 25,000 ppm female group were noted. Increased incidences of vacuolation of the olfactory nerve fiber bundle under the olfactory epithelium in the 25,000 groups of both sexes were noted.

Increased incidences of nuclear enlargement of the tracheal epithelium in the ≧ 4,000 ppm groups of both sexes were noted.

Increased incidences of nuclear enlargement of the tracheal epithelium in the lungs in the ≧ 4,000 ppm male groups and the ≧ 1,600 ppm female groups were noted. Increased incidences of bronchial epithelium degeneration and the appearance of foamy cells in the 25,000 ppm male group and the ≧ 10,000 ppm female groups were noted.

Increased incidences of hepatic single cell necrosis and centrilobular hepatic cellular swelling in the ≧ 4,000 ppm groups of both sexes were noted.

Decreased incidences of vacuolation of the proximal renal tubules were noted in the ≧ 4,000 ppm male groups.

TABLE 32 NUMBER OF MALE MOUSE WITH SELECTED LESIONS

Group (ppm)	Control	640	1600	4000	10000	25000
Number of Examined Animals	10	10	10	10	10	10
NASAL CAVITY						
Eosinophilic change:olfactory epithelium						6
Eosinophilic change:respiratory epithelium						5
Nuclear enlargement:olfactory epithelium				9	10	9
Nuclear enlargement:respiratory epithelium				2	5	1
Vacuolic change:olfactory nerve						9
TRACHEA						
Nuclear enlargement:epithelium				7	9	9
LUNG/BRONCH						
Accumuration of foamy cells						6
Nuclear enlargement:bronchial epithelium				9	9	9
Degeneration:bronchial epithelium						8
LIVER						
Necrosis:single cell				5	10	9
Swelling:central				10	10	9
KIDNEY						
Vacuolization of proximal tube	10	7	6	5		

TABLE 33 NUMBER OF FEMALE MOUSE WITH SELECTED LESIONS

Group (ppm)	Control	640	1600	4000	10000	25000
Number of Examined Animals	10	10	10	10	10	10
NASAL CAVITY						
Eosinophilic change:olfactory epithelium				1	6	6
Eosinophilic change:respiratory epithelium			1	1	5	9
Nuclear enlargement:olfactory epithelium				6	10	10
Nuclear enlargement:respiratory epithelium				3	3	7
Vacuolic change:olfactory nerve					2	8
TRACHEA						
Nuclear enlargement:epithelium			2	9	10	10
LUNG/BRONCH						
Accumulation of foamy cells					10	10
Nuclear enlargement:bronchial epithelium			10	10	10	10
Degeneration:bronchial epithelium					7	10
LIVER						
Necrosis:single cell				7	10	9
Swelling:central		1	1	10	10	9

VI Discussion

<Evaluation of the Study Results>

In the acute studies for rats, all animals in the 10,000 mg/kg male group died by two weeks after the administration. Eight animals died in the 7,143 mg/kg male group and none died in the ≦ 5,102 mg/kg male groups. All animals died in the ≧ 7,143 mg/kg female groups and five animals died in the 5,102 mg/kg female group. There were no deaths in the ≦ 3,644 mg/kg female groups. In the acute studies for mice, all animals died in the ≧ 6,923 mg/kg groups of both sexes by two weeks after the administration. Six animals died in the 5,325 mg/kg group and no animals died in the ≦ 4,096 mg/kg groups. Pathological examinations revealed effects of 1,4-dioxane on the kidney and liver for rats and the kidney for mice.

Based on the above results and taking into account water consumption by the animals, it was determined that the appropriate highest dosage concentration of 1,4-dioxane in drinking water for the two-week studies was 90,000 ppm in order to have a daily consumption of 1,4-dioxane of approximately 5,000 mg/kg (considered to be the amount at which half of the study animals would die).

In the two-week studies, the highest concentration of the substance was set to be 90,000 ppm based on the acute study results. Using a common ratio of 3.0, the exposure levels of the rest of the exposure groups were set to 30,000, 10,000, 3,330, or 1,110 ppm.

All rats in the 90,000 ppm groups of both sexes and two females in the 30,000 ppm group died. Animals in the ≧ 10,000 ppm groups of both sexes showed suppression of body weight gain during the entire study period. Animals in the ≧ 10,000 ppm groups of both sexes surviving until the time of scheduled necropsy showed nuclear enlargement of the olfactory epithelium (the supporting cells), and animals in the 30,000 ppm groups of both sexes showed centrilobular hepatic cellular swelling and vacuolar degeneration, hydropic degeneration of the proximal renal tubules, and cerebral vacuolar degeneration. Nine male mice and all female mice in the 90,000 ppm groups died. Mice in the ≧ 10,000 ppm groups of both sexes showed suppression of body weight gain during the entire study period. Mice in the 30,000 ppm groups of both sexes surviving until the time of scheduled necropsy showed centrilobular hepatic cellular swelling. Rats in the ≧ 1,100 ppm groups showed anemic-like coloration of the liver and kidney, and mice in the ≧ 3,300 ppm groups showed well defined hepatic lobule structures.

Based on the above results, it was indicated that 1,4-dioxane affected the liver, nasal cavity, brain, and kidney in rats and the liver in mice. If the highest dosage concentration were to be set to ≧ 30,000 ppm for the thirteen-week studies, it was expected that the animals would not be able to tolerate the toxicity of 1,4-dioxane. Therefore, the lower concentration was considered desirable. In order to determine a No Observed Effect Concentration (NOEC), it was also considered desirable to set the lowest dosage concentration to a concentration lower than 1,100 ppm at which effects were observed in the two-week studies.

In the thirteen-week studies, the highest concentration of the substance was set to be 25,000 ppm based on the two-week study results. Using a common ratio of 2.5, the exposure levels of the rest of the exposure groups were set to 10,000, 4,000, 1,600, and 640 ppm.

One female rat in the 25,000 ppm group died. The final body weights of the survivors of both sexes decreased ≧ 20% in comparison with those of the control groups. Water consumption decreased 26–57% in males and 42–68% in females, showing avoidance of consuming the 1,4-dioxane solution. Histopathological examinations revealed increased incidences of nuclear enlargement of the olfactory epithelium (the supporting cells) in the nasal cavity, nuclear enlargement of the respiratory and tracheal epithelium, renal granulation, centrilobular hepatic cellular swelling and vacuolar degeneration, nuclear enlargements and hydropic degeneration of the proximal renal tubules, and cerebral vacuolar degeneration in both sexes, and increased incidences of nuclear enlargement of the bronchial epithelium in females. These were considered to be the effects of 1,4-dioxane exposure.

In the 10,000 ppm groups, compared with the control groups, the final body weights stabilized at a 7% decrease in males, while there was a 12% decrease in females, beyond the reference range of 10% body weight decrease used to determine exposure concentrations for a long term toxicity study. Water consumption also decreased 17–44% in males and 25–59% in females. Histopathological examinations revealed increased incidences of nuclear enlargement of the olfactory epithelium (the supporting cells) in the nasal cavity, nuclear enlargement of the respiratory and tracheal epithelium, centrilobular hepatic cellular swelling, and nuclear enlargements of the proximal renal tubules in both sexes; and increased incidences of centrilobular hepatic vacuolar degeneration in males. These were considered to be the effects of 1,4-dioxane exposure.

In the 4,000 ppm groups, compared with the control groups, the final body weights decreased 3% in males and 6% in females, an extremely small decrease. However, water consumption decreased 9–38% in males and 3–38% in females. Histopathological examinations revealed increased incidences of nuclear enlargement of the olfactory epithelium (the supporting cells) in the nasal cavity and nuclear enlargement of the respiratory and tracheal epithelium in both sexes, and increased incidences of granulation formations in the liver and centrilobular hepatic cellular swelling in males. These changes were considered to be the effects of 1,4-dioxane exposure.

In the 1,600 ppm groups, the final body weights and water consumption hardly decreased compared with the control groups. Though to a small degree, histopathological examinations revealed increased incidences of nuclear enlargement of the respiratory epithelium in the nasal cavity in both sexes, and increased incidences of centrilobular hepatic cellular swelling in males.

In the 640 ppm groups, neither significant changes nor effects of 1,4-dioxane exposure were observed.

One male mouse in the 25,000 ppm group died. Compared with the control groups, the final body weights stabilized at a 4% decrease in females, while those in males decreased 29%. Water consumption decreased 52–93% in males and 50–76% in females. Histopathological examinations revealed eosinophilic changes in the olfactory and respiratory epithelium in the nasal cavity, nuclear enlargement of the olfactory epithelium (the supporting cells) in the nasal cavity, and vacuolation of the olfactory nerve fiber bundle, nuclear enlargement of the tracheal epithelium, the appearance of foamy cells in the lungs, nuclear enlargement of the bronchial epithelium, degeneration of the bronchial epithelium, hepatic single cell necrosis and centrilobular hepatic cellular swelling in both sexes, and nuclear enlargement of the respiratory epithelium in the nasal cavity in females. These were considered to be the effects of 1,4-dioxane exposure.

In the 10,000 ppm groups, the final body weights stabilized at a 6% decrease in males compared with the control group. Water consumption decreased 37–57% in males and 26–39% in females. Histopathological examinations revealed increased incidences of nuclear enlargement of the olfactory epithelium (the supporting cells) in the nasal cavity, nuclear enlargement of the tracheal and bronchial epithelium, hepatic single cell necrosis and centrilobular hepatic cellular swelling in both sexes; increased incidences of nuclear enlargements of the respiratory epithelium in males; and increased incidences of eosinophilic changes in the olfactory and respiratory epithelium in the nasal cavity, the appearance of foamy cells in the lungs, and degeneration of the bronchial epithelium in females. These were considered to be the effects of 1,4-dioxane exposure.

In the 4,000 ppm groups, the final body weights and water consumption hardly decreased compared with the control groups. However, histopathological examinations revealed increased incidences of nuclear enlargement of the olfactory epithelium (the supporting cells) in the nasal cavity, nuclear enlargement of the tracheal and pulmonary bronchial epithelium, hepatic single cell necrosis, and centrilobular hepatic cellular swelling in both sexes. These were considered to be the effects of 1,4-dioxane exposure.

In the 1,600 ppm groups, the final body weights and water consumption did not decrease compared with the control groups. However, histopathological examinations revealed increased incidences of nuclear enlargement of the bronchial epithelium in females.

In the 640 ppm groups, neither significant changes nor the effects of the 1,4-dioxane exposure were 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, lungs, and liver. The mice in the 1,600 ppm groups primarily had changes in the lungs.

<Setting the Exposure Levels of the Carcinogenicity Studies>

Based on the results from the thirteen-week studies described in the Evaluation of the Study Results section, rats were more sensitive to 1,4-dioxane than mice.

A carcinogenicity study by administration of drinking water with 1,4-dioxane by NCI (1978, Reference 3) 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 studies were determined with a comprehensive consideration of the above results. 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 where no severe toxic changes appeared yet carcinogenicity could be reliably proved 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 started avoiding drinking water with 1,4-dioxane at a certain concentration suggested 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.

<Conclusion>

The oral administration concentrations in the carcinogenicity studies of 1,4-dioxane (mixed with water) were determined to be 5,000, 1,000, and 200 ppm for rats; and 8,000, 2,000, and 500 ppm for mice.

V. References

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