8EHQ-0693-1047

DOW CORNING

Contains No CBI

EPA-OTS

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May 24, 1993

ATTN: TSCA SECTION 8(E) COORDINATOR
TSCA DOCUMENT PROCESSING CENTER (TS-790)
ROOM L-100
OFFICE OF PESTICIDES AND TOXIC SUBSTANCES
US ENVIRONMENTAL PROTECTION AGENCY
401 M ST SW
WASHINGTON DC 20460

Re: Followup Submission to 8EHQ-0890-1047
TSCA Section 8(e) Notification of Substantial Risk
Hexamethoxydisilylethane

Dear Sir:

Dow Corning Corporation is submitting the following pair of toxicological studies as additional followup to our Notification of Substantial Risk of August 21, 1990 (8EHQ-0890-1047).

Chemical Substance:

Hexamethoxydisilylethane
CASRN 18406-41-2

Manufacturer:

Dow Corning Corporation
2200 West Salzburg Road
Midland, Michigan 48686-0994

Introduction:

On August 10, 1990, Dow Corning submitted a Notification of Substantial Risk under TSCA Section 8(e) concerning preliminary results obtained in an acute vapor phase inhalation toxicity study of DOW CORNING® XI-6145A Additive, chemically identified as hexamethoxydisilylethane (CASRN 18406-41-2), in rats. The final report was submitted to EPA August 21, 1990. In response to the Agency's request, Dow Corning provided additional followup to 8EHQ-0890-1047 in a letter of May 30, 1991, including a description of:

> ...the nature and results, if available, of all studies (other than those submitted already to EPA or those cited in the published scientific literature) about which the company is aware or that it has conducted, is conducting,

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or plans to conduct that are designed to determine the toxicity of the chemical

As additional followup to 8EHQ-0980-1047, we are providing EPA with copies of two recently obtained Japanese toxicological studies on hexamethoxydisilylethane for which we commissioned translations of the Japanese language portions of those studies.

(1) **Submitted Study:**

FINAL REPORT - TEST TO EXAMINE THE EFFECT OF X-12-990S TO CAUSE CHROMOSOMAL ABERRATION BY USING CULTURED MAMMALIAN CELLS

Chemical Product Testing Association
Chemical Product Safety Center, Hita Laboratories
January, 1992

Executive Summary:

The test material, hexamethoxydisilylethane, was examined for its ability to induce chromosomal aberrations in Chinese hamster pulmonary fibroblast cells using both the direct (non-metabolic activation) and metabolic activation methods.

Test animals were dosed at 100, 200, and 400 µg/ml of test material in the case of a 24-hour treatment, and at 75, 150, and 300 µg/ml in the case of a 48-hour treatment in the direct method. Mitomycin C (MMC) was employed at 0.05 µg/ml as a positive control in both direct treatment regimens.

Test animals were dosed at 100, 150, and 200 µg/ml of test material in the metabolic activation method. Cyclophosphonamide (CPA) was employed at 10 µg/ml as a positive control.

The test substance did not induce any structural abnormality in chromosomes in the case of 24- and 48-hour treatments via the direct method. On addition of S9 mix in the metabolic activation method, dose-dependent structural abnormalities were observed in chromosomes at 150-200 µg/ml.

In conclusion, under the conditions of this assay and in the presence of metabolic activation, the test material induces chromosomal aberration in Chinese Hamster pulmonary fibroblast cells.

(2) **Submitted Study:**

FINAL REPORT - 28-DAY ORAL TOXICITY (REPEAT DOSE) STUDY OF X-12-990S ON RATS

Chemical Inspection Association
Chemical Safety Center, Hita Laboratories
September, 1992

Executive Summary:

A 28-day repeat dose oral toxicity study was conducted with hexamethoxydisilylethane in rats. The test material was administered to groups of six male or female Crj:CD (SJ) rats at 0 (control group), 8, 30, and 60 and 120 mg/kg body weight for either 14 or 28 days.

In the 28-day experiment, high mortality was seen at 60 and 120 kg/mg. Severe clinical signs of intoxication, increased liver weights, effects on blood chemistry, hematuria, hematological effects, and both microscopic and macroscopic incidence of liver and heart damage were seen in the 60 and 120 mg/kg dose groups. The hematological effects, hematuria, and heart pathology also were observed in the 30 mg/kg group. Increases in urinary protein were observed in male rats at all dose levels (8 mg/kg and above).

A repeat test employing a 14-day dosing period followed by a 14-day recovery period at 60 and 120 mg/kg resulted in some mortality, decreased food consumption, and pathological changes in the liver and heart, as before. In addition to the previously observed hematological, clinical chemistry, urinary findings, and increased liver weights, increased adrenal weights (60 and 120 mg/kg), heart weight (120 mg/kg) and increased creatinine concentration were observed.

Actions:

Dow Corning will notify EPA of any further pertinent information that may be developed concerning this chemical substance. Dow Corning has ceased efforts to commercialize this substance and has taken action to insure it is not present as an unintentional byproduct in other materials we make.

If you require further information concerning this notification, please contact Dr. Rhys G. Daniels, Regulatory Compliance Specialist, Dow Corning Product Safety and Regulatory Compliance Department, at the address provided below or by telephone at 517-496-4222.

Sincerely,

A E Bey

Alvin E. Bey
U.S. Area Vice-President
Corporate Director HES

Chemical Product Testing Association, Chemical Product Safety Center, Hita Laboratories: Final Report - Test to examine the effect of X-12-990S to cause chromosomal aberration by using cultured mammalian cells [Saishu Hokokusho - X-12-990S no Honyuurui Baiyosaiboo Mochiiru Senshokutai Ijo Shikenn]

Translated from Japanese by the Ralph McElroy Company, Custom Division
P.O. Box 4828, Austin, TX 78765 USA

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Code: 270-17581
Acceptance No. T91-1497
Report No. T-2932

Test Code No. K06-0265

FINAL REPORT

TEST TO EXAMINE THE EFFECT OF X-12-990S TO CAUSE CHROMOSOMAL ABERRATION BY USING CULTURED MAMMALIAN CELLS

JANUARY 1992

Chemical Product Testing Association
Chemical Product Safety Center, Hita Laboratories

Statement
Chemical Testing Association
Chemical Product Safety Center, Hita Laboratories

Test Requester: Shietsu Chemical Industry K.K.
Title: Test to examine the effect of X-12-990S to cause chromosomal aberration by using cultured mammalian cells
Test Code No.: K06-0265

The current test was carried out in conformation with "Testing facilities specified in the article 4 of the orders for testing novel chemical substances and toxicities of designated chemical substances" and "OECD Principles of Good Laboratory Practice (May 12, 1981)" according to the Environmental Safety Laws No. 39, "Yakuhatsu" No. 229, and "Kikyoku" No. 85 as of March 31, 1984, and Environmental Research Laws No. 233, "Eisei" No. 38 and 63, and "Kikyoku No. 823 as of November 18, 1988.

January 30, 1992
Operation manager: T. Yamamoto [Signature and seal]

Certificate of Accuracy

Chemical Testing Association
Chemical Product Safety Center, Hita
Laboratories

Test Requester: Shietsu Chemical Industry K.K.
Title: Test to examine the effect of X-12-990S to cause chromosomal aberration by using cultured mammalian cells
Test Code No.: K06-0265

The current test was inspected and audited by the accuracy certification department of the Chemical Product Testing Association, Chemical Product Safety Center, Hita Laboratories. The following is the date of inspection or auditing and date of reports to the operation manager and person in charge of the test.

Inspection or auditing date	Date reported to person in charge of the test	Date reported to the operation manager
9/30/1991	9/30/1991	9/30/1991
11/21/1991	11/22/1991	11/25/1991
12/16/1991	12/18/1991	12/18/1991
12/18/1991	12/18/1991	12/18/1991
1/23/1992	1/23/1992	1/23/1992
1/30/1992	1/30/1992	1/30/1992

In this report, the methods and procedures used in the test are accurately described, and the results shown in the report accurately reflect the raw data of the test.

January 30, 1992 K. Shiraishi, Accuracy Certification Dept.
January 30, 1992 H. Fujii, Manager, Accuracy Certification. Dept.

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Summary

The action of X-12-9908 inducing chromosomal aberration was examined by using pulmonary fibroblast cells (CHL cells) of Chinese hamster and mitomycin C (MMC) in the direct method (nonmetabolic activation method) or cyclophosphamide (CPA) in the metabolic activation method as a positive control.

As a result of cell proliferation inhibition and cell division inhibition tests carried out for determining suitable doses of the testing substance for testing, the dose of the testing substance for testing chromosomal aberration was set at 100, 200, and 400 μg/mL in the case of a 24-hour treatment in the

direct method and 75, 150, and 300 μg/mL in the case of a 48-hour treatment. In the metabolic activation method, it was set at 100, 150, and 200 μg/mL. The dose in the positive control was 0.05 μg/mL of MMC in the direct method 24- and 48-hour treatments and 10 μg/mL of CPA in the metabolic activation method.

The substance tested did not induce any structural abnormality in the chromosomes in the case of 24-hour and 48-hour treatments by the direct method, but in the metabolic activation method, the group with S9 Mix addition showed dose-dependent structural abnormalities of the chromosomes in a dose range of 150-200 μg/mL. On the other hand, chromosomal aberration was apparently induced in the positive control group with MMC or CPA.

From the results obtained, it was concluded that X-12-990S caused chromosomal aberration under the testing conditions employed in the current test.

Final Report

Testing Code No.: K06-^265

1. Title

Test to examine the effect of X-12-990S to cause chromosomal aberration by using cultured mammalian cells

2. Test Requester (Name): Shiatsu Chemical Industry K.K.
(Address): 2-6-1 Ohtemachi Chiyoda-Ku, Tokyo PC100
(Telephone): 03-3246-5185

3. Test Facility (Name): Chemical Product Testing Association, Chemical Product Safety Center, Hita Laboratories
(Address): 3-822 Ishii-cho, Hita City, Ohita Prefecture, 877
(Telephone): 0973-24-7211

4. Objective

The presence of any ability of the substance tested to cause chromosomal aberration by using pulmonary fibroblast cells (CHL cells) of Chinese hamster.

5. Testing Method

The testing method defined in "Partial amendment of 'Testing Methods for Novel Chemical Substances'" (Kanpogyo No. 700, Yakuhatsu No. 1039, 61 Kikyoku No. 101' of December 5, 1986) was used.

6. Good Testing Laboratory Standards

In conformation with "Testing facilities specified in the article 4 of the orders for testing novel chemical substances and toxicities of designated chemical substances" and "OECD Principles of Good Laboratory Practice (May 12, 1981)" according to the Environmental Safety Laws No. 39, "Yakuhatsu" No. 229, and "Kikyoku" No. 85 as of March 31, 1984, and Environmental Research Laws No. 233, "Eisei" No. 18 and 63, and "Kikyoku" No. 823 as of November 18, 1988.

7. Test Schedule

7.1 Starting Date October 1, 1991

7.2 Testing Period

Start of preliminary test October 7, 1991

Completion of observation December 24, 1991

7.3 Completion of testing January 30, 1992

7.4 Final Report preparation day January 30, 1992

8. Document Storage Site and Period

For the period of 10 years after the final report is submitted, the following documents and specimens will be stored at the document storage room of the Hita Laboratories of the Chemical Product Testing Association and the testing substance will be stored at the testing substance storage room. The handling after this storage period will be approved by the test requester. The storage period of some specimens, the qualities of which may deteriorate significantly during storage, is the period when they are suitable for evaluation, and in the case of disposal, the consent of the test requester will be obtained.

Test plan and any amendment or addition

Records of discussion and communication with the test requester

List of personnel involved in testing

Records related to testing substance

Records for receiving, usage, preparation, etc.

Test records, data, and chromosome specimens
Records of inspection by accuracy certification department
Copy of the final report

9. Final Report Preparer and Testing Personnel

Final report preparer

Testing manager January 30, 1992
[signature and seal]
(Manager, First Dept. for cells) M. Kajihara
Testing and Research
Dept., Hita Laboratories

Person in charge of testing January 30, 1992
[signature and seal]
S. Ogura

Management manager

Testing substance management manager S. Yoshida

10. Testing Materials and Methods

10.1 Testing materials

10.1.1 Substance to be tested (information supplied by the test requester)

(1) Name: 1,1- and 1,2-bis(trimethoxysilyl)ethane mixture:
Abbreviation: X-12-9908
(2) Lot No.: 105027
(3) Supply source: Shin-etsu Chemical Industry K.K.

(4) Structural formula

$$(CH_3O)_3\text{-}Si\text{-}CH_2CH_2\text{-}Si\text{-}(OCH_3)_3$$

$$(CH_3O)_3\text{-}Si\text{-}\underset{\underset{CH_3}{|}}{CH}\text{-}Si\text{-}(OCH_3)_3$$

(Molecular formula: $C_8H_{22}O_6Si_2$)

(5) Purity: 99% or higher

(6) Physicochemical properties

Molecular weight: 270.43

State at room temperature: colorless transparent liquid

Stability: hydrolytic

Boiling point: 103-104°C/5 mm Hg

Solubility: oil soluble

Dissolution: water --- insoluble
DMSO -- soluble
Acetone --- soluble

(7) Storage condition

Stored inside a desiccator in a cool and dark testing substance storage room.

10.1.2 Positive control substances

(1) Direct method

Name: mitomycin C (Mitomycin-Kyowa-S, MMC)

Manufacturer: Kyowa Fermentation Industry K.K.

Lot No.: 723AAC

Composition: 1 vial contains 2 mg (potency) of Nikkyoku mitomycin C and 48 mg of Nikkyoku sodium chloride

Appearance: blue-purple crystals or crystalline powder

Solubility: slightly soluble in water, methanol, or acetone, extremely insoluble in ethyl acetate or chloroform, and hardly soluble in ether or carbon tetrachloride.

Stability: stable in its crystalline form at room temperature. In the state of an aqueous solution, it is stable at pH 8.0 but if it is pH 7.0 or lower, the lower the pH value, the lower the stability.

(2) Metabolic activation method

Name: cyclophosphamide (CPA)

Manufacturer: Aldrich Chemical Co., Inc.

Lot No. 07413TX

Composition: single substance

Appearance: white crystalline powder

Purity: 98% or higher

(3) Storage conditions

Mitomycin C was stored at room temperature and cyclophosphamide was refrigerated.

10.1.3 Handling cautions

Gloves, mask, cap, and white laboratory coat were worn.

10.1.4 Cell used and medium

(1) Cell and reason for selection

Chinese hamster pulmonary fibroblast cells (CHL cells (clone No. 11), distributed from National Health Testing Center on September 28, 1988, 18th generation at the time of distribution) were used since they have been frequently used for testing chromosomal aberration, their sensitivity is high, and there is an abundance of research data available. The fundamental number chromosomes is 25 in the CHL cell, and the doubling time is about 15 hours. The frozen CHL cells were thawed and cultured. On the third day of cultivation, they were switched to the next generation, and the cells in the logarithmic proliferation period were used for testing. The number of generations was 24 in the case of 24- and 48-hour treatments in the direct method, and it was 34 in the metabolic activation method.

(2) Cultivation medium and water

Eagle's MEM cultivation medium (Nissui Pharmaceutical K.K., Lot No. 294105, 2844104) with 10% (v/v) newborn calf serum (Mitsubishi Chemical K.K, Lot No. NBJ02) added was used. It is abbreviated to 10% NCS/MEM, below.

The pure water used to prepare the cultivation medium was prepared by purifying distilled water obtained from a distilled water machine (WG-32, Yamato Scientific K.K.) by allowing it to pass through a pure-water-making machine (NANO Pure, Barnstead).

10.2 Testing methods

10.2.1 Cultivation method

On a Petri dish 6 cm in diameter (Corning Co., Ltd.), 5 mL of 10% NCS/MEM containing 1.5 X 10^4 (or 0.5 x 10^4) CHL cells/mL were spread and cultured for 2 (or 3) days. The test was carried out on a monolayer of cells in the logarithmic proliferation phase on the 2nd (or 3rd) day of cultivation.

Incidentally, the cultivation was carried out under the following conditions by using a carbon dioxide incubator.

Temperature: 37±0.5°C

Humidity: close to saturation

Gas phase: air containing 5% carbon dioxide gas

10.2.2 Testing solution

The concentrations of the substance to be tested and positive control substance were shown based on the weights of the stock samples without any purity conversion.

i) Solution to be tested

(1) Preparation method

The substance to be tested was dissolved in dimethylsulfoxide [DMSO (Donin Chemical Research Laboratory K.K., Lot No., RM142, AH176] to obtain a stock solution. Solutions of desired concentrations were prepared by diluting the stock solution with the same solvent.

(2) Preparation period

Solutions were prepared at the time of usage and used within 2 hours after preparation.

2) Amount used

The solution to be tested was added so that the concentration in the cultivation medium was 1%.

3) Positive control substance solutions

(1) Preparation method

The positive control substances were dissolved in pure water and diluted with the same solvent to solutions of desired concentrations.

(2) Preparation period

The solutions were prepared at the time of usage and used within 2 hours from the preparation.

10.2.3 S9 Mix preparation method

1) S9 (Kikkoman K.K.)

Animal used: SD rat, 7 weeks old, male

Administration method: administration into the abdominal cavity

Induction method: phenobarbital: 30 mg/kg x once, 60 mg/kg x 3 times; 5,6-benzoflavone: 80 mg/kg x once

Storage: -80°C (REVCO ultra-low temperature tank ULT-1285)

Lot No.: RAA-260

Manufacturing date: September 13, 1991

2) S9 Mix

S9	0.3 mL
20 mM HEPES (pH 7.2)	0.2 mL
50 mM $MgCl_2$	0.1 mL
330 mM KCl	0.1 mL
50 mM G-6-P	0.1 mL
40 mM NADP	0.1 mL
Pure water	0.1 mL

Incidentally, the composition of S9 Mix/MEM was as follows.

S9 Mix	0.5 mL
10% NCS/MEM	2.5 mL

10.2.4 Specimen preparation method

The cells were peeled off the Petri dish by using trypsin, the biomass was collected by centrifugation, subsequently, 0.075 M KCl was added, and a hypotonic treatment was carried out at 37°C for 15 minutes. After the hypotonic treatment, a mixture of methanol and acetic acid (3:1) was added to prepare a dispersed cell suspension, the naked eye observation of which revealed slight opaqueness. A small amount of methanol and acetic acid (3:1) was dropped on a glass slide and dried. Subsequently, it was stained with 2% Giemsa. Two specimens were prepared for each Petri dish.

10.3 Testing procedures

10.3.1 Test to set doses to be used for the substance to be tested

To determine doses to be used for the substance to be tested, the following preliminary test was carried out. First of all, a cell proliferation inhibition test was carried out to determine an approximate concentration for 50% inhibition of cell proliferation. Subsequently, a cell division inhibition test was carried out to determine the maximum dose at which the actual observation of chromosomes was possible. If no cytotoxicity was observed, the maximum dose was set at 5 mg/mL or 10 mM equivalent concentration.

1) Cell proliferation inhibition test

The cultivation medium was removed from Petri dishes on the 2nd or 3rd day of cultivation, and 5 mL each of the cultivation medium containing the substance to be tested were added to carry out cell cultivation for 24 and 48 hours in the direct method. In the metabolic activation method, the cultivation medium was removed, and subsequently, 3 mL of S9 Mix/MEM containing the substance to be tested were added to treat for 6 hours. After the treatment, S9 Mix/MEM was removed, and after the Petri dishes were washed 3 times with 2 mL of a physiological phosphate buffer [PBS(-)] containing no Ca^{2+} or Mg^{2+}, 5 mL of 10% NCS/MEM were added to carry out cultivation for 18 hours. After the cultivation, the medium was removed, the culture was peeled off from the dishes by using 2 mL of trypsin, and the number of cells was counted by using a Microcell Counter CC-130 (Toa Medical Electronics). Two dishes were used for each group, and the number of cells was counted separately.

2) Cell division inhibition test

For both direct method and metabolic activation method, the cultivation and treatment with the substance to be tested were carried out by the same procedures as those used for the above cell proliferation inhibition test. At 2 hours before completing the cultivation, 0.1 μg/mL of Colcemid was added to the cultivation medium, and chromosomal specimens were prepared to examine the presence of dividing cells.

10.3.2 Chromosomal aberration test

1) Doses

Substance to be tested

The maximum usable dose determined by the dose setting test was used as an upper limit, which was reduced by a ratio of 2 in the direct method and a difference of 50 in the metabolic activation method to set 3 levels of dose to carry out the test.

Direct method 24-hour treatment 100, 200, 400 μg/mL

Direct method 48-hour treatment 75, 150, 300 μg/mL

Metabolic activation method 100, 150, 200 μg/mL

Positive control substances

The following dose was set by utilizing the background data of the laboratory.

Direct method MMC 0.05 μg/mL

Metabolic activation method CPA 10 μg/mL

2) Chromosomal aberration test by the direct method

The medium was removed from the Petri dishes on the 3rd day of cultivation, 5 mL of the medium containing the substance to be tested were added, and the cells were cultured for 24 and 48 hours. At 2 hours before completing the cultivation, 0.1 μg/mL of Colcemid was added to the cultivation medium, and chromosomal specimens were prepared.

As a negative control, a group with solvent alone was used, and as a positive control, a group with MMC added was used.

3) Chromosomal aberration test by the metabolic activation method

The medium was removed from the Petri dishes on the 3rd day of cultivation, 3 mL of S9 Mix/MEM containing the substance to be tested were added to treat for 6 hours. After the treatment, S9 Mix/MEM was removed, and after the Petri dishes were washed

3 times with 2 mL of [PBS(-)], 5 mL of 10% NCS/MEM were added to carry out cultivation for 18 hours. After the cultivation, 0.1 μg/mL of Colcemid was added to the medium, and chromosomal specimens were prepared.

As a negative control, a group with solvent alone added was used, and as a positive control, a group with CPA added was used. Furthermore, to make the effect of metabolic activation apparent, a control group with the same dose of the substance to be tested and treatment time as those for the group with S9 Mix added but without S9 Mix added, was used.

4) Number of Petri dish and identification

Two Petri dishes were used for each group, and to avoid any partiality, dishes were randomly selected.

Each dish had the test code number, treatment method, and dose clearly written on it.

10.1.3 Observation

One hundred cells in metaphase per dish were observed to examine structural abnormalities (gaps, scission, exchange type abnormality, etc.), and quantitative abnormalities (polyploidy, intranuclear multiplication) in chromosomes and chromatids. If a cell had any of those structural and numerical abnormalities, it was considered to be an abnormal cell, and the rate of appearance of abnormal cells was determined by observing 200 cells per group. In the case of abnormality appearance rate for the structural abnormalities, the results with and without the gaps abnormality were calculated.

The gaps judgment standard was that the nonchromosomal portion was larger than the width of a chromatid, the shape was clear, and it was on the long axis of the chromatid.

Incidentally, all specimens were coded and microscopically observed without the observer knowing which treated cells he was observing.

10.3.4 Examination of the results

The results obtained were examined by grading with respect to the appearance rate (average of two dishes for each group) of cells having structural abnormalities including gaps and quantitative abnormalities as negative (-) for less than 5%, quasi-positive (±) for 5% to less than 10%, positive (+) for 10% to less than 20%, positive (++) for 20% to less than 50%, and positive (+++) for 50% and higher, and no statistical method was used. If the rate of appearance showed dose-dependency or reproducibility in two groups of positive (+) or higher, it was judged that the effect inducing chromosomal aberration was positive. Incidentally, if the result was quasi-positive (±) or positive in only a single dose, the test was repeated.

11. Results

No factor considered to adversely affect the reliability of data obtained was observed throughout the testing period. Furthermore, no change from the plan was made.

The current test was concluded to be carried out normally since no significant fluctuation in the results of two dishes of each group was found, the rate of appearance of cells having abnormalities in the negative control group was less than 5%, in the positive control group, the rate of appearance of cells having abnormalities other than gaps was higher than 10%, and no

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abnormality was observed in the cultivation conditions and testing environment.

11.1 Cell proliferation inhibition test (Table I and Figure 1) and cell division inhibition test

Table I shows the doses set in the cell proliferation inhibition test. The rate of growth for the control group with the solvent alone added was set at 100%. The 50% growth inhibition concentration of the substance to be tested was about 1,400 μg/mL in the case of 24-hour treatment in the direct method, it was about 350 μg/mL in the case of 48-hour treatment in the direct method, and in the metabolic activation method, it was about 180 μg/mL. Subsequently, for the purpose of finding out what dose could be used for the actual chromosomal aberration testing, the cell division inhibition test was carried out with concentrations in about the same range as that in the 50% proliferation inhibition test. As a result, the number of dividing cells was found to be sufficient for carrying out a chromosomal aberration test if the dose was less than 400 μg/mL in the case of 24-hour treatment in the direct method, 100 μg/mL in the case of 48-hour treatment in the direct method, or 200 μL/mL in the metabolic activation method. Therefore, the maximum dose was set at
400 μg/mL in the case of 24-hour treatment in the direct method, 100 μg/mL in the case of 48-hour treatment in the direct method, and 200 μg/mL in the metabolic activation method. The maximum doses were reduced by a ratio of 2 in the direct method and a difference of 50 in the metabolic activation method to obtain 3 doses for each group.

Incidentally, in the metabolic activation method, chromosomal aberration induction was observed, and thus, the dilution was by a difference of 50 considering the dose dependency of the substance.

11.2 Chromosomal aberration test

11.2.1 Direct method (Table II and Figure 2)

1) 24-hour treatment

The results on the rate of appearance of cells having structural abnormalities including gaps were negative with the rates of 1.5, 3.0, and 4.0% for the doses of the substance to be tested of 100, 200, and 400 μg/mL, respectively. On the other hand, in the control group with the solvent alone added, the rate of appearance of cells having structural abnormalities was 1.5% which was normal. In the positive control group with an MMC treatment, the rate was 49.0% showing apparent induction of chromosomal aberration.

Incidentally, the rate of appearance of polyploidy was negative in all groups.

2) 48-hour treatment

The results on the rate of appearance of cells having structural abnormalities including gaps were negative with the rates of 1.0, 2.0, and 2.0% for the doses of the substance to be tested of 75, 150, and 300 μg/mL, respectively. On the other hand, in the control group with the solvent alone added, the rate of appearance of cells having structural abnormalities was 1.0% which was normal. In the positive control group with an MMC treatment, the rate was 88.5% showing apparent induction of chromosomal aberration.

Incidentally, the rate of appearance of polyploidy was negative in all groups.

11.2.2 Metabolic activation method (Table III and Figure 3)

1) S9 Mix addition

The result on the rate of appearance of cells having structural abnormalities including gaps in the group with a dose of the substance to be tested of 100 μg/mL was 2.0%, which was negative, but if the doses were 150 and 200 μg/mL, the results were 19.5 and 44.5%, respectively, showing dose-dependent positive results. On the other hand, in the control group with the solvent alone added, the rate of appearance of cells having structural abnormalities was 0% which was normal. In the positive reference group with a CPA treatment, the rate was 26.5 % showing apparent induction of chromosomal aberration.

Incidentally, the rate of appearance of polyploidy was negative in all groups.

2) No addition of S9 Mix

As a control of the metabolic activation method, the cultivation medium containing no S9 Mix was used with the same dose of the substance to be tested and treatment time. In the groups with 100, 150, and 200 μg/mL of the substance to be tested, the results were 0.5, 1.0, and 1.0%, which were negative. On the other hand, in the control group with the solvent alone added and the positive control group, the results on the rate of appearance of cells having abnormalities were 1.0 and 0.5%, respectively, which were normal.

Incidentally, the rate of appearance of polyploidy was negative in all groups.

12. Conclusion

In the test to examine the effect of X-12-990S to cause chromosomal aberration carried out by using CHL cells, the results of the direct method with 24- and 48-hour treatments showed no induction of structural abnormalities, but in the groups with S9 Mix added in the metabolic activation method, dose-dependent induction of structural abnormalities in chromosomes was observed in the range of 150-200 μg/mL.

From those results obtained, it was concluded that X-12-990S can induce chromosomal aberration.

13. References

M. Ishidate, ed. (1987): "Chromosomal aberration data," LIC K.K.
M. Ishidate (1979): "Chromosomal aberration detection by using cultured cells," Soshiki Baiyo, 5 (4), 115-122.
Japanese Environmental Mutagen Research Association, Mammal Testing Group, ed. (1988): "Atlas of chromosomal aberration by chemical substances," Asakura Shoten.
M. Yoshida (1982): "Structure and abnormalities of chromosomes," Taisha, 19 (5), 427-438.
M. Yoshida (1983): "Chromosomal analysis in cultured cells," Saibo Kogaku, 2 (11), 1352-1360.
M. Sotomura ed. (1978): "Chromosomal aberration," Asakura Shoten.

Table I Results of test for cell proliferation inhibition by X-12-990S

① 直接法	24時間 ② 処理	被験物質の濃度 (μg/ml) ④	0	200	300	400	500	600	700	800	1,000	1,500**
		⑤ 増殖率 (%)*	100	90.9	82.4	81.3	62.7	57.3	55.6	51.7	58.7	65.3
	48時間 ③ 処理	被験物質の濃度 (μg/ml) ④	0	100	200	300	400	500	600	700	800	1,000
		⑤ 増殖率 (%)*	100	90.2	82.4	67.3	16.7	16.4	15.9	15.3	15.0	14.2
代謝活性化法 ⑥		被験物質の濃度 (μg/ml) ④	0	100	150	200	250	300	350	400	450	500
		⑤ 増殖率 (%)*	100	83.9	66.1	37.0	30.0	28.7	27.0	26.5	25.9	25.4

* Average of two Petri dishes
**Precipitates considered to be the substance to be tested were observed.

Key:
1 Direct method
2 24-hour treatment
3 48-hour treatment
4 Concentration of the substance to be tested
5 Rate of growth
6 Metabolic activation method

0 0 3 1

Table II Results of chromosomal aberration test (method other than the metabolic activation method)

①試験物質：X-12-6605

②時間	⑥処理時間 (h)	⑦処理濃度 (μg/ml)	⑧回数 培養時間	⑨培養容器	判定	⑩染色体構造異常細胞の出現数と出現頻度(%)* キャップ(a)	染色分体型(b)	染色体型(c)	その他(d)	合計(e) 一本	二本	判定
③ 24	6		16G	G		0	0	0	0	1	1	
			16G	G	⑤	0	0	0	0	2	2	-
			26G	G (0.0%)		0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (1.5%)	3 (1.5%)	
48	6		16G	G		0	0	0	0	0	0	
			16G	G	-	0	0	0	0	2	2	-
			26G	G (0.0%)		G (0.0%)	G (0.0%)	G (0.0%)	G (0.0%)	2 (1.5%)	2 (1.5%)	
24		100	16G	G		0	0	0	0	2	2	
			16G	G	-	0	0	0	0	1	1	-
			26G	G (0.0%)		G (0.0%)	G (0.0%)	G (0.0%)	G (0.0%)	3 (1.5%)	3 (1.5%)	
		200	16G	G		0	0	0	0	2	3	
			16G	G	-	0	0	0	0	3	3	-
			26G	G (0.0%)		G (0.0%)	G (0.0%)	G (0.0%)	G (0.0%)	5 (1.5%)	6 (1.8%)	
④ 48		400	16G	G		0	0	0	0	2	3	
			16G	G	-	0	0	0	0	3	5	-
			26G	G (0.0%)		0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (1.5%)	8 (1.8%)	
		75	16G	G		0	0	0	0	0	0	
			16G	G	-	0	0	0	0	1	2	-
			26G	G (0.0%)		G (0.0%)	G (0.0%)	G (0.0%)	G (0.0%)	1 (0.5%)	3 (1.0%)	
		150	16G	G		0	0	0	0	0	0	
			16G	G	-	0	0	0	0	3	4	-
			26G	G (0.0%)		G (0.0%)	G (0.0%)	G (0.0%)	G (0.0%)	3 (1.5%)	4 (1.8%)	
		300	16G	G		0	0	0	0	2	2	
			16G	G	-	0	0	0	0	2	2	-
			26G	G (0.0%)		0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (1.5%)	4 (1.8%)	
⑤ 24		0.05	16G	G		0	0.5	0	0	46	47	
			16G	G	-	1	0.5	0.5	0	51	51	**
			26G	G (0.0%)		1 (0.5%)	46 (23.0%)	46 (23.0%)	0 (0.0%)	97 (48.5%)	99 (49.5%)	
48		0.05	16G	0		0.5	46	0.5	0	85	89	
			16G	G	-	0	0.5	0.5	0	86	86	***
			26G	G (0.0%)		0 (0.0%)	46 (23.0%)	46 (23.0%)	0 (0.0%)	156 (48.5%)	177 (49.5%)	

⑪陽性は特に多くの培養系列(プレート)の頻度増加、陰性傾向及び出現頻度異常をもつ細胞の出現数を上段の括弧内に記載し、その合計を括弧内に表示し、それから求めた頻度をもつ細胞の出現頻度を(%)に示す。キャップ(a)には染色分体型と染色体型の再掲を含める。各欄(-)にはキャップのみをもつ細胞を除いた数値を(-)内に示す。

⑰ 個の細胞に異常の見方をもつ場合でも、1個の異常細胞として評価する。例えば、1個の細胞内に切断が2、交換がある場合には、異常細胞としては1、切断をもつ細胞としても1、交換をもつ細胞としても1として評価する。

(a)* 染色分体型切断　(b)* 染色分体型交換　(c)** 染色体型切断　(d)** 染色体型交換　その他 特殊性者を細胞化(細胞化)

Key:
1 Substance to be tested
2 Treatment
3 Solvent
4 Substance to be tested
5 Positive control
6 Treatment time
7 Treatment concentration
8 Number of cells observed
9 Number of polyploids
10 Judgment
11 Number of appearance and frequency of appearance (%)* for cells having chromosomal structural abnormalities
12 Gaps
13 Chromatid type
14 Chromosome type
15 Others
16 Total
17 For each treatment concentration, the number of cells observed for each cultivation series (plate), number of polyploids, number of appearance of cells having structural abnormalities on lines 1 and 2 with the total on the line 3, and the frequency of appearance of abnormal cells (%) determined from these results is shown inside ( ). The gaps (g) include the results for both chromatid and chromosomal types. The total (=g) shows the total number of cells with abnormalities excluding those having gaps alone and frequency of appearance (%) inside ( ).
Even in the case of several abnormalities in one cell, the computation is carried out as a single abnormal cell. For example, if a single cell has two scissions and 2 exchanges, it is counted 1 as an abnormal cell, 1 as a cell having scissions, and 1 as a cell having exchanges.
ctb: chromatic type scission, cte: cromatid type exchange, csb: chromosome type scission, cse: chromosome type exchange, others: fragmentation (excluding powdering)

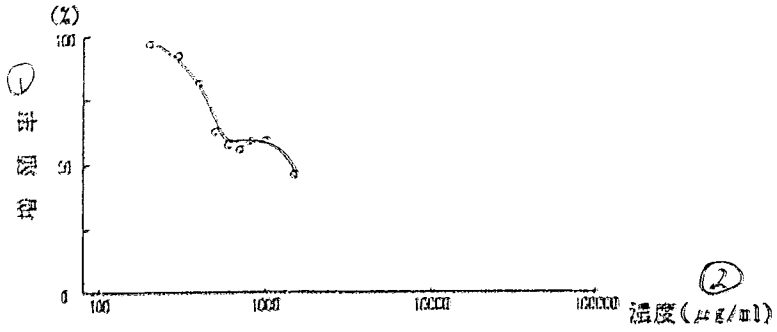
Table III Results of chromosomal aberration test (metabolic activation method)

(10) 試験 X-12-990S

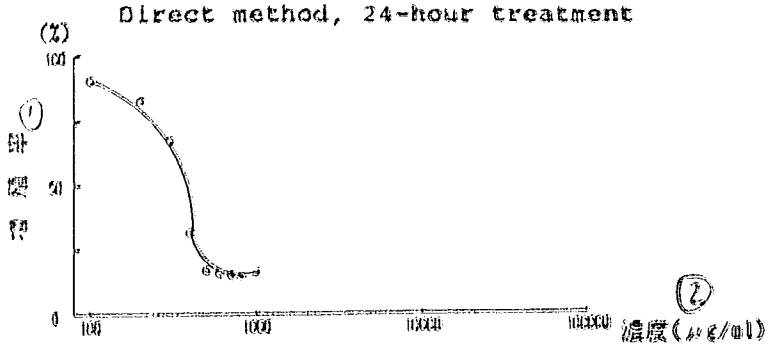
(1) 試験物質	(5) 代謝活性化	(6) 処理濃度 (μg/ml)	(7) 回数	(8) 標本作成		(11) 染色体構造異常細胞の出現数と出現頻度(%)								
						その他	染色分体型		染色体型		その他	合計		倍数性
			回目	固定	染色		CSB	CSE	CSB	CSE		-1	-2	
溶媒	-	0	1	2	(9)	0	0	0	0	0	0	2	2	-
			2	2		0	0	0	0	0	0	0	0	
			3	2		0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.0%)	2 (1.0%)	
(2)	+	0	1	2	-	0	0	0	0	0	0	0	0	-
			2	2		0	0	0	0	0	0	0	0	
			3	2		0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
溶媒	-	100	1	2	-	0	0	0	0	0	0	0	1	-
			2	2		0	0	0	0	0	0	0	0	
			3	2		0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (2.1%)	0 (0.0%)	0 (0.0%)	1 (0.5%)	
		150	1	2	-	0	0	0	0	0	0	1	1	-
			2	2		0	0	0	0	1	0	1	1	
			3	2		0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.5%)	0 (0.0%)	1 (0.5%)	1 (0.5%)	
		200	1	2	-	0	0	0	0	0	0	3	4	-
			2	2		0	0	0	0	0	0	2	2	
			3	2		0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.0%)	6 (3.0%)	
(3)	+	0	1	2	-	0	0	0	0	0	0	3	3	-
			2	2		0	0	0	0	0	0	1	1	
			3	2		0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (2.0%)	4 (2.0%)	
		150	1	2	-	2	0	0	0	0	0	18	19	-
			2	2		2	0	0	0	0	0	18	20	
			3	2		4 (2.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	36 (18.0%)	39 (19.5%)	
		200	1	2	-	1	0	0	0	0	0	43	43	-
			2	2		3	0	0	0	0	0	43	46	
			3	2		4 (2.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	86 (43.0%)	89 (44.5%)	
溶媒	-	0	1	2	-	0	0	0	0	0	0	1	1	-
			2	2		0	0	0	0	0	0	0	0	
			3	2		0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.5%)	1 (0.5%)	
(4)	+	0	1	2	-	1	4	1	4	0	0	29	30	-
			2	2		1	2	1	2	0	0	29	33	
			3	2		2 (1.0%)	6 (3.0%)	2 (1.0%)	6 (3.0%)	0 (0.0%)	0 (0.0%)	58 (29.0%)	63 (31.5%)	

(12) 用量(5%)、[illegible]計 (6%)、[illegible]の[illegible]回[illegible]

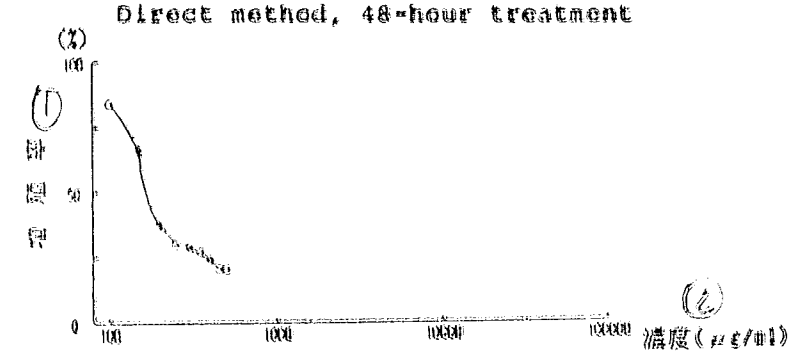
Key:
1 Treatment
2 Solvent
3 Substance to be tested
4 Positive controls
5 Presence of S9 Mix
6 Treatment concentration
7 Number of cells observed
8 Number of polyploids
9 Judgment
10 Substance to be tested
11 Number of appearance and frequency of appearance (%)* for cells having chromosomal structural abnormalities.
12 Gaps
13 Chromatid type
14 Chromosome type
15 Others
16 Total
17 Dose of sq(5%), treatment time with the substance to be tested (6 hour), cell recovery time after treating with the substance to be tested (18 hour).



Direct method, 24-hour treatment



Direct method, 48-hour treatment



Metabolic activation method

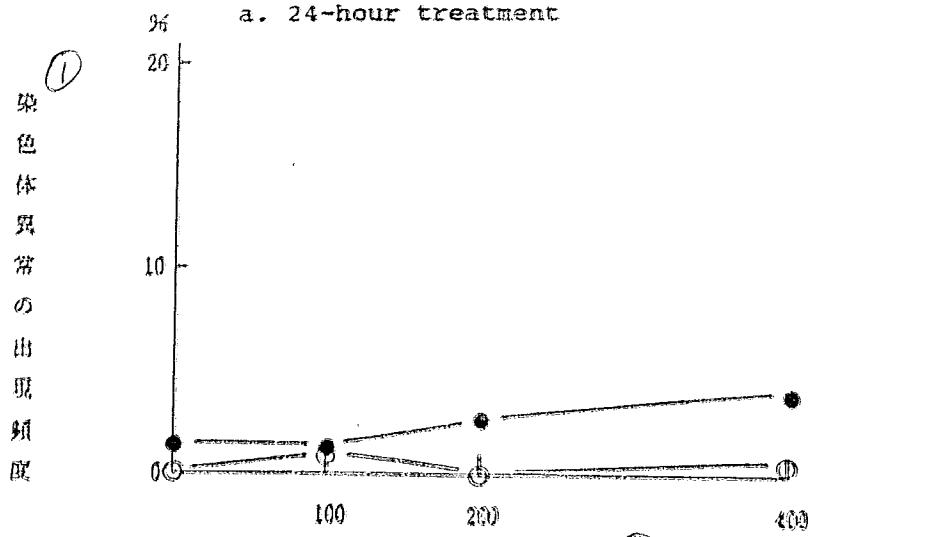
Figure 1 Results of test for cell proliferation inhibition by X-12-9908

0 0 3 6

Key: 1 Rate of growth
2 Concentration

A. method other than the metabolic activation method

a. 24-hour treatment



b. 48-hour treatment

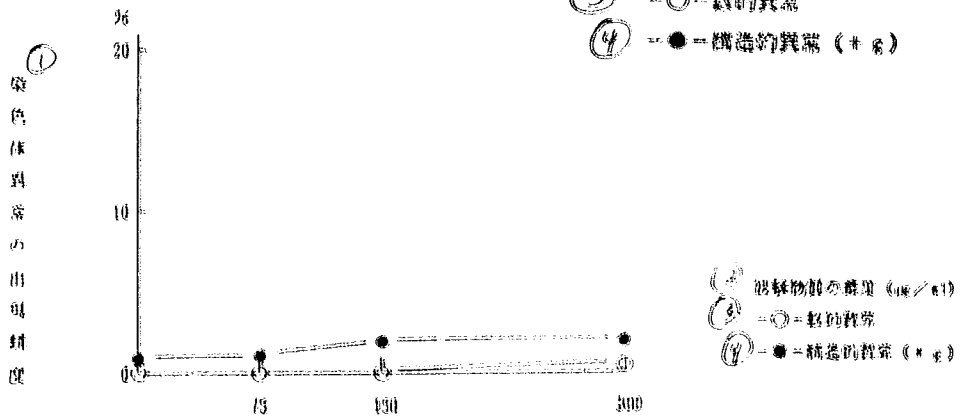


Figure 2 Results of test for chromosomal aberration caused by X-12-990S

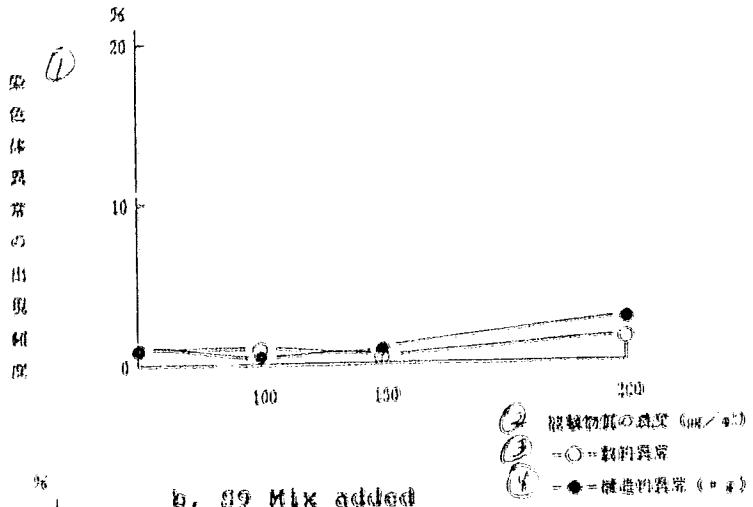
0038

Key: 1 Frequency of appearance of chromosomal aberration
2 Concentration of substance to be tested
3 Quantitative abnormalities
4 Structural abnormalities
48-hour treatment

0 0 3 4

B. Metabolic activation method

a. No S9 Mix added



b. S9 Mix added

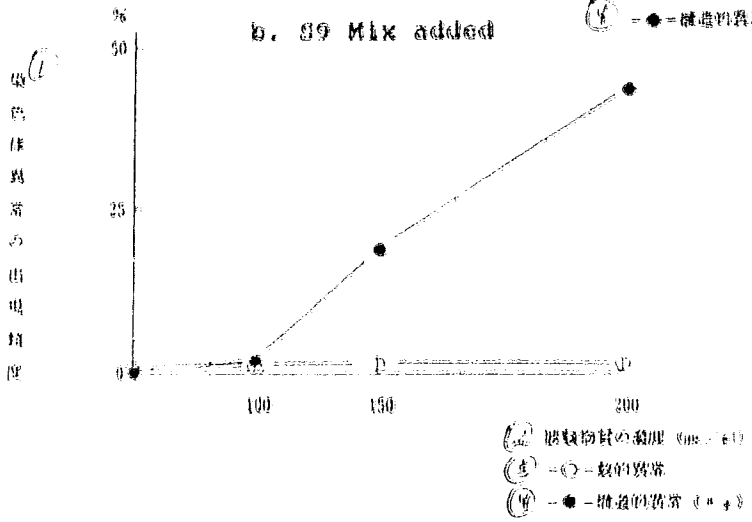


Figure 1 Results of test for chromosomal aberration caused by X-12-9905

Key:
1 Frequency of appearance of chromosomal aberration
2 Concentration of the substance to be tested
3 Quantitative abnormalities
4 Structural abnormalities

Control (metabolic activation method, control group with solvent alone added)

Structural abnormalities (metabolic activation method, 200 μg/mL), a. Chromatid scission, b. Chromatid exchange

Figure 4 Chromosomal aberration induced by X-12-9905

0 0 4 8

T-2932 (Chem.)

Report on the results of chromosomal aberration test to use a cultured mammalian cell

1. General items

新規化学物質の名称 (IUPAC命名法による) (1) (2)	1,1-及び1,2-ビス（トリメトキシシリル）エタンの混合物			
別名 (3)	X－12－990S	(11) 物理化学的性状	分子量 (5)	270.43
構造式又は示性式（いずれも不明な場合は、その製法の概要）(4) $(CH_3O)_3-Si-CH_2CH_2-Si-(OCH_3)_3$ $(CH_3O)_3-Si-CH(CH_3)-Si-(OCH_3)_3$			常温における性状 (6)	無色透明液体 (7)
			安定性 (8)	加水分解性有り (9)
			融点 (10)	——
			沸点 (12)	103～104℃ ／5mmHg
			蒸気圧 (13)	——
			分配係数 (14)	——
			溶解性 (15)	難溶性 (16)
試験に供した新規化学物質の純度 (18)	99%以上 (20)	(17) 溶解度	(18) 水	(19) 不溶
			DMSO	(20) 可溶
不純物の名称及び濃度 (25)	——		アセトン (21)	(21) 可溶
ロット番号 (26)	105027		その他 (22) (——)	——
〔備考〕(27)				

Key:

1 Novel chemical substance name (IUPAC nomenclature)
2 Mixture of 1,1- and 1,2-bis(trimethoxysilyl)ethane
3 Other name
4 Structural formula or rational formula (if not known, outline of process for the production)
5 Molecular weight
6 Property at room temperature
7 Colorless transparent liquid
8 Stability
9 Hydrolytic
10 Melting point
11 Physicochemical properties
12 Boiling point
13 Vapor pressure
14 Partition coefficient
15 Dissolution
16 Oil soluble
17 Solubility
18 Water
19 Insoluble
20 Acetone
21 Soluble
22 Others
23 Purity of the novel chemical substance to be tested
24 99% or higher
25 Impurity name and concentration
26 Lot No.
27 Notes

0 0 4 4

2. Mammalian cell and cultivation conditions

細胞名(1)	CHL	入手先(2)		国立衛生試験所(3)
種(4)	チャイニーズハムスター(5)	入手年月日(6)		昭和(7)63年 9月 28日
培養液(8)	(9)イーグルMEM (MEM)	製造元(10)		日水製薬株式会社(11)
血清(12)	10%新生仔牛血清 (NCS)(13)	製造元(Lot No.)(14)		三菱化成株式会社(15)(NBJ02)
細胞倍加時間(16)	(17)約15 h	凍結条件(18)		-196℃
継代数(19)	入手時 18代 (20)	培養条件(21)	容器(22)	直径100mmのプラスチックシャーレ(23)
			温度(24)	37±0.5℃
染色体数(27)(モード)	25 (28) 本		CO_2	5%
			湿度(25)	(26) 飽和湿度
備考(29)：*本試験時の継代数：直接法24時間処理24代、代謝活性化法34代 直接法48時間処理24代				

Key
1 Cell name
2 Source
3 National Health Testing Center
4 Species
5 Chinese hamster
6 Date obtained
7 September 28, 1988
8 Cultivation medium
9 Eagle MEM
10 Manufacturer
11 Nissui Pharmaceutical K.K.
12 Serum
13 10% newborn calf serum (NCS)
14 Manufacturer (Lot No.)
15 Mitsubishi Chemical K.K. (NBJ02)
16 Cell doubling time
17 About 15 hours
18 Freezing conditions
19 No. of generations

20 18th generation when obtained
21 Cultivation conditions
22 Container
23 Plastic Petri dish of 100 mm diameter
24 Temperature
25 Humidity
26 Almost saturation
27 Number of chromosone (mode)
28 25
29 Notes: *No. of generations at the time of testing: direct method 24-hour treatment 24th generation, direct method 48-hour treatment 24th generation, metabolic activation method 34th generation

3. S9 Mix

(1) S9 source, etc.

1. 自(1)製	製造年(3)月日	(5) ―― 年 ―― 月 ―― 日 製造
②. 購入 (2)	製造(4)元	(6) キッコーマン株式会社
	製造年(7)月日	(8) 平成 3年 9月 13日 製造
	購入年(9)月日	(10) 平成 3年 9月 27日 購入
	Lot No.	RAA-260

Key: 1 Homemade
2 Purchased
3 Date of manufacturing
4 Manufacturer
5 Manufactured on month - day - year
6 Kikkoman K.K.
7 Date of manufacturing
8 September 13, 1991
9 Date of purchasing
10 September 27, 1991

(2) S9 storage temperature, etc.

保存温	−80℃	保存機器名及び型式名	REVCO 超低温槽 ULT-1285
①		②	③

Key: 1 Storage temperature
2 Storage machine and model name
3 REVCO ultra-low-temperature tank, ULT-1285

(3) Method for S9 preparation

① 使用動物		② 誘導物質	
種・系統③	ラット・SD系④	名⑤称	⑥ PB&BF*
性②	雄⑧	投与方法⑨	⑩腹腔内投与
週⑪齢	7 ⑫週	投与期間及び投与⑬	⑭ 備考に記載
体⑮重	183〜215 g	量(g/kg体重)	
備⑯考	Induction 24hrs ↑ PB30mg/kg ↑ PB60mg/kg ↑ PB60mg/kg & BF80mg/kg ↑ PB60mg/kg ↑ S9 preparation		

Key: 1 Animal used
2 Induction substance
3 Species and type
4 Rat, SD type
5 Name
6 PB & BF*
7 Sex
8 Male

9 Administration method
10 Administration into the abdominal cavity
11 Age
12 7 weeks old
13 Administration period and dose (g/kg body weight)
14 Described in the notes
15 Body weight
16 Notes

(4) S9 Mix composition

(1) 成　分	(2) S9Mix 1 ml中の量	(3) 成　分	S9Mix 1 ml中の量 (2)
S9	0.3 ml	NADP	4 μmol
$MgCl_2$	5 μmol	(4) 緩衝液（HEPES）	4 μmol
(6) KCl	33 μmol	(5) その他（純水）	0.1 ml
グルコース－6リン酸	5 μmol	——	——
(7) 備　考	(8) S9Mix／MEMの組成：S9 Mix 0.5ml 10%NCS／MEM 2.5ml		

Key: 1 Component
2 Amount in 1 mL of S9 Mix
3 Component
4 Buffer
5 Others (pure water)
6 Glucose-6-phosphate
7 Notes
8 S9 Mix/MEM composition

(5) S9 Mix treatment conditions

	1 ①プレート法	2 ②浮遊細胞法
S9量 3 (最終濃度)	5 %	— %
S9蛋白量 4 (最終濃度)	1.38* mg/ml	— mg/ml
処理時間 5	6 h	— h
被験物質処理後の培養時間 6	18 h	— h
備考 7	* S9 Assay data (キッコーマン株式会社) による。 8	

Key:
1 (1) Plate method
2 (2) Cell suspension method
3 Amount of S9 (final concentration)
4 Amount of S9 protein (final concentration)
5 Treatment time
6 Cultivation time after treating with the substance to be tested
7 Notes
8 *S9 Assay data provided by (Kikkoman K.K.)

4. Cell proliferation inhibition test

(1) Testing conditions and preparation of solutions of the substance to be tested

実験期間 (1)		自 平成 3年10月 7日 (2) 至 平成 3年12月13日		
		(3) 代謝活性化法によらない場合		代謝活性化法による場合 (6)
		(4) 24時間処理	(5) 48時間処理	
細胞 (7)	播種細胞数 (8)	(9) 1.5×10⁴個／ml	(9) 1.5×10⁴個／ml	(9) 1.5×10⁴個／ml
	培養開始後日数 (10)	(11) 2日目	(11) 2日目	(11) 2日目
(12) 培養器	形状 (13)	(11) 円形プラスチック製シャーレ		(12) 円形プラスチック製シャーレ
	大きさ (14)	(13) 直径60mm		(13) 直径60mm
	製造元 (15)	Corning (岩城硝子) (15)		Corning (岩城硝子) (15)
	1濃度当りの培養器数 (16) (プレート数)	2		2
(20)	培養液量	(21) 5ml／培養器		(22) 3ml／培養器*
(23) 被験物質溶液の調製	溶媒の種類 (24) (25)	DMSO		DMSO
	被験物質原液の濃度	200mg／ml		100mg／ml
	被験物質量 (26)	1,000mg		300mg
	溶媒 (27) 量	5mlに定容 (28)		3mlに定容 (28)
	被験物質溶液の性状 (○で囲む) (29)	(30) (溶解)、懸濁 (31) その他 ( )		(30) (溶解)、懸濁 (31) その他 ( )
	調製後の時間 (32)	(33) 2時間以内		(33) 2時間以内
	保存方法 (34)	(35) 使用まで室温保存		(36) 使用まで室温保存
	滅菌方法 (37)	(38) 滅菌せず		(39) 滅菌せず
(41) 被験物質処理	調製溶液添加量 (42)	(43) 50ml／培養器 (44)		30ml／培養器 (45)
	処理時間 (46)	24h	48h	6h
	S9 Mix添加量 (47)	――		0.5ml／培養器 (48)
細胞数計測方法 (49)		電気式細胞計数器 (Microcell Counter CC-130) で細胞数を計測 (49)		
備考 (44)	* 被験物質による処理時の培養液量を示す。処理後は5ml／培養器で培養した。			

Key:
1 Experimental period
2 From October 7, 1991 to December 13, 1991
3 Method other than the metabolic activation method
4 24-hour treatment
5 48-hour treatment
6 Metabolic activation method
7 Cells
8 Number of cells inoculated
9 1.5×10^4 cells/mL
10 No. of cultivation days
11 2 days
12 Cultivator
13 Shape
14 Size
15 Manufacturer
16 No. of cultivators per concentration setting (No. of plates)
17 Round plastic Petri dish
18 Diameter 60 mm
19 Corning (Iwaki glass)
20 Amount of cultivation medium
21 5 mL/cultivator
22 3 mL/cultivator*
23 Preparation of solutions for the substance to be tested
24 Solvent used
25 Concentration of the stock solution of the substance to be tested
26 Amount of the substance to be tested
27 Amount of solvent
28 Made up to 5 mL
29 Made up to 3 mL
30 Property of solutions of the substance to be tested (circle one)
31 Dissolution, suspension, other ( )
32 Time after preparation
33 Within 2 hours
34 Storage method
34' Stored at room temperature before using
35' Sterilization method
35 No sterilization
36 Solutions added
37 /Cultivator

0 0 5 0

38 Treatment time
39 Amount of S9 Mix added
40 /Cultivator
41 Treatment with the substance to be tested
42 Cell counting method
43 Number of cells measured by an electric cell counter (Microcell Counter CC-130)
44 Notes *Amount of cultivation medium at the time of treatment with the substance to be tested. After the treatment, the cultivation was carried out with 5 mL/cultivator

0 0 5 8

(2) Rate of cell growth

		(4) 濃度 (μg/ml)	(5) 増殖率 (%)
(1) 代謝活性化法によらない場合	(2) 24時間処理	0	100
		200	96.9
		300	92.4
		400	81.3
		500	62.7
		600	57.3
		700	55.6
		800	58.7
		1,000	58.7
		1,500*	45.8
	(3) 48時間処理	0	100
		100	90.2
		200	82.4
		300	67.3
		400	34.7
		500	16.8
		600	15.9
		700	15.3
		800	15.0
		1,000	16.2
(6) 代謝活性化法による場合		0	100
		100	83.9
		150	66.1
		200	37.0
		250	30.0
		300	28.7
		350	27.0
		400	24.3
		450	20.9
		500	20.4

(7) 備考　* 被験物質と思われる沈殿がみられた。

Key:
1. Method other than the metabolic activation method
2. 24-hour treatment
3. 48-hou treatment
4. Concentration
5. Rate of growth (%)
6. Metabolic activation method
7. Notes *Precipitates seemed to be the substance to be tested were observed.

0 0 5 9

5. Chromosomal aberration test

(1) Testing conditions and preparation of solutions of the substance to be tested

(1) 実験期間		(2) 自 平成 3年11月21日 至 平成 3年12月18日		
		(3) 代謝活性化法によらない場合		(6) 代謝活性化法による場合
		(4) 24時間処理	(5) 48時間処理	
細胞 (7)	播種細胞(8)数	(9) 0.5×10⁶個／ml	(9) 0.5×10⁶個／ml	(9) 0.5×10⁶個／ml
	培養開始後日数	(10) 3日目 (11)	3日目 (11)	3日目 (11)
(12) 培養器	形(13)状	(14) 円形プラスチック製シャーレ		(14) 円形プラスチック製シャーレ
	大(14)きさ	(15) 直径60mm		(15) 直径60mm
	製(16)造元	Corning (岩城硝子) (17)		Corning (岩城硝子) (17)
	1濃度当りの培養器数(18)(プレート数)	2		2
	培養(20)液量	5ml／培養器 (21)		3ml／培養器* (22)
(23) 被験物質溶液の調製	溶媒の種類 (24)	DMSO		DMSO
	被験物質原液の濃度	(25) 200mg／ml		100mg／ml
	被験物質量	(26) 1,000mg		500mg
	溶媒量	(27) 5mlに定容 (28)		5mlに定容 (29)
	被験物質溶液の性状 (○で囲む)	(溶解)、懸濁 その他(　) (31)		(溶解)、懸濁 (31) その他(　)
	調製後の時間	(32) 2時間以内 (33)		2時間以内 (33)
	保存方法	(34) 使用まで室温保存 (35)		使用まで室温保存 (35)
	滅菌方法	(36) 滅菌せず (37)		滅菌せず (37)
(38) 被験物質処理	調製溶液添加量	(39) 50ml／培養器 (40)		30ml／培養器 (40)
	処理時(41)間	24h	48h	6h
	S9 Mix添加量	(42) ――		0.5ml／培養器 (43)
(44) 分裂阻害剤	種(45)類	コルセミド (46)		コルセミド (46)
	濃(47)度	0.1μg／ml		0.1μg／ml
	処理時(48)間	2h		2h
備考	* 細胞増殖阻害試験に同じ。(49)			

Key:

1 Experimental period
2 From November 21, 1991 to December 18, 1991
3 Method other than the metabolic activation method
4 24-hour treatment
5 48-hour treatment
6 Metabolic activation method
7 Cells
8 Number of cells inoculated
9 $.5 \times 10^4$ cells/mL
10 No. of cultivation day
11 3 days
12 Cultivator
13 Shape
14 Size
15 Manufacturer
16 No. of cultivators per concentration setting (No. of plates)
17 Round plastic Petri dish
18 Diameter 60 mm
19 Corning (Iwaki glass)
20 Amount of cultivation medium
21 5 mL/cultivator
22 3 mL/cultivator*
23 Preparation of solutions for the substance to be tested
24 Solvent used
25 Concentration of the stock solution of the substance to be tested
26 Amount of the substance to be tested
27 Amount of solvent
28 Made up to 5 mL
29 Made up to 3 mL
30 Property of solutions of the substance to be tested (circle one)
31 Dissolution, suspension, other ( )
32 Time after preparation
33 Within 2 hours
34' Storage method
34 Stored at room temperature before using
35' Sterilization method
35 No sterilization
36 Solutions added
37 /Cultivator

38 Treatment time
39 Amount of S9 Mix added
40 /Cultivator
41 Treatment with the substance to be tested
42 Division inhibitor
43 Kind
44 Concentration
45 Treatment time
46 Colcemid
47 Notes *Same as the cell proliferation inhibition test

(2) Results

The results obtained are shown in Table I and Table II

(3) Judgment of the results

判　(1)　定 (いずれかを○で囲むこと。)	(2) 陽　性　　(3) 陰　性

判定の理由 (4) S9 Mix添加群は被験物質の 150～ 200μg／mlで、用量依存性のある染色体の構造的異常の誘発がみられた。		
(5) D_{20}値	(6)代謝活性化 (あり) (2)　　なし (3)	153μg／ml

Key: 1 Judgment (circle one)
2 Positive
3 Negative
4 Reason - In the group with S9 Mix added, dose-dependent structural abnormalities of chromosomes were induced by using 150-200 μg/mL of the substance to be tested
5 D_{20} value
6 Metabolic activation
7 Yes
8 No

(4) Reference items

1) Cell selection reason

Frequently used for testing chromosomal aberration, the sensitivity is high, and there is an abundance of research data available.

2) Reasons for dose selection for the substance to be tested

As a result of the cell division inhibition test, the number of dividing cells was found to be sufficient for carrying out a chromosomal aberration test if the dose was less than 400 μg/mL in the case of 24-hour treatment in the direct method, 300 μg/mL in the case of 48-hour treatment in the direct method, or 200 μL/mL in the metabolic activation method. Therefore, the maximum dose was set at 400 μg/mL in the case of 24-hour treatment in the direct method, 300 μg/mL in the case of 48-hour treatment in the direct method, and 200 μg/mL in the metabolic activation method. The maximum doses were reduced by a ratio of 2 in the direct method and a difference of 50 in the metabolic activation method to obtain 3 doses for each group. The concentration for 50% inhibition of cell proliferation was found to be about 1,400 μg/mL in the case of 24-hour treatment in the direct method, it was about 350 μg/mL in the case of 48-hour treatment in the direct method, and it was about 180 μg/mL in the metabolic activation method.

3) Chromosomal aberration judgment standards (especially standards for gaps)

The gaps judgment standard was that the nonchromosomal portion was larger than the width of a chromatid, the shape was clear, and it was on the long axis of the chromatid.

The results obtained were examined by grading with respect to the appearance rate (average of two dishes for each group) of cells having structural abnormalities including gaps and quantitative abnormalities as negative (-) for less than 5%, quasi-positive (±) for 5% to less than 10%, positive (+) for 10% to less than 20%, positive (++) for 20% to less than 50%, and positive (+++)

for 50% and higher, and no statistical method was used. If the rate of appearance showed dose-dependency or reproducibility in those groups of positive (+) or higher, it was judged that the effect of inducing chromosomal aberration was positive. Incidentally, if the result was quasi-positive (±) or positive in only a single dose, the test was repeated.

4) Figures and photographs

Figure 1: results of the test for cell proliferation inhibition by X-12-990S

Figures 2 and 3: results of the test for chromosomal aberration caused by X-12-990S

Figures 4 and 5: chromosomal abnormalities induced by X-12-990S (photographs)

6. Others

(1) 染色体異常試験実施機関	(2) 名称	財団法人 (3) 化学品検査協会 化学品安全センター日田研究所
	(4) 所在地	〒877 大分県日田市石井町3丁目822番地 (5) 電話 0973(24)7211
(6) 試験責任者	(7) 職氏名	試験研究5課 縄原美次
(8) 試験実施年月日	(9) 平成 3年 10月 1日から 平成 4年 1月 30日まで	

Key:
1 Chromosomal aberration testing facility
2 Name
3 Chemical Product Testing Association, Chemical Product Safety Center, Hita Laboratories
4 Location
5 3-822 Ishii-cho, Hita City, Ohita Prefecture PC877, Telephone 0973-24-7211

0059

6 Testing manager
7 Name, position, M. Kajihara, Fifth Dept., Testing and Research,
8 Testing period
9 From October 1, 1991 to January 30, 1992

Table I Results of chromosomal abnormation test (method other than the metabolic activation method)

①試験物質：K-12-6605

②培養	③処理時間 (h)	④処理濃度 (μg/ml)	⑤培養 培地数	⑥染色体異常出現細胞数 ギャップ	染色体型	染色分体型	染色体数		その他	合計		⑧判定
										-g	+g	
⑦培養液(S9S)	24	0	100	0	1	1	0	0	0	1	1	-
			100	0	0	1	0	0	0	2	2	
			200	0 (0.0%)	1 (0.5%)	2 (1.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (1.5%)	3 (1.5%)	
	48	0	100	0	0	0	0	0	0	0	0	-
			100	0	0	1	0	1	0	0	2	
			200	0 (0.0%)	0 (0.0%)	1 (0.5%)	0 (0.0%)	1 (0.5%)	0 (0.0%)	0 (0.0%)	2 (1.0%)	
①精製水	24	100	100	0	0	0	0	1	0	2	2	-
			100	1	0	0	0	0	0	1	1	
			200	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.5%)	1 (0.5%)	
		200	100	0	0	0	0	2	0	2	3	-
			100	0	0	0	0	0	0	3	3	
			200	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.0%)	0 (0.0%)	5 (2.5%)	6 (3.0%)	
		400	100	0	0	2	0	0	0	2	3	-
			100	1	2	0	0	0	0	3	3	
			200	1 (0.5%)	2 (1.0%)	2 (1.0%)	0 (0.0%)	3 (1.5%)	0 (0.0%)	5 (2.5%)	8 (4.0%)	
	48	24	100	0	0	0	0	0	0	0	0	-
			100	0	0	0	0	0	0	1	2	
			200	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.5%)	2 (1.0%)	
		150	100	0	0	0	0	0	0	0	0	-
			100	0	0	0	0	2	0	3	4	
			200	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.0%)	0 (0.0%)	3 (1.5%)	4 (1.0%)	
		300	100	0	0	0	0	0	0	0	2	-
			100	0	0	0	0	2	0	2	2	
			200	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.0%)	0 (0.0%)	4 (1.0%)	4 (1.0%)	
②陽性対照(MMC)	24	0.05	100	0	22	53	3	2	0	46	47	+
			100	0	23	53	2	2	0	51	51	
			200	0 (0.0%)	45 (22.5%)	106 (53.0%)	5 (2.5%)	4 (2.0%)	0 (0.0%)	97 (48.5%)	98 (49.0%)	
	48	0.05	100	2	44	83	5	5	0	88	89	+++
			100	0	56	87	2	3	1	88	88	
			200	2 (1.0%)	100 (50.0%)	170 (85.0%)	7 (3.5%)	8 (4.0%)	1 (0.5%)	176 (88.0%)	177 (88.5%)	

⑨染色体異常出現細胞数、培養液数及び染色体異常をもつ細胞の出現頻度を2段ずつ行目に記載し、その合計を3行目に表示し、それから求めた頻度をもつ細胞の出現頻度%を()に示す。ギャップ(g)には染色分体型と染色体型の両者を含める。各細胞のギャップのみをもつ細胞を別に表記する。

1細胞内に数個の異常をもつ場合でも、1個の異常細胞として計算する。例えば、1個の細胞中に切断が2、交換がある場合には、異常細胞としては1、切断をもつ細胞としても1、交換をもつ細胞としても1として計算する。

⑩染色分体型切断 ⑪染色分体型交換 ⑫染色体型切断 ⑬染色体型交換 その他 倍数性等を含む(倍数性を除く)

⑰

Key:
1 Substance to be tested
2 Treatment
3 Solvent
4 Substance to be tested
5 Positive control [1-5, and 17 are partially illegible
6 Treatment time
7 Treatment concentration
8 Number of cells observed
9 Number of polyploids
10 Judgment
11 Number of appearance and frequency of appearance (%)* for cells having chromosomal structural abnormalities
12 Gaps
13 Chromatid type
14 Chromosome type
15 Others
16 Total
17 [Partially illegible] for each treatment concentration, the number of cells observed for each cultivation series (plate), number of polyploids, number of appearance of cells having structural abnormalities on lines 1 and 2 with the total on the line 3, and the frequency of appearance of abnormal cells (%) determined from these results is shown inside ( ). The gaps (g) include the results for both chromatid and chromosomal types. The total (-g) shows the total number of cells with abnormalities excluding those having gaps alone and frequency of appearance (%) inside ( ).
Even in the case of several abnormalities in [illegible] cell, the computation is carried out as a single abnormal cell. For example, if a single cell has two scissions and 2 exchanges, it is counted 1 as an abnormal cell, 1 as a cell having scissions, and 1 as a cell having exchanges.
[illegible] ctb: cromatic type scission, cte: cromatid type exchange, csb: chromosome type scission, cse: chromosome type exchange, others: fragmentation (excluding powdering)

Table II Results of chromosomal aberration test (metabolic activation method)

検体：K-12-990S

群	S9 mix	処理濃度 (μg/ml)	観察細胞数	倍数体数	判定	ギャップ g	染色分体型 ctb	染色分体型 cte	染色体型 csb	染色体型 cse	その他	合計 -g	合計 +g	判定
溶媒対照	-	0	100	1	-	0	0	1	1	0	0	2	2	-
			100	1		0	0	0	0	0	0	0	0	
			200	2, 1.0		0, 0.0	0, 0.0	1, 0.5	1, 0.5	0, 0.0	0, 0.0	2, 1.0	2, 1.0	
	+	0	100	1	-	0	0	0	0	0	0	0	0	-
			100	0		0	0	0	0	0	0	0	0	
			200	1, 0.5		0, 0.0	0, 0.0	0, 0.0	0, 0.0	0, 0.0	0, 0.0	0, 0.0	0, 0.0	
被験物質	-	100	100	1	-	1	0	0	0	0	0	0	1	-
			100	1		0	0	0	0	0	0	0	0	
			200	2, 1.0		1, 0.5	0, 0.0	0, 0.0	0, 0.0	0, 0.0	0, 0.0	0, 0.0	1, 0.5	
		150	100	0	-	0	1	0	0	0	0	1	1	-
			100	1		0	0	0	0	1	0	1	1	
			200	1, 0.5		0, 0.0	1, 0.5	0, 0.0	0, 0.0	1, 0.5	0, 0.0	2, 1.0	2, 1.0	
		200	100	0	-	1	1	1	0	1	0	3	4	-
			100	0		0	1	1	0	0	0	2	2	
			200	0, 0.0		1, 0.5	2, 1.0	2, 1.0	0, 0.0	1, 0.5	0, 0.0	5, 2.5	6, 3.0	
	+	100	100	2	-	1	1	1	1	1	0	3	3	-
			100	0		1	0	0	0	0	0	1	1	
			200	2, 1.0		1, 0.5	1, 0.5	1, 0.5	1, 0.5	1, 0.5	0, 0.0	4, 2.0	4, 2.0	
		150	100	0	+	2	1	15	1	1	0	18	19	+
			100	2		1	0	14	0	0	0	18	20	
			200	2, 1.0		4, 2.0	1, 0.5	29, 14.5	1, 0.5	1, 0.5	0, 0.0	36, 18.0	39, 19.5	
		200	100	0	-	0	22	43	0	0	0	43	43	++
			100	0		0	22	43	0	0	0	45	46	
			200	0, 0.0		0, 0.0	44, 22.0	86, 43.0	0, 0.0	0, 0.0	0, 0.0	88, 44.0	93, 46.5	
陽性対照	-	10	100	1	-	0	0	0	1	0	0	1	1	-
			100	2		0	0	0	0	0	0	0	0	
			200	3, 1.5		0, 0.0	0, 0.0	0, 0.0	1, 0.5	0, 0.0	0, 0.0	1, 0.5	1, 0.5	
	+	10	100	1	-	1	4	24	4	0	0	29	30	++
			100	0		0	7	17	2	0	0	23	23	
			200	1, 0.5		1, 0.5	11, 5.5	41, 20.5	6, 3.0	0, 0.0	0, 0.0	52, 26.0	53, 26.5	

[illegible]

Key:
1 Treatment
2 Solvent
3 Substance to be tested
4 Positive controls [1 - 4, and 17 are partially illegible]
5 Presence of S9 Mix
6 Treatment concentration
7 Number of cells observed
8 Number of polyploids
9 Judgment
10 Substance to be tested
11 Number of appearance and frequency of appearance (%)* for cells having chromosomal structural abnormalities
12 Gaps
13 Chromatid type
14 Chromosome type
15 Others
16 Total
17 [illegible] dose (5%), treatment time with the substance to be tested (6 hours), cell recovery time after treating with the substance to be tested (18 hours).

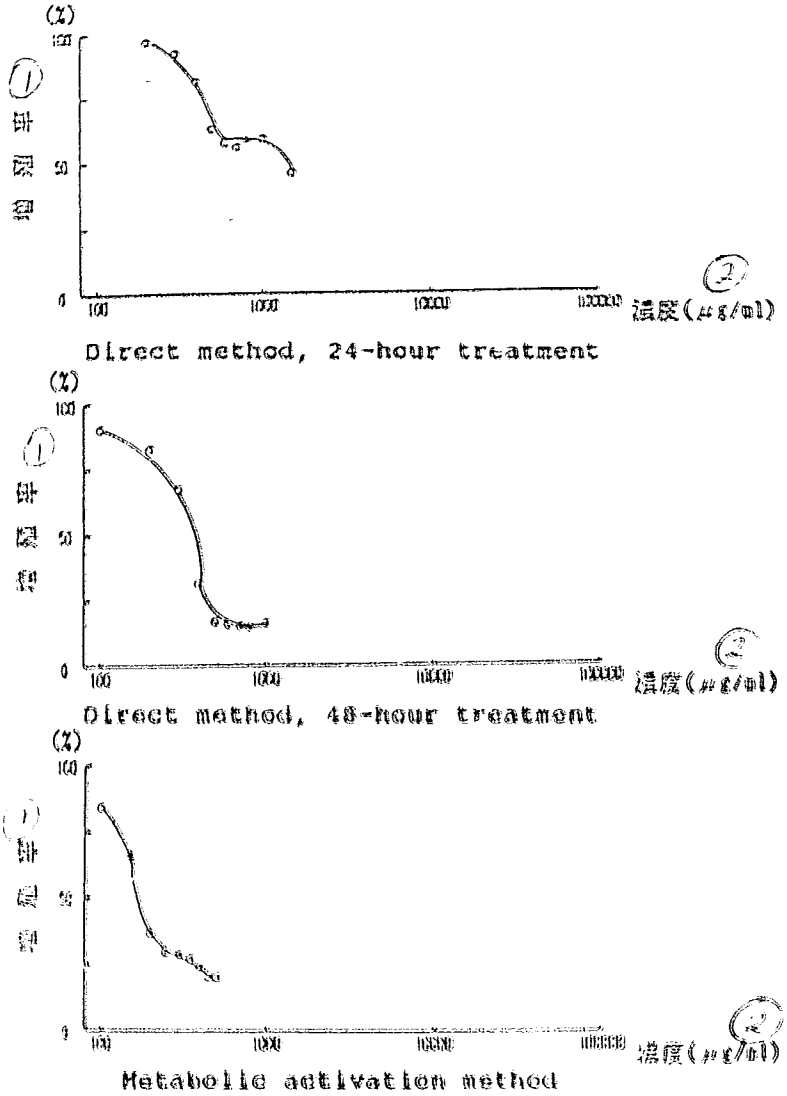
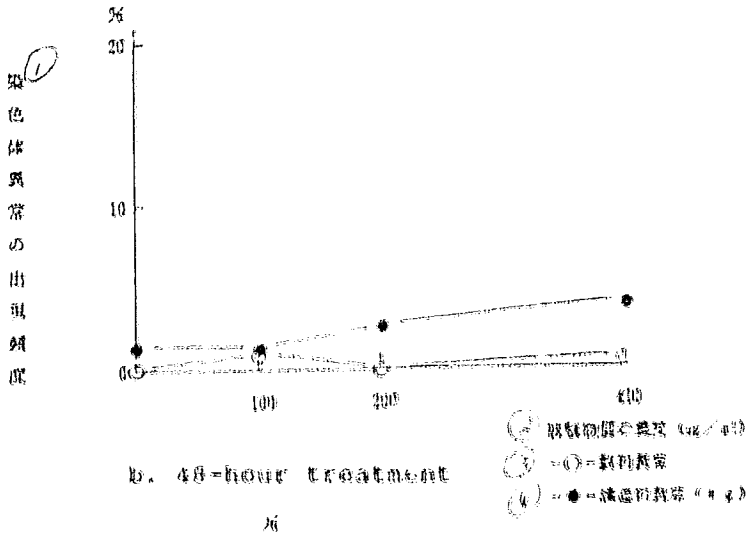


Figure 1 Results of test for cell proliferation inhibition by X-12-9909

Key: 1 Rate of growth
2 Concentration

A. method other than the metabolic activation method

a. 24-hour treatment



b. 48-hour treatment

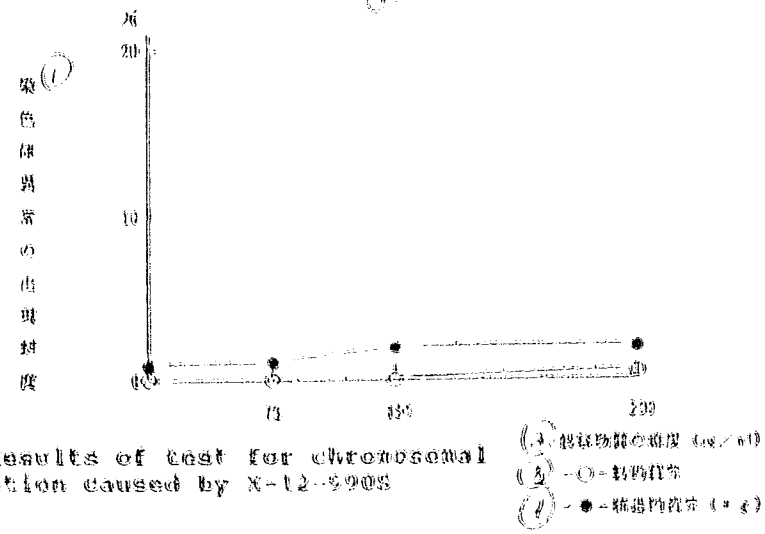
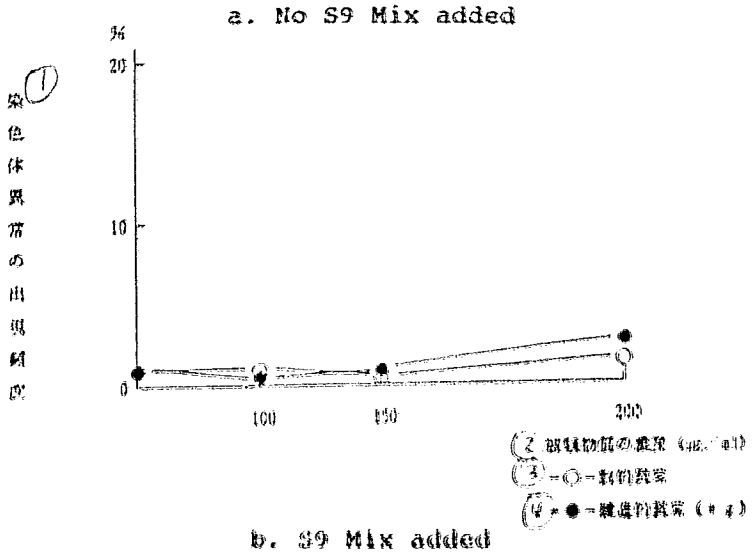


Figure 2 Results of test for chromosomal aberration caused by X-12-5905

Key:
1 Frequency of appearance of chromosomal aberration
2 Concentration of the substance to be tested
3 Quantitative abnormalities
4 Structural abnormalities

B. Metabolic activation method

a. No S9 Mix added



b. S9 Mix added

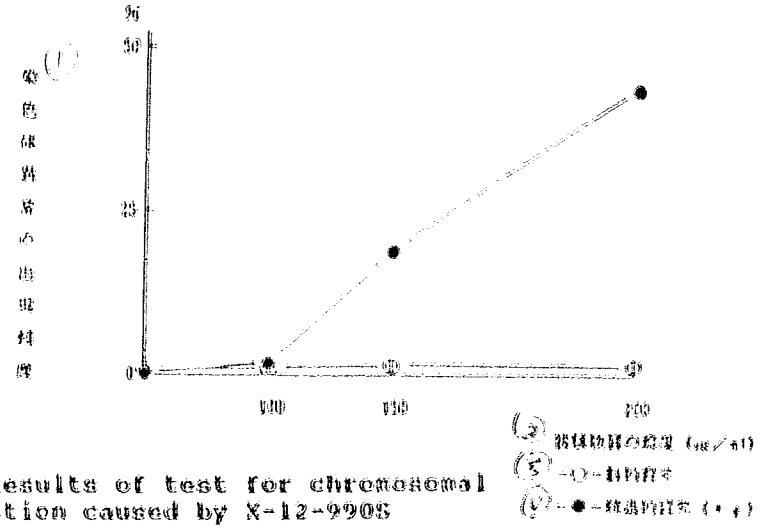


Figure 1 Results of test for chromosomal aberration caused by X-12-9905

Key:
1 Frequency of appearance of chromosomal aberration
2 Concentration of the substance to be tested
3 Quantitative abnormalities
4 Structural abnormalities

Control (metabolic activation method, control group with solvent alone added)

Structural abnormalities (metabolic activation method, 200 μg/mL), a. Chromatid scission, b. Chromatid exchange

Figure 4 Chromosomal aberration induced by X-12-9905

Work assignment

Title: Chromosomal aberration test using a cultured mammalian cell for X-12-990S

氏　名 ①	業　務　内　容 ②
試験責任者 ③ 梶　原　美　次	④ 試験の計画、実施など試験全般の管理、観察、結果の分析・評価及び報告書の作成を行う。
試験担当者 ⑤ 小　椋　正　治	⑥ 試験計画書の作成、試験物質による処理、標本の作製、観察、報告書草案の作成を行う。
細胞第1部門長 ⑦ 梶　原　美　次	⑧ 細胞第1部門のすべての業務に対し責任をもつ。作業の正確な実施、検査及び作業にたずさわる職員の教育、訓練を行う。

Key:
1 Name
2 Content of work
3 Test manager: M. Kajihara
4 Test planning, management of the overall aspect of the test, microscopic observation, analyses and evaluation of the results, and preparation of reports
5 Person in charge of test, S. Ogura
6 Preparation of document for the plan, treatment with the substance to be tested, preparation of specimens, microscopic observation, and preparation of drafts of reports
7 Cell First Division, manager, M. Kajihara
8 Responsible for all works in the Cell First Division. Accurate execution of works, and education and training of personnel involved in testing and other works

Certificate of Accuracy

Chemical Testing Association
Chemical Product Safety Center, Hita Laboratories

Test Requester: Shietsu Chemical Industry K.K.
Title: Test to examine the effect of X-12-990S to cause chromosomal aberration by using cultured mammalian cells
Test Code No.: K06-0265

The current test was inspected and audited by the accuracy certification department of the Chemical Product Testing Association, Chemical Product Safety Center, Hita Laboratories. The following is the date of inspection or auditing and date of reports to the operation manager and person in charge of the test.

Inspection or auditing date	Date reported to person in charge of the test	Date reported to the operation manager
9/30/1991	9/30/1991	9/30/1991
11/21/1991	11/22/1991	11/25/1991
12/16/1991	12/18/1991	12/18/1991
12/18/1991	12/18/1991	12/18/1991
1/23/1992	1/23/1992	1/23/1992
1/30/1992	1/30/1992	1/30/1992

In this report, the methods and procedures used in the test are accurately described, and the results shown in the report accurately reflect the raw data of the test.

January 30, 1992 K. Shiraishi, Accuracy Certification Dept.
January 30, 1992 H. Fujii, Manager, Accuracy Certifcation. Dept.

0068

受理番号	T91-1497
報告書番号	T-2932

試験コード番号　K06-0265

最終報告書

X-12-9905の経鼻類懸髄細胞を用いる染色体異常試験

1992年　1月

財団法人　化学品検査協会
化学品安全センター　財団研究所

39pgs.

0019

陳述書

財団法人　化学品検査協会
化学汎安全センター日田研究所

試験委託者：信越化学工業株式会社

試験の表題：X-12-9908 のほ乳類培養細胞を用いる染色体異常試験

試験コード番号：K06-0265

上記試験は、昭和59年3月31日付、環保業第19号、薬発第229号及び59基局第85号並びに昭和61年11月18日付、環企研第231号、衛生第18号及び61基局第821号による「新規化学物質に係る試験及び指定化学物質に係る有害性の調査の項目等を定める命令第1条に規定する試験施設について」及び「OECD Principles of Good Laboratory Practice (May 12, 1981)」に従って実施したものである。

1992年1月30日

運営管理者　山本 利人（実）

信頼性保証書

財団法人　化学品検査協会
化学品安全センター日田研究所

試験委託者：　信越化学工業株式会社

試験の表題：　X-12-9905 のは乳類培養細胞を用いる染色体異常試験

試験コード番号：　K06-0265

当試験は財団法人化学品検査協会化学品安全センター日田研究所の信頼性保証部門が監査又は査察を実施しており、監査又は査察を行った日付、運営管理者及び試験責任者に報告を行った日付は以下の通りである。

監査又は査察日	試験責任者報告日	運営管理者報告日
1991年　9月　30日	1991年　9月　30日	1991年　9月　30日
1991年　11月　21日	1991年　11月　22日	1991年　11月　25日
1991年　12月　16日	1991年　12月　18日	1991年　12月　18日
1991年　12月　18日	1991年　12月　18日	1991年　12月　18日
1992年　1月　23日	1992年　1月　23日	1992年　1月　23日
1992年　1月　30日	1992年　1月　30日	1992年　1月　30日

本報告書には、試験で使用した方法、手順が正確に記載されており報告結果は試験の生データを正確に反映している。

1992年1月30日
信頼性保証部門員　白石圭二

1992年1月30日
信頼性保証部門長　藤井甫保

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要　　約

X-12-9905 の染色体異常誘発作用について、チャイニーズハムスター肺線維芽細胞（CHL 細胞）を用い、直接法（用代謝活性化法）ではマイトマイシンC（MMC）、代謝活性化法ではシクロホスファミド（CPA）を陽性対照として検討した。

被験物質の用量設定を目的として行った細胞増殖阻害試験及び細胞分裂阻害試験の結果から、染色体異常試験での被験物質用量は、直接法24時間処理では 100、200及び 400mg／ml 等、48時間処理では75、150及び 300mg／ml 等、代謝活性化法では 100、150及び 200mg／ml を選定した。陽性対照の用量は、直接法24及び48時間処理では MMC の0.05mg／ml、代謝活性化法では CPA の10mg／ml を用いた。

被験物質は、直接法24時間処理及び48時間処理では染色体の構造的異常を誘発しなかったが、代謝活性化法の S9 Mix 添加群では 150～200mg／ml の範囲で用量依存性の多発染色体の構造的異常を誘発した。一方、陽性対照の MMC 及び CPA は明らかに染色体異常を誘発した。

以上の結果から、X-12-9905 は本試験条件下で、染色体異常を誘発するものと考えられた。

最終報告書

試験コード番号：K06-0265

1. 表　　題　　X-12-990Sのほ乳類培養細胞を用いる染色体異常試験

2. 試験委託者　（名　称）信越化学工業株式会社
（所在地）〒100　東京都千代田区大手町 2-6-1
（電　話）03-3246-5185

3. 試験施設　（名　称）財団法人化学品検査協会　化学品安全センター
日田研究所
（所在地）〒877　大分県日田市石井町 3丁目 822番地
（電　話）0973-24-7211

4. 試験目的　チャイニーズハムスターの肺線維芽細胞（CHL 細胞）を用いて、被験物質の染色体異常誘発能の有無を検討した。

5. 試験法　「『新規化学物質に係る試験の方法について』の一部改正等について」（環保業第 700号、薬発第1039号、61基局第1014号、昭和61年12月 5日）に定める試験方法に準拠した。

6. 優良試験所基準　「新規化学物質に係る試験及び指定化学物質に係る有害性の調査の項目等を定める命令第 4条に規定する試験施設について」（環企研第 233号、薬発第38号、63基局第 823号、昭和63年11月18日及び環保業第39号、薬発第 229号、59基局第85号、昭和59年 3月31日）及び「OECD Principles of Good Laboratory Practice」（May 12, 1981）に適合して行った。

7. 試験日程

7.1	試験開始日	1991年　10月　1日
7.2	試験実施期間	
	予備試験開始日	1991年　10月　7日
	観察終了日	1991年　12月　24日
7.3	試験終了日	1992年　1月　30日
7.4	最終報告書作成日	1992年　1月　30日

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2. 資料の保管場所及び保管期間

下記の資料及び標本は、当協会日田研究所の資料保管室で、また被験物質は試験物質保管室で最終報告書提出後10年間保管する。保管期限後の処置は試験委託者の承認を得る。ただし、保管期間中に品質が著しく変化する標本などの保管期間は、その品質が評価に耐え得る期間とし、廃棄に際しては試験委託者の承認を得る。

試験計画書及びその変更・追加記録
試験委託者との協議及び交信記録
試験関係者氏名リスト
被験物質に関する記録
　受領、使用、調製などの記録
試験記録、データ及び染色体標本
信頼性保証部門の査察記録
最終報告書のコピー

3. 最終報告書作成者及び試験関係者

最終報告書作成者

試験責任者：　1992年1月30日
（兼　細胞第1部門長）

梶原美次
日田研究所　試験研究部

試験担当者　1992年1月30日

小樽正浩

管理責任者

試験物質管理責任者　　吉田真和

0080

10.　試験材料及び試験方法

10.1　試験材料

10.1.1　被験物質（試験委託者提供資料）

1）名　称

1,1-及び1,2-ビス（トリメトキシシリル）エタンの混合物

略　称：　X-12-990S

2）ロット番号

105027

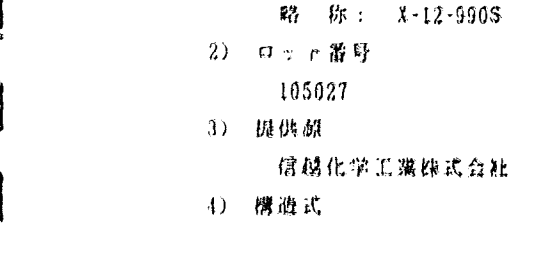
3）提供源

信越化学工業株式会社

4）構造式

$(CH_3O)_3-Si-CH_2CH_2-Si-(OCH_3)_3$

$(CH_3O)_3-Si-\underset{\displaystyle CH_3}{\underset{|}{CH}}-Si-(OCH_3)_3$



（分子式　$C_8H_{22}O_6Si_2$）

5）純　度

99%以上

6）物理化学的性状

分子量

270.43

常温における性状

無色透明液体

安定性

加水分解性有り

沸　点

103～104℃／5mmHg

溶解性

油溶性

溶解度

水	不溶
DMSO	可溶
アセトン	可溶

7）保管条件

試験物質保管室にて冷暗所、デシケーター内保管。

0 0 8 1

10.1.2 陽性対照物質

1) 直接法

名　　称	マイトマイシンC（マイトマイシン協和S、MMC）
製造元	協和発酵工業株式会社
ロット番号	723AAC
組　　成	1バイアル中に日局マイトマイシンC 2mg（力価）と日局塩化ナトリウム48mgを含有する。
外　　観	青紫色の結晶又は結晶性粉末
溶解性	水、メタノール又はアセトンに溶けにくく、酢酸エチル又はクロロホルムに極めて溶けにくく、エーテル又は四塩化炭素にほとんど溶けない
安定性	結晶の状態では常温で安定である。水溶液の状態ではpH8.0では安定であるがpH7.0以下ではpH値が低くなるにつれてその安定性は低下する。

2) 代謝活性化法

名　　称	シクロホスファミド（CPA）
製造元	Aldrich Chemical Co., Inc
ロット番号	07413TX
組　　成	単晶
外　　観	白色結晶性粉末
純　　度	98%以上

3) 保管条件

マイトマイシンCは室温保管、シクロホスファミドは冷蔵保管した。

10.1.3 取扱い上の注意

手袋、マスク、帽子及び白衣を着用した。

10.1.4 使用細胞及び培地

1) 細胞及びその選択理由

染色体異常試験によく用いられ、感受性が高く、蓄積データの豊富なチャイニーズハムスター捕獲維芽細胞（CHL細胞（クローン番号11）、国立衛生試験所から1988年9月28日に分与された。分与時の継代数18代）を使用した。CHL細胞はモード染色体数25本、倍加時間は約15時間。凍結しているCHL細胞を解凍し、増養した。増養3日目に継代し、対数増殖期にある細胞を使用した。本試験時の継代数は直接法24時間処理及び18時間処理では24代、代謝活性化法では34代であった。

0 0 8 2

2) 培地及び水

新生仔牛血清（三菱化成株式会社、ロット番号 NBJ02）を10%(v/v) 添加したイーグルMEM 培地（日水製薬株式会社、ロット番号294105、2844104）を用いた。以下、10%NCS/MEMと略記した。

培地調製用の純水は、蒸留水製造装置（WG-32、ヤマト科学株式会社）で得られた蒸留水を純水製造装置（NANO Pure, Barnstead）に通して精製した純水を使用した。

10.2 試験方法

10.2.1 培養方法

CHL 細胞を 1.5×10^{6}（又は 0.5×10^{6}）個／ml含む10%NCS/MEM 5mlを直径6cmのシャーレ（Corning Co., Ltd）に播き、2日間（又は3日間）培養した。試験は、培養2日目（又は3日目）の対数増殖期にある単層状の細胞に対して行った。

なお、培養は炭酸ガス培養器を用い次の条件で行った。

温　度　37±0.5℃

湿　度　ほぼ飽和

気　相　炭酸ガスを5%含む空気

10.2.2 試験液

被験物質及び陽性対照物質は使用に際して純度換算せず、原来重量で表示した。

1) 被験液

(1) 調製法

被験物質をジメチルスルホキシド（DMSO、株式会社 同仁化学研究所、ロット番号 RM142、AH176）に溶解し、原液を調製した。目的の濃度の溶液は原液を同溶媒で希釈し調整した。

(2) 調製時期

用時に調製し、2時間以内に使用した。

2) 被験液添加量

培地に対して1%の被験液を添加した。

3) 陽性対照液

(1) 調製法

陽性対照物質は純水に溶解し、同溶媒で希釈して目的の濃度の溶液を調製した。

(2) 調製時期

用時に調製し、2時間以内に使用した。

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10.2.3　S9 Mix調製法

1)　S9（キッコーマン株式会社）

使用動物：　SDラット、7週齢、雄

投与方法：　腹腔内投与

誘導法：　フェノバルビタール：30mg／kg×1回、60mg／kg×3回

5,6-ベンゾフラボン：80mg／kg×1回

保存：　−80℃（REVCO 超低温槽ULT-1285）

ロット番号：　RAA-260

製造年月日：　1991年 9月13日

2)　S9 Mix

S9	0.3ml
20mM HEPES（pH 7.2）	0.2ml
50mM $MgCl_2$	0.1ml
330mM KCl	0.1ml
50mM G-6-P	0.1ml
40mM NADP	0.1ml
純水	0.1ml

なお、S9 Mix/MEMの組成は以下のとおりである。

S9 Mix	0.5ml
10%NCS/MEM	2.5ml

10.2.4　標本作製方法

細胞をトリプシンでシャーレから剥がし、遠心分離して集め、次いで0.075M KCl を加え、37℃で15分間低張処理した。低張処理後、メタノール・酢酸(3:1)液を加え、細胞を固定した。細胞ペレットに少量のメタノール・酢酸(3:1)液を加え、肉眼で薄く懸濁して見える程度の細胞浮遊液を調製した。ラウンドプラスに細胞浮遊液を滴下、乾燥させた後、2%ギムザで染色した。1枚のシャーレにつき2枚の標本を作製した。

10.3　試験の実施

10.3.1　被験物質の用量設定試験

被験物質の用量設定のために、次の予備試験を行った。まず、細胞増殖が50%抑制されるおよその濃度を求めるため細胞増殖抑制試験を行った。次いで、細胞分裂抑制試験を実施し、実際に染色体の観察が可能な最高用量を決定した。細胞毒性が認められない場合は5mg／ml又は10mM相当の濃度を最高用量とした。

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0089

1) 細胞増殖抑制試験

培養 2又は 3日目のシャーレから培地を除き、直接法の場合は被験物質を含む培地を 5ml加え、細胞を24及び48時間培養した。代謝活性化法の場合は、培地を除いた後、被験物質を含むS9 Mix/MEMを 3ml加え 6時間処理した。処理後、被験物質を含むS9 Mix/MEMを除き、Ca^{2+}、Mg^{2+}不含生理的リン酸緩衝液［PBS（－）］ 2mlでシャーレを 3回洗浄後、10%NCS/MEMを 5ml加えて、18時間培養した。培養終了後培地を除き、トリプシン 2mlで細胞をシャーレから剥離させ、Microcell counter CC-130（東亜医用電子製）で細胞数を測定した。シャーレは各群 2枚を用い、それぞれ別々に計数した。

2) 細胞分裂抑制試験

直接法及び代謝活性化法とも培養及び被験物質による処理は細胞増殖抑制試験と同様に行い、双方とも培養終了 2時間前にコルセミドを 0.1μg/mlになるよう培地に加え、染色体標本を作製し、分裂細胞の有無を調べた。

10.3.2 染色体異常試験

1) 用量段階

被験物質　用量設定試験で得られた試験可能な最高用量を上限に、直接法では公比 2、代謝活性化法では公差50で希釈した 3段階の用量について試験を行った。

直接法24時間処理	100、200、400μg/ml
直接法48時間処理	75、150、300μg/ml
代謝活性化法	100、150、200μg/ml

陽性対照　当研究所のバックグラウンドデータから下記の用量で行った。

直接法	MMC	0.05μg/ml
代謝活性化法	CPA	10 μg/ml

2) 直接法による染色体異常試験

培養 3日目のシャーレから培地を除き、被験物質を含む培地を 5ml加え、細胞を24及び48時間培養した。培養終了 2時間前に、コルセミドを 0.1μg/mlになるよう培地に加え、染色体標本を作製した。

陰性対照として溶媒対照群、陽性対照としてMMC を加えた群を設けた。

3) 代謝活性化法による染色体異常試験

培養 3日目のシャーレから培地を除き、被験物質を含むS9 Mix/MEMを 3ml加えて、6時間処理した。その後、被験物質を含むS9 Mix/MEMをシャーレから除き、PBS(－) 2mlで 3回シャーレを洗浄後、10%NCS/MEMを 5ml加えて、18時間培養した。培養終了 2時間前に、コルセミドを 0.1μg/mlになるよう培地に加え、染色体標本を作製した。

陰性対照として溶媒対照群、陽性対照としてCPA を加えた群を設けた。また、代謝活性化の効果を明らかにする目的で、S9 Mixを含まない培地で被験物質用

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量及び処理時間をS9 Mix添加群と同一にした対照群を設けた。

4) シャーレの数及びシャーレの識別

各群 2枚のシャーレを用い、偏りを小さくするためシャーレはランダムに使用した。各シャーレはそれぞれ別々に計測した。

シャーレには試験コード番号、処理方法及び用量を明記した。

10.3.3 観 察

1枚のシャーレにつき 100個の細胞の分裂中期像を観察し、染色体又は染色分体にみられる構造的異常（ギャップ、切断、交換型異常など）及び数的異常（倍数性、核内倍化）を調べた。構造的異常及び数的異常について、前記の各異常のいずれかを持つ細胞を 1個の異常細胞とし、各群 200個の細胞を観察して異常細胞の出現率を求めた。構造的異常の出現率については、ギャップを含めた場合と含めない場合について算出した。

ギャップの判定基準は、非染色部分が染色分体の幅より大きく、幅が明確で染色分体の長軸上にあるものとした。

なお、標本はすべてコード化し、ブラインドで鏡検した。

10.3.4 結果の判定

結果の判定は、ギャップを含む構造的異常又は数的異常を有する細胞の出現率（2枚のシャーレの平均値）が 5%未満を陰性（−）、5%以上～10%未満を疑陽性（±）、10%以上～20%未満を陽性（+）、20%以上～50%未満を陽性（++）、50%以上を陽性（+++）とし、統計学的方法は用いなかった。陽性（+）以上を示す用量群における異常細胞の出現率に用量依存性又は再現性が認められた場合、染色体異常誘発陽性と判定した。なお、試験結果が疑陽性に終わった場合、単一濃度のみで陽性となった場合は再試験を行うこととした。

11 試験成績

データの信頼性に悪影響を及ぼしたと思われる諸要因は、試験期間を通して認められなかった。また、試験計画書からの逸脱もなかった。

本試験は、2枚のシャーレの個々の値が著しくばらつかなかったこと、陰性対照群で異常を有する細胞の出現率が 5%未満であったこと、陽性対照群でギャップ以外の異常を有する細胞の出現率が10%以上であったこと及び培養条件等試験期間に異常が認められなかったことから、正常に行われたと判断した。

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11.1　細胞増殖抑制試験（表 1及び図 1）及び細胞分裂抑制試験

細胞増殖抑制試験の設定用量は表 1の通りとした。増殖率は、溶媒対照群を100%として算出した。被験物質の50%増殖抑制濃度は直接法24時間処理で約 1,400μg／ml、直接法48時間処理で約 350μg／ml、代謝活性化法では約 180μg／mlであった。次に、実際に染色体異常試験がどの用量で可能かを知る目的で、50%増殖抑制濃度付近の用量で細胞分裂抑制試験を行った。その結果、直接法24時間処理では 400μg／ml以下、直接法48時間処理では 300μg／ml以下、代謝活性化法では 200μg／ml以下の用量で染色体異常試験を行うに足る数の分裂細胞が認められた。したがって、染色体異常試験は直接法24時間処理では 400μg／ml、直接法48時間処理では 300μg／ml、代謝活性化法では 200μg／mlを最高用量とし、直接法は公比 2で、代謝活性化法は公差50で希釈した 3段階の用量について実施した。

なお、代謝活性化法の場合には染色体異常の誘発が認められたため、用量依存性を考慮し、公差50で希釈した。

11.2　染色体異常試験

11.2.1　直接法による試験（表 2及び図 2）

1)　24時間処理

ギャップを含む染色体の構造的異常を有する細胞の出現率は、被験物質の100、 200及び 400μg／mlではそれぞれ 1.5、 3.0及び 4.0%と増加を示した。一方、溶媒対照群では構造的異常を有する細胞の出現率は、 1.5%と正常であった。また、陽性対照の MMC処理では40.0%と明らかに染色体異常の誘発が認められた。

なお、倍数体の出現率は全群で増加を示した。

2)　48時間処理

ギャップを含む染色体の構造的異常を有する細胞の出現率は、被験物質の75、 150及び 300μg／mlでは 4.0、 2.0及び 2.0%と増加をみた。一方、溶媒対照群では構造的異常を有する細胞の出現率は、 1.0%と正常であった。また、陽性対照の MMC処理では38.5%と明らかに染色体異常の誘発が認められた。

なお、倍数体の出現率は全群で増加を示した。

11.2.2　代謝活性化法による試験（表 3及び図 3）

1)　S9 Mix添加

ギャップを含む染色体の構造的異常を有する細胞の出現率は、被験物質の100μg／mlでは 2.0%で増加、 150及び 200μg／mlでは それぞれ 10.5及び 11.5%と用量依存性のある増加を示した。一方、溶媒対照群では構造的異常を有する細胞の出現率は、 0%と正常であった。また、陽性対照の CPA処理では26.5%と明らかに染色体異常の誘発が認められた。

なお、倍数体の出現率は全群で増加を示した。

0.0.8.3

2) S9 Mix非添加

代謝活性化法の対照としてS9 Mixを含まない培地で、被験物質用量及び処理時間を同一にした群では、被験物質の 100、 150及び 200μg／mlではそれぞれ 0.5、 1.0及び 3.0%と陰性を示した。一方、溶媒対照群及び陽性対照群では異常を有する細胞の出現率は、それぞれ 1.0及び 0.5%と正常であった。

なお、倍数体の出現率は全群で陰性を示した。

12. 結 論

CHL細胞を用いて行ったX-12-990S の染色体異常試験で、直接法24時間及び48時間処理では染色体の構造的異常の誘発は認められなかったが、代謝活性化法のS9 Mix添加群では、 150～ 200μg／mlの範囲で用量依存性のある染色体の構造的異常の誘発が認められた。

以上の結果から、X-12-990S は本試験条件下で、染色体異常を誘発すると考えられた。

13. 参考文献

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表1　X－12－990Sの細胞増殖抑制試験結果

直接法	24時間処理	被験物質の濃度（μg／ml）	0	200	300	400	500	600	700	800	1,000	1,500**
		増殖率（%）*	100	95.9	92.4	81.3	62.7	57.3	55.6	58.7	58.7	45.8
	48時間処理	被験物質の濃度（μg／ml）	0	100	200	300	400	500	600	700	800	1,000
		増殖率（%）*	100	90.2	82.4	67.3	34.7	16.8	15.9	15.3	15.0	16.2
代謝活性化法		被験物質の濃度（μg／ml）	0	100	150	200	250	300	350	400	450	500
		増殖率（%）*	100	93.9	66.1	37.0	30.0	28.7	27.0	24.3	20.9	20.4

*　2枚のシャーレの平均値

**　被験物質と思われる沈澱物が認められた。

被験物質名 メ-12-990S

表 2 染色体異常細胞結果（付加活性化法によらない場合）

K06-0265

処理	処理時間 (h)	処理濃度 (μg/ml)	観察細胞数	倍数体数	判定	染色体構造異常細胞の出現数と出現頻度(%)* ギャップ g	染色分体型 ctb	染色分体型 cte	染色体型 csb	染色体型 cse	その他	合計 -g	合計 +g	
溶媒 [DMSO]	24	0	100	0	-	0	1	0	0	0	0	1	1	-
			100	0		0	1	0	0	1	0	2	2	
			200	0(0.0)		0(0.0)	2(1.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	3(1.5)	3(1.5)	
	48	0	100	0	-	0	0	0	0	0	0	0	0	-
			100	0		0	1	0	1	0	0	2	2	
			200	0(0.0)		0(0.0)	1(0.5)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	2(1.0)	2(1.0)	
被験物質	24	100	100	1	-	0	0	1	0	1	0	2	2	-
			100	1		0	0	0	1	0	0	1	1	
			200	2(1.0)		0(0.0)	0(0.0)	1(0.5)	1(0.5)	1(0.5)	0(0.0)	3(1.5)	3(1.5)	
		200	100	0	-	1	0	1	0	1	0	2	2	-
			100	0		0	0	0	0	3	0	3	3	
			200	0(0.0)		1(0.5)	0(0.0)	1(0.5)	0(0.0)	4(2.0)	0(0.0)	5(2.5)	6(3.0)	
		400	100	0	-	1	2	0	0	0	0	2	3	-
			100	1		2	0	0	0	3	0	3	5	
			200	1(0.5)		3(1.5)	2(1.0)	0(0.0)	0(0.0)	3(1.5)	0(0.0)	5(2.5)	8(4.0)	
	48	75	100	0	-	0	0	0	0	0	0	0	0	-
			100	0		1	1	0	0	0	0	1	2	
			200	0(0.0)		1(0.5)	1(0.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	2(1.0)	
		150	100	0	-	0	0	0	0	0	0	0	0	-
			100	0		1	0	0	1	2	0	3	4	
			200	0(0.0)		1(0.5)	0(0.0)	0(0.0)	1(0.5)	2(1.0)	0(0.0)	3(1.5)	4(2.0)	
		300	100	0	-	0	1	0	1	0	0	2	2	-
			100	1		0	1	0	1	1	0	2	2	
			200	1(0.5)		0(0.0)	2(1.0)	0(0.0)	2(1.0)	1(0.5)	0(0.0)	4(2.0)	4(2.0)	
燐性対照 [MMS]	24	0.05	100	0	-	1	22	33	3	1	0	46	47	**
			100	0		0	25	33	2	3	0	51	51	
			200	0(0.0)		1(0.5)	47(23.5)	66(33.0)	5(2.5)	4(2.0)	0(0.0)	97(48.5)	98(49.0)	
	48	0.05	100	2	-	19	44	85	6	3	0	88	89	***
			100	0		8	58	77	2	1	1	88	88	
			200	2(1.0)		27(13.5)	162(81.0)	162(81.0)	8(4.0)	6(2.0)	1(0.5)	176(88.0)	177(88.5)	

* 処理濃度毎に各々の培養系列(プレート)の観察細胞数、倍数体数及び染色体構造異常をもつ細胞の出現数を1及び2行目に記載し、その合計を3行目に表示し、それから求めた異常をもつ細胞の出現頻度%を( )に示す。ギャップ(g)には染色分体型と染色体型の両者を含める。合計(-g)にはギャップのみをもつ細胞を除いた総数と(%)を示す。
1個の細胞中に数個の異常をもつ場合でも、1個の異常細胞として計算する。例えば、1個の細胞中に切断が2、交換が2ある場合には、異常細胞としては1、切断をもつ細胞としても1、交換をもつ細胞としても1として計算する。
ctb 染色分体型切断　cte 染色分体型交換　csb 染色体型切断　cse 染色体型交換　その他 断片化など(細胞化は除く)

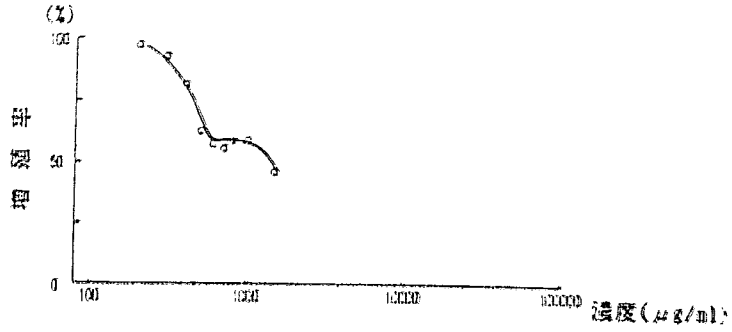
表 3　染色体異常試験結果（付加活性化法による場合）

被験物質名 X-12-990S　　X06-0265

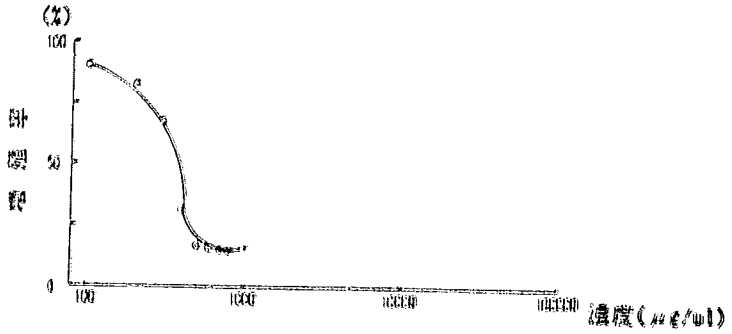
処理	59 mix 有無	処理濃度 (μg/ml)	観察 細胞数	倍数体数	判定	染色体構造異常細胞の出現数と出現頻度(%) ギャップ g	染色分体型 ctb	染色分体型 cte	染色体型 csb	染色体型 cse	その他	合計 -g	合計 +g	判定
溶媒 (DMSO)	-	0	100	1	-	0	0	1	1	0	0	2	2	-
			100	1		0	0	0	0	0	0	0	0	
			200	2 (1.0)		0 (0.0)	0 (0.0)	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)	2 (1.0)	2 (1.0)	
	*	0	100	1	-	0	0	0	0	0	0	0	0	-
			100	0		0	0	0	0	0	0	0	0	
			200	1 (0.5)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
被験物質	-	100	100	1	-	1	0	0	0	0	0	0	1	-
			100	1		0	0	0	0	0	0	0	0	
			200	2 (1.0)		1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	
		150	100	0	-	0	1	0	0	0	0	1	1	-
			100	1		0	0	0	0	1	0	1	1	
			200	1 (0.5)		0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	2 (1.0)	2 (1.0)	
		200	100	3	-	1	1	1	0	1	0	3	4	-
			100	0		0	1	1	0	0	0	2	2	
			200	3 (1.5)		1 (0.5)	2 (1.0)	2 (1.0)	0 (0.0)	1 (0.5)	0 (0.0)	5 (2.5)	6 (3.0)	
	*	100	100	2	-	0	1	0	1	1	0	3	3	-
			100	0		1	0	1	0	0	0	1	1	
			200	2 (1.0)		1 (0.5)	1 (0.5)	1 (0.5)	1 (0.5)	1 (0.5)	0 (0.0)	4 (2.0)	4 (2.0)	
		150	100	0	-	2	5	15	1	1	0	18	19	+
			100	3		2	8	14	0	0	0	18	20	
			200	3 (1.5)		4 (2.0)	13 (6.5)	29 (14.5)	1 (0.5)	1 (0.5)	0 (0.0)	36 (18.0)	39 (19.5)	
		200	100	3	-	3	15	41	0	0	0	43	43	++
			100	0		3	12	41	0	0	0	45	46	
			200	3 (1.5)		6 (3.0)	27 (13.5)	82 (41.0)	0 (0.0)	0 (0.0)	0 (0.0)	88 (44.0)	89 (44.5)	
陽性対照 (CPA)	-	10	100	1	-	0	0	0	1	0	0	1	1	-
			100	2		0	0	5	0	0	0	0	0	
			200	3 (1.5)		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.5)	1 (0.5)	
	*	10	100	1	-	1	4	24	4	0	0	29	30	++
			100	0		0	7	17	2	0	0	23	23	
			200	1 (0.5)		1 (0.5)	11 (5.5)	41 (20.5)	6 (3.0)	0 (0.0)	0 (0.0)	52 (26.0)	53 (26.5)	

59の用量(5%)、被験物質処理時間 (5h)、被験物質処理後の細胞回復時間 (18h)

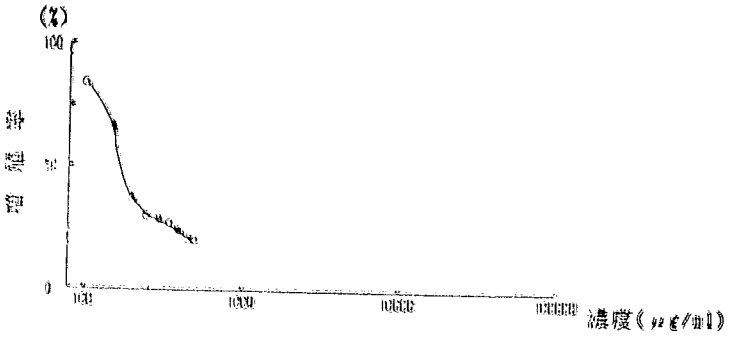
00090



直接法24時間処理



直接法48時間処理



代謝活性化法

図1　X－12－9905の細胞増殖阻害試験結果

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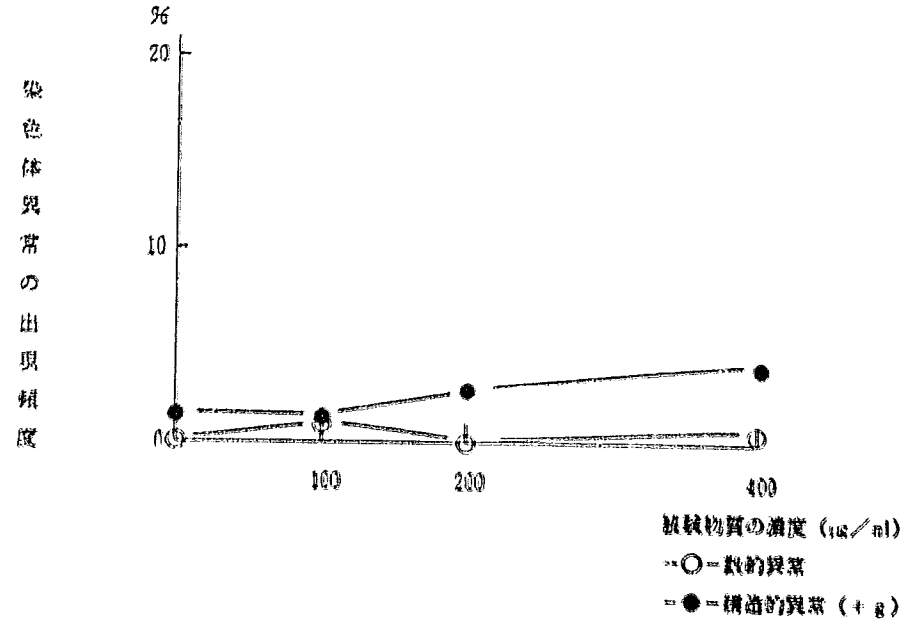
A. 代謝活性化法によらない場合

a. 24時間処理



b. 48時間処理

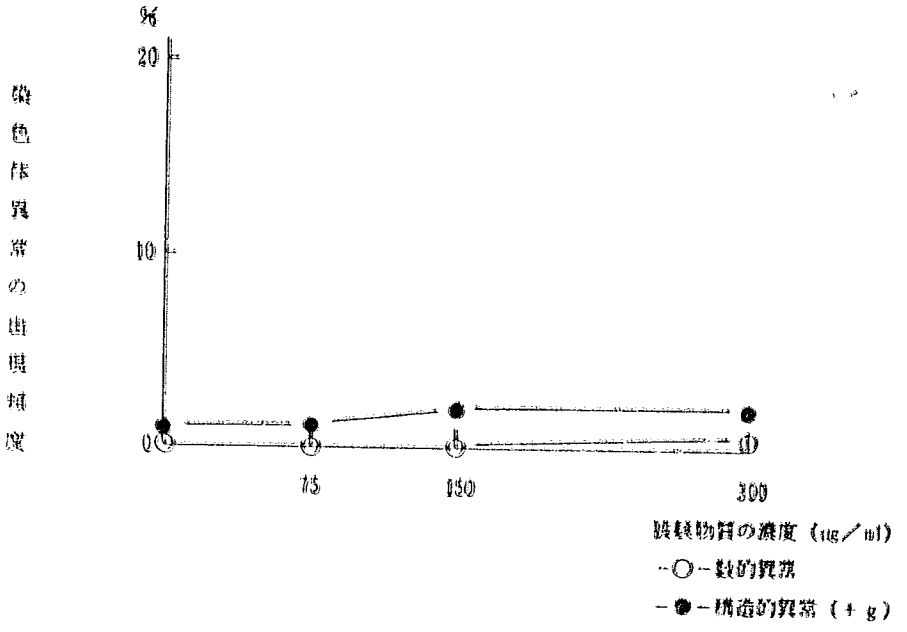
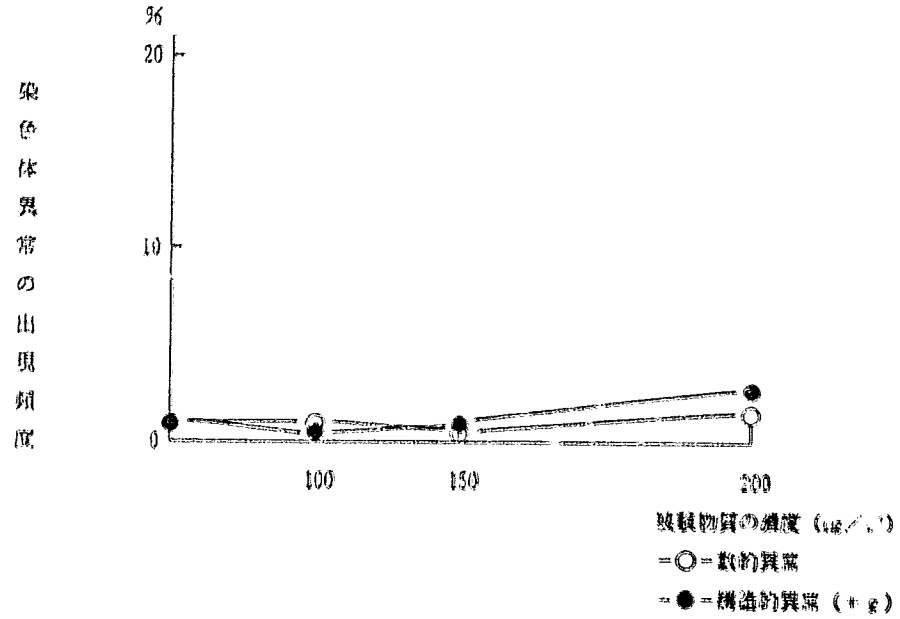


図2　X－12－9905の染色体異常試験結果

B. 代謝活性化法による場合

a. S9 Mix無



b. S9 Mix有

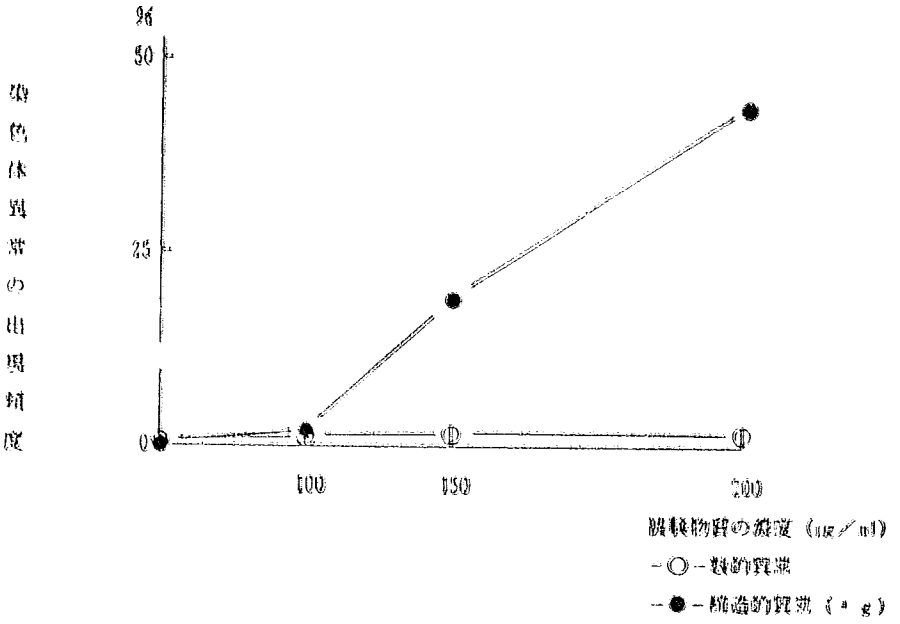


図3 X－12－990Sの染色体異常試験結果

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対照（代謝活性化剤　溶媒対照）

構造的異常（代謝活性化剤　２００μg／ml）

a、染色分体型切断

b、染色分体型交換

図4　X－12－9905により誘発された染色体異常

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0 0 9 5

T－2932号（化）

哺乳類培養細胞を用いる染色体異常試験結果報告書

1．一般的事項

新規化学物質の名称（IUPAC命名法による）	1,1-及び1,2-ビス（トリメトキシシリル）エタンの混合物			
別名	X－12－990S	物理化学的性状	分子量	270.43
構造式又は示性式（いずれも不明な場合は、その製法の概要） $(CH_3O)_3Si-CH_2CH_2-Si(OCH_3)_3$ $(CH_3O)_3Si-CH(CH_3)-Si(OCH_3)_3$			常温における性状	無色透明液体
			安定性	加水分解性有り
			融点	——
			沸点	103～104℃／5mmHg
			蒸気圧	——
			分配係数	——
			溶解性	油溶性
試験に供した新規化学物質の純度	99%以上		溶解度	水：不溶
				DMSO：可溶
不純物の名称及び濃度	——			アセトン：可溶
				その他（——）：——
ロット番号	105027			
〔備考〕				

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0096

2．細胞の種類－培養条件

細胞名	CHL	入手元		国立衛生試験所
種	チャイニーズハムスター	入手年月日		昭和 63年 9月 28日
培養液	イーグルMEM（MEM）	製造元		日水製薬株式会社
血清	10%新生仔牛血清（NCS）	製造元(Lot No.)		三菱化成株式会社（SB102）
細胞倍加時間	約15 h	凍結条件		−196℃
継代数	入手時 18代 *	培養条件	容器	直径100mmのプラスチックシャーレ
染色体数（モード）	25 本		温度	37±0．5℃
			CO_2	5%
			湿度	飽和

備考：*本試験時の継代数：直接法24時間処理24代、代謝活性化法34代
直接法48時間処理24代

3．S9Mix

（1）S9の入手方法等

1．自製	製造年月日	＝＝年＝＝月＝＝日製造
②購入	製造元	キッコーマン株式会社
	製造年月日	平成 3年 9月 13日 製造
	購入年月日	平成 3年 9月 27日 購入
	Lot No.	KAA-260

（2）S9の保存温度等

保存温	−80℃	保存機器名及び型式名	ETICO 超低温槽 ULT-1285

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（3）S9の調製方法

使用動物		誘導物質	
種・系統	ラット・SD系	名称	PB&BF*
性	雄	投与方法	腹腔内投与
週齢	7週	投与期間及び投与量（g／kg体重）	備考に記載
体重	183～215 g		
備考	Induction 24hrs PB30mg／kg　PB60mg／kg　PB60mg／kg & BF80mg／kg　PB60mg／kg　S9 preparation		

*PB…Phenobarbital, BF…5,6-Benzoflavone

（4）S9 Mixの組成

成分	S9Mix 1 ml中の量	成分	S9Mix 1 ml中の量
S9	0.3 ml	NADP	4 μmol
$MgCl_2$	8 μmol	緩衝液（HEPES）	4 μmol
KCl	33 μmol	その他（純水）	0.1 ml
グルコース-6リン酸	5 μmol	――	――
備考	S9Mix／MEMの組成：S9 Mix 0.5ml 10%NCS／MEM 2.5ml		

（5）S9 Mixの処理条件

	①プレート法	②薄層細胞法
S9量（最終濃度）	5 %	―― %
S9蛋白量（最終濃度）	1.38* mg／ml	―― mg／ml
処理時間	6 h	―― h
被験物質処理後の培養時間	18 h	―― h
備考	* S9 Assay data（キッコーマン株式会社）による。	

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4．細胞増殖抑制試験

（1）試験の条件及び被験物質溶液の調製

実験期間		自 平成 3年10月 7日 至 平成 3年12月13日		
		代謝活性化法によらない場合		代謝活性化法による場合
		24時間処理	48時間処理	
細胞	播種細胞数	1.5×10^4個／ml	1.5×10^4個／ml	1.5×10^4個／ml
	培養開始後日数	2日目	2日目	2日目
培養器	形状 大きさ 製造元 1濃度当りの培養器数（プレート数）	円形プラスチック製シャーレ 直径60mm Corning（岩城硝子） 2		円形プラスチック製シャーレ 直径60mm Corning（岩城硝子） 2
	培養液量	5ml／培養器		3ml／培養器*
被験物質溶液の調製	溶媒の種類	DMSO		DMSO
	被験物質原液の濃度	200mg／ml		100mg／ml
	被験物質量	1,000mg		300mg
	溶媒量	5mlに定容		3mlに定容
	被験物質溶液の性状（○で囲む）	溶解、懸濁 その他（　）		溶解、懸濁 その他（　）
	調製後の時間	2時間以内		2時間以内
	保存方法	使用まで室温保存		使用まで室温保存
	滅菌方法	滅菌せず		滅菌せず
被験物質処理	調製溶液添加量	50ml／培養器		30ml／培養器
	処理時間	24h	48h	6h
	SO-MIX添加量	――		0.5ml／培養器
細胞数計測方法		電気式細胞計数器（Microcell Counter（C-130））で細胞数を計測		

備考　＊ 被験物質による処理時の培養液量を示す。処理後は5ml／培養器で培養した。

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0099

（2）細胞増殖率

		濃度（mg／ml）	増殖率（%）
代謝活性化法による場合	24時間処理	0	100
		200	96.9
		300	92.4
		400	81.3
		500	62.7
		600	57.3
		700	55.6
		800	58.7
		1,000	58.7
		1,500*	45.8
	48時間処理	0	100
		100	90.2
		200	82.4
		300	67.3
		400	34.7
		500	16.8
		600	15.9
		700	15.3
		800	15.0
		1,000	16.2
代謝活性化法による場合		0	100
		100	83.9
		150	66.1
		200	37.0
		250	30.0
		300	28.7
		350	27.0
		400	24.3
		450	20.9
		500	20.4

備考　＊ 被験物質と思われる沈殿がみられた。

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5. 染色体異常試験

（1）試験の条件及び被験物質溶液の調製

実験期間		自 平成 3年11月21日 至 平成 3年12月18日		
		代謝活性化法によらない場合		代謝活性化法による場合
		24時間処理	48時間処理	
細胞	播種細胞数	0.5×10⁶個／ml	0.5×10⁶個／ml	0.5×10⁶個／ml
	培養開始後日数	3日目	3日目	3日目
培養器	形状	円形プラスチック製シャーレ		円形プラスチック製シャーレ
	大きさ	直径60mm		直径60mm
	製造元	Corning（岩城硝子）		Corning（岩城硝子）
	1濃度当りの培養器数（プレート数）	2		2
	培養液量	5ml／培養器		3ml／培養器*
被験物質溶液の調製	溶媒の種類	DMSO		DMSO
	被験物質原液の濃度	200mg／ml		100mg／ml
	被験物質量	1,000mg		500mg
	溶媒量	5mlに定容		5mlに定容
	被験物質溶液の性状（○で囲む）	(溶解)、懸濁 その他（　）		(溶解)、懸濁 その他（　）
	調製後の時間	2時間以内		2時間以内
	保存方法	使用まで室温保存		使用まで室温保存
	滅菌方法	滅菌せず		滅菌せず
被験物質処理	調製溶液添加量	50ml／培養器		30ml／培養器
	処理時間	24h	48h	6h
	S9 Mix（添加量）	――		0.5ml／培養器
分裂阻害剤	種類	コルセミド		コルセミド
	濃度	0.1μg／ml		0.1μg／ml
	処理時間	2h		2h

備考 * 細胞増殖阻害試験に同じ。

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（2）試験結果

表1及び2による。

（3）結果の判定

判　　定 （いずれかを○で囲むこと。）	(陽　性)　　陰　性

判定の理由		
S9 Mix添加群は被験物質の 150～ 200μg／mlで、用量依存性のある染色体の構造的異常の誘発がめられた。		
D_{20}値	代謝活性化 (あり)　　なし	153μg／ml

（4）参考事項

1）細胞の選択理由

染色体異常試験によく用いられ、感受性が高く、蓄積データの豊富なことによる。

2）被験物質の用量設定理由

細胞分裂抑制試験を行った結果、直接法24時間処理では 400μg／ml以下、直接法48時間処理では 300μg／ml以下、代謝活性化法では 200μg／ml以下の用量で染色体異常試験を行うに足る数の分裂細胞が認められた。よって、染色体異常試験は、直接法24時間処理では 400μg／ml、48時間処理では 300μg／ml、代謝活性化法では 200μg／mlを最高用量とし、直接法では公比 2で、代謝活性化法では公差50で希釈した 3段階の用量を設定した。なお、代謝活性化法の場合には染色体異常の誘発が認められたため、用量依存性を考慮し、公差50で希釈した。細胞増殖を50％抑制する濃度は直接法24時間処理では約 1,400μg／ml、直接法48時間処理では約 350μg／ml、代謝活性化法では約 180μg／mlであった。

3）染色体異常の判定基準（特にギャップの基準）

ギャップの判定基準は、非染色部分が染色分体の幅より大きく、形状が明確で、染色体の長軸上にあるものとした。結果の判定は、ギャップを含む染色体の構造的異常又は数的異常を有する細胞の出現率（2検の平均値）が 5％未満を陰性（－）、 5％以上～10％未満を疑陽性（±）、10％以上～20％未満を陽性（＋）、20％以上～50％未満を陽性（++）、50％以上を陽性（+++）とし、統計学的方法は用いなかった。陽性（＋）以上を示す用量群における異常細胞の出現率に用量依存性又は再現性が認められた場合、染色体異常誘発陽性とした。なお、試験結果が疑陽性に終った場合、単一濃度のみで陽性となった場合は再試験を行うこととした。

4）図及び写真

図 1：Y-12-990S の細胞増殖抑制試験結果

図 2及び 3：Y-12-990S の染色体異常試験結果

図 4及び 5：Y-12-990S により誘発された染色体異常（写真）

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6. その他

染色体異常試験実施機関	名称	財団法人 化学品検査協会 化学品安全センター日田研究所
	所在地	〒877 大分県日田市石井町3丁目822番地 電話 0973(24)7211
試験責任者	職氏名	試験研究5課 尾原美次
試験実施年月日	平成 3年 10月 1日から 平成 4年 1月 30日まで	

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0103

試験物質名 X-12-990S

表 1 染色体異常試験結果（代謝活性化法によらない場合）

K06-0265

処理	処理時間 (h)	処理濃度 (μg/ml)	観察細胞数	倍数体数	判定	染色体構造異常細胞の出現数と出現頻度(%)* ギャップ g	染色分体型 ctb	染色分体型 cte	染色体型 csb	染色体型 cse	その他	合計 -g	合計 +g	判定
溶媒 [DMSO]	24	0	100	0	-	0	1	0	0	0	0	1	1	-
			100	0		0	1	0	0	1	0	2	2	
			200	0(0.0)		0(0.0)	2(1.0)	0(0.0)	0(0.0)	1(0.5)	0(0.0)	3(1.5)	3(1.5)	
	48	0	100	0	-	0	0	0	0	0	0	0	0	-
			100	0		0	1	0	1	0	0	2	2	
			200	0(0.0)		0(0.0)	1(0.5)	0(0.0)	1(0.5)	0(0.0)	0(0.0)	2(1.0)	2(1.0)	
試験物質	24	100	100	1	-	0	0	1	0	1	0	2	2	-
			100	1		0	0	0	1	0	0	1	1	
			200	2(1.0)		0(0.0)	0(0.0)	1(0.5)	1(0.5)	1(0.5)	0(0.0)	3(1.5)	3(1.5)	
		200	100	0	-	1	0	1	0	1	0	2	3	-
			100	0		0	0	0	0	3	0	3	3	
			200	0(0.0)		1(0.5)	0(0.0)	1(0.5)	0(0.0)	4(2.0)	0(0.0)	5(2.5)	6(3.0)	
		400	100	0		1	2	0	0	0	0	2	3	-
			100	1		2	0	0	0	3	0	3	5	
			200	1(0.5)		3(1.5)	2(1.0)	0(0.0)	0(0.0)	3(1.5)	0(0.0)	5(2.5)	8(4.0)	
	48	75	100	0	-	0	0	0	0	0	0	0	0	-
			100	0		1	1	0	0	0	0	1	2	
			200	0(0.0)		1(0.5)	1(0.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(0.5)	2(1.0)	
		150	100	0	-	0	0	0	0	0	0	0	0	-
			100	0		1	0	0	1	2	0	3	4	
			200	0(0.0)		1(0.5)	0(0.0)	0(0.0)	1(0.5)	2(1.0)	0(0.0)	3(1.5)	4(2.0)	
		300	100	0	-	0	1	0	1	0	0	2	2	-
			100	1		0	1	0	1	1	0	2	2	
			200	1(0.5)		0(0.0)	2(1.0)	0(0.0)	2(1.0)	1(0.5)	0(0.0)	4(2.0)	4(2.0)	
陽性対照 [MMS]	24	0.05	100	0	-	1	22	33	3	1	0	46	47	**
			100	0		0	25	33	2	3	0	51	51	
			200	0(0.0)		1(0.5)	47(23.5)	66(33.0)	5(2.5)	4(2.0)	0(0.0)	97(48.5)	98(49.0)	
	48	0.05	100	2	-	19	44	85	6	3	0	88	89	***
			100	0		8	58	77	2	1	1	88	88	
			200	2(1.0)		27(13.5)	102(51.0)	162(81.0)	8(4.0)	4(2.0)	1(0.5)	176(88.0)	177(88.5)	

* 処理濃度毎に各々の培養系列(プレート)の観察細胞数、倍数体数及び染色体構造異常をもつ細胞の出現数を1及び2行目に記載し、その合計を3行目に表示し、それから求めた異常をもつ細胞の出現頻度%を( )に示す。ギャップ(g)には染色分体型と染色体型の両者を含める。合計(-g)にはギャップのみをもつ細胞を除いた総数と(%)を示す。
1個の細胞中に数個の異常をもつ場合でも、1個の異常細胞として計算する。例えば、1個の細胞中に切断が2、交換が2ある場合には、異常細胞としては1、切断をもつ細胞としても1、交換をもつ細胞としても1として計算する。
ctb 染色分体型切断 cte 染色分体型交換 csb 染色体型切断 cse 染色体型交換 その他 断片化など(細胞化は除く)

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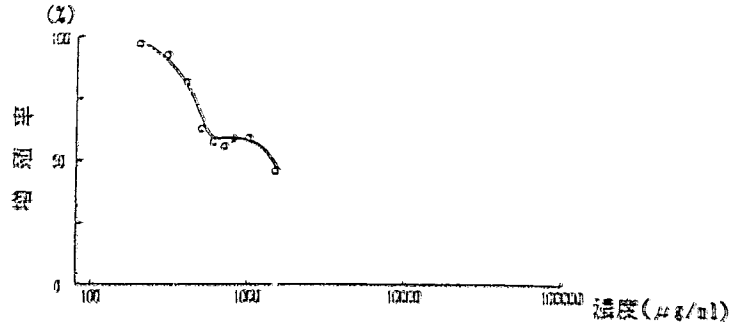
表 2　染色体異常試験結果（代謝活性化法による場合）

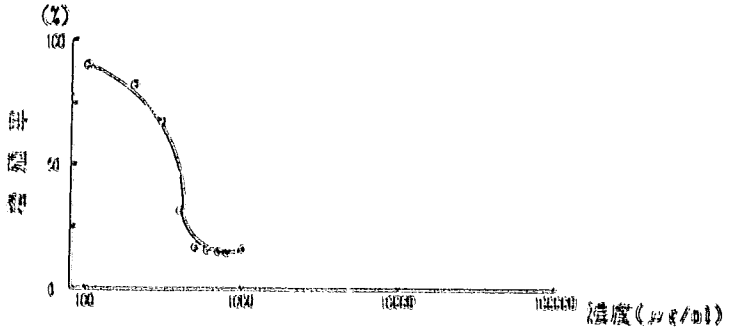
被験物質名 X-12-990S　　X06-0205

処理	S9 mix 有無	処理濃度 (μg/ml)	観察細胞数	倍数体数	判定	染色体構造異常細胞の出現数と出現頻度(%) ギャップ g	染色分体型 ctb	染色分体型 cte	染色体型 csb	染色体型 cse	その他	合計 -g	合計 +g	判定
溶媒 [DMSO]	-	0	100	1	-	0	0	1	1	0	0	2	2	-
			100	1		0	0	0	0	0	0	0	0	
			200	2 (1.0)		0 (0.0)	0 (0.0)	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)	2 (1.0)	2 (1.0)	
	*	0	100	1	-	0	0	0	0	0	0	0	0	-
			100	0		0	0	0	0	0	0	0	0	
			200	1 (0.5)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
被験物質	-	100	100	1	-	1	0	0	0	0	0	0	1	-
			100	1		0	0	0	0	0	0	0	0	
			200	2 (1.0)		1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	
		150	100	0	-	0	1	0	0	0	0	1	1	-
			100	1		0	0	0	0	1	0	1	1	
			200	1 (0.5)		0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	2 (1.0)	2 (1.0)	
		200	100	3	-	1	1	1	0	1	0	3	4	-
			100	0		0	1	1	0	0	0	2	2	
			200	3 (1.5)		1 (0.5)	2 (1.0)	2 (1.0)	0 (0.0)	1 (0.5)	0 (0.0)	5 (2.5)	6 (3.0)	
	*	100	100	2	-	0	1	0	1	1	0	3	3	-
			100	0		1	0	1	0	0	0	1	1	
			200	2 (1.0)		1 (0.5)	1 (0.5)	1 (0.5)	1 (0.5)	1 (0.5)	0 (0.0)	4 (2.0)	4 (2.0)	
		150	100	0	-	2	5	15	1	1	0	18	19	*
			100	3		2	8	14	0	0	0	18	20	
			200	3 (1.5)		4 (2.0)	13 (6.5)	29 (14.5)	1 (0.5)	1 (0.5)	0 (0.0)	36 (18.0)	39 (19.5)	
		200	100	3	-	3	15	41	0	0	0	43	43	**
			100	0		3	12	41	0	0	0	45	46	
			200	3 (1.5)		3 (1.5)	27 (13.5)	82 (41.0)	0 (0.0)	0 (0.0)	0 (0.0)	88 (44.0)	89 (44.5)	
陽性対照 [CPA]	-	10	100	1	-	0	0	0	1	0	0	1	1	-
			100	2		0	0	0	0	0	0	0	0	
			200	3 (1.5)		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	1 (0.5)	1 (0.5)	
	*	10	100	1	-	1	4	24	4	0	0	29	30	**
			100	0		0	7	17	2	0	0	23	23	
			200	1 (0.5)		1 (0.5)	11 (5.5)	41 (20.5)	6 (3.0)	0 (0.0)	0 (0.0)	52 (26.0)	53 (26.5)	

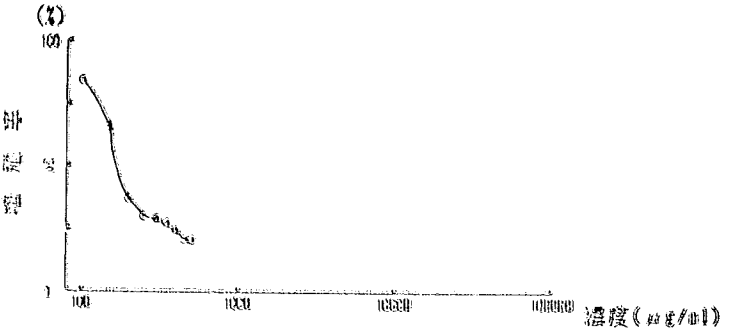
S9の用量(5%)、被験物質処理時間（6h）、被験物質処理後の細胞回復時間（18h）



直接法24時間処理



直接法48時間処理



代謝活性化法

図1　X－12－990Sの細胞増殖阻害試験結果

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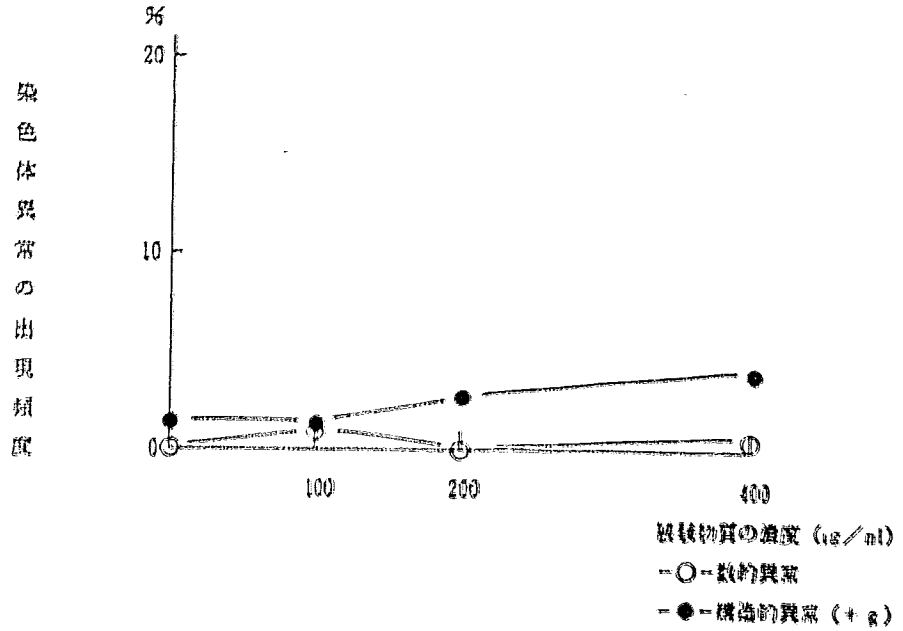
0·1·0 b

A．代謝活性化法によらない場合

a．２４時間処理



b．４８時間処理

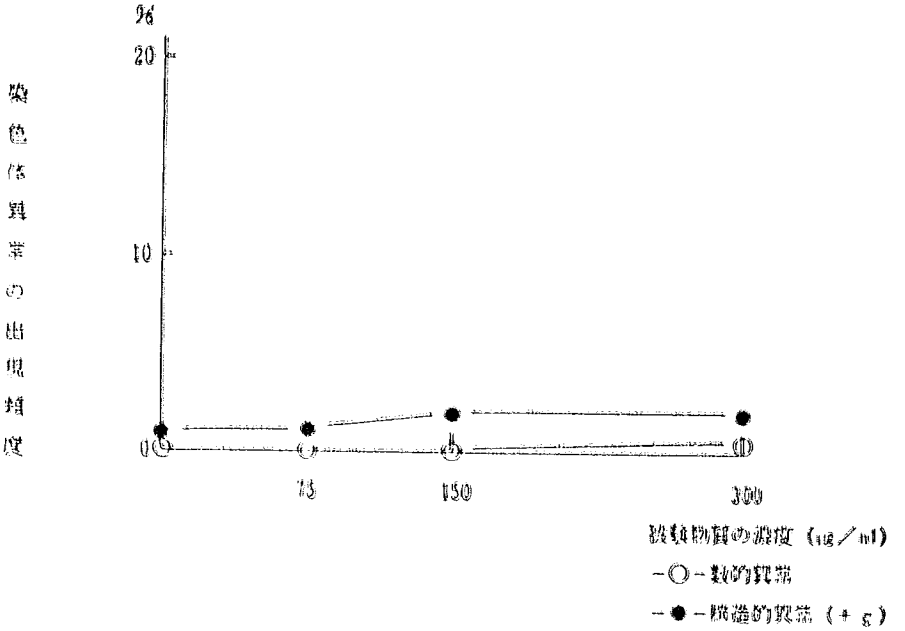
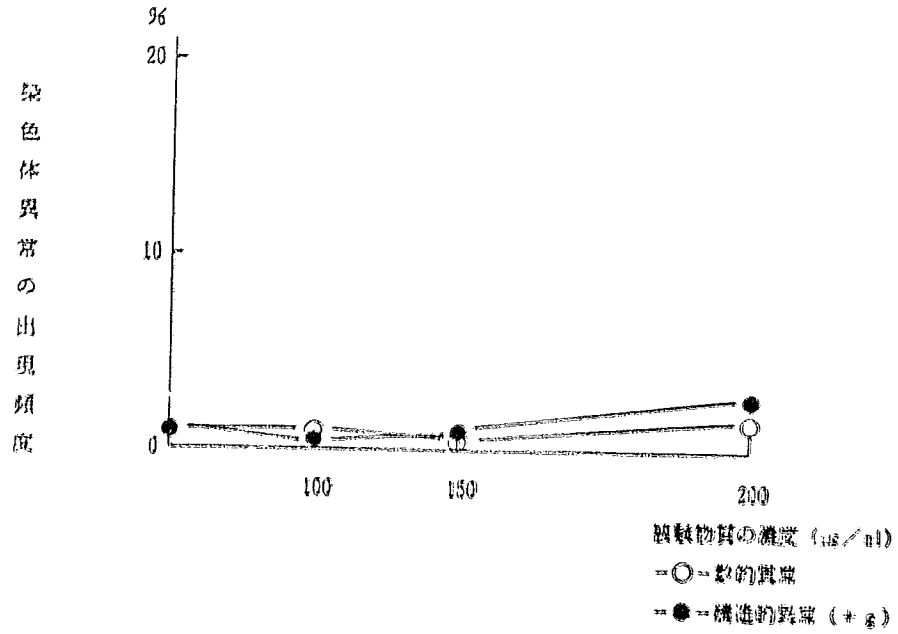


図２　Ｘ－１２－９９０５の染色体異常試験結果

B. 代謝活性化法による場合

a. S9 Mix無



b. S9 Mix有

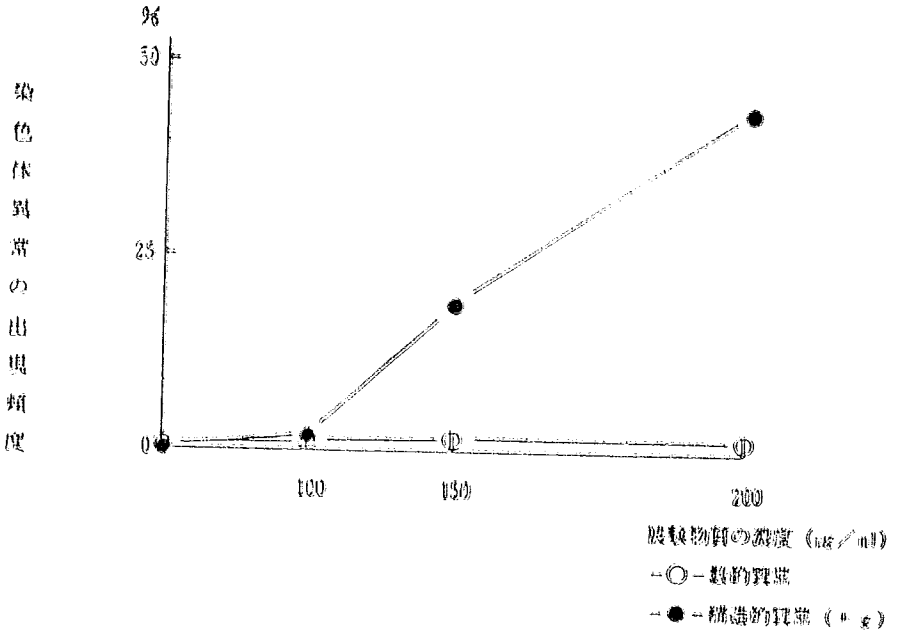
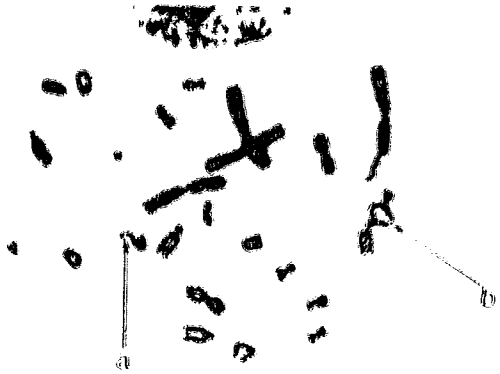


図3 X－12－990Sの染色体異常試験結果

35

0108

対　照（代謝活性化法　溶媒対照）



構造的異常（代謝活性化法　２００mg／ml）

a、染色分体型切断

b、染色分体型交換

図４　Ｘ－１２－９９０Ｓにより誘発された染色体異常

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0109

業　務　分　担　表

表　題　X－12－990Sの哺乳類培養細胞を用いる染色体異常試験

氏　　名	業　務　内　容
試験責任者 堀　原　美　次	試験の計画、実施など試験全般の管理、監査、結果の分析・評価及び報告書の作成を行う。
試験担当者 小　泉　正　造	試験計画書の作成、試験物質による処理、標本の作製、観察、報告書草案の作成を行う。
細胞第1部門長 堀　原　美　次	細胞第1部門のすべての業務に対し責任をもつ。作業の正確な実施、検査及び作業にたずさわる職員の教育、訓練を行う。

陳　述　書

財団法人　化学品検査協会
化学品安全センター日田研究所

試験委託者：信越化学工業株式会社

試験の表題：X-12-990S のほ乳類培養細胞を用いる染色体異常試験

試験コード番号：K06-0265

上記試験は、昭和59年3月31日付、環保業第39号、薬発第229号及び59基局第85号並びに昭和63年11月18日付、環企研第233号、衛生第38号及び63基局第823号による「新規化学物質に係る試験及び指定化学物質に係る有害性の調査の項目等を定める命令第4条に規定する試験施設について」及び「OECD Principles of Good Laboratory Practice (May 12, 1981)」に従って実施したものである。

1992年1月30日

運営管理者　山本武人

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信頼性保証書

財団法人　化学品検査協会
化学品安全センター日田研究所

試験委託者：　信越化学工業株式会社

試験の表題：　X-12-9908 のほ乳類培養細胞を用いる染色体異常試験

試験コード番号：　K06-0265

当試験は財団法人化学品検査協会化学品安全センター日田研究所の信頼性保証部門が監査又は査察を実施しており、監査又は査察を行った日付、運営管理者及び試験責任者に報告を行った日付は以下の通りである。

監査又は査察日	試験責任者報告日	運営管理者報告日
1991年　9月　30日	1991年　9月　30日	1991年　9月　30日
1991年　11月　21日	1991年　11月　22日	1991年　11月　25日
1991年　12月　16日	1991年　12月　18日	1991年　12月　18日
1991年　12月　18日	1991年　12月　18日	1991年　12月　18日
1992年　1月　23日	1992年　1月　23日	1992年　1月　28日
1992年　1月　30日	1992年　1月　30日	1992年　1月　30日

本報告書には、試験で使用した方法、手順が正確に記載されており報告結果は試験の生データを正確に反映している。

1992 年 1 月 30 日
信頼性保証部門員　白石圭二

1992 年 1 月 30 日
信頼性保証部門長　藤井勇作

[28-Day Oral Toxicity (Repeat Dose) Study Note: 63 Pages of Diagrams and Notes Were Removed]

Translated from Japanese by the Ralph McElroy Co., Custom Division
P. O. Box 4828, Austin, Texas 78765 USA

191 pgs

Code: 270-17582

No. D-3157 (KA)

28-day oral toxicity (repeat dose) study on mammals

1. General items

新規化学物質の名称（IUPAC命名法による）	1,1-及び1,2-ビス（トリメトキシシリル）エタンの混合物				
別名	X-12-990S	物理化学的性状	分子量		270.43
構造式又は示性式（いずれも不明の場合は、その製法の概要）	*2		常温における性状		無色透明液体
			密度		*1
			融点		—
			沸点		100～110℃/5mmHg
			蒸気圧		—
			分配係数		—
ロット番号	105027		溶解性		難溶性
			溶解度	水	不溶
試験に供した新規化学物質の純度	99%以上			DMSO	可溶
				アセトン	可溶
不純物の名称及び純度	—			その他	—

*1 Stable during the administration period

*2 Formula

$$(CH_3O)_3\text{-}Si\text{-}CH_2CH_2\text{-}Si\text{-}(OCH_3)_3$$

$$(CH_3O)_3\text{-}Si\text{-}\underset{\underset{CH_3}{|}}{CH}\text{-}Si\text{-}(OCH_3)_3$$

Key:
1 Name of new chemical (named according to IUPAC)
2 Mixture of 1,1- and 1,2-bis(trimethoxysilyl)ethane
3 Other name
4 Structural formula or empirical characteristic formula (when both are unclear, an abstract of its manufacturing method)
5 Lot No.
6 Purity of the new chemical used in experiment
7 Name and concentration of impurity
8 Over 99%
9 Physicochemical properties
10 Molecular weight
11 State at room temperature
12 Stability
13 Melting point
14 Boiling point
15 Vapor pressure
16 Distribution coefficient
17 Dissolution property
18 Solubility
19 Water
20 Acetone
21 Others
22 Colorless clear fluid
23 Oil soluble
24 Insoluble
25 Soluble

2. Acute toxicity experiment

試験 (1) No.	試験の種類 (2) 及び期間	(3) 動物種	1群当たり (4) の動物数	投与 (5) 経路	投与量 (6) (mg/kg)	LD50値又はNOEL* (7) (mg/kg)	実験 (8) 場所
1	14日間反復投与予備試験 (9)	ラット (10)	雄 4匹 (11) (12) 雌 4匹 (12) (13)	経口 (14)	1,000 250 50 (15) #対照 (mg/kg/day) (19)	異常あり(死亡) (16) 異常あり(死亡) (16) 異常あり (17) 異常なし (18) NOEL*<50mg/kg (観察項目：体重、一般状態、血液学的検査、血液化学的検査、器官重量、剖検、病理組織学的検査)	財団法人化学品検査協会 化学品安全センター目黒研究所 (20)

* NOEL : no-observed-effect level

\# Olive oil

Key:
1 Test No.
2 Type and period of test
3 Species of animal
4 Number of animals in one group
5 Means of administration
6 Dose
7 LD_{50} value or NOEL*
8 Test location
9 14-day repeat administration preparatory test
10 Rats
11 Male
12 Female
13 4 rats
14 Oral
15 Control

16 Abnormal (death)
17 Abnormal
18 Not abnormal
19 (Observation items: body weight, general state, blood examination, blood chemistry examination, organ weight, postmortem examination, histopathological examination)
20 Hita Research Lab., Chemical Safety Center, Chemical Inspection Association

General state:

(Male) Salivation for the groups with dose of 50 mg/kg or higher.

For groups with dose of 250 mg/kg or higher: decrease in spontaneous activity, reduction in breathing frequency, blepharoptosis, anemia, hypothermia, piloerection, lacrimation; for the group with dose of 250 mg/kg: crouching, nose bleeding, hardening of the covering hair, and with 4 deaths; for the group with dose of 1,000 mg/kg: pronation was observed, with 4 deaths.

(Female) Salivation for the groups with dose of 50 mg/kg or higher.

For groups with dose of 250 mg/kg or higher: decrease in spontaneous activity, reduction in breathing frequency, blepharoptosis, anemia; for the group with dose of 250 mg/kg: 4 deaths; for the group with dose of 1,000 mg/kg: pronation, hypothermia, lacrimation, and brown urine were observed, with 4 deaths.

contents in alimentary canal, red mucosa of proventriculus, red mucosa of small intestine, enlargement of adrenal gland, dropsy of pancreas. For the group with dose of 1,000 mg/kg, the following were observed: contamination of periphery of nose, intrathoracic pooling of clear fluid, disseminated white spots on liver surface, black portion of liver, black portion of glandular stomach, enlargement of lumen of duodenum, mucous-like substance in lumen, red mucosa.

(Female) For the group with dose of 50 mg/kg or higher: interlobar adhesion of liver. For the group with dose of 250 mg/kg or higher: intrathoracic pooling of clear fluid, liver sclerosis, disseminated white spots of proventriculus mucosa. For the group with dose of 250 mg/kg: intraperitoneal pooling of red fluid, intrathoracic pooling of red fluid, contamination of periphery of nose and mouth, white portion of liver, dark red portion of lung, red [portion of lung], white foam-like substance in lumen of windpipe, proventriculus mucosal excavation, red portion of mucosa, red portion of glandular stomach mucosa, red portion of mucosa of small intestine, enlargement of adrenal gland. For the group with dose of 1,000 mg/kg: red portion of glandular stomach mucosa.

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被験物質の純度 ≧99%	投与量	mg/kg	対照群 0 ♂	対照群 0 ♀	低用量群 8 ♂	低用量群 8 ♀	中用量群 30 ♂	中用量群 30 ♀	中用量群 60 ♂	中用量群 60 ♀	高用量群 120 ♂	高用量群 120 ♀	回復群 0 ♂	回復群 0 ♀	回復群 60 ♂	回復群 60 ♀	回復群 120 ♂	回復群 120 ♀
組織所見(生存例)	肝臓 うっ血		–	–	–	–	–	–	1/4	–	–	–	–	–	2/3			1/2
	腎外道血		–	–	–	–	–	–	1/4	–	–	–	–	–	–			–

Body weight:

(Male) Decrease in the body weight was observed for groups with dose of 250 mg/kg or higher.

(Female) Decrease in the body weight was observed for groups with dose of 250 mg/kg or higher.

Blood examination:

(Male) Decrease in leukocytes was observed for the group with dose of 50 mg/kg.

Blood chemistry examination:

(Female) Increase in A/G ratio was observed for the group with dose of 50 mg/kg.

Organ weight:

(Male) Relative increase in the weight of liver was observed for the group with dose of 50 mg/kg.

Postmortem examination

(Male) For the group with dose of 250 mg/kg or higher, the following were observed: contamination in the vicinity of nose and mouth, sclerosis of liver, interlobar adhesion, discoloration of heart, disseminated white spots of proventriculus mucosa, red portion of mucosa, red mucosa of glandular stomach. For the group

with dose of 250 mg/kg, the following were observed: anemia, intraperitoneal pooling of red fluid, intrathoracic pooling of red fluid, contamination of periphery of mouth, subcutaneous dropsy, black substance on the surface of liver, white portion [of liver], enlargement of spleen, kidney enlargement, dark red lung, dropsy, white foam-like substance in lumen of windpipe, red contents in alimentary canal, red mucosa of proventriculus, red mucosa of small intestine, enlargement of adrenal gland, dropsy of pancreas. For the group with dose of 1,000 mg/kg, the following were observed: contamination of periphery of nose, intrathoracic pooling of clear fluid, disseminated white spots on liver surface, black portion of liver, black portion of glandular stomach, enlargement of lumen of duodenum, mucous-like substance in lumen, red mucosa.

(Female) For the group with dose of 50 mg/kg or higher: interlobar adhesion of liver. For the group with dose of 250 mg/kg or higher: intrathoracic pooling of clear fluid, liver sclerosis, disseminated white spots of proventriculus mucosa. For the group with dose of 250 mg/kg: intraperitoneal pooling of red fluid, intrathoracic pooling of red fluid, contamination of periphery of nose and mouth, white portion of liver, dark red portion of lung, red [portion of lung], white foam-like substance in lumen of windpipe, proventriculus mucosal excavation, red portion of mucosa, red portion of glandular stomach mucosa, red portion of mucosa of small intestine, enlargement of adrenal gland. For the group with dose of 1,000 mg/kg: red portion of glandular stomach mucosa.

Histological examination

(Male) For the group with dose of 250 mg/kg or higher: centrolobular hepatic cell necrosis over wide range of liver, hemostasis, interlobar adhesion, hemostasis of lung, enlargement of cortical cells of adrenal gland, ulcer of proventriculus. For the group with dose of 250 mg/kg: extramedullary hematopoiesis of liver, increase of extramedullary hematopoiesis of spleen, epithelium cavitation of urinary microtubules of kidney, thecalutein in pulmonary alveoli of lung, bleeding of proventriculus. For the group with dose of 1,000 mg/kg: localized hepatic cell necrosis, dropsy of lung.

(Female) For the group with dose of 50 mg/kg or higher: interlobar adhesion of liver. For the group with dose of 250 mg/kg or higher: hemostasis of liver, hemostasis of lung, dropsy, cortical cell enlargement of adrenal gland, ulcer of proventriculus. For the group with dose of 250 mg/kg: centrolobular hepatic cell necrosis over wide range of liver, extramedullary hematopoiesis, increase in extramedullary hematopoiesis of spleen, epithelium cavitation of urinary microtubules, proventriculus mucosal necrosis, bleeding, mucosal necrosis of glandular stomach. For the group with dose of 1,000 mg/kg, localized hepatic cell necrosis.

3.　２８日間反復投与毒性試験 (1)

被験物質投与期間 (1a)	自平成　4年　3月　4日 (2)　至平成　4年　3月31日	
使用動物種 (3) 系統	ラット，Sprague-Dawley　Cr (4)	1群当たりの動物数 (5)
投与経 (6) 路	強制経口投与（オリーブ油） (7)	雄：　6匹 (5)　雌：　6匹

被験物質の純度 ≧99% (9)	投与量 (15) mg/kg	対照群 (26) ♂	対照群 ♀	低用量群 8 (26) ♂	低用量群 ♀	中用量群 30 (31) ♂	中用量群 ♀	中用量群 60 (31) ♂	中用量群 ♀	高用量群 120 (32) ♂	高用量群 ♀	回復群 (33) 0 ♂	回復群 ♀	回復群 60 ♂	回復群 ♀	回復群 120 ♂	回復群 ♀
体重変化 (16) (投与期間)				−	−	−	−	−	−	−	−						
体重変化 (11) (回復期間)	day5、10													−		↓	−
	day14													−			↓
摂餌量 (12) (投与期間)				−	−	−	−	−	−	−	−						
摂餌量 (13) (回復期間)	day 8													↓		↓	−
	day 14													↓			−
一般状態 (14)	流涙 (16)	−	−	1/6	3/6	5/6	2/6	9/12	9/12	8/12	7/12	−	−	−	−	−	−
	自発運動低下 (17)	−	−	1/6	−	−	−	2/12	5/12	10/12	10/12	−	−	1/4	1/2	1/1	1/3
	うずくまり (18)	−	−	−	−	−	−	1/12	3/12	1/12	1/12	−	−	−	−	1/1	−
	腹臥 (19)	−	−	1/6	−	−	−	2/12	3/12	2/12	2/12	−	−	−	−	−	−
	呼吸数減少 (20)	−	−	−	−	−	−	−	2/12	4/12	4/12	−	−	−	−	−	1/3
	深大呼吸 (21)	−	−	−	−	−	−	3/12	6/12	2/12	5/12	−	−	2/4	1/2	1/1	1/3
	呼吸数増加 (22)	−	−	−	−	−	−	−	−	1/12	−	−	−	1/4	−	−	−
	貧血 (23)	−	−	−	−	−	−	4/12	8/12	6/12	3/12	−	−	2/4	2/2	1/1	2/3
	体温低下 (24)		−	−	−	−	−	4/12	7/12	5/12	3/12	−	−	2/4	2/2	1/1	1/3
	立毛 (25)	−	−	−	−	−	−	2/12	2/12	1/12	1/12	−	−	2/4	−	1/1	1/3
	流涙 (26)	−	−	−	−	−	−	−	3/12	3/12	2/12	−	−	−	−	1/1	1/3
	紅涙 (27)	−	−	−	−	−	−	−	1/12	−	−	−	−	−	−	1/1	−
	鼻出血 (28)	−	−	−	−	−	−	2/12	1/12	−	−	−	−	2/4	−	1/1	−

(34) **：　被験物質投与期間＜（自平成 4年 3月 4日　至平成 4年 3月17日）（14日間、15日目切迫屠殺）＞
統計学的な有意差検定は行われていない。

***：　被験物質投与期間＜（自平成 4年 3月 4日　至平成 4年 3月17日）（14日間）＞

0 1 2 3

Key to previous page:

1 3. 28-day toxicity (repeat dose) study
1A Period of administration of test substance
2 March 4, 1992 to March 31, 1992
3 Species/system of test animal
4 Rats
5 Number of animals of each group
6 Method of administration
7 Forced oral administration (olive oil)
8 Male: 6 rats; female: 6 rats
9 Purity of test substance ≥ 99%
10 Variation in body weight (during administration)
11 Variation in body weight (recovery period)
12 Food consumption (during the administration period)
13 Food consumption (during recovery period)
14 General state
15 Dose
16 Salivation
17 Decrease in spontaneous activity
18 Crouching
19 Pronation
20 Decrease in breathing frequency
21 Deep breathing
22 Increase of breathing frequency
23 Anemia
24 Hypothermia
25 Piloerection
26 Lacrimation
27 Red tears
28 Nose bleeding
29 Control group
30 Low-dose group
31 Medium-dose group
32 High-dose group
33 Recovery group
34 **: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days, sacrificed on the 15th day by manual strangulation)).
Statistically significant difference test was not performed.
***: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days)).
The recovery period (from March 18, 1992 to March 31, 1992 (a period of 14 days)).
Statistically significant difference test was not performed.

被験物質の純度 ≥99%	投与量 mg/kg	対照群 0 ♂	対照群 0 ♀	低用量群 8 ♂	低用量群 8 ♀	中用量群 30 ♂	中用量群 30 ♀	中用量群 60 ♂	中用量群 60 ♀	高用量群 120** ♂	高用量群 120** ♀	回復群 0 ♂	回復群 0 ♀	回復群 60 ♂	回復群 60 ♀	回復群 120*** ♂	回復群 120*** ♀
一般状態	混濁及び(又は)赤色尿	–	–	–	–	–	–	1/12	1/12	–	–	–	–	1/4	–	1/1	–
	眼瞼下垂	–	–	–	–	–	–	–	1/12	–	–	–	–	–	–	–	–
	耳介の発赤	–	–	–	–	–	–	–	1/12	–	–	–	–	–	–	–	–
	脱毛	2/12	2/12	–	–	–	–	2/12	–	–	–	2/6	1/6	2/4	–	–	–
	軟便	–	–	–	–	–	–	–	–	–	1/12	–	–	–	–	–	–
	異常呼吸音	1/12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	異常呼吸	1/12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	あえぎ呼吸	–	–	1/6	–	–	–	–	–	–	–	–	–	–	–	–	–
	チアノーゼ	–	–	1/6	–	–	–	–	–	–	–	–	–	–	–	–	–
	切迫屠殺	–	–	–	–	–	–	–	–	1/12	2/12	–	–	–	–	–	–
	死亡	1/12	–	–	–	–	–	4/12	9/12	10/12	7/12	–	–	1/4	2/2	1/1	1/3
血液学的検査	赤血球数			–	–	–	–	–	–	–	–			–			↓
	白血球数			–	–	–	–	–	–	↑	–			–			↓
	ヘモグロビン濃度			–	–	–	–	–	–	–	–			–			↓
	ヘマトクリット値			–	–	–	–	–	–	–	–			–			↓
	平均赤血球容積			–	–	▽	–	▼	–	–	–			–			–
	平均赤血球ヘモグロビン量			–	–	–	–	▽	–	–	–			–			–
	平均赤血球ヘモグロビン濃度			–	▽	–	▽	–	–	–	–			–			–
	血小板数			–	–	–	▽	↓	–	–	–			▽			↓
	網状赤血球数			–	–	–	–	–	–	↑	↑			–			–
	プロトロンビン時間			–	–	–	–	↑	–	–	–			↑			↑
	活性化部分トロンボプラスチン時間			–	–	–	–	↑	–	–	–			↑			↑
	リンパ球比率			–	–	–	–	–	–	–	–			–			↓
	分葉核好中球比率			–	–	–	–	–	–	–	–			–			↑

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***: 被験物質投与期間 < (自平成 4年 3月 4日 至平成 4年 3月17日) (14日間) >

Key to previous page

1 Purity of test substance
2 Dose
3 Control group
4 Low-dose group
5 Medium-dose group
6 High-dose group
7 Recovery group
8 General state
9 Blood examination
10 Cloudy and/or red urine
11 Blepharoptosis
12 Flushing of auricle
13 Trichomadesis
14 Soft stool
15 Abnormal breathing sound
16 Abnormal breathing
17 Gasping breathing
18 Cyanosis
19 Sacrifice by manual strangulation
20 Death
21 Red blood cell count
22 White blood cell count
23 Hemoglobin concentration
24 Hematocrit value
25 Mean red blood cell volume
26 Mean red blood cell hemoglobin level
27 Mean red blood cell hemoglobin concentration
28 Platelet count
29 Reticular red blood cell count
30 Prothrombin time
31 Activated partial thromboplastin time
32 Lymphocyte proportion
33 Segmented neutrophil proportion
34 **: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days, sacrificed on the 15th day by manual strangulation)).
Statistically significant difference test was not performed.
***: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days)).
The recovery period (from March 18, 1992 to March 31, 1992 (a period of 14 days)).
Statistically significant difference test was not performed.

被験物質の純度 ≧99%	投与量 mg/kg	対照群 0 ♂	対照群 0 ♀	低用量群 8 ♂	低用量群 8 ♀	中用量群 30 ♂	中用量群 30 ♀	中用量群 60 ♂	中用量群 60 ♀	高用量群 120** ♂	高用量群 120** ♀	回復群 0 ♂	回復群 0 ♀	回復群 60 ♂	回復群 60 ♀	回復群 120 ♂	回復群 120 ♀
血液化学的検査	GOT			−	−	−	−	↑	−	−	↑			↑			↑
	GPT			−	−	−	−	↑	−	−	−			△			↑
	アルカリ性フォスファターゼ			−	−	−	−	↑	−	−	−			−			−
	γ-GTP			−	−	−	−	−	−	−	−			▼			−
	総コレステロール			−	▽	−	−	−	−	−	−			−			−
	尿素窒素			−	−	−	−	↑	−	−	−			△			↑
	クレアチニン			−	−	−	−	−	−	−	−			△			−
	クロライド			−	▽	−	−	▽	−	−	−			▽			−
尿検査	蛋白の増加			○	−	○	−	○	−	*	*			○			−
	潜血			−	−	○	−	○	−	*	*			−			−
	尿の濁り			−	−	−	−	−	−	*	*			○			−
器官重量(絶対重量)	副腎			−	−	−	−	−	−	−	−			▲			↑
器官重量(相対重量)	肝臓			−	−	−	−	△	−	↑	↑			−			↑
	心臓			−	−	−	−	−	−	*	*			−			↑
	副腎			−	−	−	−	−	−	−	−			−			↑
剖検所見(生存例)	貧血	−	−	−	−	−	−	1/4	−	−	−	−	−	1/3			1/2
	脱毛	−	1/6	−	−	−	−	−	−	−	−	−	−	−			−
	鼻周囲の汚染	−	−	−	−	−	−	−	−	−	−	−	−	1/3			−
	胸腹内透明液貯留	−	−	−	−	−	−	1/4	−	−	−	−	−	2/3			1/2

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***: 被験物質投与期間 <(自平成4年3月4日 至平成4年3月17日)(14日間)>
回復期間 <(自平成4年3月18日 至平成4年3月31日)(14日間)>
統計学的な有意差検定は行われていない。

Key to previous page

1 Purity of test substance
2 Dose
3 Control group
4 Low-dose group
5 Medium-dose group
6 High-dose group
7 Recovery group
8 Blood chemistry examination
9 Urine examination
10 Organ weight (absolute weight)
11 Organ weight (relative weight)
12 Findings of postmortem examination (surviving cases)
13 Alkaline phosphatase
14 Total cholesterol
15 Urea nitrogen
16 Creatinine
17 Chloride
18 Increase in protein
19 Occult blood
20 Cloudiness of urine
21 Adrenal gland
22 Liver
23 Heart
24 Adrenal gland
25 Anemia
26 Trichomadesis
27 Contamination of periphery of nose
28 Intrathoracic pooling of clear fluid
29 **: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days, sacrificed on the 15th day by manual strangulation)).
Statistically significant difference test was not performed.
***: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days)).
The recovery period (from March 18, 1992 to March 31, 1992 (a period of 14 days)).
Statistically significant difference test was not performed.

被験物質の純度 ≧99%	投与量 mg/kg	対照群 0 ♂	対照群 0 ♀	低用量群 8 ♂	低用量群 8 ♀	中用量群 30 ♂	中用量群 30 ♀	中用量群 60 ♂	中用量群 60 ♀	高用量群 120** ♂	高用量群 120** ♀	回復群 0 ♂	回復群 0 ♀	回復群 60 ♂	回復群 60 ♀	回復群 120 ♂	回復群 120 ♀
剖検所見 (生存例)	肝臓																
	播種性白色斑	–	–	–	–	–	–	2/4	–	–	–	–	–	1/3		–	
	播種性白色点	–	–	–	–	–	–	–	–	–	–	–	–	–			1/2
	腫大	–	–	–	–	–	–	3/4	–	–	–	–	–	–			1/2
	表面線維状物付着	–	–	–	–	–	–	–	–	–	–	–	–	1/3		–	
	硬化	–	–	–	–	–	–	1/4	–	–	–	–	–	1/3		–	
	葉間歯肉	–	–	–	–	–	–	3/4	–	1/1	2/2	–	–	3/3		–	
	白色部	–	–	–	–	–	–	1/4	–	1/1	–	–	–	–		–	
	腎臓																
	腎盂拡張	–	–	–	–	–	–	–	–	–	–	1/6	–	–		–	
	心臓																
	退色	–	–	–	–	–	–	2/4	–	–	–	–	–	2/3			1/2
	白色部	–	–	–	–	1/6	–	–	–	–	–	–	–	–			–
	白色点	–	–	–	–	–	–	–	–	–	1/2	–	–	–			–
	腺胃																
	粘膜黒色部	1/5	–	–	–	1/6	–	1/4	–	–	–	–	–	–			–
	粘膜陥凹部	–	–	–	1/6	–	–	–	–	–	–	–	–	–			–
	膵臓																
	水腫	–	–	–	–	–	–	–	–	–	–	–	–	1/3			–
	胸腺																
	水腫	–	–	–	–	–	–	–	–	–	–	–	–	1/3			–
	腹腔リンパ節																
	腫大	–	–	–	–	–	–	1/4	–	–	–	–	–	–			–
剖検所見 (死亡例)	貧血	–						–	–	2/10	1/7			–	–	–	–
	鼻出血	–						–	–	–	–			–	–	1/1	–
	腹腔内赤色液貯留	–						–	–	2/10	–			–	–	–	–
	胸腔内赤色液貯留	–						–	–	2/10	–			–	–	1/1	–

**: 被験物質投与期間 < (自平成 4年 3月 4日 至平成 4年 3月17日) (14日間)、15日目切迫屠殺 >
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***: 被験物質投与期間 < (自平成 4年 3月 4日 至平成 4年 3月17日) (14日間) >
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統計学的な有意差検定は行われていない。

Key to previous page

1 Purity of test substance
2 Dose
3 Control group
4 Low-dose group
5 Medium-dose group
6 High-dose group
7 Recovery group
8 Findings of postmortem examination (surviving cases)
9 Findings of postmortem examination (dead cases)
10 Liver:
11 Disseminated white portion
12 Disseminated white spots
13 Enlargement
14 Attachment of fiber-like substance on surface
15 Sclerosis
16 Interlobar adhesion
17 White portion
18 Kidney: pyelectasis
19 Heart: discoloration
20 White portion
21 White spots
22 Glandular stomach: mucosal black portion
23 Mucosal excavation
24 Pancreas: dropsy
25 Thymus gland: dropsy
26 Abdominal cavity lymphocyte: enlargement
27 Anemia
28 Nose bleeding
29 Intraperitoneal pooling of red fluid
30 Intrathoracic pooling of red fluid
31 **: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days, sacrificed on the 15th day by manual strangulation)).
Statistically significant difference test was not performed.
***: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days)).
The recovery period (from March 18, 1992 to March 31, 1992 (a period of 14 days)).
Statistically significant difference test was not performed.

被験物質の純度 ≥99% (8)	投与量 mg/kg	対照群 (3) 0 ♂	対照群 (3) 0 ♀	低用量群 (4) 8 ♂	低用量群 (4) 8 ♀	中用量群 (5) 30 ♂	中用量群 (5) 30 ♀	中用量群 (5) 60 ♂	中用量群 (5) 60 ♀	高用量群 (6) 120** ♂	高用量群 (6) 120** ♀	回復群 (7) 0 ♂	回復群 (7) 0 ♀	回復群 (7) 60 ♂	回復群 (7) 60 ♀	回復群 (7) 120 ♂	回復群 (7) 120 ♀
剖検所見 (死亡例) (8)	紅斑 (9)	–						–	–	–	–			–	–	1/1	–
	鼻周囲の汚染 (10)	–						1/4	1/9	–	–			–	–	–	–
	鼻・口周囲の汚染 (11)	–						–	–	1/10	–			–	–	–	–
	腹腔内透明液貯留 (12)	–						–	2/9	1/10	–			1/1	–	1/1	–
	胸腔内透明液貯留 (13)	–						3/4	3/9	6/10	3/7			1/1	–	–	1/1
	肝臓 (14) 褐色化	–						–	–	1/10	–			–	–	–	–
	斑状性白色部 (15)	–						–	–	1/10	–			–	–	–	–
	斑状性白色点 (16)	–						–	–	–	–			1/1	1/2	–	–
	腫大 (17)	–						4/4	8/9	9/10	5/7			1/1	2/2	–	1/1
	表面線維状物付着 (18)	–						–	–	1/10	–			–	–	–	–
	表面顆粒状化 (19)	–						–	1/9	–	–			–	–	–	–
	硬化 (20)	–						3/4	2/9	8/10	4/7			1/1	–	1/1	1/1
	境界不鮮明 (21)	–						3/4	8/9	10/10	5/7			–	–	1/1	–
	白色部 (22)	–						3/4	2/9	7/10	1/7			–	–	–	–
	脾臓 (23) 黒色部	–						1/4	–	–	–			–	–	–	–
	腫大 (24)	–						–	–	5/10	–			–	–	–	–
	萎縮 (25)	–						–	–	–	–			–	–	1/1	–
	腎臓 (26) 腎盂拡張	–						–	1/9	–	–			–	–	–	–
	腫大 (27)	–						1/4	–	–	–			–	–	–	–
	心臓 (28) 退色	–						2/4	8/9	1/10	1/7			–	2/2	–	–
	斑状性白色点 (29)	–							–	–	–			1/1	–	–	–
	白色部 (30)	–						–	–	1/10	–			–	–	–	–

(31)

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回復期間 <(自平成 4年 3月18日 至平成 4年 3月31日) (14日間) >

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Key to previous page

1 Purity of test substance
2 Dose
3 Control group
4 Low-dose group
5 Medium-dose group
6 High-dose group
7 Recovery group
8 Findings of postmortem examination (dead cases)
9 Red tears
10 Contamination of periphery of nose
11 Contamination of periphery of nose and mouth
12 Intraperitoneal pooling of clear fluid
13 Intrathoracic pooling of clear fluid
14 Liver: Brown coloration
15 Disseminated white portion
16 Disseminated white spots
17 Enlargement
18 Attachment of fiber-like substance on surface
19 Granulation of surface
20 Sclerosis
21 Interlobar adhesion
22 White portion
23 Spleen: black portion
24 Enlargement
25 Becoming shorter
26 Kidney: pyelectasis
27 Enlargement
28 Heart: discoloration
29 Disseminated white spots
30 White portion
31 **: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days, sacrificed on the 15th day by manual strangulation)).
Statistically significant difference test was not performed.
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被験物質の純度 ≧99%	投与量 mg/kg	対照群 0 ♂	対照群 0 ♀	低用量群 8 ♂	低用量群 8 ♀	中用量群 30 ♂	中用量群 30 ♀	中用量群 60 ♂	中用量群 60 ♀	高用量群 120** ♂	高用量群 120** ♀	回復群*** 0 ♂	回復群*** 0 ♀	回復群*** 60 ♂	回復群*** 60 ♀	回復群*** 120 ♂	回復群*** 120 ♀
剖検所見(死亡例)	肺																
	暗赤色化	–						1/4	2/9	4/10	4/7			1/1	–	1/1	–
	割面海綿状物	–						–	–	–	–			–	1/2	–	–
	赤色化	1/1						–	–	1/10	–			–	–	–	–
	気管																
	管腔内白色泡沫状物	–						–	1/9	2/10	1/7			1/1	–	–	–
	腺胃																
	粘膜黒色部	–						–	–	–	1/7			–	–	–	–
	粘膜黒色物付着	–						1/4	1/9	1/10	–			–	–	–	–
	粘膜暗赤色部	–						–	–	1/10	–			–	–	–	–
	粘膜淡赤色部	–						–	–	1/10	–			–	–	–	–
	粘膜赤色化	–						–	–	–	1/7			–	–	–	–
	粘膜赤色部	–						–	–	2/10	2/7			–	–	–	–
	空腸																
	管腔内赤褐色粘液状物	–						–	1/9					–	–	–	–
	回腸																
	管腔内赤褐色粘液状物	–						–	1/9	–	–			–	–	–	–
	精巣																
	暗赤色化	–						1/4		–				–	–	–	–
	矮小化	–						–		–				1/1		–	
	卵巣																
	暗赤色化								1/9		–				2/2		–
	副腎																
	腫大	–						–	–	–	–			1/1	–	–	–
	胸腺																
	矮小化	–						–	–	–	–			1/1	–	–	–

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Key to previous page

1 Purity of test substance
2 Dose
3 Control group
4 Low-dose group
5 Medium-dose group
6 High-dose group
7 Recovery group
8 Findings of postmortem examination (dead cases)
9 Lung: dark red coloration
10 Foam-like substance on cross section
11 Red coloration
12 Windpipe: white foam-like substance in lumen
13 Glandular stomach: mucosal black portion
14 Attachment of mucosal black substance
15 Mucosal dark red portion
16 Mucosal light red portion
17 Mucosal red coloration
18 Mucosal red portion
19 Jejunum: red-brown mucilform substance in lumen
20 Ileum: red-brown mucilform substance in lumen
21 Testicle: dark red coloration
22 Becoming shorter and smaller
23 Ovary: dark red coloration
24 Adrenal gland: enlargement
25 Thymus: becoming shorter and smaller
26 **: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days, sacrificed on the 15th day by manual strangulation)).
Statistically significant difference test was not performed.
***: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days)).
The recovery period (from March 18, 1992 to March 31, 1992 (a period of 14 days)).
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被験物質の純度 ≧99%	投与量 mg/kg	対照群 0 ♂	対照群 0 ♀	低用量群 8 ♂	低用量群 8 ♀	中用量群 30 ♂	中用量群 30 ♀	中用量群 60 ♂	中用量群 60 ♀	高用量群 120** ♂	高用量群 120** ♀	回復群 0 ♂	回復群 0 ♀	回復群 60 ♂	回復群 60 ♀	回復群 120 ♂	回復群 120 ♀
組織所見(生存例)	肝臓																
	うっ血	–	–	–	–	–	–	1/4	–	–	–	–	–	2/3			1/2
	髄外造血	–	–	–	–	–	–	1/4	–	–	–	–	–	–			–
	石灰化を伴う肉芽組織	–	–	–	–	–	–	2/4	–	1/1	–	–	–	–			–
	出血	–	–	–	–	–	–	1/4	–	–	–	–	–	1/3			–
	肝細胞内脂肪滴	3/5	4/6	3/6	5/6	3/6	4/6	3/4	1/1	–	2/2	2/6	2/6	2/3			1/2
	小肉芽腫	–	–	–	–	–	1/6	–	–	–	–	–	1/6	–			–
	肝細胞壊死	–	–	–	–	1/6	–	2/4	–	–	–	–	–	2/3			1/2
	被膜上結合組織増殖及びフィブリン滲出	–	–	–	–	–	–	–	–	1/1	1/2	–	–	2/3			1/2
	肝細胞腫大	–	–	–	–	–	–	3/4	–	1/1	–	–	–	2/3			–
	脾臓																
	髄外造血	–	–	*	*	–	–	1/4	–	–	–	–	–	–			–
	心臓																
	細胞変性を伴う筋線維壊死	–	–	–	–	3/6	1/6	2/4	–	1/1	1/2	–	–	1/3			1/2
	筋線維空胞化	–	–	–	–	–	–	2/4	–	1/1	–	–	–	2/3			1/2
	肺																
	細胞浸潤	–	1/6	*	*	–	–	–	–	–	–	–	–	–			–
	出血	–	1/6	*	*	–	–	–	–	–	–	–	–	–			–
	腺胃																
	粘膜壊死	1/5	–	*	–	1/6	–	1/4	–	–	–	–	–	–			–
	膵臓	*	*	*	*	*	*	*	*	*	*	*	*	–			*
	胸腺	*	*	*	*	*	*	*	*	*	*	*	*	–			*
	腹腔リンパ節洞内赤血球	*	*	*	*	*	*	1/1	*	*	*	*	*	*			*

**: 被験物質投与期間 <(自平成 4年 3月 4日 至平成 4年 3月17日) (14日間、15日目の剖検)>
統計学的な有意差検定は行われていない。

***: 被験物質投与期間 <(自平成 4年 3月 4日 至平成 4年 3月17日) (14日間)>
回復期間 <(自平成 4年 3月18日 至平成 4年 3月31日) (14日間)>
統計学的な有意差検定は行われていない。

Key to previous page

1 Purity of test substance
2 Dose
3 Control group
4 Low-dose group
5 Medium-dose group
6 High-dose group
7 Recovery group
8 Findings of postmortem examination (surviving cases)
9 Liver: Hemostasis
10 Extramedullary hematopoiesis
11 Granulation tissue accompanying petrifaction
12 Bleeding
13 Fatty droplets in hepatic cells
14 Small granuloma
15 Necrosis of hepatic cells
16 Hyperplasia of on-capsular connective tissue and fibrin seepage
17 Enlargement of hepatic cells
18 Spleen: extramedullary hematopoiesis
19 Heart: necrosis of muscle fibers accompanying cell reaction
20 Cavitation of muscle fibers
21 Lung: cell infiltration
22 Bleeding
23 Glandular stomach: necrosis of mucosa
24 Pancreas
25 Thymus
26 Peritoneal lymph node: intracavity red blood cells
27 **: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days, sacrificed on the 15th day by manual strangulation)).
Statistically significant difference test was not performed.
***: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days)).
The recovery period (from March 18, 1992 to March 31, 1992 (a period of 14 days)).
Statistically significant difference test was not performed.

被験物質の純度 ≧99%	投与量 mg/kg	対照群 0 ♂	対照群 0 ♀	低用量群 8 ♂	低用量群 8 ♀	中用量群 30 ♂	中用量群 30 ♀	中用量群 60 ♂	中用量群 60 ♀	高用量群 120 ♂	高用量群 120 ♀	回復群 0 ♂	回復群 0 ♀	回復群 60 ♂	回復群 60 ♀	回復群 120 ♂	回復群 120 ♀
組織所見（生存例）	皮膚																
	斑状形成	*	1/1	*	*	*	*	*	*	*	*	*	*	*			*
組織所見（死亡例）	肝臓																
	うっ血	–						4/4	9/9	10/10	7/7			1/1	2/2	1/1	1/1
	髄外造血	–						–	1/9	5/10	2/7			–	–	–	–
	石灰化を伴う肉芽組織	–						3/4	–	2/10	–			–	–	–	–
	出血	–						4/4	8/9	9/10	6/7			1/1	1/2	1/1	1/1
	肝細胞内脂肪滴	–						1/4	9/9	–	6/7			–	2/2	–	1/1
	肝細胞壊死	–						4/4	8/9	9/10	6/7			1/1	1/2	1/1	1/1
	被膜上結合組織増殖及びフィブリン滲出	–						2/4	5/9	10/10	5/7			1/1	–	1/1	1/1
	肝細胞壊死	–						–	–	1/10	–			–	–	–	–
	脾臓																
	髄外造血	–						2/4	1/9	5/10	3/7			–	–	–	–
	組織球性細胞増加							–	–	1/10	–			–	–	–	–
	腎臓																
	嚢胞形成	1/1						–	–	–	–			–	–	–	–
	心臓																
	細胞反応を伴う筋線維壊死	–						4/4	9/9	8/10	1/7			1/1	2/2	–	1/1
	血栓	–						1/4	–	–	–			–	–	–	–
	筋線維空胞化	–						1/4						1/1	–	1/1	–

**: 被験物質投与期間 <（自平成 1年 3月 4日 至平成 1年 3月17日）（14日間、150日切迫屠殺）>
統計学的な有意差検定は行われていない。

***: 被験物質投与期間 <（自平成 1年 3月 4日 至平成 1年 3月17日）（14日間）>
回復期間 <（自平成 1年 3月18日 至平成 1年 3月31日）（14日間）>
統計学的な有意差検定は行われていない。

Key to previous page

1 Purity of test substance
2 Dose
3 Control group
4 Low-dose group
5 Medium-dose group
6 High-dose group
7 Recovery group
8 Histological findings (surviving cases)
9 Histological findings (dead cases)
10 Skin: crust formation
11 Liver: hemostasis
12 Extramedullary hematopoiesis
13 Granulation tissue accompanying petrifaction
14 Bleeding
15 Fatty droplets in hepatic cells
16 Necrosis of hepatic cells
17 Hyperplasia of on-capsular connective tissue and fibrin seepage
18 Enlargement of hepatic cells
19 Spleen: extramedullary hematopoiesis
20 Increase of histiocytes
21 Kidney: formation of cytocyst
22 Heart: necrosis of muscular fiber accompanying cell reaction
23 Thrombus
24 Cavitation of muscle fibers
25 **: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days, sacrificed on the 15th day by manual strangulation)).
Statistically significant difference test was not performed.
***: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days)).
The recovery period (from March 18, 1992 to March 31, 1992 (a period of 14 days)).
Statistically significant difference test was not performed.

被験物質の純度 ≧99%	投与量 mg/kg	対照群 0 ♂	対照群 0 ♀	低用量群 8 ♂	低用量群 8 ♀	中用量群 30 ♂	中用量群 30 ♀	中用量群 60 ♂	中用量群 60 ♀	高用量群 120** ♂	高用量群 120** ♀	回復群 0 ♂	回復群 0 ♀	回復群 60 ♂	回復群 60 ♀	回復群 120 ♂	回復群 120 ♀
組織所見（死亡例）	肺 うっ血	−						2/4	2/9	6/10	5/7			1/1	1/2	1/1	1/1
	水腫	−						1/4	2/9	4/10	3/7			1/1	1/2	−	−
	出血	−						−	1/9	−	−			−	−	−	−
	腺胃 粘膜壊死	−						−	−	2/10	1/7			−	−	−	−
	精巣 精子形成減少	*						−		*				1/1		*	
	出血	*						1/1		*				−		*	
	卵巣 うっ血								1/1		*				2/2		*
	副腎 皮質細胞腫大	−						1/4	−	2/10	−			1/1	−	−	−
	胸腺 リンパ球減少	*						*	*	*	*			1/1	*	*	*
NOEL（mg/kg）		8mg/kg/day未満															
NOELの推定 根拠とした変化		一般状態において雌雄共8mg/kg以上の群で流涎、尿検査において雄8mg/kg以上の群で尿蛋白の増加がみられたことによる。															

−：異常なし
○：異常あり
▽or△：危険率（P）＜0．05
▼or▲：危険率（P）＜0．01
↑or↓：統計学的有意差がみられないもの
＊：検査せず
＊＊：被験物質投与期間＜（自平成4年3月4日 至平成4年3月17日）（14日間、15日目切迫解殺）＞
統計学的な有意差検定は行われていない。
＊＊＊：被験物質投与期間＜（自平成4年3月4日 至平成4年3月17日）（14日間）＞
回復期間 ＜（自平成4年3月18日 至平成4年3月31日）（14日間）＞
統計学的な有意差検定は行われていない。

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Key to previous page

1 Purity of test substance
2 Dose
3 Control group
4 Low-dose group
5 Medium-dose group
6 High-dose group
7 Recovery group
8 Histological findings (dead cases)
9 Liver: hemostasis
10 Dropsy
11 Bleeding
12 Glandular stomach: necrosis of mucosa
13 Testicle: decrease in sperm formed
14 Bleeding
15 Ovary: hemostasis
16 Adrenal gland: enlargement of cortical cells
17 Thymus: decrease in lymphocytes
18 Less than 8 mg/kg/day
19 Variation as base for estimating NOEL
20 As far as the general state is concerned, for both male and female groups with dose of 8 mg/kg or higher, salivation was observed; in urine examination, for the male group with dose of 8 mg/kg or higher, increase in urinary protein was observed.
21 Not abnormal
22 Abnormal
23 Risk rate
24 Risk rate
25 With no statistically significant difference.
26 *: Not examined.
**: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days, sacrificed on the 15th day by manual strangulation)).
Statistically significant difference test was not performed.
***: The administration period of the test substance (from March 4, 1992 to March 17, 1992 (a period of 14 days)).
The recovery period (from March 18, 1992 to March 31, 1992 (a period of 14 days)).
Statistically significant difference test was not performed.

4. Others

反復投与毒性試験実施機関 (1)	名称 (2)	(3) 財団法人 化学品検査協会 化学品安全センター日田研究所
	所在地 (4)	(5) 大分県日田市石井町3丁目822番地 電話 0973(24)7211
試験責任者 (6)	職氏名 (8)	馬野 高昭 (10) (印)
試験実施年月日 (7)	(9) 平成 4年 1月 6日 から 平成 4年 9月21日 まで	

Key: 1 Institution performing the repeat administration toxicity study
2 Name
3 Hita Research Lab., Chemical Safety Center, Chemical Inspection Association
4 Location
5 3-822 Ishii-cho, Hita-shi, Oaibu-shi, Phone: 0973(24)7211
6 Person in charge of study
7 Test date
Year Month Day
8 Name
9 From January 6, 1992 to September 21, 1992
10 Takaaki Mano [seal]

Work Distribution table

Title: 28-day oral toxicity (repeat dose) study of X-12-990S on rats

氏　名 (1)	(2) 業務内容
試験責任者 (3) 馬野高昭	試験の計画、実施など試験全般の管理、結果の総合的な分析・評価及び報告書の作成を行う。
動物試験部門長 (4) 今田中伸哉	動物試験部門のすべての業務に対し責任をもつ。作業の正確な実施、検査及び作業にたずさわる職員の教育、訓練を行う。
病理部門長 (5) 山崎寛治	病理形態学的分野の業務を行う。剖検及び検査の業務に対し責任を持つ。
臨床化学部門長 (6) 白石啓三	血液学的検査、血液化学的検査及び尿検査のほか、生体試料の生化学的分析に関連した業務を行う。
製剤・分析部門長 (7) 松浦孝雄	被験物質の安定性、調製液の安定性・均一性に関する分析を行う。

Key: 1 Name
2 Work contents
3 Person in charge of study: Takaaki Mano
4 Director of Animal Test Division: Nobiya Imatanaka
5 Director of Pathology Division: Hirokaru Yamasaki
6 Director of Clinical Chemistry Division: Keiji Shiroishi
7 Director of Formulation/Analysis Division

8 Management of general study, such as test plan, implementation, etc., general analysis/evaluation of results, and preparation of report.

9 Responsible for all of the work of the animal test department. In charge of correct implementation and inspection of operation, as well as education and training of the staff performing the inspection of operation.

10 In charge of work in the pathology field. Responsible for the work of postmortem examination and microscopic examination.

11 In charge of the work of blood examination, blood chemical examination, urine examination, as well as biochemical examination of the biosamples.

12 In charge of examination of the stability of substance to be inspected, as well as stability/uniformity of the solution prepared.

Reception No. D91-0946
Report No. D-3157

Test Code No. B11-0153

Final report

28-day oral toxicity (repeat dose) study of X-12-9908 on rats

September, 1992
Hita Research Lab., Chemical Safety Center,
Chemical Inspection Association [seal]

Statement

Hita Research Lab., Chemical Safety Center, Chemical Inspection Association

Requester of the test: Shin Etsu Chemical Industry Co., Ltd.
Title of the test: 28-day oral toxicity (repeat dose) study of X-12-9908 on rats
Test code No. 811-0153

The aforementioned test was carried out according to "Test facility defined in command No. 4 for determining the test item related to the novel chemicals and the examination item of the harmful effects of assigned chemicals" drafted according to the following codes: KANKIKEN No. 233, HSHI No. 38, and 63 KIKYOKU No. 823 dated November 18, 1988; KANHOGYO No. 39, YAKUHO No. 229, 59 KIKYOKU No. 85 dated March 31, 1984; and "OECD principles of good laboratory practice" (May 12, 1981)

September 21, 1992
Tadaaki Anan (seal), manager of operation

Warranty of reliability

Hita Research Lab., Chemical Safety Center, Chemical Inspection Association

Requester of test: Shin Etsu Chemical Industry Co., Ltd.
Title of test: 28-day oral toxicity (repeat dose) study of X-12-990S on rats
Test Code No. B11-0153

This test was performed under monitoring or inspection by the reliability warranty department of Hita Research Lab., Chemical Safety Center, Chemical Inspection Association. The following are the dates on which the monitoring or inspection was performed and report was lodged to the manager of the operation and the persons in charge of the test.

① 監査又は査察日			② 試験責任者報告日			③ 運営管理者報告日		
1991年	12月	25日	1991年	12月	25日	1991年	12月	25日
1992年	2月	25日	1992年	2月	26日	1992年	2月	26日
1992年	3月	3日	1992年	3月	5日	1992年	3月	5日
1992年	3月	4日	1992年	3月	6日	1992年	3月	6日
1992年	3月	13日	1992年	3月	13日	1992年	3月	16日
1992年	3月	18日	1992年	3月	19日	1992年	3月	19日
1992年	4月	1日	1992年	4月	2日	1992年	4月	2日
1992年	4月	2日	1992年	4月	6日	1992年	4月	6日
1992年	4月	15日	1992年	4月	16日	1992年	4月	16日
1992年	4月	30日	1992年	5月	1日	1992年	5月	1日
1992年	6月	4日	1992年	6月	4日	1992年	6月	4日
1992年	9月	10日	1992年	9月	10日	1992年	9月	10日
1992年	9月	18日	1992年	9月	21日	1992年	9月	21日
1992年	9月	21日	1992年	9月	21日	1992年	9月	21日

This report is a correct reflection of the method and the procedure used in the test as well as the raw data of the test results.
September 21, 1992, Keiji Shiroi (seal), staff of Reliability Warranty Department
September 21, 1992, Hiroyasu Fujii (seal), director of Reliability Warranty Department

Key:

1	Monitoring or examination date
2	Date of report to the person in charge of the test
3	Date of report to the manager of operation
4	December 25, 1991
5	December 25, 1991
6	December 25, 1991
7	February 25, 1992
8	February 26, 1992
9	February 26, 1992
10	March 3, 1992
11	March 5, 1992
12	March 5, 1992
13	March 4, 1992
14	March 6, 1992
15	March 6, 1992
16	March 13, 1992
17	March 13, 1992
18	March 16, 1992
19	March 18, 1992
20	March 19, 1992
21	March 19, 1992
22	April 1, 1992
23	April 2, 1992
24	April 2, 1992
25	April 2, 1992
26	April 6, 1992
27	April 6, 1992
28	April 15, 1992
29	April 16, 1992
30	April 16, 1992
31	April 30, 1992
32	May 1, 1992
33	May 1, 1992
34	June 4, 1992
35	June 4, 1992
36	June 4, 1992
37	September 10, 1992
38	September 10, 1992
39	September 10, 1992
40	September 18, 1992
41	September 21, 1992
42	September 21, 1992
43	September 21, 1992
44	September 21, 1992
45	September 21, 1992

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Affiliated data: Physicochemical Test Report

Abstract

28-day oral toxicity (repeat dose) study and 14-day repeat test of X-12-990S were performed using 5-week-old Crj:CD (SD) species of rats, with 6 rats in each group of male and female.

The doses adopted include the following 4 levels: 120 mg/kg as the high dose, as well as 8, 30, and 60 mg/kg. Also tested are control group, as well as recovery groups with doses of 60 and 120 mg/kg, respectively.

For the group with dose of 120 mg/kg, many rats died during the administration period. Consequently, for the group with dose of 120 mg/kg, 14-day repeat administration was performed. For the 1 male and 2 females surviving, they were sacrificed by manual strangulation on the next day.

As far as the recovery test is concerned, for the high-dose (120 mg/kg) group, after the 14-day repeat administration, for 1 male and 3 females, 14-day recovery test was performed; for the medium-dose (60 mg/kg) group, after 28-day repeat administration, for 4 males and 2 females, 14-day recovery test was performed.

1. Results of the 28-day repeat administration

1) General state

During the administration period, for both females and males, for the groups with dose of 8 mg/kg or higher, salivation was observed; for the groups with dose of 60 mg/kg, the following symptoms were observed: decrease of spontaneous activity, crouching, prouation, deep breathing, anemia, hyperthermia, and

piloerection, and the general state was deteriorated. During 9th-28th days, 4 males and 9 females died.

2) Blood examination

At the end of the administration period, for the male group with dose of 10 mg/kg or higher, decrease in the mean red blood cell volume was observed; for the [male] group with dose of 60 mg/kg, the following symptoms were observed: mean red blood cell hemoglobin level, decrease in platelet count, prolongation of prothrombin time and activated partial thromboplastin time.

3) Blood chemistry examination

At the end of the administration period, for the male group with dose of 60 mg/kg, the following symptoms were observed: increase in GOT, GPT, alkaline phosphatase and urea nitrogen, and decrease in chloride.

4) Urinary examination

At the end of the administration period, for the male group with dose of 8 mg/kg or higher, increase in urinary protein was observed; for the [male] groups with doses of 10 mg/kg and 60 mg/kg, occult blood was observed.

5) Organ weight

At the end of the administration period, for the male group with dose of 60 mg/kg, increase in the liver weight was observed.

6) Findings of postmortem examination

For the surviving males with dose of 60 mg/kg and the dead females and males in the groups with dose of 60 mg/kg, the following symptoms were observed: discoloration of heart, enlargement of liver, sclerosis of [liver], interlobar adhesion [of liver], and white portion [on liver].

7) Histopathological examination

For both male and female groups with dose of 30 mg/kg, necrosis of muscle fibers accompanying cell reaction of heart was observed; for the males [of the group with dose of 30 mg/kg], necrosis of hepatic cells was observed; for the male group with dose of 60 mg/kg, the following symptoms were observed: hemostasis in liver, extramedullary hematopoiesis, granulation accompanying petrifaction, bleeding, necrosis of hepatic cells, enlargement of hepatic cells, extramedullary hematopoiesis of spleen, necrosis of muscle fibers accompanying the cell reaction of heart, and cavitation of muscule cells. In the cases of death, for both female and male groups with dose of 60 mg/kg, the following symptoms were observed: hemostasis of liver, bleeding [of liver], necrosis of hepatic cells, on-capsular connective tissue hyperplasia and fibrin seepage, extramedullary

hematopoiesis of spleen, necrosis of muscle fibers accompanying the cell reaction of heart, hemostasis of lung, dropsy; for males [in the group with dose of 60 mg/kg], the following symptoms were observed: granulation tissue accompanying petrifaction of liver, thrombus of heart, cavitation of muscle fibers, bleeding of testicle, cortical cell enlargement of adrenal gland; for females [in the group with dose of 60 mg/kg], the following symptoms were observed: extramedullary hematopoiesis of liver, pulmonars bleeding, hemostasis of ovary.

2. Results of high-dose (120 mg/kg) group

During the administration period, the same deterioration of the general state as that for the 60-mg/kg groups was observed. By the 14th day, 10 males and 7 females died. The abnormalities observed with respect to the blood examination, blood chemistry examination, organ weight, postmortem examination of dead and surviving rats, and the histopathological examination were also identical to those for the groups with dose of 60 mg/kg. The changes observed are believed to be caused by the pathological changes in the liver and heart.

3. Results of the recovery test

In the recovery period, for both male and female groups with doses of 60 mg/kg and 120 mg/kg, the same deterioration in the general state was observed as that in the administration period. For the groups with dose of 60 mg/kg, by the 9th recovery day,

1 male and 2 females died; for the groups with dose of 120 mg/kg, by the 11th day of recovery, 1 male and 1 female died.

As far as the change in body weight is concerned, for both the male and female groups with dose of 120 mg/kg, a decrease in the body weight was observed.

As far as the food consumption is concerned, for male groups with doses of 60 mg/kg and 120 mg/kg, there was a tendency of decrease in the food consumption.

The results of the examination at the end of the recovery period are as follows: for the male group with dose of 60 mg/kg, in addition to the original symptoms, such as increase in GOT, GPT, urea nitrogen and urinary protein, decrease in platelet count and chloride, and prolongation of the prothrombin time and activated partial thromboplastin time, the following new symptoms were also observed: increase in creatinine, cloudiness of urine, and increase in the weight of adrenal gland. For the female group with dose of 120 mg/kg, in addition to increase in GOT, GPT, urea nitrogen, segmented neutrophil proportion, and decrease in red blood cell count, white blood cell count, hemoglobin concentration, hematocrit level, platelet count, and lymphocyte proportion, and a tendency of prolongation of the prothrombin time and activated partial thromboplastin time, there was also a tendency of increase in the weights of liver, heart, and adrenal gland.

In the pathological examination performed for the dead cases and surviving cases, the pathological changes in the liver and heart were the main residual symptoms observed.

Consequently, in this round of tests for X-12-9906, for the target organs of liver and heart, there was no recovery for

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changes caused by the pathological changes of these organs. For NOEL, it is believed that [the level] is lower than 8 mg/kg of body weight/day.

Final report

1. Title: 28-day oral toxicity (repeat dose) study of X-12-9908 on rats

2. Requester of the test: Shin Etsu Chemical Industry Co., Ltd.
Address: 2-6-1 Otemachi, Chiyoda-ku, Tokyo, 100 Japan

3. Test facility: Hita Research Lab., Chemical Safety Center, Chemical Inspection Association
Address: 1-822 Ishii-cho, Hita-shi, Oaibu-ken, 877 Japan

4. Test purpose: 28-day oral toxicity (repeat dose) study was performed for the substance to be tested on animals. By observing the changes in the function and state of the living body in the administration period, the toxicity of the test substance was clarified.

5. Test method: It was based on "407. Repeat dose oral toxicity-rodent: 28-day or 14-day study" defined in "OECD guidelines for testing of chemicals" (May 12, 1981) and on "28-day oral toxicity (repeat dose) study on mammals" defined in "On partial revision of "on the test method concerning new chemical substance" (Kanhoyyo No. 700,

Yakuhatsu No. 1039, 61 Kikyoku No. 1014, December 5, Sho 61 [1986]).

6. Standards of excellent test institution

It was implemented on the base of "Test facility defined in command No. 4 for determining the test item related to the novel chemicals and the examination item of the harmful effects of assigned chemicals" (drafted according to the following codes: KANKIKEN No. 233, ESEI No. 38, and 63 KIKYOKU No. 823 dated November 18, 1988; KANHOGYO No. 39, YAKUHO No. 229, 59 KIKYOKU No. 85, March 31, 1984). and "OECD principles of good laboratory practice" (May 12, 1981).

7. Test period

Test start date: January 6, 1992
Animal procurement date: February 25, 1992
Period of administration (28-day): March 4, 1992 to March 31, 1992
(14-day): March 4, 1992 to March 17, 1992
Recovery period end date (28-day): April 14, 1992
(14-day): March 31, 1992
Test end date: September 21, 1992

8. Stored data and storage period

For 10 years after the final report is lodged, the data, formalin-fixed specimens, paraffin blocks, slice specimens, smear samples, etc., are to be kept at the data archives of Hita Research Lab. of this association, and the samples for each lot of the test substance are to be kept at the Test Substance Storage Chamber. The treatment after the aforementioned storage period should be approved by the requester of the test. However, for the wet specimens, for which there might be a significant change in the quality, the storage period is set as the period suitable for the quality evaluation. When such specimens are to be disposed of, the approval by the requester of the test is needed.

9. Preparers and managers of the final report

9.1 Signatures and seals of preparers of the final report

Person in charge of the test: Takaaki Mano [seal], Test Research Department, Hita Research Lab., September 21, 1992

Director of Animal Test Department: Nobiya Imatanaka [seal], September 21, 1992

Director of Pathology Department: Hiroharu Yamasaki [seal], September 21 1992

Director of Clinical Chemistry Department: Keiji Shirai [seal], September 21, 1992

9.2 Managers and director of department

Manager of test substance: Masakazu Yoshida
Manager of test animals: Yoshinobu Taniya
Director of Formulation/Analysis Department: Takanori Matsumoto

10. Test substance and method

10.1 Test substance

Name

Chemical name*: a mixture of 1,1- and 1,2-bis(trimethoxysilyl)ethane
Abbreviation*: X-12-9909
Lot No.*: 105027
Supplier*: Shin Etsu Chemical Industry Co., Ltd.
Structural formula:

$$(CH_3O)_3\text{-}Si\text{-}CH_2CH_2\text{-}Si\text{-}(OCH_3)_3$$

$$(CH_3O)_3\text{-}Si\text{-}\underset{\underset{CH_2}{\|}}{CH}\text{-}Si\text{-}(OCH_3)_3$$

(Molecular formula: $C_8H_{22}O_6Si_2$)
Purity*: over 99%
Physicochemical properties*

molecular weight: 270.43
Form at room temperature: colorless clear liquid
Melting point: --
Boiling point: 103-104°C/5 mm Hg
Vapor pressure: --
Distribution coefficient: --
Dissolution property: oil soluble
Solubility
Water: insoluble
DMSO: soluble
Acetone: soluble
Others: --

Storage conditions: Stored in dark and cold place (within a desiccator)

Stability test: It was confirmed as stable during the period of administration as checked by the Chemical Inspection Association

* According to the data provided by the test requester.

10.2 Animals used in test

Crj:CD (SD) species of rats (SPF) produced by Nippon Charles River Co., Ltd. (transliteration) (Hino Animal Raising Center, 715 Shitakomatsuge, Hino-cho, Gamo-gun, Shiga-ken, 529-16, Japan) were procured, quarantined, and tamed. Those with good growth and good general state were selected using a random extraction method for different body weights, and were grouped with nearly identical mean body weight for the various groups. The animals were 5 weeks old when the administration was started. The body

weights were in the range of 131.0-159.6 g for males, and in the range of 105.2-134.4 g for females. Earmarks were made to identify the animals.

10.3 Raising method

During the test period, the animals were raised individually in stainless steel bracket cages (products of Tokiwa Scientific Instrument Co., Ltd., measuring 165 W x 300 D x 150 H mm) in a growth chamber with intervening walls with temperature set at 23 ± 2°C, relative humidity set at 55 ± 10%, ventilation frequency set at 10-15 rounds/h, and light/dark cycle set at 12 h (light for 7 h, dark for 19 h). The cages were exchanged once a week, and the racks and cages were identified by labels. MF solid fodder (product of Oriental Yeast Industry Co., Ltd.) was fed as fodder, and drink water of the tap water of Hita-shi (water with added chlorine) was made available all the time using an automatic water feed unit. The fodder and feed equipment were sterilized using an autoclave at 121°C for 30 min. For the mixtures of the fodder and drink water, analysis was performed to make sure that there was no problem for the test.

During the period of quarantine and taming and the test period, no variation that may have influenced the test results was observed in the aforementioned raising environmental factors.

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10.4 Test method

1) Group configuration

The group configuration is as follows:

試験群 (1)	投与量 (2) (mg/kg/day)	投与容量 (3) (ml/kg)	投与濃度 (4) (%)	動物数 (5) 雄 (6) (動物番号) (7)	動物数 (5) 雌 (8) (動物番号) (7)
媒体対照群 (9)	0	10	(0)	6 (1 − 6)	6 (49 − 54)
媒体対照回復群 (10)	0	10	(0)	6 (7 − 12)	6 (55 − 60)
低用量群 (11)	(8)	(10)	(0.08)	6 (13 − 18)	6 (61 − 66)
中用量 (1) 群 (12)	(30)	(10)	(0.3)	6 (19 − 24)	6 (67 − 72)
中用量 (2) 群 (13)	(60)	(10)	(0.6)	6 (25 − 30)	6 (73 − 78)
中用量(2) 回復群 (14)	(60)	(10)	(0.6)	6 (31 − 36)	6 (79 − 84)
高用量群 (15)	(120)	(10)	(1.2)	6 (37 − 42)	6 (85 − 90)
高用量回復群 (16)	(120)	(10)	(1.2)	6 (43 − 48)	6 (91 − 96)

Key:
1 Test group
2 Dose
3 Volumetric dose
4 Concentration of dose
5 Animal number
6 Male
7 (Animal Nos.)
8 Female
9 Medium control group
10 Medium control recovery group
11 Low-dose group
12 Medium-dose group (1)
13 Medium-dose group (2)
14 Medium-dose recovery group (2)

15 High-dose group
16 High-dose recovery group

Reasons for setting dose of administration: the 14-day repeat administration preparatory test was performed with three doses: 50, 250, and 1,000 mg/kg. The results indicate that for the general state, postmortem examination findings, blood examination, blood chemistry examination, organ weight, and histopathological examination, abnormality was observed up to the group with dose of 50 mg/kg; for the change in body weight, abnormality was observed up to the group with dose of 250 mg/kg; and for groups with doses of 250 and 1,000 mg/kg, all the rats died. Consequently, for this test, 4 doses were set, with 120 mg/kg set as the high dose, followed by doses of 60, 30, and 8 mg/kg. Also, the recovery groups were set as the groups with doses of 60, 120 mg/kg and the medium control group.

In this test, for the high-dose group and the high-dose recovery group (with dose of 120 mg/kg), 9 males (Nos. 38, 40-47) and 7 females (Nos. 85, 87, 88, 89, 91, 95, and 96) died during the administration period before the 14th day, making the 28-day repeat administration difficult. Consequently, for this dose, 14-day repeat administration was performed, and the surviving rats in the high-dose group, including one male (No. 37) and two females (Nos. 86, 90) were sacrificed by manual strangulation on the next day. For the high-dose repeat test, as one male (No. 48) in the high-dose recovery group was in a near-death state, one male (No. 36) in the high-dose group was taken as the recovery group together with 3 females (Nos. 92, 93, 94) in the high-dose recovery group for 14-day repeat administration.

followed by 14-day recovery test. For the other dose groups, the test was carried out as planned. Also, in the sacrifice by manual strangulation, no control group was set.

2) Preparation method and stability test of test solution

(1) Preparation method

The test substance was measured accurately, and olive oil (product of Fujizawa Astra Co., Ltd.) was added while stirred for dissolution to a solution with a concentration of 1.2% (w/v). The solutions with concentrations of 0.6, 0.3, and 0.08% (w/v) were prepared by diluting the solution with concentration of 1.2% (w/v). The preparation was performed once a week.

(2) Stability test

The stability of the test substance in this preparation method was tested by the Chemical Inspection Association, and the test substance was confirmed to be stable.

3) Administration

For the groups with dose of 120 mg/kg, forced oral administration was performed for 14 days. For the other test groups, forced oral administration was performed for 28 days, each day in the morning with the aid of Nélaton's catheter (product of Terumo Co., Ltd.) and syringe (product of Terumo Syringe Co., Ltd.). For the medium control recovery group and

recovery groups with dose of 120 and 60 mg/kg, a recovery period of 14 days was set.

4) Observation/measurement/examination

The counting method of days and weeks is as follows: the day when the administration is started is taken as day 1, the day before the administration start day is taken as day -1, the week of start of administration and the week when the recovery started are taken as week 1, and the last day of the administration is taken as recovery day 0.

(1) Observation of the general state of animals

For all the cases:
Once a day or more frequent.

(2) Body weight measurement (times)

For all the cases:
Before administration: day-2 (when groups are divided)
Period of administration (14-day): day 1
(administration (start) day) 3, 5, 8, 10, 12, 14
(measurement was manually performed before fast)
Period of administration (28-day: day 1)
(administration (start) day), 3, 5, 8, 10, 12 15, 17, 19, 22, 24, 26, 29
Recovery period: day 1 (recovery (start day)), 3, 5, 8, 10, 12, 14

In order to calculate the relative weights of the organs, measurement was performed once right before the postmortem examination.

(3) Measurement of food consumption

For all the case:
Before administration: once
During administration and recovery periods: twice a week *
* Although this is not in agreement with the test plan, there is no influence on the test results.

(4) Blood examination

For all the cases except the cases of death (Nos. 6, 27, 29, 32, 34, 35, 38-48, 74-85, 87, 88, 89, 91, 92, 95, 96), after the end of the administration period and after the end of the recovery period, after one night of fasting (16-20 h), under anesthesia, blood was collected from the abdominal aorta. Except for the examination items of prothrombin time and the activated partial thromboplastin time, EDTA-2K was used as the antiagglutinin, and the examination was performed for the obtained blood and serum samples with respect to the following items.

1 赤血球数(RBC) (×10⁴ /mm³)	電気抵抗変化検出方式 2	Microcell counter M-2000、東亜医用電子 3
4 白血球数(WBC) (×10³ /mm³)	電気抵抗変化検出方式 2	Microcell counter M-2000、東亜医用電子 3
5 ヘモグロビン濃度 (Hb) (g/dl)	Oxyhemoglobin 法 6	Microcell counter M-2000、東亜医用電子 3
7 ヘマトクリット値 (Ht) (%)	Pulse 検出方式 8	Microcell counter M-2000、東亜医用電子 3

Key: 1 Red blood cell count
2 Method of detection of change in electrical resistance
3 Toa Medical Electronics Co., Ltd.
4 White blood cell count
5 Hemoglobin concentration
6 Method
7 Hematocrit value
8 Detection method

平均赤血球容積 (MCV) (μm³)	Ht / RBC	×10³	Microcell counter M-2000、東亜医用電子
平均赤血球ヘモグロビン量(MCH)(pg)	Hb / RBC	×10³	Microcell counter M-2000、東亜医用電子
平均赤血球ヘモグロビン濃度(MCHC) (%)	Hb / Ht	×10²	Microcell counter M-2000、東亜医用電子
血小板数(Platelet)電気抵抗変化検出方式 (×10⁴ ／mm³)			Microcell counter M-2000、東亜医用電子
網状赤血球数比率 (Reticulo) (‰)	Neomethylene blue 染色法		MICROS BEC-120A、OTSUKA
白血球百分率 (%)			
単　球 (Mono)			
好塩基球(Baso)			
好酸球 (Eosino)			
リンパ球(Lymph)	Wright染色法		MICROS BEC-120A、OTSUKA
桿状核好中球 (Stab)			
分節核好中球 (Seg)			
プロトロンビン時間 (PT) (sec)	磁気センサー方式 (クエン酸ナトリウム加血漿を用いた)		MC-10A、AMELUNG
活性化部分トロンボプラスチン時間(APTT)(sec)	磁気センサー方式 (クエン酸ナトリウム加血漿を用いた)		MC-10A、AMELUNG

Key to previous page

1 Mean red blood cell volume
2 Toa Medical Electronics Co., Ltd.
3 Mean red blood cell hemoglobin level
4 Mean red blood cell hemoglobin concentration
5 Platelet count
6 Detection method of electrical resistance
7 Reticular red blood cell count proportion
8 Staining method
9 White blood cell percentage
10 Monocyte
11 Basophil
12 Eosinophil
13 Lymphocyte
14 Stab neutrophil
15 Segmented neutrophil
16 Prothrombin time
17 Magnetic sensor method (using sodium citrate additive to serum)
18 Activated partial thromboplastin time
19 Magnetic sensor method (using sodium citrate additive to serum)

(5) 血液化学的検査

血液学的検査時の採取血液から血清を分離し、得られた血清について下記項目の検査を行った。

GOT (IU／ℓ)	UV法	7150 Automatic Analyzer、日立
GPT (IU／ℓ)	UV法	7150 Automatic Analyzer、日立
アルカリ性フォスファターゼ (ALP) (IU／ℓ)	P-Nitrophenyl Phosphate 法	7150 Automatic Analyzer、日立
γ-GTP (IU／ℓ)	L-γ-Glutamyl-p-nitroanilide法	7150 Automatic Analyzer、日立
コリンエステラーゼ(ChE)(IU／ℓ)	Butyrylthiocholine iodide 法	7150 Automatic Analyzer、日立
総ビリルビン (T-Bil)(mg／dℓ)	Diazo 法	7150 Automatic Analyzer、日立
総コレステロール (T-Cho)(mg／dℓ)	酵素法	7150 Automatic Analyzer、日立
トリグリセライド (TG) (mg／dℓ)	酵素法	7150 Automatic Analyzer、日立
血糖 (Glucose) (mg／dℓ)	Glucokinase-G-6-PDH 法	7150 Automatic Analyzer、日立
総蛋白(T-Protein) (g／dℓ)	Biuret法	7150 Automatic Analyzer、日立
アルブミン (Albumin) (g／dℓ)	Bromocresol green 法	7150 Automatic Analyzer、日立
A／G比 (A/G ratio)	Albumin / T-Protein − Albumin	
尿素窒素 (BUN) (mg／dℓ)	Urease indophenol 法	7150 Automatic Analyzer、日立
クレアチニン (Creatinine) (mg／dℓ)	Jaffé 法	7150 Automatic Analyzer、日立
カルシウム(Ca) (mg／dℓ)	OCPC法	7150 Automatic Analyzer、日立

Key to previous page

1 (5) Blood chemistry examination
From the blood collected for the blood examination, the serum was separated and was used for examination of the following items.

2 Hitachi Ltd.
3 Method
4 Alkaline phosphatase
5 Cholinesterase
6 Total bilirubin
7 Total cholesterol
8 Enzyme method
9 Triglyceride
10 Enzyme method
11 Blood glucose
12 Total protein
13 Albumin
14 A/G ratio
15 Urea nitrogen
16 Creatinine
17 Calcium

無機リン(IP) (mg/dl)	Fiske-Subbarow法	7150 Automatic Analyzer、日立
ナトリウム(Na) (mEq/l)	炎光光度法	775 形炎光光度計、日立
カリウム(K) (mEq/l)	炎光光度法	775 形炎光光度計、日立
クロライド(Cl) (mEq/l)	電極法	CL-6M 形クロライドカウンタ、電器産業

Key: 1 Organic phosphorus
2 Method
3 Hitachi Ltd.
4 Sodium
5 Potassium
6 Chloride
7 Flame photometry method
9 Model 775 flame photometer, product of Hitachi Ltd.
10 Model CL-6M chloride counter, product of Hiranuma Industry Co., Ltd.

(6) Urine examination

For all the cases except the dead cases (Nos. 6, 27, 29, 32, 34, 35, 38-48, 74-85, 87, 88, 89, 91, 92, 95, 96) and the group with rats sacrificed by manual strangulation (with dose of 120 mg/kg):

Period of administration (28-day): 1 round (day 28)

Recovery period: 1 round (day 14)

For the 16-h urine sampled in the individual metabolic cages, in addition to the urine amount and tone, a type of test paper (N-Multisticks®, product of Miles-Sankyo [transliteration] K.K.) was used to examine the following items: pH, protein, ketone body, bilirubin, occult blood, sugar, and urobilinogen.

(7) Postmortem examination

For all the cases
The corpses were observed carefully and recorded in detail.

(8) Measurement of organ weight

For all of the cases, the wet weight was measured for the following organs:

Brain, liver, spleen, kidney, heart (excluding the dead cases taking place on or before day 28), adrenal gland, testicle (or, ovary)

(9) Histopathological postmortem examination

For all the cases, the following organs and tissues were fixed by formalin.

Brain, pituitary body, eyeballs, thyroid (including epithelioid body), heart, lung, liver, kidney, spleen, adrenal gland, stomach, intestine (duodenum-rectum), testicle (or ovary), bladder, marrow (thigh bone), pathologically changed portions visible to naked eyes.

For the following groups, paraffin-embedded slice specimens were prepared for the organs and tissues. After hematoxylin-eosin staining, they were examined on an optical microscope.

In the period of administration

For medium control group, and groups with doses of 30, 60, and 120 mg/kg: liver, heart, kidney, spleen accessary, stomach, intestines (duodenum, jejunum, ileum, cecum, colon, rectum), lung

For the group with dose of 8 mg/kg: liver, heart

For the other pathologically changed portions visible to the naked eye:

Male: For the group with dose of 60 mg/kg (No. 29): testicle

For the group with dose of 60 mg/kg (No. 30): peritoneal lymph nodes

Female: For the medium control group (No. 51): skin

For the group with dose of 8 mg/kg (No. 61): stomach

For the group with dose of 60 mg/kg (No. 77): ovary

In the recovery period

For the medium control group, 60 and 120-mg/kg recovery groups: liver, heart, kidney, spleen, adrenal gland, stomach, intestines (duodenum, jejunum, ileum, cecum, colon, rectum), lung (female of medium control recovery group, female and male of 60 and 120-mg/kg recovery group)

For the other pathologically changed portions visible to naked eyes

Male: For the recovery group with dose of 60 mg/kg (No. 33): thymus, pancreas

For the recovery group with dose of 60 mg/kg (No. 34): thymus, testicle

Female: For the recovery group with dose of 60 mg/kg (No. 81): ovary

For the recovery group with dose of 60 mg/kg (No. 84): ovary

5) Statistical processing

For the body weight, food consumption, blood examination, blood chemistry examination, urine amount, and organ weight, the Bartlett method was used to perform one-dimensional variance analysis. When one-dimensional variance was observed with 5% level of significance, one-dimensional configuration dispersion analysis was performed. When a significant difference was observed in the dispersion analysis, if the case number of the medium control group is identical to that of the various administration groups, assay was performed using the Dunnett method; if they are not identical to each other, the Scheffé method was used to perform the assay.

When one-dimensional variance was not observed, Kruskal-Wallis assay was performed. When significant difference was observed, if the case number of the medium control group is identical to that of the various administration groups, assay was performed using the nonparametric Dunnett method; if they are not identical to each other, the nonparametric Scheffé method was used to perform the assay.

11. Test results

For the medium control group, 1 male (No. 6) died midway; for the 60-mg/kg group, 5 males (Nos. 27, 29, 32, 34, 35) and 11 females (Nos. 74-84) died midway; for the 120-mg/kg group, 11 males (Nos. 38-48) and 8 females (Nos. 85, 87, 88, 89, 91, 92, 95, 96) died midway. These dead cases were excluded from the items which require statistical study (not including the body weight and food consumption). For the 120-mg/kg group and 120-mg/kg recovery group sacrificed by manual strangulation, the statistically significant difference assay was not carried out. For the purpose of reference, the data at the end of 14-day administration and at the end of 14-day recovery period are listed in the text, table and addendum.

11.1 General state (Table 1, Addendum 1)

1) During the administration period

(14-day)

Male: For the group with dose of 120 mg/kg, the following symptoms were observed: salivation (8/12), decrease in spontaneous activity (10/12), crouching (1/12), prone position (2/12), decrease in breathing frequency (4/12), deep breathing (2/12), increase in breathing frequency (1/12), anemia (6/12), hyperthermia (5/12), piloerection (1/12), lacrimation (3/12). In days 5-14, ten rats (Nos. 38, 40-48) died.

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After administration for 14 days, on the 15th day one rat (No. 37) was sacrificed by manual strangulation; for one rat (No. 39), 14-day recovery test was performed.

Female: For the group with dose of 120 mg/kg, the following symptoms were observed: salivation (7/12), decrease in spontaneous activity (10/12), crouching (1/12), pronation (2/12), decrease in breathing frequency (4/12), deep breathing (5/12), anemia (3/12), hyperthermia (3/12), piloerection (1/12), lacrimation (2/12). In days 10-14, 7 rats (Nos. 85, 87-89, 91, 95, 96) died.

In addition, soft stool was observed (1/12).

After administration for 14 days, 2 rats (Nos. 86, 90) were sacrificed by manual strangulation on day 15, and for 3 rats (Nos. 92, 93, 94), 14-day recovery test was performed.

(28-day)

Male: For the group with dose of 8 mg/kg, the following symptom was observed: salivation (1/6). For the group with dose of 30 mg/kg, salivation (5/6) was observed. For the group with dose of 60 mg/kg, the following symptoms were observed: salivation (9/12), decrease in spontaneous activity (2/12), crouching (1/12), pronation (2/12), deep breathing (3/12), anemia (4/12), hyperthermia (4/12), piloerection (2/12), nose bleeding (2/12), cloudy urine and/or red urine (1/12). In days 11-26, 4 rats (Nos. 27, 29, 32, 35) died.

In addition, for the medium control group, the following symptoms were observed: abnormal breathing sound (1/12), abnormal breathing (1/12), trichomadesis (2/12), death (1/12). For the

group with dose of 8 mg/kg, the following symptoms were observed: decrease in spontaneous activity (1/6), pronation (1/6), gasping breathing (1/6), cyanosis (1/6). For the group with dose of 60 mg/kg, trichomadesis was observed (2/12).

Female: for the group with dose of 8 mg/kg, salivation (3/6) was observed. For the group with dose of 30 mg/kg, salivation (2/6) was observed. For the group with dose of 60 mg/kg, the following symptoms were observed: salivation (9/12), decrease in spontaneous activity (5/12), crouching (3/12), pronation (3/12), decrease in breathing frequency (2/12), deep breathing (6/12), anemia (8/12), temperature decrease (7/12), piloerection (2/12), lacrimation (3/12), red tears (1/12), nose bleeding (1/12), cloudy and/or red urine (1/12), blepharoptosis (1/12), flushing of auricle (1/12). In days 9-28, 9 rats (Nos. 74-80, 82, 83) died.

In addition, for the medium control group, trichomadesis (2/12) was observed.

2) During the recovery period

Male: For the group with dose of 60 mg/kg, the following symptoms were observed: decrease in spontaneous activity (1/4), deep breathing (2/4), increase in breathing frequency (1/4), anemia (2/4), temperature decrease (2/4), piloerection (2/4), nose bleeding (2/4), cloudy and/or red urine (1/4). On day 9, one rat (No. 34) died.

For the group with dose of 120 mg/kg, the following symptoms were observed: decrease in spontaneous activity (1/1), crouching (1/1), deep breathing (1/1), anemia (1/1), hyperthermia (1/1),

piloerection (1/1), lacrimation (1/1), red tears (1/1), nose bleeding (1/1), cloudy and/or red urine (1/1). On day 11, one rat (No. 39) died.

In addition, for the medium control group, trichomadesis (2/6) was observed. For the group with dose of 60 mg/kg, trichomadesis (2/4) was observed.

Female: For the group with dose of 60 mg/kg, the following symptoms were observed: decrease in spontaneous activity (1/2), deep breathing (1/2), anemia (2/2), hyperthermia (2/2). On days 2 and 3, 2 rats (Nos. 81, 84) died.

For the group with dose of 120 mg/kg, the following symptoms were observed: decrease in spontaneous activity (1/3), decrease in breathing frequency (1/3), deep breathing (1/3), anemia (2/3), hyperthermia (1/3), piloerection (1/3), lacrimation (1/3). On day 4, one rat (No. 92) died.

In addition, for the medium control group, trichomadesis (1/6) was observed.

11.2 Body weight (Table II, Addendum 2, Figure 1)

1) During the administration period

For both male and female, no abnormality was observed.

2) During the recovery period

Male: For the group with dose of 120 mg/kg, decrease was observed on days 5 and 10.

Female: For the group with dose of 120 mg/kg, decrease was observed on day 14.

11.3 Food consumption (Table III, Addendum 3, Figure 2

1) During the administration period

For both male and female, no abnormality was observed.

2) During the recovery period

Male: For the group with dose of 60 mg/kg, decrease was observed on days 8 and 14. For the group with dose of 120 mg/kg, decrease was observed on day 8.

Female: No abnormality was observed.

11.4 Blood examination (Table IV, Addendum 4)

1) At the end of administration period

(14-day)

Male: For the group with dose of 120 mg/kg, there was a tendency of increase in the white blood cell count and reticular red blood cell count.

Female: For the group with dose of 120 mg/kg, there was a tendency of increase in the reticular red blood cell count.

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(28-day)

Male: For the group with dose of 30 mg/kg, there was a tendency of decrease in the mean red blood cell volume. For the group with dose of 60 mg/kg, there was a tendency of decrease in the mean red blood cell volume and mean red blood cell hemoglobin level and there was a tendency of decrease in the platelet count. Also, there was a tendency of prolongation of the prothrombin time and activated thromboplastin time.

Female: For the group with dose of 8 mg/kg, there was a tendency of decrease in the mean red blood cell hemoglobin concentration. For the group with dose of 30 mg/kg, there was a tendency of decrease in the mean red blood cell hemoglobin concentration and platelet count. For the group with dose of 60 mg/kg, no abnormality was observed in any case.

2) At the end of recovery period

Male: For the group with dose of 60 mg/kg, in addition to a decrease in the platelet count, there was a tendency of prolongation of the prothrombin time and activated partial thromboplastin time.

Female: For the group with dose of 120 mg/kg, there was a tendency of decrease in red blood cell count, white blood cell count, hemoglobin concentration, hematocrit value, and platelet count, there was a tendency of prolongation of the prothrombin time and activated partial thromboplastin time, and there was a tendency of decrease in the lymphocyte proportion and

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an increase in the segmented neutrophile with respect to the white blood cell percentage.

11.5 Blood chemistry examination (Table V, Addendum 5)

1) At the end of administration period

(14-day)

Male: No abnormality was observed.

Female: For the group with dose of 120 mg/kg, there was a tendency of increase in GOT.

(28-day)

Male: For the group with dose of 60 mg/kg, there was a tendency of increase in GOT, GPT, alkaline phosphatase, and urea nitrogen, and a tendency of decrease in chloride.

Female: For the group with dose of 8 mg/kg, there was a tendency of decrease in the total cholesterol and chloride.

2) At the end of the recovery period

Male: For the group with dose of 60 mg/kg, there was a tendency of GOT, GPT, urea nitrogen, and creatinine, a decrease in chloride, as well as a decrease in γ-GTP.

Female: For the group with dose of 120 mg/kg, there was a tendency of increase in GOT, GPT, and urea nitrogen.

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11.6 Urine examination (Table VI, Addendum 6)

1) Period of administration

(14-day)

For both male and female, no examination was performed.

(28-day)

Male: For the group with dose of 8 mg/kg, increase in protein was observed. For the group with dose of 30 mg/kg, increase in protein as well as occult blood were observed. For the group with dose of 60 mg/kg, increase in protein as well as occult blood were observed.

Female: No abnormality was observed.

2) Recovery period

Male: For the group with dose of 60 mg/kg, cloudy urine and increase in protein were observed.

Female: No abnormality was observed.

11.7 Organ weight (Tables VII, VIII, Addenda 7, 8)

1) At the end of administration period

(14-day)

Male: For the group with dose of 120 mg/kg, there was a tendency of increase in the relative weight of liver.

Female: For the group with dose of 120 mg/kg, there was a tendency of increase in the relative weight of liver.

(28-day)

Male: For the group with dose of 60 mg/kg, there was a tendency of increase in the relative weight of liver.

Female: No abnormality was observed.

2) At the end of recovery period

Male: For the group with dose of 60 mg/kg, there was a tendency of increase in the relative weight of adrenal gland.

Female: For the group with dose of 120 mg/kg, there was a tendency of increase in the relative weight of liver, a tendency of increase in the relative weight of heart, and a tendency of increase in the absolute and relative weight of the adrenal gland.

11.8 Postmortem examination (Table IX, Addendum 9)

1) At the end of the administration period

(14-day)

Surviving rats: Male: For the group with dose of 120 mg/kg, interlobar adhesion (1/1) and white portion (1/1) of liver were observed.

Female: For the group with dose of 120 mg/kg, interlobar adhesion of liver (2/2) and white spots of heart (1/2) were observed.

Dead rats: Male: For the group with dose of 120 mg/kg, the following symptoms were observed: anemia (2/10), intraperitoneal pooling of red fluid (3/10), intrathoracic pooling of red fluid (2/10), contamination of periphery of nose and mouth (1/10), intraperitoneal pooling of clear fluid (1/10), intrathoracic pooling of clear fluid (6/10), brown coloration of liver (1/10), disseminated white portion (1/10), enlargement (9/10), attachment of fiber-like substance on surface (1/10), sclerosis (8/10), interlobar adhesion (10/10), white portion [of liver] (7/10), enlargement of spleen (5/10), discoloration of heart (1/10), white portion [of heart] (1/10), dark red coloration of lung (4/10), red coloration (1/10), white foam-like substance in lumen of windpipe (2/10), black substance attachment of glandular stomach mucosa (1/10), mucosal dark red portion (1/10), mucosal light red portion (1/10), mucosal red portion (2/10).

Female: For the group with dose of 120 mg/kg, the following symptoms were observed: anemia (1/7), intrathoracic pooling of clear fluid (3/7), enlargement of liver (5/7), sclerosis of liver (4/7), interlobar adhesion (5/7), white portion (1/7), discoloration of heart (1/7), dark red coloration of lung (4/7), white foam-like substance in lumen of windpipe (1/7), glandular stomach mucosal black portion (1/7), mucosal red coloration (1/7), mucosal red portion (2/7).

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(28-day)

Surviving cases: Male: For the group with dose of 30 mg/kg, the following symptoms were observed: white portion of heart (1/6) and glandular stomach mucosal black portion (1/6). For the group with dose of 60 mg/kg, the following symptoms were observed: anemia (1/4), intrathoracic pooling of clear fluid (1/4), disseminated white portion of liver (2/4), enlargement (3/4), sclerosis (1/4), interlobar adhesion (3/4), white portion (1/4), discoloration of heart (2/4), glandular stomach mucosal black portion (1/4), intraperitoneal lymph node enlargement (1/4). In addition, for the medium control group, glandular stomach's mucosa black portion (1/5) was observed.

Female: For the group with dose of 8 mg/kg, mucosal excavation of the glandular stomach (1/6) was observed. In addition, for the medium control group, trichomadesis (1/6) was observed.

Dead cases: Male: For the group with dose of 60 mg/kg, the following symptoms were observed: contamination of periphery of nose (1/4), intrathoracic pooling of clear fluid (3/4), enlargement of liver (4/4), sclerosis (3/4), interlobar adhesion (3/4), white portion (3/4), black portion of spleen (1/4), enlargement of kidney (1/4), discoloration of heart (2/4), dark red coloration of lung (1/4), glandular stomach mucosal black substance attachment (1/4), dark red coloration of testicle (1/4). For the medium control group, red coloration of lung (1/1) was observed.

Female: For the group with dose of 60 mg/kg, the following symptoms were observed: contamination of periphery of nose (1/9),

intraperitoneal pooling of clear fluid (2/9), intrathoracic pooling of clear fluid (3/9), enlargement of liver (8/9), surface granulation (1/9), sclerosis (2/9), interlobar adhesion (1/9), white portion (2/9), discoloration of heart (8/9), dark red coloration of lung (2/9), white foam-like substance in lumen of windpipe (1/9), black substance attachment to glandular stomach mucosa (1/9), red-brown muciform substance in lumen of jejunum (1/9), red-brown muciform substance in lumen of ileum (1/9), dark red coloration of ovary (1/9). In addition for the 60-mg/kg group, pyelectasis of kidney (1/9) was observed.

2) At the end of the recovery period

Surviving cases: Male: For the group with dose of 60 mg/kg, the following symptoms were observed: anemia (1/3), contamination of periphery of nose (1/3), intrathoracic pooling of clear fluid (2/3), disseminated white portion of liver (1/3), attachment of fiber-like substance on the surface (1/3), sclerosis (1/3), interlobar adhesion (3/3), discoloration of heart (2/3), pancreas dropsy (1/3), dropsy of thymus (1/3). For the medium control group, pyelectasis (1/6) was observed.

Female: For the group with dose of 120 mg/kg, the following symptoms were observed: anemia (1/2), intrathoracic pooling of clear fluid (1/2), disseminated white portion of liver (1/2), enlargement (1/2), discoloration of heart (1/2).

Dead cases: Male: For the group with dose of 60 mg/kg, the following symptoms were observed: intraperitoneal pooling of clear fluid (1/1), intrathoracic pooling of clear fluid (1/1), disseminated white spots of liver (1/1), enlargement (1/1),

sclerosis (1/1), disseminated white spots of heart (1/1), dark red coloration of lung (1/1), white foam-like substance in lumen of windpipe (1/1), shrinkage of testicle (1/1), enlargement of adrenal gland (1/1), shrinkage of thymus (1/1). For the group with dose of 120 mg/kg, the following symptoms were observed: nose bleeding (1/1), intrathoracic pooling of red fluid (1/1), red tears (1/1), intraperitoneal pooling of clear fluid (1/1), sclerosis of liver (1/1), interlobar adhesion (1/1), shrinkage of spleen (1/1), dark red coloration of lung (1/1).

Female: For the group with dose of 60 mg/kg, the following symptoms were observed: disseminated white spots of liver (1/2), enlargement (2/2), discoloration of heart (2/2), foam-like substance on cross section of lung (1/2), dark red coloration of ovary (2/2). For the group with dose of 120 mg/kg, the following symptoms were observed: intrathoracic pooling of clear fluid (1/1), enlargement of liver (1/1), sclerosis (1/1).

11.9 Histopathological examination (Table X, Addendum 9)

1) At the end of the administration period

(14-day)

Surviving cases: Male: For the group with dose of 120 mg/kg, the following symptoms were observed: granulation tissue accompanying petrifaction of liver (1/1), on-capsular connective tissue hyperplasia and fibrin seepage (1/1), hepatic cell enlargement (1/1), necrosis of muscle fibers accompanying cell reaction of heart (1/1), cavitation of muscle fibers (1/1).

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Female: For the group with dose of 120 mg/kg, the following symptoms were observed: on-capsular connective tissue hyperplasia and fibrin seepage (1/2), necrosis of muscle fibers accompanying cell reaction of heart (1/2), and fatty droplets in hepatic cells (2/2).

Dead cases: Male: For the group with dose of 120 mg/kg, the following symptoms were observed: hemostasis (10/10), extramedullary hematopoiesis (5/10), granulation tissue accompanying petrifaction (2/10), bleeding (9/10), necrosis of hepatic cells (9/10), on-capsular connective tissue hyperplasia and fibrin seepage (10/10), enlargement of hepatic cells (1/10), extramedullary hematopoiesis of spleen (5/10), increase of histocytes (1/10), necrosis of muscle fibers accompanying cell reaction of heart (8/10), hemostasis of lung (6/10), dropsy (4/10), mucosal necrosis of glandular stomach (2/10), enlargement of cortical cells of adrenal gland (2/10).

Female: For the group with dose of 120 mg/kg, the following symptoms were observed: hemostasis of liver (7/7), extramedullary hematopoiesis (2/7), bleeding (6/7), necrosis of hepatic cells (6/7), on-capsular connective tissue hyperplasia and fibrin seepage (5/7), extramedullary hematopoiesis of spleen (3/7), necrosis of muscle fibers accompanying cell reaction of heart (7/7), hemostasis of lung (5/7), dropsy (3/7), mucosal necrosis of glandular stomach (1/7), and fatty droplets in hepatic cells of liver (6/7).

(28-day)

Surviving cases: Male: For the group with dose of 30 mg/kg, the following symptoms were observed: necrosis of hepatic cells of liver (1/6), necrosis of muscle fibers accompanying cell reaction of heart (3/6). For the group with dose of 60 mg/kg, the following symptoms were observed: hemostasis of liver (1/4), extramedullary hematopoiesis (1/4), granulation tissue accompanying petrifaction (2/4), bleeding (1/4), necrosis of hepatic cells (2/4), enlargement of hepatic cells (3/4), extramedullary hematopoiesis of spleen (1/4), necrosis of muscle fibers accompanying cell reaction of heart (2/4), cavitation of muscle fibers (2/4), intracavity red blood cells of peritoneal lymph nodes (1/1). For the medium control group, the following symptoms were observed: fatty droplets in hepatic cells of liver (3/5), necrosis of mucosa of glandular stomach (1/5). For the group with dose of 8 mg/kg, fatty droplets in hepatic cells of liver (3/6) were observed. For the group with dose of 30 mg/kg, fatty droplets in hepatic cells of liver (3/6) and mucosal necrosis of glandular stomach (1/6) were observed. For the group with dose of 60 mg/kg, fatty droplets in hepatic cells of liver (3/4) and mucosal necrosis of glandular stomach (1/4) were observed.

Female: For the group with dose of 30 mg/kg, necrosis of muscle fibers accompanying cell reaction of heart (1/6) was observed. For the medium control group, the following symptoms were observed: fatty droplets in hepatic cells of liver (4/6), cell infiltration of lung (1/6), bleeding (1/6), and formation of crust on skin (1/1). For the group with dose of 8 mg/kg, fatty

droplets in hepatic cells of liver (5/6) were observed. For the group with dose of 30 mg/kg, fatty droplets in hepatic cells of liver (4/6) and small granuloma (1/6) were observed. For the group with dose of 60 mg/kg, fatty droplets in hepatic cells of liver (1/1) were observed.

Dead cases: Male: For the group with dose of 60 mg/kg, the following symptoms were observed: hemostasis of liver (4/4), granulation tissue accompanying petrifaction (3/4), bleeding (4/4), necrosis of hepatic cells (4/4), on-capsular connective tissue hyperplasia and fibrin seepage (2/4), extramedullary hematopoiesis of spleen (2/4), necrosis of muscle fibers accompanying cell reaction of heart (4/4), thrombus (1/4), cavitation of muscle fibers (1/4), homostasis of lung (2/4), dropsy (1/4), bleeding of testicle (1/1), enlargement of cortical cells of adrenal gland (1/4). For the medium control group, cytocyst formation of kidney was observed (1/1). For the group with dose of 60 mg/kg, fatty droplets in hepatic cells of liver (1/4) were observed.

Female: For the group with dose of 60 mg/kg, the following symptoms were observed: hemostasis of liver (9/9), extramedullary hematopoiesis (1/9), bleeding (8/9), necrosis of hepatic cells (8/9), on-capsular connective tissue hyperplasia and fibrin seepage (5/9), extramedullary hematopoiesis of spleen (1/9), necrosis of muscle fibers accompanying cell reaction of heart (9/9), hemostasis of lung (2/9), dropsy (2/9), bleeding (1/9), hemostasis of ovary (1/1), fatty droplets in hepatic cells of liver (9/9).

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2) At the end of recovery period

Surviving cases: Male: For the group with dose of 60 mg/kg, the following symptoms were observed: hemostasis of liver (2/3), bleeding (1/3), necrosis of hepatic cells (2/3), on-capsular connective tissue hyperplasia and fibrin seepage (2/3), enlargement of hepatic cells (2/3), necrosis of muscle fibers accompanying cell reaction of heart (1/3), cavitation of muscle fibers (2/3). For the medium control group, fatty droplets in hepatic cells of liver was observed (2/6). For the group with dose of 60 mg/kg, fatty droplets in hepatic cells of liver (2/3) were observed.

Female: For the group with dose of 120 mg/kg, the following symptoms were observed: hemostasis of liver (1/2), necrosis of hepatic cells (1/2), on-capsular connective tissue hyperplasia and fibrin seepage (1/2), necrosis of muscle fibers accompanying cell reaction of heart (1/2), cavitation of muscle fibers (1/2). For the medium control group, the following symptoms were observed: fatty droplets in hepatic cells of liver (2/6), small granuloma (1/6). For the group with dose of 120 mg/kg, fatty droplets in hepatic cells of liver (1/2) were observed.

Dead cases: Male: For the group with dose of 60 mg/kg, the following symptoms were observed: hemostasis (1/1), bleeding (1/1), necrosis of hepatic cells (1/1), on-capsular connective tissue hyperplasia and fibrin seepage (1/1), necrosis of muscle fibers accompanying cell reaction of heart (1/1), cavitation of muscle fibers (1/1), hemostasis of lung (1/1), dropsy (1/1), decrease in formation of sperm of testicle (1/1), enlargement of cortical cells of adrenal gland (1/1), decrease in lymphocytes

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of thymus (1/1). For the group with dose of 120 mg/kg, the following symptoms were observed: hemostasis of liver (1/1), bleeding (1/1), necrosis of hepatic cells (1/1), on-capsular connective tissue hyperplasia and fibrin seepage (1/1), cavitation of muscle fibers of heart (1/1), hemostasis of lung (1/1).

Female: For the group with dose of 60 mg/kg, the following symptoms were observed: hemostasis of liver (2/2), bleeding (1/2), necrosis of hepatic cells (1/2), necrosis of muscle fibers accompanying cell reaction of heart (2/2), hemostasis of lung (1/2), dropsy (1/2), hemostasis of ovary (2/2). For the group with dose of 120 mg/kg, the following symptoms were observed: hemostasis of liver (1/1), bleeding (1/1), necrosis of hepatic cells (1/1), on-capsular connective tissue hyperplasia and fibrin seepage (1/1), necrosis of muscle fibers accompanying cell reaction of heart (1/1), hemostasis of lung (1/1). In addition, for the 60-mg/kg group, fatty droplets in hepatic fibers of liver (2/2) was observed. For the group with dose of 120 mg/kg, fatty droplets in the hepatic cells of liver (1/1) were observed.

12. Discussion

We have performed 28-day oral toxicity (repeat dose) study of X-12-9908 on SD-species rats with doses of 3, 30, 60, and 120 mg/kg/day. Also, 14-day recovery test was performed.

However, for the high-dose groups (120 mg/kg), many rats died during the administration period, and it was believed that the 28-day repeat administration is difficult. Consequently, for the group with dose of 120 mg/kg, 14-day repeat administration

was performed, and the rats were sacrificed by manual strangulation on the next day for the surviving cases. For the other dose groups, 28-day repeat administration was carried out.

In the recovery test, for the high-dose (120 mg/kg) group, after 14 days of repeat administration, 14-day recovery test was performed. For the medium-dose (60 mg/kg) group, after the 28 days of administration, [14-day] recovery test was performed.

1. Test results and main influences of 28-day repeat administration

As far as the effects of the test substance are concerned, during the administration period, for both male and female, for the group with dose of 8 mg/kg, salivation was observed. For the group with dose of 60 mg/kg, the following symptoms were observed: decrease in spontaneous activity, crouching, pronation, deep breathing, anemia, hyperthermia, piloerection, nose bleeding, cloudy and/or red urine. For females, there are also the following symptoms: decrease in breathing frequency, lacrimation, red tears, blepharoptosis, flushing of auricle. In days 9-28, 4 males and 2 females died.

As far as the blood examination is concerned, at the end of the administration period, for the male groups with 30 mg/kg or higher, decrease in the mean red blood cell volume was observed. For the group with dose of 60 mg/kg, the following symptoms were observed: decrease in the mean red blood cell hemoglobin level, and platelets, and prolongation of prothrombin time and activated partial thromboplastin time.

As far as the blood chemistry examination is concerned, at the end of administration period, for the male 60 mg/kg, increase in GOT, GPT, alkaline phosphatase, and urea nitrogen, as well as chloride were observed.

In the urine examination, at the end of the administration period, for the male groups with dose of 8 mg/kg or higher, increase in the urinary protein was observed. For the groups with doses of 30 mg/kg and 60 mg/kg, occult blood was observed.

As far as the organ weight is concerned, at the end of the administration period, increase in the weight of liver was observed for the male 60 mg/kg.

In the postmortem examination, at the end of the administration period, for the male group with dose of 30 mg/kg was observed. For the group with dose of 60 mg/kg, the following symproms were observed: anemia, intrathoracic pooling of clear fluid, disseminated white portion of liver, enlargement, sclerosis, interlobar adhesion, white portion, and discoloration of heart. For the dead cases, for both male and female, for the group with dose of 60 mg/kg, the following symptoms were observed: contamination of periphery of nose, intrathoracic pooling of clear fluid, enlargement of liver, sclerosis, interlobar adhesion, white portion, discoloration of heart, dark red coloration of lung, black substance attachment to glandular stomach mucosa. For males, the following symptoms were observed: black portion of spleen, enlargement of kidney, dark red coloration of testicle. For females, the following symptoms were observed: intraperitoneal pooling of clear fluid, liver surface granulation, white foam-like substance in lumen of windpipe, red

brown muciform substance in lumen of jejunum, red brown muciform substance in lumen of ileum, dark red coloration of ovary.

As far as the histopathological examination is concerned, for both females and males, for the groups with dose of 30 mg/kg, necrosis of muscle fibers accompanying cell reaction of heart was observed. For the males, necrosis of heparic cells of liver was observed. For the male group with dose of 60 mg/kg, the following symptoms were observed: hemostasis of liver, extramedullary hematopoiesis, granulation tissue accompanying petrifaction, bleeding, necrosis of hepatic cells, enlargement of hepatic cells, extramedullary hematopoiesis of spleen, necrosis of muscle fibers accompanying cell reaction of heart, and cavitation of muscle fibers. For the dead cases, for both male and female groups with dose of 60 mg/kg, the following symptoms were observed: hemostasis of liver, bleeding, necrosis of hepatic cells, on-capsular connective tissue hyperplasia and fibrin seepage, extramedullary hematopoiesis of spleen, necrosis of muscle fibers accompanying cell reaction of heart, hemos is of lung, dropsy. For males: the following symptoms were observed: granulation tissue accompanying petrifaction of liver, thrombus of heart, cavitation of muscle fibers, bleeding of testicle, enlargement of cortical cells of adrenal gland. For females, the following symptoms were observed: extramedullary hematopoiesis of liver, bleeding of lung, hemostasis of ovary.

From the aforementioned results, it is believed that the main effects of the X-12-9905 are on liver and heart. The problems of the other organs are also induced by the liver and heart problems. The abnormal values observed in the blood

examination, blood chemistry examination, and urine examination are believed to be caused by liver and heart problems.

2. On the results of the high-dose (120 mg/kg) groups

As the effects of the test substance, for the 14-day administration, in the period of administration, for both males and females, the following symptoms were observed: salivation, decrease in spontaneous activity, crouching, pronation, decrease in breathing frequency, deep breathing, anemia, hypothermia, piloerection, lacrimation. For males, increase in breathing frequency was observed. By day 14, 10 males and 7 females died.

In the blood examination, for both males and females, there is a tendency of increase in the reticular red blood cells. For males, there is a tendency of increase in the white blood cell count.

In the blood chemistry examination, for females, there is a tendency of increase in GOT.

For the organ weight, for both males and females, there is a tendency of increase in the weight of liver.

The abnormal findings in the postmortem examination of both dead cases and surviving cases and in the histopathological examination are the same as those for the group with dose of 60 mg/kg in the aforementioned 28-day repeat administration test. They are mainly changes in liver and heart as well as problems caused by heart and liver problems.

3. On the repeat test

The repeat test was performed for 4 males and 2 females in the groups with dose of 60 mg/kg, and for 1 case of male and 3 cases of females in the groups with dose of 120 mg/kg.

In the recovery period, for both the males and females in the groups with doses of 60 mg/kg and 120 mg/kg, the same deterioration in the general state as that in the administration period was observed. For the group with dose of 60 mg/kg, by recovery day 9, 1 male and 2 females died. For the group with dose of 120 mg/kg, by recovery day 11, 1 male and 1 female died.

As far as change in the body weight is concerned, for both males and females in the group with dose of 120 mg/kg, there was a decrease in the body weight observed.

As far as food consumption is concerned, for the males in the groups with doses of 60 mg/g and 120 mg/kg, there is a tendency of decrease.

1) 60-mg/kg recovery group (at the end of the recovery period)

For males, in addition to the residual effects of increase in GOT, GPT, urea nitrogen and urinary protein, decrease in the platelet count and chloride, and prolongation of the prothrombin time and activated partial thromboplastin time, the following new symptoms were observed: increase in creatinine, cloudiness of urine, increase in the weight of adrenal gland.

The histopathology examination on the dead cases and surviving cases also indicates that the residual problems are mainly related to liver and heart.

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2) 120-mg/kg recovery group (at the end of the recovery period)

For females, the following symptoms were observed: increase in GOT, GPT, urea nitrogen and segmented neutrophil proportion, decrease in the red blood cell count, white blood cell count, hemoglobin concentration, hematocrit value, platelet count and lymphocyte proportion, and prolongation of the prothrombin time and activated partial thromboplastin time. Also for the females, there is a tendency of increase in the weight of liver, heart, and adrenal gland.

For the dead cases and surviving cases, the results of histopathology examination also indicate that the changes are mainly problems related to liver and heart.

3) The aforementioned results indicate that there is no recovery property for the abnormalities caused by administration of X-12-9908

4. On the no-effect level

For the groups with dose of 8 mg/kg or higher, for both males and females, salivation was observed. For males, increase in the urinary protein was observed. Consequently, for X-12-9908, the NOEL is estimated as lower than 8 mg/kg/day.

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English Language Data

* Figures 1 - 2
* Tables 1 - 10
* Photos 1 - 7
* Addenda 1 - 9 (Observations and Findings)

The data appended after page 74 and before page 75 of the translated version of this toxicological study appeared as sixty-three (63) pages of English language materials in the original Japanese language report.

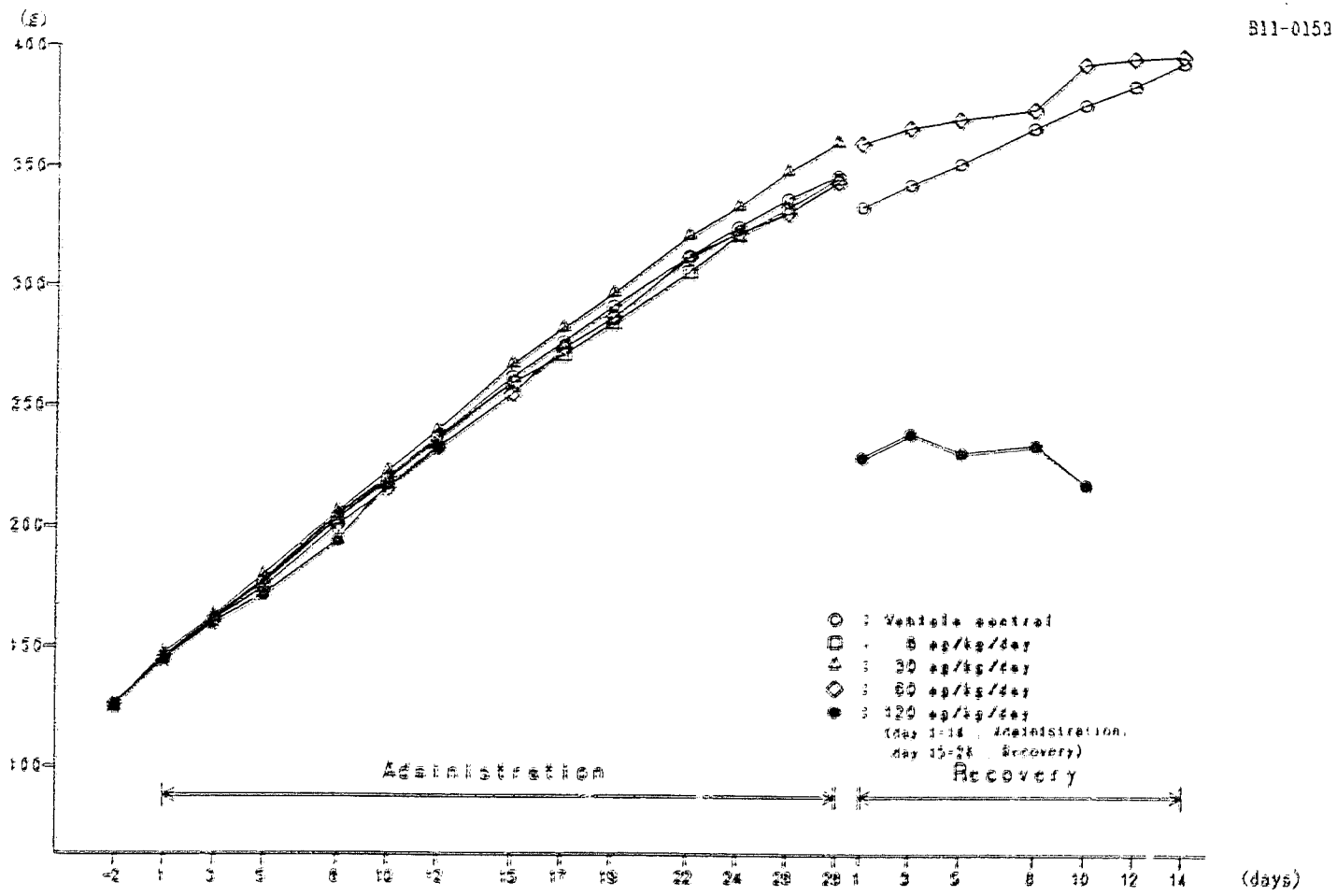

Figure 1 28-day repeated-dose oral toxicity study in rats
Mean body weights : Male

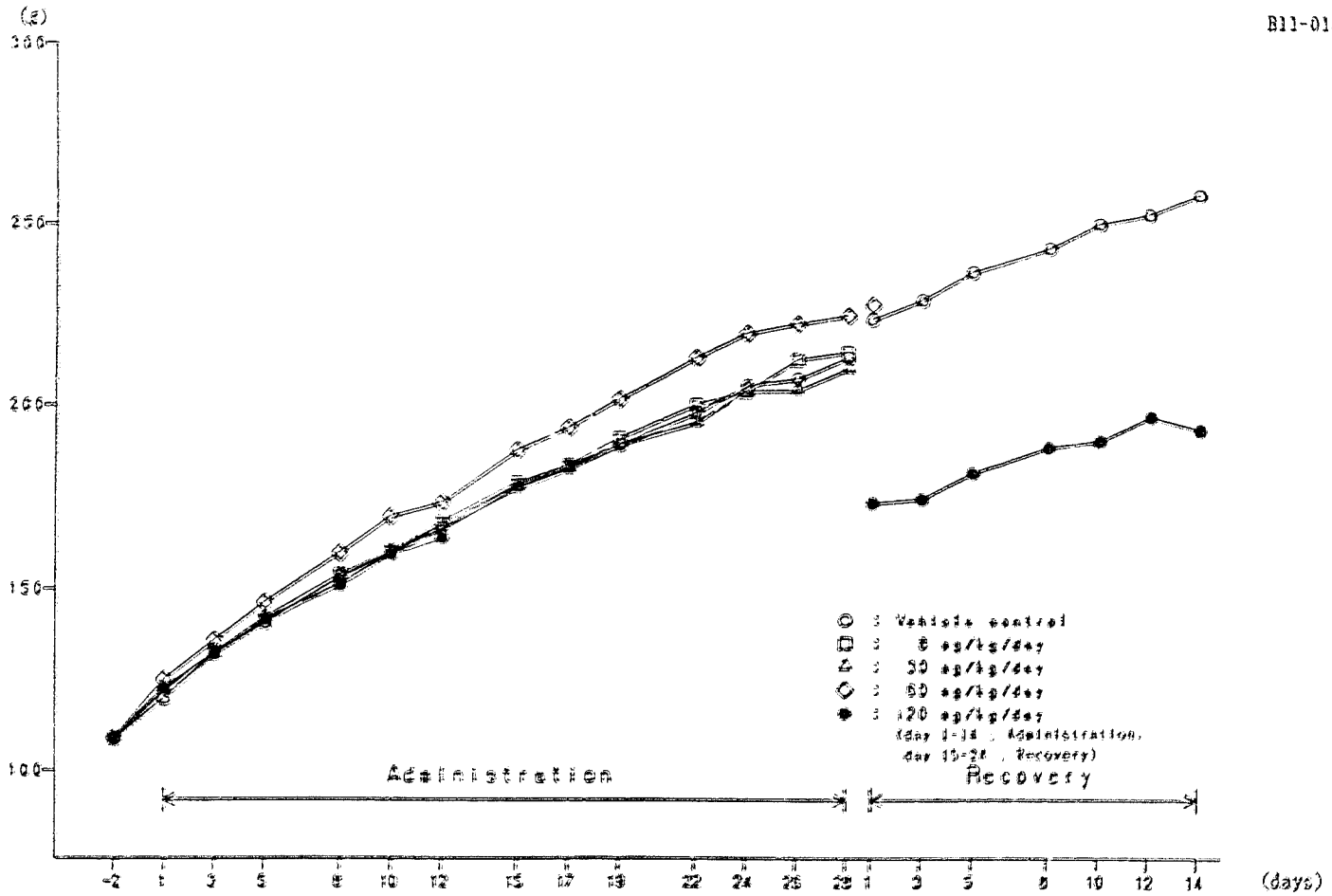

Figure 1 -Continued
Mean body weights : Female

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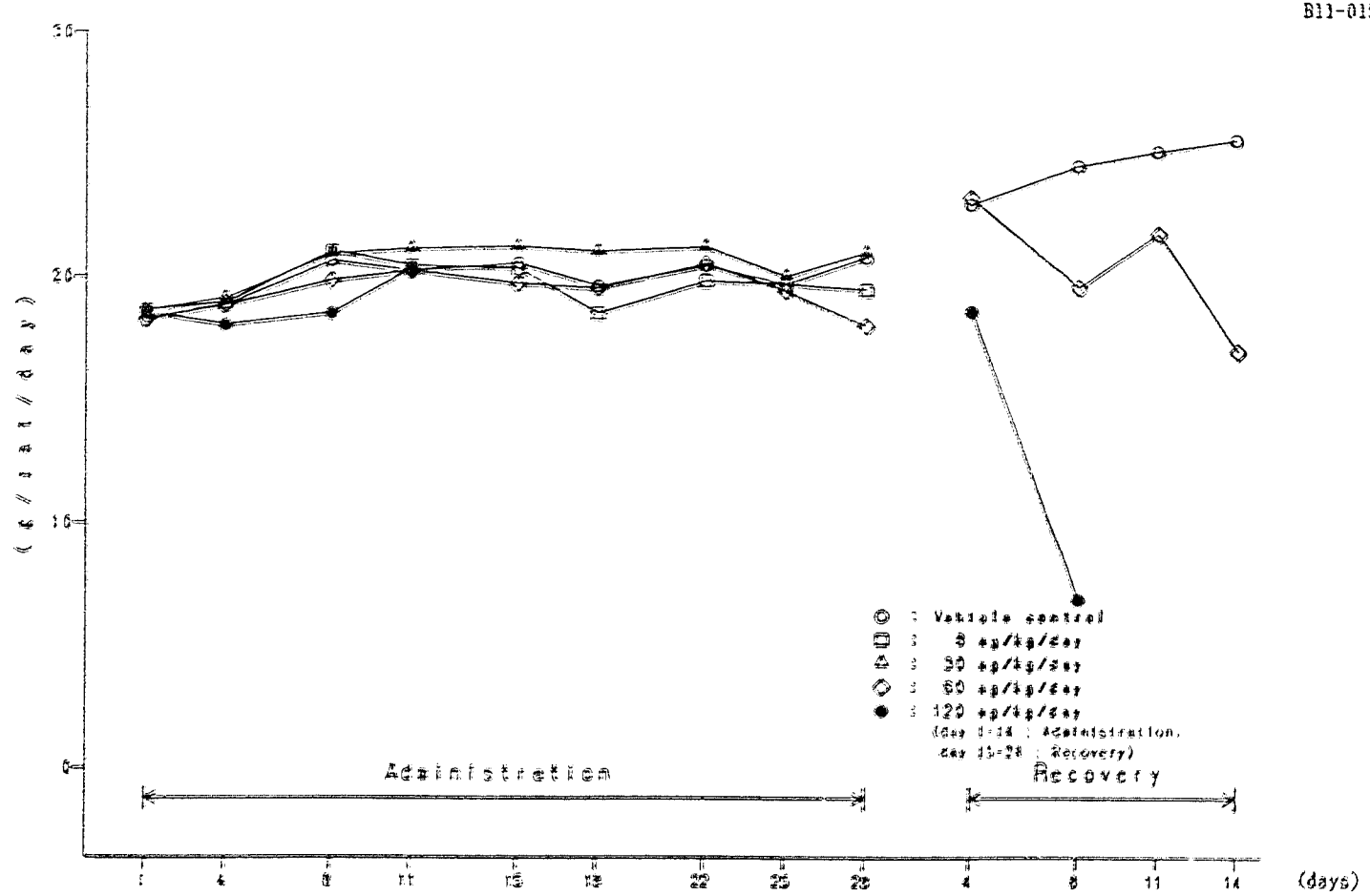

Figure 2 28-day repeated-dose oral toxicity study in rats
Mean food consumption : Male

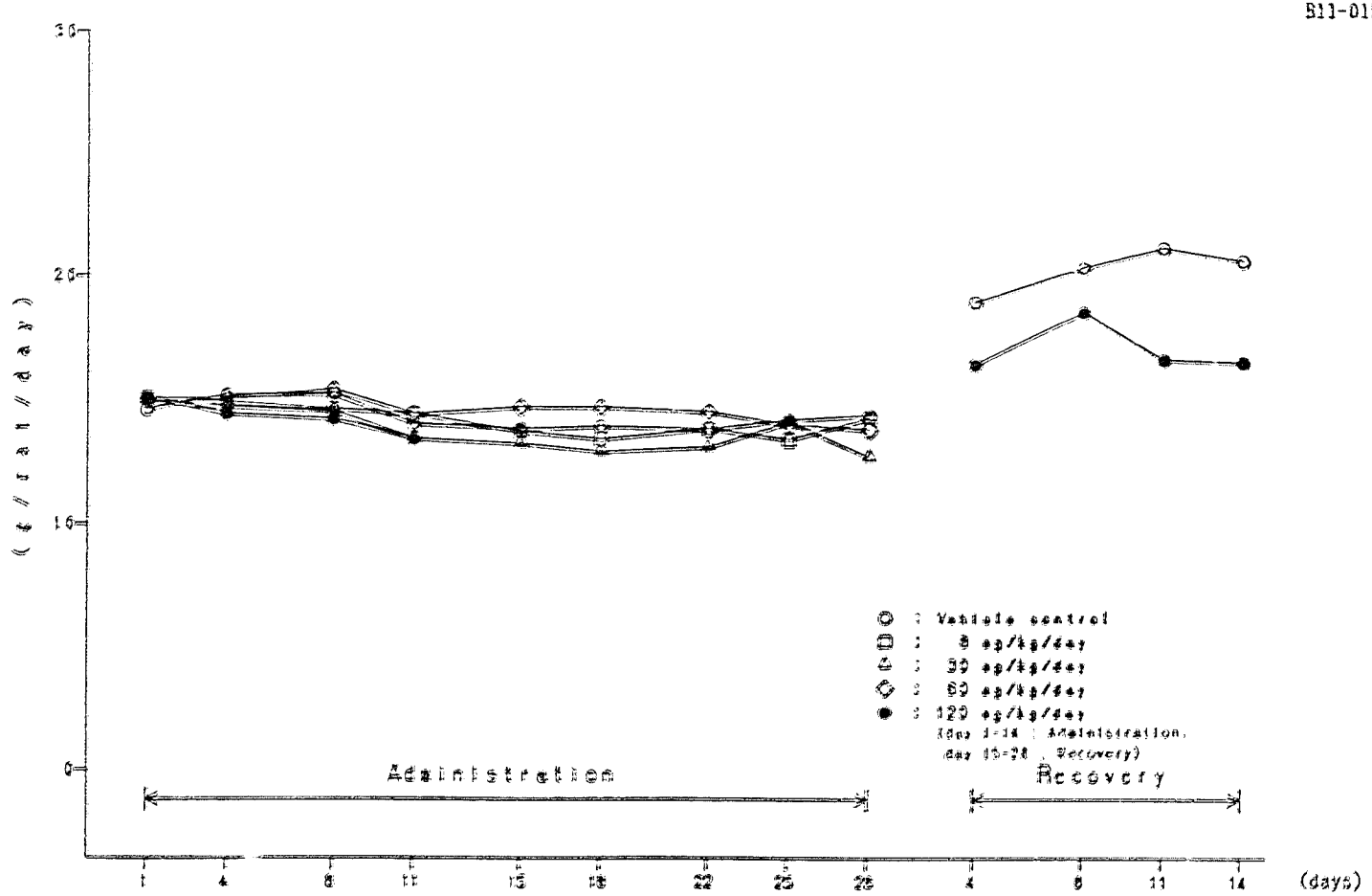

Figure 2 -Continued
Mean food consumption : Female

Table 1 28-day repeated-dose oral toxicity study in rats
Clinical signs

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Sex	Signs	No. of animals (5 animals / group)										
		Administration period								Recovery period		
		Vehicle control	Vehicle control (Recovery)	3	30	60	60 (Recovery)	120*	120 (Recovery)	Vehicle control	60	120
												(mg/kg/day)
Male	No abnormalities detected	5	4	4	5	1				4	1	
	Salivation			1	5	3	5	4	4			
	Decreased spontaneous locomotion			1		1	1	5	5		1	1
	Convulsing						1		1			1
	Prone position			1		1	1	1	1			
	Decreased respiratory rate							2	1			
	Deep breath					1	2		2		2	1
	Increased respiratory rate								1		1	
	Anemia					2	2	2	4		2	1
	Subnormal temperature					2	2	2	3		2	1
	Piloerection					2	1	1			2	1
	Lacrimation							1	2			1
	Reddish tear											1
	Nasal bleeding					1	1				2	1
	Cloudy and/or red urine					1					1	1
	Loss of hair		2				2			2	2	
	Abnormal respiratory sound	1										
	Abnormal respiration	1										
	Gasping			1								
	Cyanosis			1								
	Sacrifice							1				
	Death	1				2	2	4	5		1	1

* Sacrifice at 14days after dosing

Table 1 – Continued
Clinical signs

811-0153

Sex	Signs	No. of animals (5 animals / group)										
		Administration period								Recovery period		
		Vehicle control	Vehicle control (Recovery)	6	30	60	60 (Recovery)	120*	120 (Recovery)	Vehicle control	60	120
		(mg/kg/day)										
	No abnormalities detected	5	5	3	4	2				5		1
	Salivation			3	2	3	5	4	3			
	Decreased spontaneous locomotion					2	2	4	5		1	1
	Crouching						3	1				
	Prone position					2	1	1	1			
	Decreased respiratory rate					2		2	2			1
	Deep breath					3	3	3	2		1	1
	Ataxia					2	3	2	2		2	2
	Subnormal temperature					4	3	2	2		2	1
	Fibrillation					2	2		1			1
	Lacrimation					2	2	2	3			1
Female	Reddish tear						2					
	Nasal bleeding					1						
	Cloudy and/or red urine						1					
	Ptosis						2					
	Redness of auricle						1					
	Loss of hair	1	2							1		
	Soft stool							1				
	Sacrifice							2				
	Death					3	4	4	3		2	1

Table 2 18-day repeated-dose oral toxicity study in rats
Mean body weights(g)

B11-0153

Sex	Exp.group (mg/kg/day)	Number of animals	Administration -2	1	3	5	8	10	12 (days)
Male	Vehicle control	12	125.5 ± 5.3	145.3 ± 6.3	161.0 ± 8.4	177.5 ± 9.8	204.3 ± 11.8(11)	220.0 ± 15.1(11)	237.1 ± 17.5(11)
	6	6	125.1 ± 5.7	144.4 ± 6.1	161.0 ± 9.5	176.1 ± 12.7	202.6 ± 19.3	218.5 ± 21.7	238.2 ± 23.8
	30	6	125.4 ± 6.0	147.8 ± 7.4	162.6 ± 8.0	178.6 ± 10.0	205.8 ± 11.8	222.4 ± 15.0	238.6 ± 17.4
	60	12	125.8 ± 5.1	145.2 ± 6.6	160.5 ± 7.1	174.0 ± 7.4	200.3 ± 10.6	215.2 ± 12.4	232.3 ± 12.0(11)
	120	12	126.0 ± 4.0	144.0 ± 5.8	158.7 ± 7.2	172.6 ± 9.1(10)	194.0 ± 16.4(8)	217.0 ± 17.6(3)	253.0 ± 19.1(3)
Female	Vehicle control	12	108.4 ± 6.7	118.6 ± 7.1	132.8 ± 5.8	145.2 ± 7.3	152.7 ± 9.2	158.8 ± 9.1	158.2 ± 11.3
	6	6	108.1 ± 5.4	122.6 ± 4.3	132.3 ± 4.7	141.5 ± 5.2	153.8 ± 7.7	160.5 ± 9.4	168.2 ± 10.8
	30	6	108.3 ± 6.4	121.6 ± 6.6	132.2 ± 4.4	142.0 ± 4.2	153.7 ± 5.7	160.3 ± 6.7	166.9 ± 8.2
	60	12	108.0 ± 6.4	124.0 ± 5.7	133.5 ± 6.3	146.2 ± 5.6	158.8 ± 9.8	165.0 ± 8.8(11)	172.4 ± 8.3(10)
	120	12	108.1 ± 5.3	122.7 ± 5.0	131.7 ± 6.3	141.2 ± 8.0	152.0 ± 12.5	158.4 ± 11.4(9)	164.1 ± 16.1(6)

Mean ±S.D.
Figures in parentheses indicate number of animals used for mean calculation.
* : Significantly different from Vehicle control at P<0.05.
** : Significantly different from Vehicle control at P<0.01.

Table 2 -Continued
Mean body weights(g)

811-0153

Sex	Exp.group (mg/kg/day)	Number of animals	Administration 15	17	19	22	24	26	28 (days)
Male	Vehicle control	11	261.8 ± 20.7	276.4 ± 23.1	291.3 ± 24.2	312.4 ± 29.7	325.0 ± 32.3	337.1 ± 33.8	347.0 ± 36.6
	6	6	259.4 ± 25.6	271.2 ± 29.3	284.5 ± 32.3	305.7 ± 34.3	321.5 ± 37.3	333.2 ± 38.0	345.0 ± 41.4
	30	6	267.2 ± 20.3	282.1 ± 21.7	296.6 ± 23.1	321.0 ± 25.0	333.5 ± 25.6	348.5 ± 27.2	360.7 ± 27.1
	60	11	255.0 ± 20.0	273.9 ± 14.6(10)	288.6 ± 18.5(10)	311.8 ± 20.4(9)	322.3 ± 22.2(9)	330.8 ± 31.3(9)	344.3 ± 34.2(8)
Female	Vehicle control	12	178.1 ± 11.1	183.2 ± 12.1	189.2 ± 12.2	197.9 ± 13.9	205.6 ± 16.7	207.4 ± 15.9	213.3 ± 17.5
	6	6	178.9 ± 13.1	183.7 ± 8.5	190.3 ± 10.6	200.3 ± 12.4	204.1 ± 13.6	212.8 ± 12.0	214.8 ± 14.3
	30	6	177.2 ± 10.1	182.4 ± 11.4	189.1 ± 10.4	195.2 ± 11.9	204.1 ± 11.9	204.2 ± 14.7	210.3 ± 14.1
	60	6	187.7 ± 7.4	193.8 ± 6.4	201.6 ± 7.1	213.2 ± 5.5	220.0 ± 8.3	222.8 ± 8.2	225.0 ± 7.9(5)

Mean ± S.D.
Figures in parentheses indicate number of animals used for mean calculation.
* : Significantly different from Vehicle control at P<0.05.
** : Significantly different from Vehicle control at P<0.01.

Table 2 -Continued
Mean body weights(g)

B11-0153

Sex	Exp.group (mg/kg/day)	Number of animals	Recovery 1	3	5	8	10	12	14 (days)
Male	Vehicle control	6	333.6 ± 42.3	343.2 ± 43.9	352.2 ± 45.5	367.0 ± 47.7	376.6 ± 51.0	384.8 ± 50.5	394.7 ± 51.9
	60	4	366.5 ± 23.3	367.1 ± 22.5	370.5 ± 32.6	374.7 ± 42.4	393.8 ± 25.5(3)	396.2 ± 36.1(3)	397.4 ± 46.0(3)
Female	Vehicle control	6	224.0 ± 6.4	228.4 ± 11.3	237.1 ± 9.5	243.7 ± 13.4	250.6 ± 13.9	253.3 ± 15.9	258.9 ± 13.3
	60	2	226.0 ± 7.0	-	-	-	-	-	-

Mean ± S.D.
Figures in parentheses indicate number of animals used for mean calculation.
* : Significantly different from Vehicle control at P<0.05.
** : Significantly different from Vehicle control at P<0.01.

Table 2 -Continued

Mean body weights(g) : (administration for 14 days)

B11-0153

Sex	Dig group (mg/kg/day)	Number of animals	Recovery						
			1	3	5	8	10	12	14 (days)
Male	120	1	226.3 -	236.1 -	231.1 -	234.2 -	218.0 -	- -	- -
Female	120	3	173.5 ± 15.2	174.7 ± 6.2	181.9 ± 12.4(2)	188.7 ± 8.14(2)	190.5 ± 6.2(3)	187.1 ± 1.9(2)	193.5 ± 2.0(2)

Mean ±S.E.

Figures in parentheses indicate number of animals used for mean calculation.

Table 3 28-day repeated-dose oral toxicity study in rats
Mean food consumption(g/rat/day)

Sex	Exp.group (mg/kg/day)	Number of animals	Administration 1	4	8	11	15	18	22	25	28 (days)
Male	Vehicle control	12	18.1 ± 0.9	18.6 ± 1.6	20.6 ± 1.6(11)	20.2 ± 2.8(11)	20.5 ± 2.1(11)	19.6 ± 2.0(11)	20.4 ± 2.5(11)	19.[illegible] ± 2.8(11)	20.8 ± 3.0(11)
	6	6	18.6 ± 1.1	18.9 ± 1.4	21.0 ± 2.9	20.4 ± 2.8	20.3 ± 2.5	18.5 ± 3.4	19.8 ± 2.6	19.7 ± 2.5	19.5 ± 2.5
	30	6	18.6 ± 0.9	19.1 ± 1.4	20.9 ± 1.6	21.1 ± 2.5	21.2 ± 2.2	21.0 ± 2.7	21.2 ± 1.8	20.0 ± 1.4	21.0 ± 2.1
	60	12	18.2 ± 1.4	18.6 ± 1.3	19.8 ± 1.6	20.2 ± 1.7(11)	19.7 ± 2.6(12)	19.[illegible] ± 2.6(12)	20.5 ± 2.3(9)	19.4 ± 2.7(9)	18.0 ± 5.8(8)
	120	12	18.5 ± 1.1	18.0 ± 2.8(6)	18.5 ± 2.0(5)	20.3 ± 2.1(3)	- -	- -	- -	- -	- -
Female	Vehicle control	12	14.6 ± 2.6	15.2 ± 1.4	15.2 ± 1.6	14.0 ± 1.4	13.8 ± 1.4	13.9 ± 1.7	13.8 ± 1.8	14.2 ± 2.4	14.4 ± 1.8
	6	6	15.1 ± 1.0	14.9 ± 1.2	14.6 ± 1.3	14.4 ± 1.5	13.7 ± 1.4	13.4 ± 0.7	13.8 ± 0.8	13.4 ± 1.2	14.3 ± 1.4
	30	6	14.5 ± 1.3	14.7 ± 0.4	14.5 ± 0.9	13.4 ± 1.1	13.2 ± 1.4	12.9 ± 1.3	13.1 ± 0.8	14.1 ± 0.0	12.7 ± 2.0
	60	12	15.0 ± 1.0	15.1 ± 1.0	15.4 ± 1.3	14.4 ± 1.2(11)	14.7 ± 0.9(6)	14.7 ± 0.6(6)	14.5 ± 1.5(6)	14.0 ± 1.3(6)	13.8 ± 1.3(4)
	120	12	15.1 ± 0.6	14.4 ± 1.3	14.2 ± 1.6	13.4 ± 0.6(7)	- -	- -	- -	- -	- -

Mean ±S.D.
Figures in parentheses indicate number of animals used for mean calculation.
* : Significantly different from Vehicle control at P<0.05.
** : Significantly different from Vehicle control at P<0.01.

Table 3 -Continued

Mean food consumption(g/rat/day)

Sex	Exp.group (mg/kg/day)	Number of animals	Recovery 4	8	11	14 (days)
Male	Vehicle control	6	23.0 ± 3.7	24.6 ± 3.0	24.2 ± 2.6	25.7 ± 3.0
	60	4	23.7 ± 2.1	18.8 ± 13.0	21.8 ± 6.3(3)	17.0 ± 11.4(3)
Female	Vehicle control	6	18.6 ± 1.7	20.4 ± 1.7	21.2 ± 1.8	20.7 ± 1.9
	60	0	- -	- -	- -	- -

Mean ±S.D.

Figures in parentheses indicate number of animals used for mean calculation.

* : Significantly different from Vehicle control at P<0.05.

** : Significantly different from Vehicle control at P<0.01.

Table : -Continued
Mean food consumption(g/rat/day) : (administration for 14 days)

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Sex	Dig group (mg/kg/day)	Number of animals	Recovery 4	8	11	14 (days)
Male	120	1	18.6 -	6.6 -	- -	- -
Female	120	2	16.5 ± 3.1	18.6 ± 0.2	16.7 ± 1.2	16.8 ± 3.7

Mean ±S.D.

Table 4 28-day repeated-dose oral toxicity study in rats
Hematology

Sex	Exp.group (mg/kg/day)	Number of animals	RBC ($\times 10^4/mm^3$)	WBC ($\times 10^2/mm^3$)	Hb (g/dl)	Ht (%)	MCV (μm^3)	MCH (pg)	MCHC (%)	Platelet ($\times 10^4/mm^3$)	Reticulo (‰)	P T (sec)	APTT (sec)
Male	Vehicle control	5	723 ± 23	117 ± 24	14.3 ± 0.3	44.2 ± 0.4	61.0 ± 1.8	19.9 ± 0.8	32.5 ± 0.9	112.2 ± 10.8	17 ± 3	13.9 ± 1.4	26.3 ± 4.0
	6	5	737 ± 39	99 ± 23	14.5 ± 0.4	43.9 ± 2.2	59.6 ± 1.3	19.7 ± 0.7	33.1 ± 1.2	110.2 ± 8.7	17 ± 6	12.8 ± 0.9	27.4 ± 3.7
	30	6	739 ± 31	109 ± 22	14.2 ± 0.5	42.8 ± 1.4	58.0* ± 1.3	19.2 ± 0.7	33.1 ± 0.6	117.0 ± 11.2	17 ± 7	13.1 ± 0.8	26.6 ± 2.2
	60	4	766 ± 57	126 ± 25	13.8 ± 0.8	43.2 ± 2.8	56.9** ± 1.6	18.2* ± 0.9	31.9 ± 1.1	81.3 ± 32.1	24 ± 3	14.1 ± 1.5	34.3 ± 10.4
	Recovery Vehicle control	6	841 ± 57	106 ± 16	14.9 ± 0.5	46.5 ± 1.8	55.4 ± 2.2	17.7 ± 0.7	32.0 ± 0.5	113.2 ± 4.0	22 ± 8	13.9 ± 1.3	27.8 ± 3.0
	60	3	862 ± 30	100 ± 36	15.1 ± 0.8	48.6 ± 4.2	56.4 ± 1.1	17.6 ± 0.7	31.2 ± 0.7	71.6* ± 40.2	22 ± 8	14.8 ± 0.5	37.5 ± 16.6
Female	Vehicle control	6	731 ± 29	79 ± 13	14.5 ± 0.7	41.3 ± 1.8	56.5 ± 1.3	19.9 ± 0.8	35.2 ± 1.1	141.4 ± 13.0	11 ± 3	12.2 ± 0.2	22.9 ± 2.2
	6	6	761 ± 34	81 ± 14	14.5 ± 0.5	42.8 ± 1.5	57.0 ± 1.0	19.3 ± 0.4	33.9* ± 0.7	138.8 ± 9.4	16 ± 7	11.7 ± 1.1	24.1 ± 2.0
	30	6	755 ± 38	74 ± 19	14.5 ± 0.6	42.6 ± 1.9	56.8 ± 1.7	19.2 ± 0.5	33.8* ± 0.5	124.0* ± 10.9	13 ± 5	11.8 ± 0.7	24.3 ± 1.3
	60	1	764 -	93 -	15.0 -	43.3 -	56.7 -	19.6 -	34.6 -	140.9 -	18 -	11.6 -	26.9 -
	Recovery Vehicle control	6	792 ± 36	88 ± 12	14.3 ± 0.5	43.2 ± 1.9	54.5 ± 1.5	18.1 ± 0.4	33.2 ± 0.5	129.6 ± 14.5	18 ± 7	11.7 ± 0.3	21.6 ± 2.2
	60	0	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -

Mean ± S.D.
* : Significantly different from Vehicle control at P<0.05.
** : Significantly different from Vehicle control at P<0.01.

Table 4 Continued
Hematology

Sex	Exp.group (mg/kg/day)	Number of animals	Differentiation of Leukocyte (%)					
			Mono	Baso	Eosino	Lymph	Stab	Seg
Male	Vehicle control	5	0.0 ±0.0	0.0 ±0.0	0.0 ±0.4	80.0 ± 3.1	0.0 ±0.0	8.2 ± 3.3
	6	6	0.2 ±0.3	0.0 ±0.0	0.0 ±0.6	85.6 ± 3.2	0.3 ±0.3	13.3 ± 3.1
	30	6	0.0 ±0.0	0.0 ±0.0	0.4 ±0.5	83.8 ± 5.9	0.3 ±0.3	15.5 ± 6.1
	60	4	0.3 ±0.5	0.0 ±0.0	0.1 ±0.3	86.6 ± 7.0	0.0 ±0.0	13.0 ± 8.7
	Recovery							
	Vehicle control	6	0.1 ±0.2	0.0 ±0.0	0.2 ±0.4	88.3 ± 5.8	0.0 ±0.0	11.5 ± 6.0
	60	3	0.0 ±0.0	0.0 ±0.0	0.3 ±0.3	85.0 ±11.7	0.0 ±0.0	14.7 ±11.5
Female	Vehicle control	6	0.0 ±0.0	0.0 ±0.0	0.3 ±0.3	89.8 ± 6.6	0.1 ±0.2	10.1 ± 6.4
	6	6	0.0 ±0.0	0.0 ±0.0	0.3 ±0.4	86.8 ± 3.6	0.2 ±0.3	12.8 ± 3.9
	30	6	0.0 ±0.0	0.0 ±0.0	0.0 ±0.4	88.8 ± 2.3	0.0 ±0.0	10.5 ± 2.2
	60	1	0.0 -	0.0 -	1.0 -	81.0 -	0.0 -	18.0 -
	Recovery							
	Vehicle control	6	0.0 ±0.0	0.0 ±0.0	0.7 ±0.5	86.8 ± 6.2	0.0 ±0.0	12.5 ± 6.8
	60	0	- -	- -	- -	- -	- -	- -

Mean ±S.D.
* : Significantly different from Vehicle control at P<0.05.
** : Significantly different from Vehicle control at P<0.01.

Table 4 -Continued

Hematology : (administration for 14 days)

Sex	Dos.group (mg/kg/day)	Number of animals	RBC ($\times 10^4/mm^3$)	WBC ($\times 10^2/mm^3$)	Hb (g/dl)	Ht (%)	MCV (μm^3)	MCH (pg)	MCHC (%)	Platelet ($\times 10^4/mm^3$)	Reticulo (‰)	P T (sec)	APTT (sec)
Male	120	1	722	233	14.2	46.3	64.0	19.5	30.5	121.5	50	13.5	22.4
			-	-	-	-	-	-	-	-	-	-	-
	Recovery 120	6	-	-	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-	-	-
Female	120	2	724	66	14.0	44.5	61.5	19.3	31.4	120.1	25	12.3	10.5
			± 7	± 32	± 0.2	± 0.6	± 0.2	± 0.1	± 0.1	± 23.2	± 4	± 0.2	± 3.3
	Recovery 120	2	766	57	13.3	39.3	65.2	18.9	33.8	82.0	13	13.7	41.3
			± 63	± 42	± 1.7	± 3.7	± 0.3	± 0.6	± 1.1	± 80.9	± 1	± 3.0	110.0

Mean ± S.D.

Table 4 -Continued
Hematology : (administration for 14 days)

Sex	Exp.group (mg/kg/day)	Number of animals	Differentiation of Leukocyte (%) Mono	Baso	Eosino	Lymph	Stab	Seg
Male	120	1	0.0	0.0	0.5	94.0	0.5	5.0
			-	-	-	-	-	-
	Recovery 120	0	-	-	-	-	-	-
			-	-	-	-	-	-
Female	120	2	0.0	0.0	0.0	94.5	0.5	5.5
			±0.0	±0.0	±0.0	± 1.2	±0.4	± 1.4
	Recovery 120	2	0.0	0.0	0.5	91.0	0.0	8.0
			±0.0	±0.0	±0.4	± 0.1	±0.0	± 0.5

Mean ±S.D.

Table 6 28-day repeated-dose oral toxicity study in rats
Blood chemistry

B11-0153

Sex	Exp. group (mg/kg/day)	Number of animals	GOT (IU/l)	GPT (IU/l)	ALP (IU/l)	ChE (IU/l)	γ-GTP (IU/l)	T-Cho (mg/dl)	TG (mg/dl)	Glucose (mg/dl)	T-protein (g/dl)	Albumin (g/dl)	A/G ratio
Male	Vehicle control	6	69 ± 8	24 ± 3	492 ± 66	51 ± 11	0.6 ±0.3	54 ± 7	65 ±15	162.8 ± 24.7	5.3 ±0.2	3.0 ±0.1	1.25 ±0.11
	8	6	71 ± 7	25 ± 4	527 ±103	53 ± 10	0.6 ±0.1	50 ± 9	60 ±12	145.5 ± 13.0	5.4 ±0.2	3.0 ±0.2	1.28 ±0.09
	30	6	65 ± 6	26 ± 4	534 ± 54	59 ± 11	0.7 ±0.3	45 ± 4	65 ±14	147.2 ± 12.4	5.6 ±0.2	3.0 ±0.1	1.16 ±0.08
	60	4	417 ±574	216 ±367	636 ±206	65 ± 16	0.6 ±0.1	60 ± 8	91 ±11	175.5 ± 39.4	5.6 ±0.4	3.1 ±0.2	1.26 ±0.06
	Recovery												
	Vehicle control	6	77 ± 13	25 ± 4	312 ± 32	55 ± 11	0.6 ±0.2	55 ±13	65 ±13	130.7 ± 11.4	5.6 ±0.5	3.0 ±0.1	1.15 ±0.06
	60	3	553 ±793	305* ±454	356 ± 75	45 ± 4	0.2** ±0.1	47 ± 3	53 ± 8	151.6 ± 38.0	5.5 ±0.3	2.9 ±0.1	1.13 ±0.11
Female	Vehicle control	6	63 ± 9	20 ± 3	315 ± 55	228 ± 75	1.1 ±0.4	58 ± 7	27 ± 2	141.9 ± 12.9	5.5 ±0.1	3.3 ±0.1	1.47 ±0.11
	8	6	63 ± 6	20 ± 3	287 ± 75	237 ±115	1.0 ±0.3	48* ± 5	27 ± 4	146.6 ± 19.7	5.6 ±0.2	3.2 ±0.1	1.31 ±0.13
	30	6	66 ± 9	20 ± 1	293 ± 41	284 ±104	0.9 ±0.2	48 ± 6	30 ± 8	147.3 ± 14.9	5.6 ±0.2	3.3 ±0.2	1.39 ±0.15
	60	1	86 -	18 -	363 -	253 -	1.6 -	53 -	25 -	145.9 -	6.0 -	3.5 -	1.40 -
	Recovery												
	Vehicle control	6	67 ± 8	23 ± 8	191 ± 16	357 ±105	0.6 ±0.2	64 ±13	31 ± 5	139.7 ± 20.8	5.9 ±0.1	3.2 ±0.2	1.23 ±0.06
	60	0	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -

Mean ± S.D.
* : Significantly different from Vehicle control at P<0.05.
** : Significantly different from Vehicle control at P<0.01

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Table 5 -Continued
Blood chemistry

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Sex	Dosage group (mg/kg/day)	Number of animals	BUN (mg/dl)	Creatinine (mg/dl)	T-Bil (mg/dl)	Ca (mg/dl)	IP (mg/dl)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)
Male	Vehicle control	5	11.4 ± 0.9	0.45 ±0.07	0.23 ±0.02	9.8 ± 0.2	7.8 ±0.5	140 ± 1	4.4 ±0.4	108.7 ± 3.7
	6	6	10.8 ± 1.0	0.46 ±0.04	0.20 ±0.03	9.7 ± 0.2	7.7 ±0.9	140 ± 1	4.5 ±0.4	108.8 ± 1.5
	30	6	11.4 ± 1.3	0.42 ±0.10	0.20 ±0.05	9.6 ± 0.3	7.9 ±0.3	139 ± 2	4.1 ±0.4	108.1 ± 0.5
	60	4	14.3 ± 7.6	0.52 ±0.06	0.16 ±0.04	10.1 ± 0.4	7.8 ±0.2	139 ± 6	4.3 ±0.7	103.8* ± 2.8
	Recovery									
	Vehicle control	6	14.6 ± 1.1	0.47 ±0.06	0.19 ±0.04	9.4 ± 0.3	8.0 ±0.4	141 ± 1	4.3 ±0.4	107.5 ± 1.4
	60	3	28.3* ±15.2	0.53* ±0.04	0.16 ±0.01	9.7 ± 0.3	7.1 ±0.8	139 ± 4	4.8 ±0.8	103.5* ± 3.7
Female	Vehicle control	6	12.5 ± 2.3	0.46 ±0.07	0.25 ±0.02	9.7 ± 0.2	7.8 ±0.7	139 ± 1	4.2 ±0.2	107.0 ± 1.8
	6	6	12.4 ± 1.3	0.47 ±0.08	0.22 ±0.03	9.8 ± 0.2	7.4 ±0.5	137 ± 2	3.9 ±0.3	105.1* ± 0.8
	30	6	12.7 ± 1.5	0.48 ±0.03	0.25 ±0.03	9.6 ± 0.2	7.3 ±0.7	139 ± 1	4.0 ±0.4	108.0 ± 0.9
	60	1	15.8 =	0.68 =	0.11 =	10.0 =	7.6 =	138 =	3.9 =	104.7 =
	Recovery									
	Vehicle control	6	16.8 ± 5.5	0.52 ±0.07	0.20 ±0.03	9.4 ± 0.3	8.5 ±0.5	139 ± 3	4.2 ±0.4	105.5 ± 2.1
	60	6	= =	= =	= =	= =	= =	= =	= =	= =

Mean ±S.D.
* : Significantly different from Vehicle control at P<0.05.
** : Significantly different from Vehicle control at P<0.01.

Table 5 -Continued

Blood chemistry : (administration for 14 days)

811-0153

Sex	Exp.group (mg/kg/day)	Number of animals	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	ChE (IU/L)	γ-GTP (IU/L)	T-Cho (mg/dl)	TG (mg/dl)	Glucose (mg/dl)	T-protein (g/dl)	Albumin (g/dl)	A/G ratio
Male	120	1	76	28	605	52	0.8	49	72	152.7	5.7	3.2	1.28
			-	-	-	-	-	-	-	-	-	-	-
	Recovery 120	6	-	-	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-	-	-
Female	120	2	133	22	451	300	0.9	77	45	158.6	5.7	3.4	1.43
			± 183	± 6	± 37	± 8	±0.1	± 2	±20	± 11.4	±0.6	±0.1	±0.08
	Recovery 120	2	1267	664	327	306	1.0	52	42	157.1	5.6	3.3	1.40
			±1656	±692	± 45	±274	±0.2	± 0	± 0	± 39.7	±0.0	±0.2	±0.22

Mean ±S.D.

Table 5 -Continued
Blood chemistry : (administration for 14 days)

B11-0153

Sex	Exp. group (mg/kg/day)	Number of animals	BUN (mg/dl)	Creatinine (mg/dl)	T-Bil (mg/dl)	Ca (mg/dl)	P (mg/dl)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)
Male	125	1	11.1	0.42	0.17	9.5	8.8	143	4.2	101.8
			-	-	-	-	-	-	-	-
	Recovery									
	125	6	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-
Female	125	2	16.7	0.39	0.23	10.0	7.0	141	4.2	104.5
			± 3.6	±0.03	±0.02	± 0.6	±1.1	± 2	±0.1	± 0.4
	Recovery									
	125	2	20.7	0.56	0.17	9.5	7.4	135	4.5	105.4
			± 6.6	±0.06	±0.02	± 0.6	±0.0	± 1	±0.1	± 3.7

Mean ±S.D.

Table 6 28-day repeated-dose oral toxicity study in rats
Urinalysis

B11-0153

Sex	Exp. group (mg/kg/day)	Number of animals	Volume[a] (ml)	Color SY	Y	(T)	pH 5.0	6.0	6.5	Protein −	±	+	++	Ketones −	±	Bilirubin −	Occult Blood −	±	+	Glucose −	Urobilinogen (EU/dl) 0.1	1
Male	Vehicle control	5	11 ± 5	3	2		2	2	3	0	4	1	0	2	3	5	2	3	0	5	3	2
	6	6	6 ± 3	0	6		0	4	2	0	2	2	2	3	3	6	3	3	0	6	2	4
	30	6	8 ± 3	0	6		0	4	2	0	1	2	3	3	3	6	2	3	1	6	2	4
	60	4	9 ± 6	1	3		0	3	1	0	2	1	1	0	4	4	1	1	2	4	3	1
	Recovery																					
	Vehicle control	6	12 ± 6	2	4		0	3	3	0	3	3	0	1	5	6	5	1	0	6	5	1
	60	3	6 ± 1	0	3	(1)	0	2	1	0	0	1	2	2	1	3	1	2	0	3	2	1
Female	Vehicle control	6	5 ± 5	2	4		2	4	1	1	1	1	3	6	0	6	5	1	0	6	2	4
	6	6	6 ± 4	2	4		0	4	2	1	1	2	1	6	0	6	5	1	0	6	2	4
	30	6	6 ± 5	2	4		0	4	2	1	4	0	1	6	0	6	3	3	0	6	3	3
	60	1	3 ±	0	1		1	0	0	0	0	0	1	1	0	1	1	0	0	1	0	1
	Recovery																					
	Vehicle control	6	8 ± 3	1	5		0	3	3	0	5	2	1	6	0	6	4	2	0	6	3	3
	60	0	±	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

a) : Mean ± S. D.
SY : Slightly yellow
Y : Yellow
(T) : Turbid

Table 6 – Continued

Urinalysis (administration for 14 days)

B11-0155

Sex	Dig. group (mg/kg/day)	Number of animals	Volume[a] (ml)	Color SY	Color Y	pH 6.5	Protein –	Protein ±	Ketones –	Bilirubin –	Occult Blood –	Glucose –	Urobilinogen (EU/dl) 0.1	Urobilinogen (EU/dl) 1
Male	120	0	– –	0	0	0	0	0	0	0	0	0	0	0
	Recovery 120	0	– –	0	0	0	0	0	0	0	0	0	0	0
Female	120	0	– –	0	0	0	0	0	0	0	0	0	0	0
	Recovery 120	2	0 ± 6	1	1	2	2	2	2	2	2	2	1	1

a) : Mean ± S.D.

SY : Slightly yellow

Y : Yellow

Table 7 28-day repeated-dose oral toxicity study in rats
Absolute organ weights

Sex	Dos.group (mg/kg/day)	Number of animals	Spleen (g)	Liver (g)	Kidney (g)	Heart (g)	Brain (g)	Testis (g)	Adrenal gland (mg)	Ovary (mg)	Body weight (g)
Male	Vehicle control	6	0.66 ± 0.06	11.18 ± 1.14	2.41 ± 0.22	1.70 ± 0.08	1.81 ± 0.05	2.88 ± 0.18	53.1 ± 5.8		345.0 ± 18.0
	6	6	0.64 ± 0.12	10.40 ± 1.76	2.35 ± 0.33	1.13 ± 0.06	1.81 ± 0.11	2.90 ± 0.17	54.7 ± 5.6		327.3 ± 40.0
	30	6	0.63 ± 0.06	11.71 ± 1.52	2.53 ± 0.21	1.16 ± 0.13	1.80 ± 0.06	3.10 ± 0.16	53.4 ± 8.8		340.0 ± 38.2
	60	4	0.64 ± 0.15	12.45 ± 1.40	3.28 ± 0.28	1.21 ± 0.10	1.67 ± 0.10	3.11 ± 0.12	56.0 ± 8.8		318.7 ± 31.8
	Recovery										
	Vehicle control	6	0.62 ± 0.10	10.67 ± 1.60	2.54 ± 0.36	1.31 ± 0.14	1.83 ± 0.14	3.02 ± 0.20	61.9 ± 6.9		365.8 ± 47.1
	60	3	0.63 ± 0.22	12.67 ± 2.68	2.66 ± 0.33	1.40 ± 0.15	1.97 ± 0.03	3.16 ± 0.43	70.4** ± 6.1		376.8 ± 34.4
Female	Vehicle control	6	0.45 ± 0.05	6.28 ± 0.74	1.56 ± 0.09	0.78 ± 0.06	1.80 ± 0.04		57.8 ± 5.9	98.6 ± 12.5	193.4 ± 17.2
	6	6	0.45 ± 0.10	6.00 ± 0.66	1.59 ± 0.11	0.80 ± 0.06	1.77 ± 0.05		66.6 ± 4.7	102.7 ± 11.2	203.6 ± 12.2
	30	6	0.40 ± 0.06	6.41 ± 0.32	1.66 ± 0.11	0.81 ± 0.11	1.78 ± 0.08		63.7 ± 10.2	93.4 ± 4.2	199.4 ± 14.8
	60	1	0.47 ± -	7.15 ± -	1.55 ± -	0.87 ± -	1.66 ± -		69.0 ± -	106.2 ± -	218.2 ± -
	Recovery										
	Vehicle control	6	0.48 ± 0.02	6.87 ± 0.84	1.61 ± 0.12	0.83 ± 0.08	1.84 ± 0.05		76.2 ± 4.2	100.2 ± 12.0	237.1 ± 12.0
	60	0	- ± -	- ± -	- ± -	- ± -	- ± -		- ± -	- ± -	- ± -

Mean ± S.D.
* : Significantly different from vehicle control at P<0.05.
** : Significantly different from vehicle control at P<0.01.

Table 7 - Continued

Absolute organ weights : (administration for 14 days)

B11-0153

Sex	Exp. group (mg/kg/day)	Number of animals	Spleen (g)	Liver (g)	Kidney (g)	Heart (g)	Brain (g)	Testis (g)	Adrenal gland (mg)	Ovary (mg)	Body weight (g)
Male	125	1	0.63	9.44	2.10	-	1.87	2.17	44.0	-	234.3
			-	-	-	-	-	-	-	-	-
	Recovery 125	0	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-
Female	125	2	0.40	5.82	1.45	-	1.74	-	48.2	67.0	151.0
			± 0.61	± 0.24	± 0.66	-	± 0.02	-	± 2.5	± 0.8	± 2.7
	Recovery 125	2	0.15	6.75	1.45	0.84	1.74	-	75.7	66.0	183.3
			± 0.11	± 1.33	± 0.66	± 0.02	± 0.02	-	± 0.8	± 15.0	± 0.2

Mean ± S.D.

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Table 4 90-day repeated-dose oral toxicity study in rats
Relative organ weights

B11-0153

Sex	Dose group (mg/kg/day)	Number of animals	Spleen (g/100g)	Liver (g/100g)	Kidney (g/100g)	Heart (g/100g)	Brain (g/100g)	Testis (g/100g)	Adrenal gland (mg/100g)	Ovary (mg/100g)	Body weight (g)
Male	Vehicle control	6	0.20 ±0.02	3.24 ±0.16	0.70 ±0.04	0.35 ±0.04	0.53 ±0.03	0.87 ±0.06	15.4 ± 1.2		345.0 ± 18.0
	6	6	0.17 ±0.03	3.13 ±0.20	0.71 ±0.04	0.35 ±0.02	0.53 ±0.05	0.80 ±0.10	16.9 ± 2.7		327.3 ± 40.0
	30	6	0.19 ±0.01	3.43 ±0.13	0.75 ±0.06	0.35 ±0.03	0.56 ±0.04	0.81 ±0.08	15.7 ± 2.0		340.0 ± 20.2
	60	4	0.20 ±0.04	3.32* ±0.15	0.72 ±0.05	0.39 ±0.03	0.59 ±0.08	0.88 ±0.07	17.7 ± 3.1		318.7 ± 31.6
	Recovery Vehicle control	6	0.17 ±0.01	2.92 ±0.17	0.71 ±0.04	0.36 ±0.02	0.53 ±0.05	0.83 ±0.08	17.1 ± 2.1		365.8 ± 47.1
	60	3	0.17 ±0.05	3.18 ±0.17	0.70 ±0.03	0.37 ±0.04	0.53 ±0.04	0.83 ±0.04	20.5 ± 3.5		378.8 ± 34.4
Female	Vehicle control	6	0.21 ±0.01	3.24 ±0.23	0.76 ±0.06	0.40 ±0.03	0.54 ±0.08		30.0 ± 2.0	51.4 ± 8.3	193.4 ± 17.2
	6	6	0.22 ±0.04	3.36 ±0.15	0.78 ±0.03	0.43 ±0.01	0.55 ±0.05		32.8 ± 2.1	54.4 ± 4.8	203.8 ± 12.2
	30	6	0.21 ±0.03	3.22 ±0.15	0.78 ±0.02	0.41 ±0.04	0.50 ±0.06		33.1 ± 5.9	47.0 ± 2.9	190.4 ± 14.8
	60	1	0.22 -	3.44 -	0.68 -	0.41 -	0.67 -		52.3 -	51.2 -	213.3 -
	Recovery Vehicle control	6	0.19 ±0.01	2.96 ±0.17	0.77 ±0.05	0.38 ±0.03	0.58 ±0.05		31.5 ± 4.9	44.5 ± 5.9	237.1 ± 12.0
	60	0	- -	- -	- -	- -	- -		- -	- -	- -

Mean ± S.D.
* : Significantly different from Vehicle control at P<0.05
** : Significantly different from Vehicle control at P<0.01

Table 8 -Continued

Relative organ weights : (administration for 14 days)

B11-0153

Sex	Exp. group (mg/kg/day)	Number of animals	Spleen (g/100g)	Liver (g/100g)	Kidney (g/100g)	Heart (g/100g)	Brain (g/100g)	Testis (g/100g)	Adrenal gland (mg/100g)	Ovary (mg/100g)	Body weight (g)
Male	120	1	0.27	4.0[illegible]	0.80	-	0.80	0.83	18.8	-	234.3
			-	-	-	-	-	-	-	-	-
	Recovery 120	6	-	-	-	-	-	-	-	-	-
			-	-	-	-	-	-	-	-	-
Female	120	2	0.2[illegible]	3.86	0.86	-	1.15	-	31.9	44.4	151.0
			±0.01	±0.23	±0.08	-	±0.02	-	± 2.3	± 0.4	± 2.7
	Recovery 120	2	0.24	3.68	0.78	0.48	0.85	-	41.3	47.0	153.3
			±0.06	±0.72	±0.04	±0.02	±0.02	-	± 0.6	± 8.1	± 0.2

Mean ±S.S.

Table 6 28-day repeated-dose oral toxicity study in rats

Gross pathological findings

B11-0153

Findings	Male												Female										
	Vehicle control		Vehicle control (Recovery)	5	10	40	40 (Recovery)			200[a] (Recovery)		200[a] (Recovery)	Vehicle control	Vehicle control (Recovery)	5	10	40	40 (Recovery)			200[a] (Recovery)		200[a] (Recovery) (mg/kg/day)
	ss[*]	fd	ta	ta	ta	ta	fd	ta	fd	fd	ss	fd	ta	ta	ta	ta	ta	fd	fd	fd	ss	ta	fd
	ss: 5		6	6	5	4	4	3	1	10	1	1	6	6	6	6	1	9	2	7	2	2	1
Anemia	0	0	0	0	0	1	0	1	0	2	0	0	0	0	0	0	0	0	0	1	0	1	0
Loss of hair	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Nasal hemorrhage	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Reddish fluid in abdominal cavity	0	0	0	0	0	0	1	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
Reddish fluid in thoracic cavity	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0
Reddish tear	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Stains around nose	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Stains around nose and mouth	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Transparent fluid in abdominal cavity	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	2	0	0	0	0	0
Transparent fluid in thoracic cavity	0	0	0		0	1	3	2	1	5	0	0	0	0	0	0	0	3	0	3	0	1	1
Liver																							
Brownish change	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
Disseminated whitish region	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Disseminated whitish spots	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	
Enlargement	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	
Fibrous substance on surface	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Granular surface	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
Induration	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	2	0	1	0	0	1
Interlobar adhesion	0	0	0	0	0	0	0	0	0	10	0	1	0	0	0	0	0	1	0	0	2	0	0
Whitish region	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	0	2	0	1	0	0	0

* ta: terminal autopsy, fd: found dead, ss: autopsied for moribund state

ss: No. of animals examined

(a): Administration for 14 days

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Table 3 - Continued

Cross pathological findings

B11-0153

Findings	Male												Female										
	Vehicle control		Vehicle control (Recovery)	8	30	60		60 (Recovery)		120[a]		120[a] (Recovery)	Vehicle control	Vehicle control (Recovery)	8	30	60		60 (Recovery)		120[a]	120[a] (Recovery)	(mg/kg/day)
	ta*	fd	ta	ta	ta	ta	fd	ta	fd	fd	aa	fd	ta	ta	ta	ta	ta	fd	fd	fd	aa	ta	fd
	5**	1	6	6	6	4	4	3	2	10	1	2	6	6	6	6	1	8	2	7	2	2	1
Spleen																							
Blackish region	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enlargement	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0
Small	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Kidney																							
Dilatation of pelvis	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Enlargement	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heart																							
Discoloration	0	0	0	0	0	2	2	2	0	1	0	0	0	0	0	0	0	8	2	1	0	1	0
Disseminated whitish spots	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Whitish region	0	0	0	0	1	0	0	0	0	[illegible]	0	1	0	0	0	0	0	0	0	0	0	0	0
Whitish spots	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Lung																							
Dark reddish change	0	0	0	1	0	0	1	0	1	4	0	1	0	0	0	0	0	2	0	4	0	0	0
Roamy substance on cut surface	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Reddish change	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0

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Table 5 — Continued

Gross pathological findings

B11-0153

Findings	Male												Female											
	Vehicle control		Vehicle control (Recovery)	8	30	60		60 (Recovery)		120[a]		120[a] (Recovery)	Vehicle control	Vehicle control (Recovery)	8	30	60		60 (Recovery)		120[a]	120[a] (Recovery)		(mg/kg/day)
	ta*	ft	ta	ta	ta	ta	ft	ta	fd	ft	aa	fd	ta	ta	ta	ta	ta	fd	fd	fd	aa	ta	fd	
	5**	1	6	6	6	4	4	3	1	10	1	1	6	6	6	6	1	5	2	7	2	2	1	
Trachea																								
Whitish foamy substance in lumen	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	1	0	1	0	0	0	
Glandular stomach																								
Blackish region of mucosa	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	
Blackish substance on mucosa	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	
Dark reddish region of mucosa	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
Depressed region of mucosa	0	0	0	[illegible]	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	
Pale reddish region of mucosa	0	0	0		0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
Reddish change of mucosa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	
Reddish region of mucosa	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2	0	0	0	
Jejunum																								
Reddish brownish mucoid substance in lumen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	
Ileum																								
Reddish brownish mucoid substance in lumen	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	

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Table 5 – Continued

Gross pathological findings

B11-0153

Findings	Male												Female											
	Vehicle control		Vehicle control (Recovery)	0	30	60		60 (Recovery)		120[a]		120[a] (Recovery)	Vehicle control	Vehicle control (Recovery)	0	30	60		60 (Recovery)		120[a]		120[a] (Recovery)	(mg/kg/day)
	ta*	fd	ta	ta	ta	ta	fd	ta	fd	fd	aa	fd	ta	ta	ta	ta	ta	fd	fd	fd	aa	ta	fd	
	5**		6	6	6	4	4	3	1	10	1	1	6	6	6	6	3	9	2	7	2	2	1	
Testis																								
Dark reddish change	0	0	0	0	0	0	1	0	0	0	0	0												
Small	0	0	0	0	0	0	0	0	1	0	0	0												
Ovary																								
Dark reddish change													0	0	0	0	0	1	2	0	0	0	0	
Adrenal																								
Enlargement	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Pancreas																								
Edema	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Thymus																								
Edema	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Small	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Cilliac lymph nodes																								
Enlargement	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

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Table 16 28-day repeated-dose oral toxicity study in rats

Histopathological findings

B11-0153

Findings	Male												Female										
	Vehicle control	Vehicle control (Recovery)	8	30	60			60 (Recovery)			120[a]	120[a] (Recovery)	Vehicle control	Vehicle control (Recovery)	8	30	60		60 (Recovery)		120[a]	120[a] (Recovery)	(mg/kg/day)
	ta*	fd	ta	ta	ta	ta	fd	ta	fd	fd	ma	fd	ta	ta	ta	ta	ta	fd	fd	fd	ma	ta	fd
	5**	1	6	6	6	4	4	3	2	10	2	1	6	6	6	6	1	9	2	7	2	2	1
Liver																							
No abnormalities detected	2/5***	1/1	4/6	3/6	2/6	0/4	0/4	0/3	0/2	0/10	0/2	0/1	2/6	3/6	1/6	2/6	0/1	0/9	0/2	0/7	0/2	1/2	0/1
Congestion	0/5	0/1	0/6	1/6	0/6	1/4	4/4	2/3	1/2	10/10	2/2	1/1	0/6	0/6	0/6	0/6	0/1	9/9	2/2	7/7	0/2	1/2	1/1
Extramedullary hematopoiesis	0/5	0/1	0/6	0/6	0/6	1/4	0/4	0/3	0/2	5/10	0/2	0/1	0/6	0/6	0/6	0/6	0/1	1/9	1/2	2/7	0/2	0/2	0/1
Granulation tissue with calcification	0/5	0/1	0/6	0/6	0/6	2/4	3/4	0/3	0/2	2/10	1/2	0/1	0/6	0/6	0/6	0/6	0/1	0/9	0/2	0/7	0/2	0/2	0/1
Bile stasis	0/5	0/1	0/6	0/6	0/6	2/4	4/4	1/3	1/2	9/10	0/2	1/1	0/6	0/6	0/6	0/6	0/1	8/9	1/2	6/7	0/2	0/2	1/1
Lipid droplets in hepatocytes	3/5	0/1	2/6	3/6	3/6	3/4	2/4	2/3	0/2	0/10	0/2	0/1	4/6	3/6	5/6	4/6	1/1	9/9	2/2	6/7	2/2	1/2	1/1
Microgranuloma	0/5	0/1	0/6	0/6	0/6	0/4	0/4	0/3	0/2	0/10	0/2	0/1	0/6	1/6	1/6	1/6	0/1	0/9	0/2	0/7	0/2	0/2	0/1
Necrosis of hepatocytes	0/5	0/1	0/6	0/6	0/6	2/4	2/4	2/3	1/2	9/10	0/2	0/1	0/6	0/6	0/6	0/6	0/1	8/9	1/2	6/7	0/2	1/2	1/1
Proliferation of connective tissue and ossification of fibrin on capsule	0/5	0/1	0/6	0/6	0/6	0/4	2/4	2/3	1/2	10/11	1/2	1/1	0/6	0/6	0/6	0/6	0/1	1/9	0/2	5/7	1/2	1/2	1/1
Swelling of hepatocytes	0/5	0/1	0/6	0/6	0/6	3/4	0/4	2/3	0/2	10/10	1/2	0/1	0/6	0/6	0/6	0/6	0/1	0/9	0/2	0/7	0/2	0/2	0/1

*: ta ; terminal autopsy

**: No. of animals autopsied

***: No. of animals affected / No. of animals examined

—: Not examined

(a): Administration for 14 days

Table 15 — Continued

Histopathological findings

Findings	Male												Female										
	Vehicle control		Vehicle control (Recovery)		5	30	60			60 (Recovery)		120[a]	120[a] (Recovery)		Vehicle control	Vehicle control (Recovery)	5	30	60		60 (Recovery)	120[a]		120[a] (Recovery)	(mg/kg/day)
	ta	fd	ta	fd	ta	ta	ta	fd	ta	fd	fd	ta	fd		ta	ta	ta	ta	ta	fd	fd	fd	ma	ta	fd
	5	1	6	6	6	4	4	3	1	10	1	1			6	6	6	6	1	9	2	7	2	2	1
Spleen																									
No abnormalities detected	5/5	1/1	6/6	=	6/6	3/4	2/4	3/3	1/1	8/10	1/1	1/1			6/6	6/6	=	6/6	1/1	8/9	2/2	4/7	2/2	2/2	1/1
Extramedullary hematopoiesis	0/5	0/1	0/6	=	0/6	1/4	2/4	0/3	0/1	3/10	0/1	0/1			0/6	0/6	=	0/6	0/1	1/9	0/2	3/7	0/2	0/2	0/1
Increased histiocytic cells	0/5	0/1	0/6	=	0/6	0/4	0/4	0/3	0/1	1/10	0/1	0/1			0/6	0/6	=	0/6	0/1	0/9	0/2	0/7	0/2	0/2	0/1
Kidney																									
No abnormalities detected	5/5	0/1	6/6	=	6/6	4/4	4/4	3/3	1/1	10/10	1/1	1/1			6/6	6/6	=	6/6	1/1	9/9	2/2	7/7	2/2	2/2	1/1
Cyst formation	0/5	1/1	0/6	=	0/6	0/4	0/4	0/3	0/1	0/10	0/1	0/1			0/6	0/6	=	0/6	0/1	0/9	0/2	0/7	0/2	0/2	0/1
Heart																									
No abnormalities detected	5/5	1/1	6/6	6/6	3/6	0/4	0/4	3/3	0/1	2/10	0/1	0/1			6/6	6/6	6/6	5/6	1/1	0/9	0/2	0/7	1/2	1/2	0/1
Necrosis of muscular fibers with cell reaction	0/5	0/1	0/6	0/6	3/6	2/4	4/4	3/3	1/1	8/10	1/1	0/1			0/6	0/6	0/6	1/6	0/1	9/9	2/2	7/7	1/2	1/2	1/1
Thrombus	0/5	0/1	0/6	0/6	0/6	0/4	1/4	0/3	0/1	0/10	0/1	0/1			0/6	0/6	0/6	0/6	0/1	0/9	0/2	0/7	0/2	0/2	0/1
Vacuolization of muscular fibers	0/5	0/1	0/6	0/6	0/6	2/4	1/4	2/3	1/1	0/10	1/1	1/1			0/6	0/6	0/6	0/6	0/1	0/9	0/2	0/7	0/2	1/2	0/1

Table 18 – Continued

Histopathological findings

B11-0153

Findings	Male												Female										
	Vehicle control		Vehicle control (Recovery)	8	30	60		60 (Recovery)		120[a]	120[a] (Recovery)		Vehicle control	Vehicle control (Recovery)	8	30	60	60 (Recovery)		120[a]	120[a] (Recovery) (mg/kg/day)		
	ta	fd	ta	ta	ta	ta	fd	ta	fd	fd	ma	fd	ta	ta	ta	ta	ta	fd	fd	fd	ma	ta	fd
	6	1	6	6	6	4	4	3	1	10	1	1	6	6	6	6	1	9	2	7	2	2	1
Lung																							
No abnormalities detected	5/5	1/1	6/6	=	6/6	4/4	2/4	3/3	0/1	4/10	1/1	0/1	5/6	6/6	=	6/6	1/1	6/9	1/2	2/7	2/2	2/2	0/1
Cell infiltration	0/5	0/1	0/6	=	0/6	0/4	0/4	0/3	0/1	0/10	0/1	0/1	1/6	0/6	=	0/6	0/1	0/9	0/2	0/7	0/2	0/2	0/1
Congestion	0/5	0/1	0/6	=	0/6	0/4	2/4	0/3	0/1	0/10	0/1	1/1	0/6	0/6	=	0/6	0/1	2/9	1/2	5/7	0/2	0/2	1/1
Edema	0/5	0/1	0/6	=	0/6	0/4	1/4	0/3	0/1	4/10	0/1	0/1	0/6	0/6	=	0/6	0/1	2/9	1/2	3/7	0/2	0/2	0/1
Hemorrhage	0/5	0/1	0/6	=	0/6	0/4	0/4	0/3	0/1	0/10	0/1	0/1	1/6	0/6	=	0/6	0/1	1/9	0/2	0/7	0/2	0/2	0/1
Forestomach																							
No abnormalities detected	5/5	1/1	6/6	=	6/6	4/4	4/4	3/3	1/1	10/10	1/1	1/1	6/6	6/6	=	6/6	1/1	9/9	2/2	7/7	2/2	2/2	1/1
Glandular stomach																							
No abnormalities detected	4/5	1/1	6/6	=	5/6	3/4	4/4	3/3	1/1	8/10	1/1	1/1	6/6	6/6	1/1	6/6	1/1	9/9	2/2	6/7	2/2	2/2	1/1
Necrosis of mucosa	1/5	0/1	0/6	=	1/6	1/4	0/4	0/3	0/1	2/10	0/1	0/1	0/6	0/6	0/1	0/6	0/1	0/9	0/2	1/7	0/2	0/2	0/1
Duodenum																							
No abnormalities detected	5/5	1/1	6/6	=	6/6	4/4	4/4	3/3	1/1	10/10	1/1	1/1	6/6	6/6	=	6/6	1/1	9/9	2/2	7/7	2/2	2/2	1/1
Jejunum																							
No abnormalities detected	5/5	1/1	6/6	=	6/6	4/4	4/4	3/3	1/1	10/10	1/1	1/1	6/6	6/6	=	6/6	1/1	9/9	2/2	7/7	2/2	2/2	1/1
Ileum																							
No abnormalities detected	5/5	1/1	6/6	=	6/6	4/4	4/4	3/3	1/1	10/10	1/1	1/1	6/6	6/6	=	6/6	1/1	9/9	2/2	7/7	2/2	2/2	1/1

Table 15 – Continued

Histopathological findings

Findings	Male												Female										
	Vehicle control		Vehicle control (Recovery)		1	30	60		60 (Recovery)		120[a]	120[a] (Recovery)	Vehicle control	Vehicle control (Recovery)	1	30	60	60 (Recovery)		120[a]	120[a] (Recovery) (mg/kg/day)		
	ta	fd	ta	fd	ta	ta	fd	ta	fd	fd	aa	fd	ta	ta	ta	ta	ta	fd	fd	fd	aa	ta	fd
	5	1	6	6	6	4	1	3	1	11	1	1	6	6	6	6	1	9	2	7	2	2	1
Cecum																							
No abnormalities detected	5/5	1/1	6/6	–	6/6	4/4	4/4	3/3	1/1	10/10	1/1	1/1	6/6	6/6	–	6/6	1/1	9/9	2/2	7/7	2/2	2/2	1/1
Colon																							
No abnormalities detected	5/5	1/1	6/6	–	6/6	4/4	4/4	3/3	1/1	10/10	1/1	1/1	6/6	6/6	–	6/6	1/1	9/9	2/2	7/7	2/2	2/2	1/1
Rectum																							
No abnormalities detected	5/5	1/1	6/6	–	6/6	4/4	4/4	3/3	1/1	10/10	1/1	1/1	6/6	6/6	–	6/6	1/1	9/9	2/2	7/7	2/2	2/2	1/1
Testis																							
Decreased spermatogenesis	–	–	–	–	–	–	1/1	–	1/1	–	–	–											
Hemorrhage	–	–	–	–	–	–	1/1	–	0/1	–	–	–											
Ovary																							
Congestion													–	–	–	–	–	1/1	2/2	–	–	–	–
Adrenal																							
No abnormalities detected	5/5	1/1	6/6	–	6/6	4/4	3/4	3/3	0/1	8/10	1/1	1/1	6/6	6/6	–	6/6	1/1	9/9	2/2	7/7	2/2	2/2	1/1
Swelling of cortical cells	0/5	0/1	0/6	–	0/6	0/4	1/4	0/3	1/1	2/10	0/1	0/1	0/6	0/6	–	0/6	0/1	0/9	0/2	0/7	0/2	0/2	0/1
Pancreas																							
No abnormalities detected	–	–	–	–	–	–	–	0/1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 15 - Continued

Histopathological Findings

B11-0153

Findings	Male												Female										
	Vehicle control		Vehicle control (Recovery)	5	30	60		60 (Recovery)		120[a]		120[a] (Recovery)	Vehicle control	Vehicle control (Recovery)	5	30	60	60 (Recovery)		120[a]		120[a] (Recovery) (mg/kg/day)	
	ta	fd	ta	ta	ta	ta	fd	ta	fd	fd	aa	fd	ta	ta	ta	ta	ta	fd	fd	id	aa	ta	fd
	5	1	6	6	6	4	4	3	1	10	1	1	6	6	6	6	1	9	2	1	2	2	1
Thymus																							
No abnormalities detected	=	=	=	=	=	=	=	2/2	0/2	=	=	=	=	=	=	=	=	=	=	=	=	=	=
Decreased lymphocytes	=	=	=	=	=	=	=	0/2	2/2	=	=	=	=	=	=	=	=	=	=	=	=	=	=
Cervical lymph nodes																							
Erythrocytes in sinus	=	=	=	=	-	1/1	-	-	=	=	=	=	=	=	=	=	=	=	=	=	=	=	=
Skin																							
Scab formation	=	=	=	=	-	=	-	=	=	=	=	=	1/1	=	=	=	-	=	=	=	=	=	=

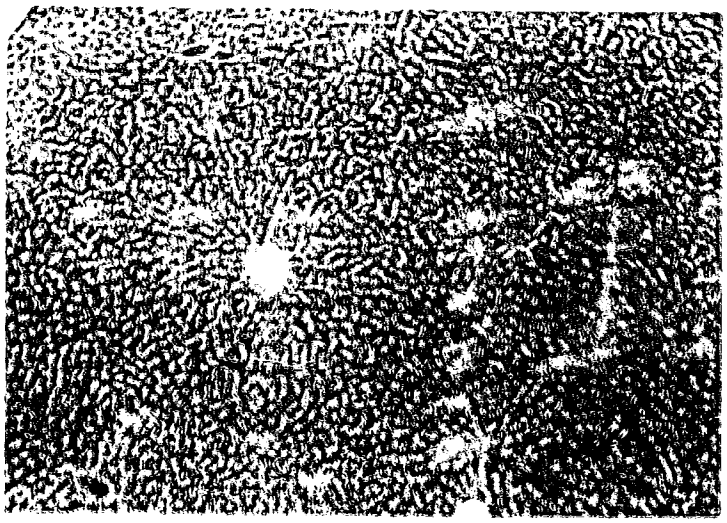

Photo 1 Liver of a male rat administered orally with vehicle for 28 days
No abnormalities detected
No 5 animal HE. × 40

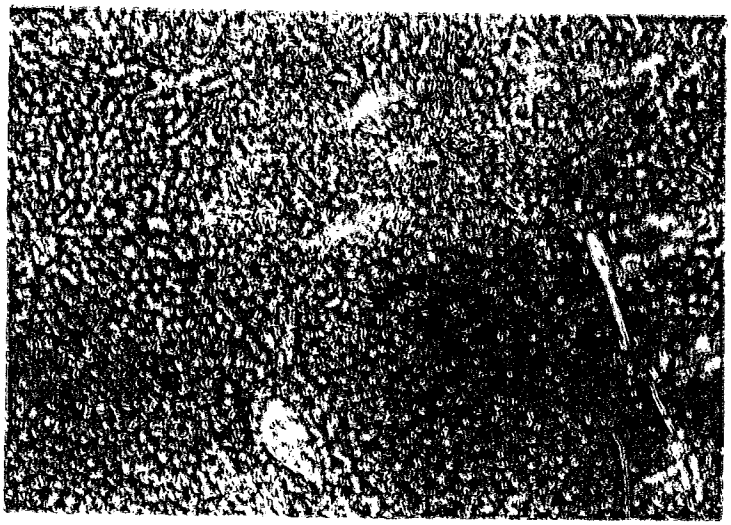

Photo 2 Liver of a male rat administered orally with test substance at dose level of 60mg/kg/day for 28 days
Necrosis of hepatocytes
No 28 animal HE. × 40

0 2 9 5

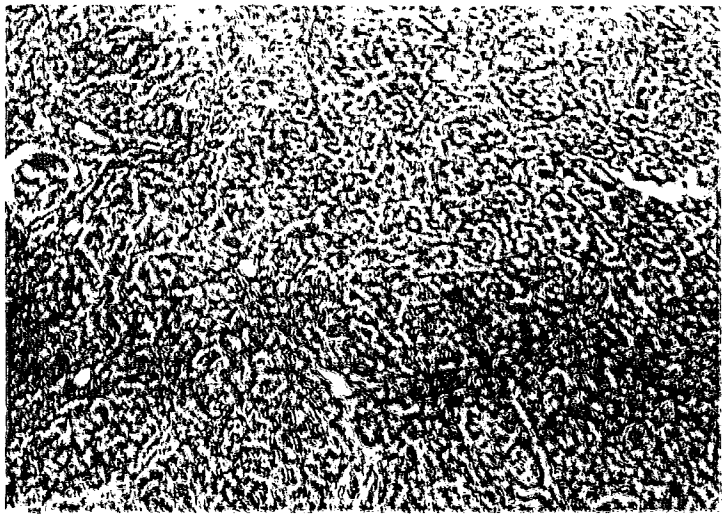

Photo 3 Liver of a male rat administered orally with test substance at dose level of 60mg/kg/day for 28 days
Granulation tissue with calcification
No. 30 animal HE. × 90

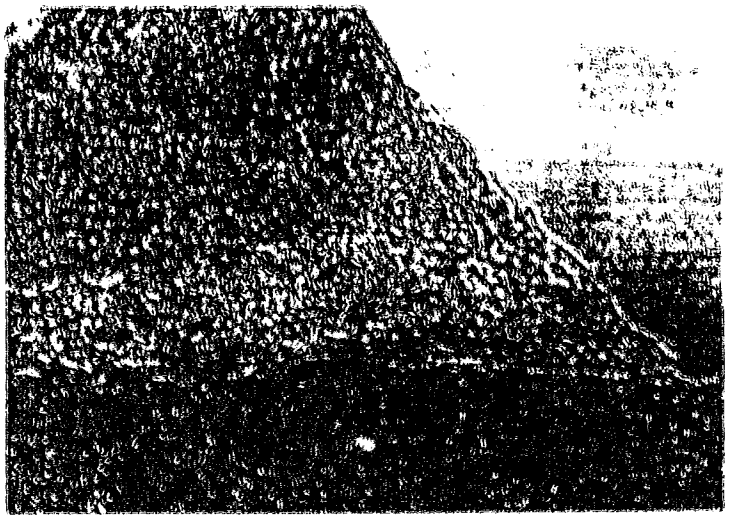

Photo 4 Liver of a dead male rat administered orally with test substance at dose level of 60mg/kg/day
Exudation of fibrin on capsule
No. 27 animal HE. × 90.

112

0 2 9 6

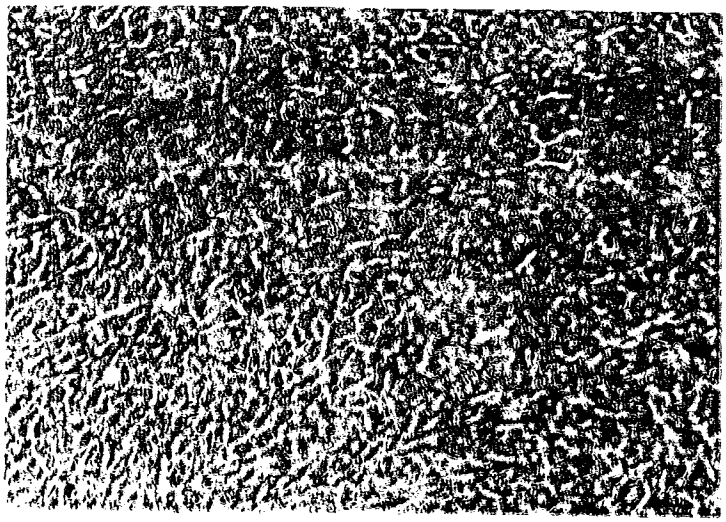

Photo 5 Heart of a male rat administered orally with vehicle for 28 days
No abnormalities detected
No 5 animal HE. ×180

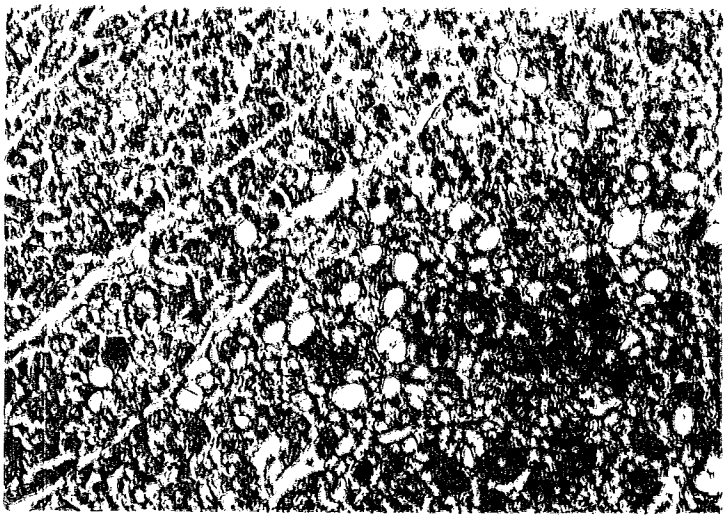

Photo 6 Heart of a male rat administered orally with test substance at dose level of 60mg/kg/day for 28 days
Vacuolization of muscular fibers
No 25 animal HE. ×180

113

Photo 7 Heart of a dead male rat administered orally with test substance at dose level of 60mg/kg/day
Necrosis of muscular fibers with cell reaction
No 27 animal HE. ×180.

114

Addendum 1 28-day repeated-dose oral toxicity study in rats
Clinical signs
Vehicle control

B11-0153

Signs	Sex	Administration 1	2	3	4 (weeks)	Recovery 1	2 (weeks)
No abnormalities detected	Male	1-5, 7-12*	1-5, 7-12	1-5, 7-12	1-5, 8-17, 12	8-12, 12	7, 12
	Female	49-60	[illegible]	49-60	49, 51, 52-58, 60	55-58, 60	55-60
Abnormal respiratory sound	Male	6					
	Female						
Abnormal respiration	Male	6					
	Female						
Loss of hair	Male				7, 11	7, 11	
	Female				57, 59	59	
Death	Male	5					
	Female						

* Animal number.

115

Addendum 1 - Continued
8 mg/kg

B11-0153

Signs	Sex	Administration 1	Administration 2	Administration 3	Administration 4 (weeks)	Recovery 1	Recovery 2 (weeks)
No abnormalities detected	Male	13-18*	13-15, 17, 18	13, 14, 17, 18	13-15, 17, 18		
	Female	61-66	61, 62, 64, 66	61, 64-66	61, 62, 64-66		
Salivation	Male		16	16	16		
	Female		63, 65	62, 63	63		
Decreased spontaneous locomotion	Male			15			
	Female						
Prone position	Male			15			
	Female						
Gasping	Male			15			
	Female						
Cyanosis	Male			15			
	Female						

* Animal number.

116

Addendum 1 - Continued
10mg/kg

B11-0153

Signs	Sex	Administration 1	2	3	4 (weeks)	Recovery 1	2 (weeks)
No abnormalities detected	Male	19, 24*	19, 20, 22, 24	22, 24	19, 20, 22, 23		
	Female	67-72	68-72	68-72	67-72		
Salivation	Male		21, 23	20-22, 24	22, 24		
	Female		67	67, 72	72		

* Animal number.

Addendum 1 - Continued
50mg/kg

Signs	Sex	Administration 1	Administration 2	Administration 3	Administration 4 (weeks)	Recovery 1	Recovery 2 (weeks)
No abnormalities detected	Male	25-30*	25, 30, 36	25, 30, 32	25	31, 33	31, 36
	Female	73-77, 79, 80, 82	73, 82	73, 83	83		
Salivation	Male		25, 28, 29, 31-34, 36	25, 28, 29, 31, 33-36	25, 28, 31-34, 36		
	Female	73, 81, 83, 84	74, 77, 79-81, 83-84	78, 82, 84	78, 80-84		
Decreased spontaneous locomotion	Male			25, 35		34	
	Female		74, 75, 77, 79		82	81	
Crouching	Male				30		
	Female		79		82, 83		
Prone position	Male			25	30		
	Female		75, 77		83		
Decreased respiratory rate	Male						
	Female		75, 76				
Deep breath	Male			25, 35	32	34	33, 34
	Female		74, 75, 77, 79		82, 83	81	
Increased respiratory rate	Male					34	34
	Female						
Anemia	Male			25, 35	30, 32	34	33, 34
	Female		74-77, 79		78, 82, 83	81, 84	
Subnormal temperature	Male			25, 35	30, 32	34	33, 34
	Female		74-77, 79		82, 83	81, 84	
Piloerection	Male			25, 35		34	33, 34
	Female		79		82		

* Animal number.

Addendum 1 - Continued
50mg/kg

811-0153

Signs	Sex	Administration 1	Administration 2	Administration 3	Administration 4 (weeks)	Recovery 1	Recovery 2 (weeks)
Lacrimation	Male						
	Female		[illegible]				
Reddish tear	Male						
	Female		[illegible]				
Nasal bleeding	Male			[illegible]			[illegible]
	Female		74				
Ptosis	Male						
	Female				[illegible]		
Cloudy and/or [illegible]	Male				[illegible]		[illegible]
	Female				[illegible]		
Redness of auricle	Male						
	Female			[illegible]			
Loss of hair	Male			[illegible]	[illegible]	[illegible]	
	Female						
Death	Male		[illegible]	[illegible]	[illegible]		[illegible]
	Female		[illegible]	[illegible]	[illegible]	[illegible]	

* Animal number.

Addendum 1 – Continued (administration for 14 days)
125mg/kg

B11-0153

Signs	Sex	Administration 1	Administration 2	Recovery 1	Recovery 2 (weeks)
No abnormalities detected	Male				
	Female	86, 93*		85, 94	33
Salivation	Male	37-39, 42, 45-48	37, 39, 42, 47, 48		
	Female	87, 90, 94, 96	86-88, 90, 92, 96		
Decreased spontaneous locomotion	Male	37-39, 41, 42, 44-48	37-39, 41, 42, 44, 47, 48		33
	Female	85-88, 91, 92, 94, 95	85-94, 90-96	95	
Crouching	Male		44		33
	Female		88		
Prone position	Male		47, 48		
	Female		88, 91		
Decreased respiratory rate	Male	37, 41, 47	41, 42		
	Female	86, 93	88, 91	95	
Deep breath	Male		44, 48		33
	Female	85	85, 88, 91, 96	95	
Increased respiratory rate	Male	45			
	Female				
Anemia	Male	45, 47	41, 42, 44, 48	38	33
	Female		88, 91, 96	92	34
Subnormal temperature	Male	45	41, 42, 44, 48		33
	Female		88, 91, 96	92	
Piloerection	Male		42		33
	Female		91	92	

* Animal number.

Addendum 1 - Continued (administration for 14 days)
120mg/kg

B11-0153

Signs	Sex	Administration 1	Administration 2	Recovery 1	Recovery 2 (weeks)
Lacrimation	Male	45*	41, 44		39
	Female		88, 91	92	
Reddish tear	Male				39
	Female				
Nasal bleeding	Male				39
	Female				
Cloudy and/or Red urine	Male				39
	Female				
Soft stool	Male				
	Female	88			
Sacrifice	Male		37		
	Female		86, 90		
Death	Male	40, 43, 48	38, 41, 42, 44, 45, 47, 48		39
	Female		85, 87-89, 91, 95, 96	92	

* Animal number.

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Addendum 2 28-day repeated-dose oral toxicity study in rats 811-0153

Body weights(g)

Sex	Exp.group (mg/kg/day)	Animal No.	Administration -2	1	3	5	8	10	12 (days)
Male	Vehicle control	1	128.8	149.5	164.5	180.7	205.0	227.0	243.0
		2	131.2	152.8	175.0	189.5	219.0	237.0	259.0
		3	125.7	146.3	162.2	176.4	203.0	216.5	234.1
		4	119.4	139.2	159.4	172.3	206.7	225.5	24[illegible].1
		5	126.4	146.7	169.3	188.2	216.1	232.2	251.1
		6	123.2	143.8	159.6	170.3	- b)	-	-
		7	125.0	142.0	151.2	169.4	197.6	212.2	230.4
		8	126.7	147.0	167.8	178.5	205.7	222.4	232.9
		9	133.4	154.1	172.4	189.8	216.5	232.7	248.0
		10	123.1	146.0	168.1	178.5	194.4	209.7	225.[illegible]
		11	134.3	146.0	161.0	178.6	200.0	220.5	236.7
		12	119.8	131.5	152.4	155.4	178.4	182.7	183.[illegible]
	8	13	123.7	146.4	160.5	178.8	202.[illegible]	220.9	236.3
		14	130.0	148.6	165.2	184.0	210.8	225.[illegible]	247.0
		15	124.3	139.6	160.1	175.5	204.8	222.8	235.8
		16	131.4	154.3	169.3	188.8	213.7	232.5	247.2
		17	125.8	147.1	167.8	189.1	217.7	231.4	252.2
		18	115.7	131.0	149.2	161.5	184.0	170.3	183.0
	30	19	126.4	144.6	161.9	178.7	198.2	198.7	217.8
		20	117.5	138.1	157.5	178.1	206.0	226.8	246.5
		21	130.5	151.6	164.9	183.8	206.7	212.0	241.5
		22	125.8	144.6	160.8	183.4	214.1	222.7	239.2
		23	126.3	141.8	157.3	171.3	198.4	214.2	231.5
		24	133.5	153.6	177.6	187.2	215.6	240.8	265.3
	60	25	125.3	149.5	157.9	171.1	201.1	219.5	237.9
		26	127.2	146.6	159.8	176.7	199.8	216.7	240.3
		27	122.5	135.1	152.2	164.7	187.5	194.1	- b)
		28	132.3	155.7	172.1	179.5	213.0	229.5	[illegible]
		29	129.5	151.9	163.9	178.5	204.2	219.8	237.9
		30	118.4	135.2	151.0	162.2	185.7	201.0	210.7
		31	125.5	148.8	164.2	181.0	210.4	232.5	247.5
		32	118.7	147.7	162.2	175.2	203.0	214.7	228.9
		33	127.0	148.7	163.8	181.1	202.8	218.6	232.2
		34	139.4	142.8	156.3	180.8	190.9	208.5	229.1
		35	121.8	137.0	153.0	160.5	185.4	200.1	210.4
		36	131.3	152.5	165.3	184.0	210.0	226.7	249.4
	120	37	131.8	150.0	169.2	182.1	212.1	229.0	243.9
		38	128.4	145.1	162.6	171.2	198.4	- b)	-
		39	121.8	138.0	152.5	164.2	181.0	199.7	210.9
		40	130.9	151.0	162.5	- b)	-	-	-
		41	118.3	133.1	149.0	163.7	174.2	- b)	-
		42	125.0	143.7	160.5	173.2	195.3	- b)	-
		43	120.3	135.5	153.2	- b)	-	-	-
		44	130.4	149.4	163.0	181.0	205.3	- b)	-
		45	127.7	145.5	158.1	185.4	- b)	-	-
		46	123.3	145.8	157.3	164.9	- b)	-	-
		47	125.3	141.9	154.1	162.0	179.0	- b)	-
		48	132.3	152.5	174.1	195.7	214.4	227.5	244.2

b) Dead animal.

b) Dead animal.

Addendum 2 -Continued
Body weight (g)

111-0153

Sex	Exp.group (mg/kg/day)	Animal No.	Administration 15	17	19	22	24	26	28 (days)
Male	Vehicle control	1	266.2	285.2	299.4	320.7	331.1	344.0	348.1
		2	264.6	304.6	314.8	348.1	361.1	374.2	388.4
		3	260.3	272.7	288.3	313.3	325.6	336.6	349.5
		4	274.6	292.0	309.5	341.4	356.2	373.4	384.5
		5	272.5	287.3	301.9	323.4	339.2	351.1	357.7
		6	= b)	=	=	=	=	=	=
		7	254.3	267.6	285.3	298.1	313.1	325.4	338.1
		8	255.3	267.9	282.4	300.9	312.9	319.3	333.0
		9	260.6	294.2	309.2	324.1	339.4	349.3	360.3
		10	253.6	269.2	285.5	308.1	321.6	337.4	346.5
		11	266.7	282.5	301.4	322.5	337.9	351.6	367.1
		12	268.1	217.1	226.2	235.6	239.6	249.3	251.6
	6	13	260.6	281.3	293.1	319.1	333.9	345.9	360.4
		14	268.3	280.9	295.3	315.7	333.4	348.3	352.6
		15	268.4	286.3	275.4	299.6	313.4	327.7	344.6
		16	271.1	285.1	302.2	317.4	335.5	342.9	356.2
		17	282.9	298.3	316.6	341.1	359.6	376.0	392.0
		18	207.3	215.2	224.6	241.2	251.4	260.5	268.0
	30	19	235.4	247.7	256.0	293.1	299.5	300.8	313.7
		20	273.7	287.7	307.4	335.2	349.2	369.6	361.7
		21	275.5	287.0	299.1	324.1	339.2	352.7	365.7
		22	265.0	280.0	294.2	318.2	331.1	347.9	360.0
		23	259.2	275.3	292.3	313.3	328.5	341.6	352.0
		24	299.5	314.6	328.8	355.3	364.4	378.6	391.0
	60	25	268.1	285.0	301.1	329.6	343.3	369.2	379.0
		26	256.4	272.1	285.1	305.8	315.8	329.3	336.3
		27	= b)	=	=	=	=	=	=
		28	264.5	279.3	294.7	318.8	324.3	338.4	346.2
		29	252.9	266.6	257.2	= b)	=	=	=
		30	235.6	248.9	259.6	277.4	283.8	287.9	273.4
		31	273.4	291.9	306.4	330.2	341.5	354.0	366.1
		32	253.4	263.4	275.6	292.4	302.4	280.1	= b)
		33	260.2	273.0	288.1	308.5	318.1	331.7	339.6
		34	249.5	264.1	279.6	304.6	315.4	320.8	334.1
		35	207.9	= b)	=	=	=	=	=
		36	282.5	296.3	316.5	343.2	355.9	372.0	365.0

b) : Dead animal.

Attachment 2 -Continued
Body weights(g)

Sex	Exp.group (mg/kg/day)	Animal No.	Administration -3	1	3	5	8	10	12 (days)
Female	Vehicle control	49	113.4	124.5	130.7	144.2	155.8	159.9	171.5
		50	108.6	115.7	127.0	131.4	144.4	146.1	154.0
		51	110.9	127.1	135.1	144.1	158.1	160.3	188.9
		52	95.1	110.4	121.1	127.2	139.6	145.9	147.4
		53	110.6	127.3	132.3	146.1	160.3	164.8	178.2
		54	102.6	116.0	126.7	132.3	141.2	151.6	150.2
		55	110.7	124.7	133.2	145.1	158.2	167.8	170.2
		56	108.1	123.3	130.9	144.5	162.1	172.4	180.7
		57	111.3	124.4	138.5	146.2	163.1	170.0	178.7
		58	104.3	121.7	133.3	143.8	152.0	161.4	163.7
		59	103.2	114.6	122.3	131.4	138.5	153.0	160.3
		60	116.1	105.2	135.6	147.8	156.8	167.3	172.0
	6	61	107.7	123.5	132.3	138.4	153.6	160.7	169.7
		62	115.7	126.8	135.3	146.2	155.8	158.0	169.0
		63	110.6	125.1	139.8	149.2	166.3	170.9	187.8
		64	114.1	125.5	128.8	139.3	146.7	157.1	164.0
		65	101.7	110.8	127.3	135.1	145.7	154.5	158.8
		66	104.8	117.4	130.0	138.9	150.8	153.8	160.1
	30	67	102.8	116.0	128.2	138.7	140.0	152.2	161.7
		68	108.5	122.0	134.4	145.4	160.8	166.5	178.8
		69	112.6	124.9	134.5	146.5	147.4	159.2	164.0
		70	116.4	132.2	138.5	148.7	154.7	165.7	178.1
		71	108.3	123.0	130.8	140.7	150.1	146.6	158.2
		72	99.2	115.8	126.6	137.1	154.9	153.8	162.7
	60	73	106.4	121.7	137.4	142.1	150.6	165.2	177.9
		74	107.6	124.6	140.5	155.6	169.9	161.1	179.6
		75	104.7	119.9	132.1	140.9	147.5	- b)	-
		76	102.8	118.9	121.8	137.3	140.3	158.4	164.1
		77	118.7	134.4	140.9	154.4	165.4	173.8	170.4
		78	112.9	133.5	140.2	152.3	166.4	174.4	179.7
		79	111.1	130.9	135.8	145.8	157.1	164.3	169.4
		80	114.4	129.1	140.7	154.1	166.1	171.4	- b)
		81	107.5	124.4	134.2	146.1	161.3	166.0	175.3
		82	95.2	114.8	125.5	137.0	149.0	160.7	167.1
		83	117.2	123.7	141.8	148.1	163.0	172.5	180.9
		84	105.8	123.4	130.4	142.4	151.7	165.8	171.0
	120	85	109.4	120.4	129.9	139.2	148.1	- b)	-
		86	107.0	119.8	128.2	134.7	149.8	152.3	153.3
		87	108.9	122.9	128.5	133.7	141.1	- b)	-
		88	116.7	129.5	137.4	149.8	162.0	166.7	177.6
		89	103.9	128.0	138.3	148.7	154.0	159.1	- b)
		90	102.4	118.8	129.8	138.3	147.9	154.8	162.3
		91	116.1	126.3	138.7	142.8	156.8	- b)	-
		92	113.3	129.3	138.8	154.3	168.3	178.3	184.5
		93	99.2	112.7	115.5	127.1	129.1	140.9	143.5
		94	103.8	119.4	128.3	134.1	143.7	153.8	163.6
		95	107.3	122.5	133.8	145.1	159.1	158.1	- b)
		96	100.1	124.0	130.8	148.7	161.9	171.5	- b)

b) : Dead animal.

Addendum 2 -Continued
Body weights(g)

Sex	Exp.group (mg/kg/day)	Animal No.	Administration 14	17	19	22	24	26	28 (days)
Female	Vehicle control	49	165.5	167.7	194.0	195.6	211.7	203.7	218.3
		50	165.1	167.4	177.1	186.1	194.2	196.4	204.7
		51	160.3	166.6	191.1	201.6	212.3	210.6	217.2
		52	158.3	159.3	167.7	167.8	171.7	171.3	175.8
		53	166.0	166.0	202.4	205.3	218.3	216.1	227.0
		54	165.5	169.2	172.4	181.9	181.3	193.0	189.5
		55	160.5	192.4	191.3	201.9	205.4	209.5	213.2
		56	135.3	196.6	208.4	219.8	231.0	232.3	236.7
		57	192.6	190.4	201.4	208.8	214.8	215.8	222.1
		58	176.7	183.4	186.7	197.3	193.0	209.2	207.5
		59	175.5	178.8	186.1	198.5	206.0	201.5	215.1
		60	178.3	181.1	191.6	210.1	217.8	228.0	230.8
	5	61	181.7	180.9	192.9	200.2	209.2	214.4	220.8
		62	174.3	183.0	188.6	194.4	203.3	210.5	217.7
		63	202.0	200.3	209.6	224.2	227.7	236.[illegible]	236.5
		64	180.2	183.1	192.0	198.5	200.3	209.8	208.0
		65	171.9	177.7	183.6	196.0	198.2	204.3	206.8
		66	163.4	177.0	178.8	188.4	188.1	201.3	197.8
	30	67	177.5	174.7	186.9	194.2	200.1	199.8	210.7
		68	183.2	193.1	187.9	199.4	214.5	214.0	223.2
		69	182.1	183.7	192.5	203.0	201.4	205.7	201.4
		70	189.5	188.7	201.7	207.7	221.5	222.9	228.1
		71	162.2	169.2	173.3	173.7	188.5	179.4	189.5
		72	168.8	177.0	182.5	192.9	198.8	203.2	208.6
	60	73	168.5	166.6	20[illegible].5	210.7	217.2	223.5	227.2
		74	= b)	=	=	=	=		=
		75	= b)	=	=	=	=	=	=
		76	= b)	=	=	=	=	=	=
		77	= b)		=	=	=	=	=
		78	168.2	186.7	200.1	215.5	220.7	226.1	223.7
		79	= b)	=	=	=	=	=	=
		80	= b)	=	=		=	=	=
		81	167.4	188.[illegible]	205.4	220.2	232.0	235.7	237.4
		82	176.2	183.9	191.3	200.0	211.9	215.8	218.6
		83	199.2	201.2	212.5	217.0	227.0	215.9	= b)
		84	167.9	194.0	198.6	209.0	211.4	217.0	218.1

b) : Dead animal.

Attachment 2 -Continued

Body weight(g)

Sex	Exp.group (mg/kg/day)	Animal No.	Days 1	3	6	8	10	12	14 (days)
Male	Vehicle control	7	337.1	345.1	355.0	372.0	383.4	388.8	403.4
		8	335.0	343.0	353.4	364.0	373.8	382.8	391.0
		9	363.2	374.5	386.4	405.4	418.4	423.2	434.3
		10	348.5	358.1	370.2	385.4	395.7	408.0	418.0
		11	366.3	377.2	384.5	397.4	411.1	421.1	428.0
		12	241.4	256.4	263.8	274.8	278.5	287.3	293.8
	60	31	366.5	377.0	383.8	394.0	392.4	398.0	405.5
		32	= B)	-	-	-	-	-	-
		33	344.7	353.1	363.0	370.0	366.0	350.0	345.0
		34	338.0	344.2	328.0	327.4	= B)	-	-
		35	= B)	-	-	-	-	-	-
		36	389.7	393.2	404.0	415.0	418.5	432.0	440.0
Female	Vehicle control	55	219.0	220.0	232.0	237.4	241.3	240.7	249.2
		56	235.3	247.0	254.4	263.3	268.0	275.2	277.0
		57	224.5	232.0	234.1	243.5	243.7	240.0	250.1
		58	216.1	225.2	236.1	233.0	244.3	244.0	254.7
		59	211.0	219.2	228.1	232.3	237.5	235.5	248.0
		60	234.3	233.0	247.7	257.5	267.0	270.0	273.0
	60	76	= B)	-	-	=	=	=	=
		80	= B)	=	=	=	=	=	=
		61	232.0	= B)	=	=	=	=	=
		62	= B)	=	=	=	=	=	=
		63	= B)	-	=	=	=	=	=
		64	223.0	= B)	=	=	=	=	=

B) : Dead animal.

Addendum 2 -Continued

Body weights(g) : (administration for 14 days)

Sex	Exp.group (mg/kg/day)	Animal No.	Recovery 1	3	5	8	10	12	14 (days)
Male	120	37	- a)	-	-	-	-	-	-
		38	- b)	-	-	-	-	-	-
		39	223.3	219.1	231.1	234.2	218.0	- b)	-
		40	- b)	-	-	-	-	-	-
		41	- b)	-	-	-	-	-	-
		42	- b)	-	-	-	-	-	-
		43	- b)	-	-	-	-	-	-
		44	- b)	-	-	-	-	-	-
		45	- b)	-	-	-	-	-	-
		46	- b)	-	-	-	-	-	-
		47	- b)	-	-	-	-	-	-
		48	- b)	-	-	-	-	-	-
Female	120	65	- b)	-	-	-	-	-	-
		66	- a)	-	-	-	-	-	-
		67	- b)	-	-	-	-	-	-
		68	- b)	-	-	-	-	-	-
		69	- b)	-	-	-	-	-	-
		70	- a)	-	-	-	-	-	-
		71	- b)	-	-	-	-	-	-
		72	167.6	162.7	- b)	-	-	-	-
		73	157.4	155.4	173.1	183.0	180.7	184.4	194.0
		74	175.4	175.1	180.6	184.4	181.0	185.7	192.3
		75	- b)	-	-	-	-	-	-
		76	- b)	-	-	-	-	-	-

a) : Sacrificed animal.
b) : Dead animal.

Attachment 3 28-day repeated-dose oral toxicity study in rats
Food consumption(g/rat/day)

Sex	Exp.group (mg/kg/day)	Animal no.	Administration 1	4	8	11	15	18	22	25	28 (days)
Male	Vehicle control	1	16.5	19.6	20.4	20.5	20.7	19.3	21.4	19.4	21.1
		2	16.4	19.7	22.2	22.5	23.4	22.2	22.2	21.3	23.1
		3	16.0	17.4	19.3	19.0	18.9	18.9	19.3	18.3	20.0
		4	17.2	20.0	22.5	23.0	22.9	21.2	23.3	22.1	24.3
		5	16.4	19.0	21.7	21.8	20.9	19.3	20.0	20.0	20.5
		6	16.2	20.9	- b)	-	-	-	-	-	-
		7	18.1	18.7	19.6	18.6	22.8	18.2	18.5	19.4	17.9
		8	16.4	17.2	20.5	19.8	17.9	16.5	16.9	16.5	17.4
		9	19.0	19.6	21.5	20.4	21.8	20.8	21.5	20.3	22.1
		10	17.8	18.3	19.1	18.8	19.2	20.0	21.0	19.8	21.3
		11	19.4	20.8	22.5	21.5	23.0	22.8	23.8	25.1	25.2
		12	17.6	18.3	17.9	17.8	16.8	16.8	18.1	14.4	15.5
	6	13	18.1	18.5	20.8	20.4	19.8	21.4	22.2	21.8	20.7
		14	19.7	19.3	22.1	20.8	20.8	19.1	20.0	18.9	17.9
		15	17.1	19.4	22.1	21.4	22.4	14.4	19.5	19.9	21.2
		16	16.8	19.4	21.9	21.5	21.0	19.5	19.4	19.8	18.9
		17	19.4	20.4	23.8	23.1	22.9	22.1	22.5	22.5	23.6
		18	17.3	15.8	14.3	14.9	14.8	14.3	15.3	15.5	15.7
	30	19	18.8	16.7	18.2	18.9	17.2	16.9	17.7	17.5	17.3
		20	16.1	19.6	21.9	21.9	22.0	21.8	22.0	20.8	23.2
		21	19.1	16.9	21.1	22.[illegible]	21.2	22.0	21.7	[illegible].5	20.8
		22	17.8	19.8	20.8	21.[illegible]	21.2	20.8	21.8	[illegible].8	22.9
		23	17.7	19.5	19.9	21.8	20.9	21.4	21.5	20.1	21.0
		24	20.2	21.0	23.5	24.5	23.9	23.5	22.0	19.6	20.7
	60	25	18.8	19.3	19.7	21.1	21.4	21.2	21.0	21.5	22.0
		26	17.5	17.5	19.1	19.5	19.0	18.7	18.5	17.3	17.3
		27	17.1	18.8	17.2	- b)	-	-	-	-	-
		28	18.1	19.2	20.3	20.1	20.3	20.2	21.0	21.4	17.7
		29	16.4	18.7	19.2	19.9	19.3	19.2	- b)	-	-
		30	16.1	19.7	19.3	18.3	18.7	17.9	18.8	15.7	4.7
		31	19.5	19.5	21.7	23.0	21.7	22.7	21.7	22.0	19.6
		32	19.7	19.7	19.5	19.0	18.9	18.1	18.8	15.5	- b)
		33	18.4	19.3	21.6	20.9	21.1	20.[illegible]	21.2	19.5	20.6
		34	18.1	17.9	19.5	19.9	19.3	20.5	20.8	18.1	18.5
		35	16.7	19.2	18.3	18.4	17.3	- b)	-	-	-
		36	23.8	22.1	23.7	22.5	23.1	22.5	24.7	22.0	23.8
	120	37	20.0	21.2	21.3	21.9					
		38	19.1	19.2	18.1	- b)					
		39	17.4	19.7	19.5	17.9					
		40	18.3	19.1	- b)	-					
		41	18.5	19.8	17.0	- b)					
		42	19.1	18.9	19.7	- b)					
		43	17.0	- c)	- b)	-					
		44	19.4	- c)	- c)	- b)					
		45	19.8	- c)	- b)	-					
		46	18.9	- c)	- b)	-					
		47	17.1	- c)	- c)	- b)					
		48	19.2	- c)	- c)	21.1					

b) : Dead animal.
c) : Missing value.

Addendum 3 -Continued
Food consumption(g/rat/day)

Sex	Exp.group (mg/kg/day)	Animal No.	Administration 1	4	8	11	15	18	22	25	28 (days)
Female	Vehicle control	49	17.2	15.0	16.3	14.6	14.4	14.0	15.4	16.2	15.9
		50	13.7	13.7	12.8	12.2	12.3	11.9	12.1	13.1	13.8
		51	14.9	14.3	14.8	12.5	13.1	13.3	14.3	14.5	13.8
		52	13.1	13.1	13.5	12.0	11.2	10.9	9.5	9.5	11.5
		53	16.3	16.3	16.3	15.8	15.8	16.2	15.4	18.5	15.0
		54	15.0	14.6	14.8	13.7	13.5	14.1	12.0	13.0	14.0
		55	15.8	15.8	15.1	13.8	13.6	13.5	13.8	12.3	12.7
		56	16.5	17.0	17.7	16.3	15.7	15.0	16.9	15.7	17.0
		57	16.4	15.7	16.0	15.2	15.1	13.5	14.1	13.8	14.7
		58	15.4	14.8	15.4	14.8	13.2	13.2	13.3	12.7	12.8
		59	14.6	13.6	13.4	14.2	13.2	13.3	13.8	13.8	13.6
		60	16.5	17.6	17.4	13.8	14.2	16.0	15.4	17.0	16.8
	8	61	14.1	14.0	14.8	13.0	13.0	13.4	13.3	14.5	15.8
		62	14.1	14.0	13.5	12.5	11.5	12.9	12.4	14.3	13.5
		63	16.5	16.8	16.2	17.1	15.8	14.5	14.7	14.0	16.5
		64	16.8	13.5	12.8	13.8	13.7	12.5	14.1	13.8	13.7
		65	15.2	14.8	14.8	14.1	14.0	13.2	14.3	13.7	13.3
		66	14.7	15.5	15.8	14.7	13.7	13.8	13.9	11.2	13.3
	35	67	16.0	14.7	14.8	14.4	13.0	12.1	12.8	14.7	13.9
		68	13.0	15.0	15.3	14.0	13.0	12.0	12.9	13.5	13.1
		69	16.6	15.0	14.0	14.0	14.4	12.0	13.9	15.3	9.3
		70	16.5	14.0	14.3	13.9	14.8	14.0	14.2	14.4	14.0
		71	14.6	14.4	13.1	11.4	11.0	11.5	12.0	14.0	11.4
		72	16.0	15.2	15.7	13.7	12.0	12.0	13.0	12.9	13.5
	66	73	13.0	14.0	14.8	12.7	13.7	13.8	12.9	12.2	14.7
		74	14.4	16.0	17.8	16.1	= (b)	=	=	=	=
		75	14.7	13.0	13.1	= (b)	=	=	=	=	=
		76	14.4	14.1	14.8	13.9	= (b)	=	=	=	=
		77	15.8	15.7	14.8	14.4	= (b)	=	=	=	=
		78	17.1	16.9	17.4	15.2	14.8	15.5	15.4	15.0	12.0
		79	16.0	14.4	15.9	14.5	= (b)	=	=	=	=
		80	14.2	17.2	16.2	12.3	= (b)	=	=	=	=
		81	14.0	15.3	15.8	14.8	14.7	15.0	15.7	15.3	14.0
		82	13.3	14.3	14.8	13.8	13.8	13.8	14.3	13.3	=
		83	14.7	15.8	15.5	15.2	15.7	14.0	13.0	13.0	=
		84	16.1	15.8	15.0	15.3	15.7	14.3	15.8	13.7	13.0
	120	85	13.2	14.3	15.1	= (b)					
		86	14.0	14.1	13.7	13.4					
		87	13.8	12.1	11.9	= (b)					
		88	14.8	14.0	14.5	12.7					
		89	15.5	14.8	14.7	= (b)					
		90	15.8	14.1	14.5	14.0					
		91	15.8	14.8	13.8	= (b)					
		92	16.5	15.0	16.2	14.4					
		93	14.2	12.5	12.4	12.7					
		94	17.1	13.0	13.0	13.7					
		95	15.2	15.4	15.8	= (b)					
		96	15.6	17.1	16.8	13.7					

b) : Dead animal.

Addendum 3 Continued 311-0153

Food consumption/g/rat/day.

Sex	Exp. group (mg/kg/day)	Animal No.	Recovery 4	8	11	14 (days)
Male	Vehicle control	7	20.6	23.9	25.3	25.3
		8	20.6	22.8	24.4	24.4
		9	25.3	28.0	27.4	26.4
		10	24.6	25.5	25.7	26.6
		11	26.3	27.5	28.2	28.6
		12	18.6	20.1	20.3	20.5
	60	31	23.2	24.6	21.6	16.2
		32	- b)	-	-	-
		33	24.2	25.4	18.7	5.0
		34	20.3	1.6	-	-
		35	- b)	-	-	-
		36	25.3	26.6	28.8	27.8
Female	Vehicle control	55	17.5	16.7	10.6	20.0
		56	21.1	22.3	24.1	21.2
		57	19.5	19.3	20.6	16.7
		58	16.2	19.6	21.3	20.2
		59	16.9	19.5	19.0	18.7
		60	20.5	22.6	22.4	24.1
	60	70	- b)	-	-	-
		80	- b)	-	-	-
		81	- b)	-	-	-
		82	- b)	-	-	-
		83	b)	-	-	-
		84	- b)	-	-	-

b) Dead animal.

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Addendum 1 -Continued

Food consumption(g/rat/day) : (administration for 14 days)

311-0143

Sex	Dig.group (mg/kg/day)	Animal No.	Recovery 4	8	12	14 (days)
Male	120	37	- a)	-	-	-
		38	- b)	-	-	-
		39	18.3	6.8	- b)	-
		40	- b)	-	-	-
		41	- b)	-	-	-
		42	- b)	-	-	-
		43	- b)	-	-	-
		44	- b)	-	-	-
		45	- b)	-	-	-
		46	- b)	-	-	-
		47	- b)	-	-	-
		48	- b)	-	-	-
Female	120	85	- b)	-	-	-
		86	- b)	-	-	-
		87	- b)	-	-	-
		88	- b)	-	-	-
		89	- b)	-	-	-
		90	- b)	-	-	-
		91	- b)	-	-	-
		92	- b)	-	-	-
		93	16.5	16.4	17.5	18.2
		94	16.4	18.7	15.8	14.0
		95	- b)	-	-	-
		96	- b)	-	-	-

a) : Sacrificed animal.
b) : Dead animal.

Sex	Dosage (mg/kg/day)	Animal No	RBC ($\times 10^4/mm^3$)	Hb ($\times 10^2/mm^3$)	Ht (g/dl)	Hct (%)	MCV (μm^3)	MCH (pg)	MCHC (%)	Platelet ($\times 10^4/mm^3$)	Reticulo (‰)	P.T (sec)	APTT (sec)
Male	Vehicle control	1	721	117	15.2	44.2	61.3	16.7	30.1	100.2	22	15.8	28.1
		2	732	116	14.8	44.7	61.1	16.1	31.3	106.5	15	13.8	30.0
		3	738	110	14.3	43.6	59.3	16.4	32.8	128.7	18	14.7	30.4
		4	684	113	14.4	43.7	63.9	21.1	33.0	110.0	15	12.3	24.4
		5	716	112	14.8	44.0	59.5	20.9	33.8	115.8	17	12.8	28.4
		6 **	-	-	-	-	-	-	-	-	-	-	-
		Recovery											
		7	642	94	14.8	48.7	55.1	17.5	31.7	116.8	26	12.8	24.5
		8	767	133	14.9	44.3	58.3	17.6	31.7	112.8	43	15.7	30.0
		9	734	109	14.8	45.8	61.9	18.6	31.6	121.7	28	14.2	27.5
		10	617	93	14.8	45.8	55.8	18.1	32.5	113.7	11	13.3	24.0
		11	863	119	15.3	46.7	54.1	17.7	32.8	121.2	18	15.0	31.5
		12	949	126	15.6	48.1	52.2	16.8	31.8	121.1	18	12.4	29.0
	6	13	662	144	14.3	40.9	60.1	20.7	34.5	119.3	19	12.5	30.7
		14	727	96	14.7	43.2	59.4	20.1	34.0	88.4	8	13.9	30.1
		15	733	122	14.5	41.3	61.6	19.6	32.0	106.0	13	11.8	30.8
		16	721	95	14.0	42.7	63.1	19.4	32.8	106.9	13	13.5	27.5
		17	793	76	14.8	47.0	59.1	18.6	31.5	109.2	22	13.1	29.1
		18	764	77	15.3	44.2	57.8	18.3	33.9	122.2	35	12.0	26.4
	36	19	752	102	14.4	43.6	57.9	19.6	33.0	119.7	14	13.8	27.0
		20	702	101	13.5	40.7	58.0	19.2	33.2	123.3	32	14.0	25.7
		21	735	145	13.8	42.1	57.3	19.7	32.8	111.1	14	12.2	23.4
		22	714	93	14.7	43.2	60.5	20.6	34.0	100.3	13	13.6	27.5
		23	728	116	13.6	42.0	57.6	18.6	32.0	133.1	16	12.8	26.1
		24	785	84	14.7	44.7	56.9	18.7	32.9	114.7	13	12.3	20.0
	60	25	635	120	13.5	41.7	59.4	19.4	32.7	107.5	25	13.9	27.3
		26	747	115	13.5	41.3	55.0	18.1	32.8	99.5	26	12.8	30.8
		27 **	-	-	-	-	-	-	-	-	-	-	-
		28	634	102	15.0	47.3	56.7	18.0	31.7	83.5	20	13.7	29.4
		29 **	-	-	-	-	-	-	-	-	-	-	-
		30	762	93	13.5	43.1	56.6	17.3	30.4	35.8	24	16.2	40.7
		Recovery											
		31	798	134	14.4	45.4	56.8	18.0	31.7	92.1	14	13.1	22.8
		32 **	-	-	-	-	-	-	-	-	-	-	-
		33	863	138	15.2	53.3	55.2	16.8	30.4	70.1	20	25.0	55.5
		34 **	-	-	-	-	-	-	-	-	-	-	-
		35 **	-	-	-	-	-	-	-	-	-	-	-
		36	822	154	14.6	47.0	57.2	18.0	31.5	113.6	22	17.4	34.1

** : Dead animal

Addendum 4 -Continued
Hematology

811-0253

Sex	Exp. group (mg/kg/day)	Animal No.	Differentiation of Leucocyte (%) Mono	Baso	Eosino	Lymph	Stab	Seg
Male	Vehicle control	1	0.0	0.0	1.0	89.5	0.0	9.5
		2	0.0	0.0	1.0	88.5	0.0	10.5
		3	0.0	0.0	1.0	95.5	0.0	3.5
		4	0.0	0.0	1.0	88.5	0.0	10.5
		5	0.0	0.0	1.0	88.0	0.0	12.0
		6 **	-	-	-	-	-	-
		Recovery						
		7	0.0	0.0	0.0	86.5	0.0	13.5
		8	0.0	0.0	0.0	78.5	0.0	21.5
		9	0.0	0.0	0.0	92.5	0.0	7.5
		10	0.0	0.0	0.0	93.0	0.0	7.0
		11	0.0	0.0	1.0	93.5	0.0	5.5
		12	0.5	0.0	0.0	85.5	0.0	14.0
	6	13	0.0	0.0	1.0	81.5	0.5	17.0
		14	0.5	0.0	0.0	89.0	0.5	10.0
		15	0.0	0.0	0.5	88.5	0.0	11.0
		16	0.0	0.0	0.0	88.0	0.5	11.5
		17	0.0	0.0	0.5	82.5	0.0	17.0
		18	0.5	0.0	1.5	85.0	0.0	13.0
	30	19	0.0	0.0	1.0	89.5	0.5	9.0
		20	0.0	0.0	0.5	85.5	0.5	13.5
		21	0.0	0.0	0.0	77.0	0.0	23.0
		22	0.0	0.0	0.0	81.0	0.0	19.0
		23	0.0	0.0	1.0	78.5	0.5	20.0
		24	0.0	0.0	0.0	91.5	0.0	8.5
	90	25	0.0	0.0	0.0	90.5	0.0	9.5
		26	0.0	0.0	0.0	86.0	0.0	14.0
		27 **	=	=	=	=	=	=
		28	0.5	0.0	0.0	93.0	0.0	6.5
		29 **	=	=	=	=	=	=
		30	0.5	0.0	0.5	77.0	0.0	22.0
		Recovery						
		31	0.0	0.0	0.5	91.5	0.0	8.0
		32 **	=	=	=	=	=	=
		33	0.0	0.0	0.5	71.5	0.0	28.0
		34 **	=	=	=	=	=	=
		35 **	=	=	=	=	=	=
		36	0.0	0.0	0.0	91.0	0.0	8.0

** : Dead animal.

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Addendum 4 -Continued
Hematology

Sex	Exp. group (mg/kg/day)	Animal No.	RBC ($\times 10^6$/mm^3)	mHC ($\times 10^6$/mm^3)	Hb (g/dl)	Hc (%)	mCV (μm^3)	mCH (pg)	mCHC (%)	Platelet ($\times 10^4$/mm^3)	Reticulo (%)	P T (sec)	APTT (sec)
Female	Vehicle control	49	71[illegible]	76	14.9	42.3	59.1	20.8	35.2	119.3	6	12.1	22.5
		50	666	67	13.8	3[illegible].3	55.5	19.7	35.6	154.9	14	11.8	23.1
		51	757	43	14.1	42.5	56.1	18.6	33.2	134.0	15	12.4	22.4
		52	717	63	14.6	40.1	55.9	20.4	36.4	147.6	12	12.5	24.5
		53	723	79	14.1	40.6	56.2	19.5	34.7	150.3	7	12.2	25.8
		54	775	69	15.6	43.6	56.3	20.1	35.8	142.1	12	12.2	19.2
		Recovery											
		55	776	102	14.5	42.9	55.1	18.6	33.8	122.3	21	11.8	22.4
		56	791	66	14.4	42.8	54.1	18.2	33.6	144.8	8	12.1	18.9
		57	663	73	15.2	46.0	53.3	17.8	33.0	130.3	9	11.4	19.0
		58	777	79	13.8	41.2	53.0	17.6	33.5	147.6	24	11.3	23.4
		59	760	62	14.5	44.5	57.1	18.6	32.6	123.0	21	11.9	21.4
		60	764	67	13.5	41.3	54.1	17.7	32.7	109.8	22	11.5	23.9
	6	61	712	65	13.6	41.3	58.0	19.1	32.9	149.2	15	11.8	21.1
		62	726	63	14.3	40.9	56.3	19.7	35.0	136.0	11	11.9	20.1
		63	723	114	14.4	42.4	58.6	19.8	34.0	142.3	27	12.7	24.5
		64	773	62	14.8	43.3	56.0	19.1	34.2	1[illegible].6	12	12.8	25.2
		65	775	61	14.8	44.1	56.9	19.[illegible]	33.6	140.6	18	10.2	25.7
		66	764	71	15.1	44.8	5[illegible].4	19.[illegible]	33.7	126.4	9	10.6	22.1
	30	67	665	61	13.8	40.8	59.6	20.1	33.8	129.1	20	12.1	23.8
		68	753	57	14.5	42.2	55.6	19.1	34.4	117.3	9	12.0	25.2
		69	760	75	14.9	44.1	56.5	19.1	33.8	130.3	13	12.2	25.9
		70	746	47	14.0	42.0	56.1	18.7	33.3	118.9	14	12.6	22.0
		71	759	74	14.4	41.7	54.9	19.0	34.5	139.3	7	11.3	24.1
		72	796	109	15.3	46.1	57.9	19.2	33.2	109.2	12	10.7	24.6
	60	73	764	67	15.0	43.3	56.7	19.6	34.6	140.9	16	11.6	26.9
		74 **	-	-	-	-	-	-	-	-	-	-	-
		75 **	-	-	-	-	-	-	-	-	-	-	-
		76 **	-	-	-	-	-	-	-	-	-	-	-
		77 **	-	-	-	-	-	-	-	-	-	-	-
		78 **	-	-	-	-	-	-	-	-	-	-	-
		Recovery											
		79 **	-	-	-	-	-	-	-	-	-	-	-
		80 **	-	-	-	-	-	-	-	-	-	-	-
		81 **	-	-	-	-	-	-	-	-	-	-	-
		82 **	-	-	-	-	-	-	-	-	-	-	-
		83 **	-	-	-	-	-	-	-	-	-	-	-
		84 **	-	-	-	-	-	-	-	-	-	-	-

** : Dead animal.

0258

Sex	Dose group (mg/kg/day)	Animal No.	Differentiation of tumor cells (%)					
			Mucin	Acini	Solid	Papill	Stab	Sag
Female	Vehicle control	49	0.0	0.0	0.0	80.0	0.0	20.0
		50	0.0	0.0	0.0	80.0	0.0	20.0
		51	0.0	0.0	0.0	80.0	0.0	20.0
		52	0.0	0.0	0.0	80.0	0.0	20.0
		53	0.0	0.0	0.0	80.0	0.0	20.0
		54	0.0	0.0	0.0	75.0	0.0	25.0
		Recovery						
		55	0.0	0.0	0.0	77.0	0.0	23.0
		56	0.0	0.0	0.0	100.0	0.0	0.0
		57	0.0	0.0	0.0	95.0	0.0	5.0
		58	0.0	0.0	0.0	83.0	0.0	17.0
		59	0.0	0.0	0.0	89.0	0.0	11.0
		60	0.0	0.0	0.0	100.0	0.0	0.0
	6	61	0.0	0.0	0.0	100.0	0.0	0.0
		62	0.0	0.0	0.0	83.0	0.0	17.0
		63	0.0	0.0	0.0	87.0	0.0	13.0
		64	0.0	0.0	0.0	89.0	0.0	11.0
		65	0.0	0.0	0.0	92.0	0.0	8.0
		66	0.0	0.0	0.0	89.0	0.0	11.0
	30	67	0.0	0.0	0.0	88.0	0.0	12.0
		68	0.0	0.0	0.0	88.0	0.0	8.0
		69	0.0	0.0	0.0	90.0	0.0	10.0
		70	0.0	0.0	0.0	92.0	0.0	8.0
		71	0.0	0.0	0.0	88.0	0.0	12.0
		72	0.0	0.0	0.0	90.0	0.0	10.0
		73	0.0	0.0	0.0	91.0	0.0	10.0
	60	74 **	-	-	-	-	-	-
		75 **	-	-	-	-	-	-
		76 **	-	-	-	-	-	-
		77 **	-	-	-	-	-	-
		78 **	-	-	-	-	-	-
		Recovery						
		79 **	-	-	-	-	-	-
		80 **	-	-	-	-	-	-
		81 **	-	-	-	-	-	-
		82 **	-	-	-	-	-	-
		83 **	-	-	-	-	-	-
		84 **	-	-	-	-	-	-

** : Dead animal.

135

Addendum 4 -Continued

Hematology : (administration for 14 days)

B11-0153

Sex	Exp.group (mg/kg/day)	Animal No.	RBC ($x10^6/mm^3$)	WBC ($x10^2/mm^3$)	Hb (g/dl)	Hc (%)	MCV (μm^3)	MCH (pg)	MCHC (%)	Platelet ($x10^4/mm^3$)	Reticula (%)	P T (sec)	APTT (sec)
Male	12%	37 a)	723	158	14.1	46.3	64.0	19.5	30.5	121.5	60	13.6	22.4
		38 b)	-	-	-	-	-	-	-	-	-	-	-
		40 b)	-	-	-	-	-	-	-	-	-	-	-
		41 b)	-	-	-	-	-	-	-	-	-	-	-
		42 b)	-	-	-	-	-	-	-	-	-	-	-
		43 b)	-	-	-	-	-	-	-	-	-	-	-
		44 b)	-	-	-	-	-	-	-	-	-	-	-
		45 b)	-	-	-	-	-	-	-	-	-	-	-
		46 b)	-	-	-	-	-	-	-	-	-	-	-
		47 b)	-	-	-	-	-	-	-	-	-	-	-
		48 b)	-	-	-	-	-	-	-	-	-	-	-
		Recovery											
		38 b)	-	-	-	-	-	-	-	-	-	-	-

a) : Sacrificed animal.
b) : Dead animal.

Addendum 4 -Continued

Hematology : (administration for 14 days)

Sex	Exp.group (mg/kg/day)	Animal No.	Differentiation of Leukocyte (%) Mono	Baso	Eosino	Lymph	Stab	Seg
Male	120	37 a)	0.0	0.0	0.5	84.0	0.5	5.0
		38 b)	-	-	-	-	-	-
		40 b)	-	-	-	-	-	-
		41 b)	-	-	-	-	-	-
		42 b)	-	-	-	-	-	-
		43 b)	-	-	-	-	-	-
		44 b)	-	-	-	-	-	-
		45 b)	-	-	-	-	-	-
		46 b)	-	-	-	-	-	-
		47 b)	-	-	-	-	-	-
		48 b)	-	-	-	-	-	-
		Recovery						
		38 b)	-	-	-	-	-	-

a) : Sacrificed animal.
b) : Dead animal.

(37)

Addendum 4 -Continued

Hematology : (administration for 14 days)

B11-0163

Sex	Exp. group (mg/kg/day)	Animal No.	RBC ($\times 10^6/mm^3$)	WBC ($\times 10^3/mm^3$)	Hb (g/dl)	Hc (%)	MCV (μm^3)	MCH (pg)	MCHC (%)	Platelet ($\times 10^4/mm^3$)	Reticulo (‰)	P T (sec)	APTT (sec)
Female	126	65 b)	-	-	-	-	-	-	-	-	-	-	-
		66 a)	723	122	14.1	44.9	61.6	19.3	31.4	103.7	28	12.4	21.8
		67 b)	-	-	-	-	-	-	-	-	-	-	-
		68 b)	-	-	-	-	-	-	-	-	-	-	-
		69 b)	-	-	-	-	-	-	-	-	-	-	-
		30 a)	716	68	13.8	44.2	61.3	19.2	31.3	136.5	22	12.1	17.5
		31 b)	-	-	-	-	-	-	-	-	-	-	-
		35 b)	-	-	-	-	-	-	-	-	-	-	-
		33 b)	-	-	-	-	-	-	-	-	-	-	-
		Recovery											
		32 b)	-	-	-	-	-	-	-	-	-	-	-
		33	753	65	14.5	41.9	55.6	19.3	34.6	139.2	12	10.9	27.2
		34	655	27	12.1	36.7	56.0	18.5	33.0	24.8	14	18.4	55.4

a) : Sacrificed animal.

b) Dead animal.

Addendum 4 -Continued

Hematology : (administration for 16 days)

311-C153

Sex	Dep. group (mg/kg/day)	Animal No.	Differentiation of Leukocyte (%)					
			Mono	Baso	Eosino	Lymph	Stab	Seg
Female	120	65 b)	-	-	-	-	-	-
		66 a)	0.0	0.0	0.0	85.5	0.0	5.5
		67 b)	-	-	-	-	-	-
		68 b)	-	-	-	-	-	-
		69 b)	-	-	-	-	-	-
		70 a)	0.0	0.0	0.0	85.0	0.5	4.5
		71 b)	-	-	-	-	-	-
		72 b)	-	-	-	-	-	-
		73 b)	-	-	-	-	-	-
		Recovery						
		82 b)	-	-	-	-	-	-
		83	0.0	0.0	0.5	77.5	0.0	22.0
		84	0.0	0.0	0.0	85.0	0.0	34.0

a) : Sacrificed animal.
b) : Dead animal.

Sex	Exp group (mg (g/kg))	Animal No	BUN (mg/dl)	Creatinine (mg/dl)	T-Bil (mg/dl)	Ca (mg/dl)	P (mg/dl)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)
		1	11.1	0.40	0.21	9.5	7.2	140	4.0	105.2
		2	11.0	0.45	0.21	9.7	7.6	142	4.3	110.3
		3	11.8	0.44	0.25	9.3	7.9	139	5.0	104.9
		4	12.3	0.37	0.22	9.7	8.5	141	4.1	106.8
		5	10.0	0.44	0.23	9.7	7.8	140	4.4	108.3
	Vehicle control	6 **	-	-	-	-	-	-	-	-
		Average	11.4	0.42	0.23	9.9	7.4	140	4.6	105.3
		7	14.2	0.41	0.18	9.4	7.0	142	5.6	108.2
		8	15.2	0.42	0.21	9.6	5.0	143	3.8	108.2
		10	10.0	0.43	0.13	9.3	5.5	141	4.3	108.5
		11	13.4	0.42	0.20	9.4	6.7	141	3.9	108.3
		12	12.7	0.42	0.21	9.5	8.4	141	4.4	108.4
		13	11.1	0.43	0.25	9.3	8.0	138	4.0	104.8
		14	9.3	0.44	0.20	9.8	7.1	138	4.0	105.8
	6	15	10.5	0.40	0.24	9.8	7.2	140	4.5	106.7
		16	11.0	0.51	0.16	9.6	7.2	138	4.8	108.7
		17	11.8	0.41	0.16	10.0	8.4	141	4.8	107.0
Male		18	11.5	0.45	0.23	9.8	7.5	138	4.5	108.3
		19	12.1	0.32	0.26	9.5	8.5	142	5.4	105.7
		20	10.1	0.37	0.22	9.6	7.4	138	4.1	107.7
	60	21	13.7	0.45	0.25	10.1	8.2	140	4.1	105.5
		22	10.5	0.40	0.14	9.8	7.8	138	4.3	106.8
		23	10.3	0.46	0.16	9.7	8.0	139	4.5	105.6
		24	10.3	0.43	0.21	10.1	7.8	138	4.4	105.1
		25	10.1	0.42	0.15	9.9	7.7	139	4.0	105.6
		26	10.2	0.47	0.21	10.5	7.8	140	3.9	105.9
		27 **	-	-	-	-	-	-	-	-
		28	12.3	0.40	0.17	10.4	7.3	139	4.4	105.1
		29 **	-	-	-	-	-	-	-	-
	60	30	36.1	0.74	0.15	9.9	7.4	138	5.9	99.0
		Average								
		31	12.8	0.42	0.16	10.1	8.0	142	4.9	106.9
		32 **	-	-	-	-	-	-	-	-
		33	15.4	0.74	0.16	9.6	6.9	134	5.6	99.5
		34 **	-	-	-	-	-	-	-	-
		35 **	-	-	-	-	-	-	-	-
		36	16.6	0.74	0.22	9.6	6.6	139	4.0	103.0

** Dead animal.

Addendum 6 -Continued
Blood chemistry

811-0153

Sex	Exp group (mg/kg/day)	Animal No.	GOT (IU/l)	GPT (IU/l)	ALP (IU/l)	ChA (IU/l)	γ-GTP (IU/l)	T-Cho (mg/dl)	TG (mg/dl)	Glucose (mg/dl)	T-protein (g/dl)	Albumin (g/dl)	A/G ratio
Female	Vehicle control	49	71	14	369	123	0.9	59	30	132.1	5.4	3.1	1.35
		50	51	18	327	338	1.0	49	28	161.2	5.8	3.4	1.55
		51	57	21	281	201	0.9	52	25	148.8	5.5	3.4	1.62
		52	57	16	346	210	1.2	57	25	146.1	5.3	3.2	1.52
		53	66	17	230	208	0.7	58	28	135.8	5.7	3.3	1.37
		54	74	21	317	286	1.7	63	28	126.8	5.6	3.3	1.43
		Recovery											
		55	66	20	164	336	0.8	62	27	132.6	5.9	3.1	1.11
		56	60	18	165	506	0.8	50	30	116.5	5.7	3.1	1.19
		57	65	19	186	375	0.5	52	39	156.7	6.1	3.5	1.35
		58	66	18	168	308	0.6	57	28	145.1	5.8	3.2	1.23
		59	66	17	202	185	0.5	68	27	118.4	5.8	3.2	1.23
		60	65	23	201	416	0.3	76	33	166.6	5.9	3.3	1.27
	6	61	64	21	266	205	0.8	50	27	176.4	5.7	3.2	1.28
		62	66	21	281	195	0.8	38	22	120.6	5.4	3.1	1.35
		63	58	20	407	203	1.1	53	27	153.7	5.7	3.0	1.11
		64	53	16	247	185	0.7	49	34	151.3	5.5	3.1	1.20
		65	72	24	268	435	1.5	48	25	126.8	5.5	3.3	1.50
		66	64	16	223	288	0.8	72	25	149.7	6.0	3.4	1.31
	30	67	60	21	312	201	0.8	51	23	154.9	5.5	3.1	1.29
		68	61	16	308	291	0.8	57	28	154.2	5.7	3.2	1.28
		69	63	16	348	378	1.1	56	35	145.8	6.0	3.5	1.40
		70	56	16	231	268	0.9	56	42	153.0	5.5	3.4	1.62
		71	73	20	282	414	0.7	58	23	157.1	5.3	3.2	1.52
		72	75	21	283	127	0.7	58	26	117.9	5.6	3.1	1.24
	60	73	65	16	303	259	1.0	53	[illegible]	145.9	6.0	3.5	1.40
		74 **	-	-	-	-	-	-	-	-	-	-	-
		75 **	-	-	-	-	-	-	-	-	-	-	-
		76 **	-	-	-	-	-	-	-	-	-	-	-
		77 **	-	-	-	-	-	-	-	-	-	-	-
		78 **	-	-	-	-	-	-	-	-	-	-	-
		Recovery											
		79 **	-	-	-	-	-	-	-	-	-	-	-
		80 **	-	-	-	-	-	-	-	-	-	-	-
		81 **	-	-	-	-	-	-	-	-	-	-	-
		82 **	-	-	-	-	-	-	-	-	-	-	-
		83 **	-	-	-	-	-	-	-	-	-	-	-
		84 **	-	-	-	-	-	-	-	-	-	-	-

** : Dead animal.

142

Addendum 5 -Continued
Blood chemistry

Sex	Exp. group (mg/kg/day)	Animal No.	BUN (mg/dl)	Creatinine (mg/dl)	T-Bil (mg/dl)	Ca (mg/dl)	iP (mg/dl)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)
Female	Vehicle control	49	12.7	0.42	0.21	9.7	6.4	139	4.2	107.5
		50	16.6	0.56	0.22	9.4	6.9	137	4.1	108.0
		51	11.5	0.48	0.20	9.6	6.0	141	4.2	109.0
		52	11.0	0.41	0.21	10.0	7.0	136	4.1	107.9
		53	10.5	0.41	0.22	9.7	7.6	136	4.7	104.2
		54	12.1	0.48	0.17	9.6	7.7	136	4.0	105.8
		Recovery								
		55	15.3	0.54	0.19	9.0	5.2	139	3.5	107.1
		56	18.6	0.53	0.21	9.4	5.5	137	4.2	108.4
		57	18.2	0.52	0.19	9.6	5.3	136	4.1	107.1
		58	15.6	0.49	0.21	9.4	4.8	143	4.7	101.8
		59	15.4	0.45	0.25	9.3	5.5	143	4.6	104.3
		60	18.4	0.67	0.18	9.6	6.3	137	4.1	106.5
	6	61	14.4	0.51	0.20	9.2	7.1	134	4.3	105.1
		62	16.6	0.38	0.25	9.9	6.7	137	3.7	108.1
		63	12.4	0.41	0.21	9.7	8.1	136	4.3	104.5
		64	11.2	0.44	0.16	9.4	7.3	136	3.6	105.0
		65	12.6	0.41	0.21	9.6	7.7	136	3.9	104.1
		66	12.6	0.53	0.19	9.7	7.2	136	3.6	105.0
	30	67	13.1	0.40	0.22	9.8	6.9	137	4.3	106.0
		68	9.6	0.51	0.16	9.9	6.5	139	3.4	106.4
		69	14.3	0.54	0.19	9.8	6.9	140	3.7	107.1
		70	13.5	0.46	0.20	9.9	7.8	139	4.5	105.8
		71	12.7	0.57	0.15	9.5	8.3	140	4.1	105.7
		72	12.8	0.46	0.23	9.8	7.5	137	4.1	104.4
	60	73	10.4	0.66	0.11	10.0	7.9	136	3.9	104.7
		74 **	-	-	-	-	-	-	-	-
		75 **	-	-	-	-	-	-	-	-
		76 **	-	-	-	-	-	-	-	-
		77 **	-	-	-	-	-	-	-	-
		78 **	-	-	-	-	-	-	-	-
		Recovery								
		79 **	-	-	-	-	-	-	-	-
		80 **	-	-	-	-	-	-	-	-
		81 **	-	-	-	-	-	-	-	-
		82 **	-	-	-	-	-	-	-	-
		83 **	-	-	-	-	-	-	-	-
		84 **	-	-	-	-	-	-	-	-

** : Dead animal.

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Attachment 5 -Continued

Blood chemistry : (administration for 14 days)

B11-0153

Sex	Dosage (mg/kg/day)	Animal No.	GOT (IU/L)	GPT (IU/L)	ALP (IU/L)	ChE (IU/L)	γ-GTP (IU/L)	T-Cho (mg/dl)	TG (mg/dl)	Glucose (mg/dl)	T-protein (g/dl)	Albumin (g/dl)	A/G ratio
Male	126	37 a)	70	25	605	52	0.6	48	92	152.7	5.7	3.2	1.28
		38 b)	-	-	-	-	-	-	-	-	-	-	-
		40 b)	-	-	-	-	-	-	-	-	-	-	-
		41 b)	-	-	-	-	-	-	-	-	-	-	-
		42 b)	-	-	-	-	-	-	-	-	-	-	-
		43 b)	-	-	-	-	-	-	-	-	-	-	-
		44 b)	-	-	-	-	-	-	-	-	-	-	-
		45 b)	-	-	-	-	-	-	-	-	-	-	-
		46 b)	-	-	-	-	-	-	-	-	-	-	-
		47 b)	-	-	-	-	-	-	-	-	-	-	-
		48 b)	-	-	-	-	-	-	-	-	-	-	-
		Recovery 39 b)	-	-	-	-	-	-	-	-	-	-	-

a) : Sacrificed animal.
b) : Dead animal.

Addendum 6 -Continued

Blood chemistry : (administration for 24 days.

311-0153

Sex	Dosage (mg/kg/day)	Animal No.	BUN (mg/dl)	Creatinine (mg/dl)	T-Bil (mg/dl)	Ca (mg/dl)	IP (mg/dl)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)
male	125	37 a)	11.1	0.42	0.17	6.6	6.8	143	4.2	101.0
		38 b)	-	-	-	-	-	-	-	-
		40 b)	-	-	-	-	-	-	-	-
		41 b)	-	-	-	-	-	-	-	-
		42 b)	-	-	-	-	-	-	-	-
		43 b)	-	-	-	-	-	-	-	-
		44 b)	-	-	-	-	-	-	-	-
		45 b)	-	-	-	-	-	-	-	-
		46 b)	-	-	-	-	-	-	-	-
		47 b)	-	-	-	-	-	-	-	-
		48 b)	-	-	-	-	-	-	-	-
		Recovery								
		39 b)	-	-	-	-	-	-	-	-

a) : Sacrificed animal.
b) : Dead animal.

Addendum 6 -Continued

Blood chemistry : (administration for 14 days)

R11-0153

Sex	Exp.group (mg/kg/day)	Animal No.	GOT (IU/l)	GPT (IU/l)	ALP (IU/l)	ChE (IU/l)	γ-GTP (IU/l)	T-Cho (mg/dl)	TG (mg/dl)	Glucose (mg/dl)	T-protein (g/dl)	Albumin (g/dl)	A/G ratio
Female	120	65 b)	-	-	-	-	-	-	-	-	-	-	-
		66 a)	205	26	435	294	0.9	75	52	164.6	5.7	3.3	1.37
		67 b)	-	-	-	-	-	-	-	-	-	-	-
		68 b)	-	-	-	-	-	-	-	-	-	-	-
		69 b)	-	-	-	-	-	-	-	-	-	-	-
		60 a)	60	16	467	308	0.8	78	38	148.5	5.7	3.4	1.48
		61 b)	-	-	-	-	-	-	-	-	-	-	-
		65 b)	-	-	-	-	-	-	-	-	-	-	-
		66 b)	-	-	-	-	-	-	-	-	-	-	-
		Recovery											
		62 b)	-	-	-	-	-	-	-	-	-	-	-
		63	84	33	359	430	0.8	52	42	129.0	5.8	3.1	1.24
		64	2446	1234	295	181	1.1	52	43	185.2	5.8	3.4	1.55

a) : Sacrificed animal.

b) : Dead animal.

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Attachment 6 -Continued

Blood chemistry : (Administration for 14 days)

Sex	Exp.group (mg/kg/day)	Animal No.	BUN (mg/dl)	Creatinine (mg/dl)	T-Bil (mg/dl)	Ca (mg/dl)	IP (mg/dl)	Na (mEq/l)	K (mEq/l)	Cl (mEq/l)
		65 b)	-	-	-	-	-	-	-	-
		66 a)	18.4	0.42	0.22	10.4	8.5	139	4.3	104.7
		67 b)	-	-	-	-	-	-	-	-
		68 b)	-	-	-	-	-	-	-	-
		69 b)	-	-	-	-	-	-	-	-
Female	120	60 a)	13.0	0.39	0.23	9.6	7.0	142	4.1	104.2
		61 b)	-	-	-	-	-	-	-	-
		62 b)	-	-	-	-	-	-	-	-
		63 b)	-	-	-	-	-	-	-	-
		Recovery								
		62 b)	-	-	-	-	-	-	-	-
		63	16.8	0.62	0.18	9.4	7.0	134	4.4	105.8
		64	24.5	0.63	0.15	10.3	7.8	138	4.8	108.0

a) : Sacrificed animal.
b) : Dead animal.

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Addendum 5 22-day repeated-dose oral toxicity study in rats

Urinalysis

R11-0153

Sex	Exp. group (mg/kg/day)	Animal No.	Volume (ml)	Color	pH	Protein	Ketones	Bilirubin	Occult Blood	Glucose	Urobilinogen (EU/dl)
Male	Vehicle control	1	15	SY	6.5	±	-	-	±	-	1
		2	4	*	6.5	-	-	-	-		1
		3	14	SY	6.5	±	±	-	-	-	0.1
		4	14	SY	6.0	±	-	-	±	-	0.1
		5	6	*	6.5	±	±	-	±	-	0.1
		6a)	—	—	—	—	—	—	—	—	—
		Recovery									
		7	15	SY	6.5	±	±	-	-	-	0.1
		8	14	*	6.5	±	±	-	±	-	0.1
		9	9	*	6.0	-	±	-	-	-	0.1
		10	13	SY	6.5	±	±	-	-	-	0.1
		11	8	*	6.5	-	-	-	-	-	0.1
		12	7	*	6.0	-	±	-	-	-	1
	2	13	4	*	6.0	**	-	-	*	-	1
		14	4	*	6.0	**	-	-		-	1
		15	5	*	6.0	-	-	-	-	-	1
		16	9	*	6.5	±	±	-	±	-	0.1
		17	6	*	6.0	-	±	-	-	-	1
		18	10	*	6.5	±	±	-	±	-	0.1

a) : Dead animal

SY : Slightly yellow

* : Yellow

Addendum 6 – Continued

Urinalysis

511-0153

Sex	Exp. group (mg/kg/day)	Animal No.	Volume (ml)	Color	pH	Protein	Ketones	Bilirubin	Occult Blood	Glucose	Urobilinogen (EU/dl)
		15	8	✓	6.5	*	±	–	±	–	0.1
		20	5	✓	6.0	**	±	–	–	–	1
	30	21	4	✓	6.0	**	–	–	±	–	1
		22	8	✓	6.0	*	–	–	–	–	0.1
		23	13	✓	6.5	±	±	–	*	–	1
		24	8	✓	6.0	**	–	–	±	–	1
		25	12	✓	6.0	*	±	–	–	–	0.1
		26	14	SY	6.5	±	±	–	*	–	0.1
		27a)	—	—	—	—	—	—	—	—	—
Male		28	4	✓	6.0	**	±	–	±	–	1
		29a)	—	—	—	—	—	—	—	—	—
		30	6	✓	6.0	±	±	–	*	–	0.1
	60										
		Recovery									
		31	7	✓	6.0	**	–	–	±	–	0.1
		32a)	—	—	—	—	—	—	—	—	—
		33	5	✓(T)	6.0	**	–	–	±	–	0.1
		34a)	—	—	—	—	—	—	—	—	—
		35a)	—	—	—	—	—	—	—	—	—
		36	7	✓	6.0	*	±	–	–	–	1

a) : Dead animal

SY : Slightly yellow

✓ : Yellow

(T) : Turbid

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Addendum 6 – Continued

Urinalysis

B11-0153

Sex	Exp. group (mg/kg/day)	Animal No.	Volume (ml)	Color	pH	Protein	Ketones	Bilirubin	Occult Blood	Glucose	Urobilinogen (Eu/dl)
		49	3	*	6.0	**	–	–	±	–	1
		50	3	*	6.0	*	–	–	–	–	1
		51	7	SY	6.0	±	–	–	–	–	0.1
		52	[illegible]	*	6.0	**	–	–	–	–	1
		53	15	SY	6.5	–	–	–	–	–	0.1
		54	2	*	6.0	**	–	–	–	–	1
	Vehicle control	Sec. ary									
		55	8	*	6.5	±	–	–	–	–	0.1
		56	7	*	6.0	±	–	–	±	–	1
Female		57	5	*	6.5	±	–	–	–	–	0.1
		58	7	*	6.5	±	–	–	–	–	1
		59	[illegible]	SY	6.0	±	–	–	–	–	0.1
		60	4	*	6.0	**	–	–	±	–	1
		61	1	*	6.5	*	–	–	–	–	1
		62	[illegible]	SY	6.5	–	–	–	±	–	0.1
	8	63	4	*	6.0	*	–	–	–	–	1
		64	10	SY	6.0	±	–	–	–	–	0.1
		65	2	*	6.0	*	–	–	–	–	1
		66	2	*	6.0	**	–	–	–	–	1

SY Slightly yellow

* Yellow

DEL 4

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Addendum 5 - Continued

Urinalysis

B11-0152

Sex	Exp. group (µg/kg/day)	Animal No.	Volume (ml)	Color	pH	Protein	Ketones	Bilirubin	Occult Blood	Glucose	Urobilinogen (EU/dl)
		67	7	±	6.5	±	-	-	±	-	1
		68	7	±	6.0	±	-	-	-	-	1
	30	69	2	±	6.0	++	-	-	-	-	1
		70	11	±±	6.0	-	-	-	-	-	0.1
		71	5	±	6.0	±	-	-	±	-	0.1
		72	16	±±	6.5	±	-	-	±	-	0.1
		73	7	±	6.0	++	-	-	-	-	1
		74a)	—	—	—	—	—	—	—	—	—
		75a)	—	—	—	—	—	—	—	—	—
Female		76a)	—	—	—	—	—	—	—	—	—
		77a)	—	—	—	—	—	—	—	—	—
		78b)	7	±	6.5	*	-	-	*	-	0.1
	60										
		Recovery									
		79a)	—	—	—	—	—	—	—	—	—
		80a)	—	—	—	—	—	—	—	—	—
		81a)	—	—	—	—	—	—	—	—	—
		82a)	—	—	—	—	—	—	—	—	—
		83a)	—	—	—	—	—	—	—	—	—
		84a)	—	—	—	—	—	—	—	—	—

a) Dead animal

b) Dead animal (in metabolic cage)

±± Slightly yellow

± Yellow

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Addendum 5 - Continued

Urinalysis (administration for 14 days)

B11-0153

Sex	Exp. group (mg/kg/day)	Animal No.	Volume (ml)	Color	pH	Protein	Ketones	Bilirubin	Occult Blood	Glucose	Urobilinogen (Fu/ml)
		37a)	—	—	—	—	—	—	—	—	—
		38b)	—	—	—	—	—	—	—	—	—
		40b)	—	—	—	—	—	—	—	—	—
		41b)	—	—	—	—	—	—	—	—	—
		42b)	—	—	—	—	—	—	—	—	—
		43b)	—	—	—	—	—	—	—	—	—
Male	120	44b)	—	—	—	—	—	—	—	—	—
		45b)	—	—	—	—	—	—	—	—	—
		46b)	—	—	—	—	—	—	—	—	—
		47b)	—	—	—	—	—	—	—	—	—
		48b)	—	—	—	—	—	—	—	—	—
		Recovery									
		39b)	—	—	—	—	—	—	—	—	—

a) Sacrificed animal

b) Dead animal

Appendix 6 - Continued

Urinalysis (administration for 14 days)

311-0153

Sex	Exp. group (mg/kg/day)	Animal No.	Volume (ml)	Color	pH	Protein	Ketones	Bilirubin	Occult Blood	Glucose	Urobilinogen (EU/dl)
		85(a)	—	—	—	—	—	—	—	—	—
		86(a)	—	—	—	—	—	—	—	—	—
		87(a)	—	—	—	—	—	—	—	—	—
		88(a)	—	—	—	—	—	—	—	—	—
		89(a)	—	—	—	—	—	—	—	—	—
		90(a)	—	—	—	—	—	—	—	—	—
Female	120	91(b)	—	—	—	—	—	—	—	—	—
		92(b)	—	—	—	—	—	—	—	—	—
		93(b)	—	—	—	—	—	—	—	—	—
		Recovery									
		92(b)	—	—	—	—	—	—	—	—	—
		93	+	+	6.5	+	+	+	+	+	1
		94	12	SY	6.5	+	+	+	+	+	0.1

a) Sacrificed animal

b) Dead animal

SY Slightly yellow

Y Yellow

Addendum 7 28-day repeated-dose oral toxicity study in rats
Absolute organ weights

311-0153

Sex	Exp group (mg/kg/day)	Animal No.	Spleen (g)	Liver (g)	Kidney (g)	Heart (g)	Brain (g)	Testis (g)	Adrenal gland (mg)	Ovary (mg)	Body weight (g)
Male	Vehicle control	1	0.67	10.60	2.16	1.34	1.86	3.08	50.9	-	328.0
		2	0.72	13.62	2.41	1.15	1.86	2.85	54.8	-	388.8
		3	0.67	10.24	2.33	1.15	1.93	3.12	44.8	-	329.1
		4	0.60	11.56	2.75	1.18	1.97	2.78	59.4	-	364.3
		5	0.75	10.56	2.49	1.20	1.95	3.07	55.8	-	335.9
		6 b)	0.45	10.20	2.19	-	1.62	[illegible]	39.1	-	168.4
		Recovery									
		7	0.55	10.93	2.40	1.26	1.87	3.29	58.1	-	369.9
		8	0.70	10.28	2.[illegible]4	1.24	2.02	3.25	66.4	-	369.5
		9	0.70	12.62	[illegible]	1.40	1.91	3.21	60.2	-	399.3
		10	0.67	10.41	2.[illegible]3	1.40	1.96	2.99	69.2	-	385.3
		11	0.65	11.66	2.98	1.47	2.10	2.85	61.3	-	386.8
		12	0.46	7.98	2.03	1.08	1.69	2.55	56.0	-	273.0
	6	13	0.58	11.11	2.26	1.15	1.85	2.93	47.2	-	341.5
		14	0.56	11.53	2.42	1.21	1.90	2.74	55.2	-	336.2
		15	0.68	10.48	2.48	1.13	1.96	3.10	60.4	-	328.5
		16	0.58	10.52	2.59	1.15	2.06	2.83	55.5	-	338.8
		17	0.59	12.11	2.63	1.19	1.97	3.08	60.5	-	369.6
		18	0.33	7.14	1.73	0.96	1.74	2.71	49.5	-	251.1
	30	19	0.49	9.31	2.41	1.01	1.85	3.13	46.6	-	295.5
		20	0.69	12.06	2.92	1.33	1.80	3.36	57.6	-	382.6
		21	0.67	13.87	2.32	1.25	1.78	2.91	49.8	-	345.6
		22	0.65	11.23	2.45	1.04	1.95	3.04	65.1	-	338.5
		23	0.59	11.29	2.48	1.26	1.89	2.91	52.0	-	335.2
		24	0.71	12.60	2.56	1.16	2.01	3.25	48.0	-	369.8
	60	25	0.64	13.76	2.35	1.19	1.75	3.17	46.5	-	354.7
		26	0.66	10.69	2.30	1.20	1.97	3.13	61.5	-	317.0
		27 b)	0.61	12.57	2.25	-	1.85	1.88	62.5	-	163.2
		28	0.61	13.46	2.58	1.35	1.92	3.21	65.1	-	325.0
		29 b)	0.61	16.62	3.38	-	1.89	2.63	66.6	-	256.5
		30	0.44	11.65	1.81	1.10	1.65	2.93	50.7	-	278.1
		Recovery									
		31	0.72	12.66	2.69	1.57	1.99	3.28	77.7	-	385.3
		32 b)	0.62	15.72	2.23	-	1.96	2.53	53.7	-	280.1
		33	0.38	9.76	2.31	1.29	1.93	2.68	61.7	-	339.0
		34 b)	0.37	17.10	3.29	1.72	2.00	0.49	101.2	-	312.1
		35 b)	0.30	14.00	2.09	-	1.80	1.86	58.2	-	215.8
		36	0.80	13.79	2.87	1.35	1.99	3.51	69.7	-	406.2

b) : Dead animal.

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Addendum 7 -Continued
Absolute organ weights

Sex	Exp.group (mg/kg/day)	Animal No.	Spleen (g)	Liver (g)	Kidney (g)	Heart (g)	Brain (g)	Testis (g)	Adrenal gland (mg)	Ovary (mg)	Body weight (g)
Female	Vehicle control	49	0.40	5.36	1.49	0.75	1.80	-	56.5	105.0	203.8
		50	0.38	6.17	1.36	0.78	1.76	-	52.8	120.0	188.8
		51	0.40	6.54	1.60	0.73	1.79	-	62.0	98.8	204.3
		52	0.34	5.16	1.43	0.75	1.77	-	54.4	84.5	187.3
		53	0.50	7.45	1.58	0.88	1.84	-	67.6	84.7	213.8
		54	0.40	6.14	1.54	0.75	1.85	-	54.5	80.5	181.4
		Recovery									
		55	0.46	8.74	1.71	0.78	1.78	-	70.8	116.8	234.7
		56	0.49	7.08	1.73	0.82	1.81	-	73.3	80.5	253.8
		57	0.44	6.71	1.71	0.88	1.84	-	59.8	88.8	228.2
		58	0.45	6.60	1.84	0.81	1.83	-	83.8	109.6	231.4
		59	0.46	8.24	1.84	0.87	1.81	-	80.8	111.8	224.4
		60	0.48	8.07	2.03	1.04	1.89	-	83.1	110.8	250.2
	6	61	0.38	7.2[illegible]	1.67	0.80	1.82	-	61.1	103.3	207.6
		62	0.49	6.45	1.62	0.84	1.72	-	68.3	105.7	208.0
		63	0.61	8.03	1.74	0.88	1.82	-	74.0	113.4	222.7
		64	0.46	6.45	1.45	0.85	1.75	-	64.4	88.7	199.1
		65	0.42	6.34	1.54	0.82	1.81	-	63.4	112.5	183.4
		66	0.31	6.23	1.51	0.80	1.72	-	68.4	91.5	180.5
	60	67	0.51	[illegible]	1.52	0.74	1.74	-	57.9	98.8	187.7
		68	0.38	[illegible]	1.68	0.87	1.77	-	62.5	89.8	215.1
		69	0.34	[illegible]	1.55	0.72	1.80	-	82.8	81.2	190.0
		70	[illegible]	6.9[illegible]	1.65	0.82	1.78	-	60.0	88.8	218.1
		71	0.41	6[illegible]	1.33	0.70	1.82	-	53.6	89.1	179.4
		72	0.30	[illegible]	1.52	0.83	1.75	-	84.4	84.3	198.0
	600	73	0.47	[illegible]	1.43	0.87	1.80	-	69.0	109.3	219.3
		74 b)	0.33	[illegible].84	2.03	-	1.81	-	74.5	85.3	188.3
		75 b)	0.34	10.62	1.86	-	1.74	-	48.4	82.5	143.4
		76 b)	[illegible]	8.86	1.73	-	1.77	-	48.8	83.0	154.1
		77 b)	[illegible].4	11.30	2.20	-	1.84	-	69.9	109.1	188.5
		78 b)	0.34	12.48	1.75	1.54	1.74	-	84.5	105.9	211.8
		Recovery									
		79 b)	0.31	11.57	1.84	-	1.82	-	80.5	48.4	181.8
		80 b)	0.31	12.84	1.75	-	1.87	-	71.0	72.9	188.4
		81 b)	0.41	12.22	2.15	1.40	1.91	-	87.2	108.0	218.7
		82 b)	0.33	11.83	1.95	-	1.81	-	68.5	94.8	219.7
		83 b)	0.58	14.40	2.33	-	1.95	-	79.8	83.9	215.9
		84 b)	0.51	14.18	1.92	1.68	1.83	-	79.8	103.0	218.7

b) : Dead animal.

Addendum 7 -Continued

Absolute organ weights : (administration for 14 days)

Sex	Exp.group (mg/kg/day)	Animal No.	Spleen (g)	Liver (g)	Kidney (g)	Heart (g)	Brain (g)	Testis (g)	Adrenal gland (mg)	Ovary (mg)	Body weight (g)
Male	120	37 a)	0.63	8.44	2.10	-	1.87	2.17	44.0	-	234.3
		38 b)	0.75	12.04	2.02	-	1.88	2.05	42.9	-	185.8
		40 b)	0.47	11.03	1.87	-	2.20	1.30	45.9	-	147.5
		41 b)	0.78	11.61	1.75	-	1.83	1.43	38.7	-	172.0
		42 b)	0.71	12.75	2.09	-	1.90	1.59	43.8	-	180.3
		43 b)	0.51	10.23	1.82	-	1.72	1.22	49.5	-	142.3
		44 b)	0.73	13.46	2.04	-	1.82	1.74	49.5	-	183.1
		45 b)	0.62	10.79	1.94	-	1.85	1.34	38.8	-	141.8
		46 b)	0.64	10.03	1.72	-	1.83	1.48	38.1	-	187.2
		47 b)	0.90	12.04	1.95	-	1.81	1.82	39.1	-	185.2
		48 b)	0.58	14.33	2.43	-	1.99	2.02	55.3	-	232.7
		Recovery									
		39 b)	0.17	8.68	2.04	-	1.92	2.49	81.0	-	213.3

a) : Sacrificed animal.
b) : Dead animal.

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Sex	Dose group (mg/kg/day)	Animal No.	Spleen (g/100g)	Liver (g/100g)	Kidney (g/100g)	Heart (g/100g)	Brain (g/100g)	Testis (g/100g)	Adrenal gland (mg/100g)	Ovary (mg/100g)	Body weight (g)
Male	Vehicle control	1	0.20	3.22	0.66	0.41	0.57	0.94	15.5	-	329.0
		2	0.20	3.55	0.68	0.31	0.51	0.78	14.5	-	388.8
		3	0.20	3.11	0.71	0.35	0.59	0.85	13.8	-	320.1
		4	0.16	3.17	0.75	0.32	0.54	0.75	18.3	-	384.3
		5	0.22	3.13	0.74	0.36	0.56	0.81	18.8	-	335.9
		6 b)	0.27	6.66	1.30	-	1.08	0.78	23.2	-	168.4
		Recovery									
		7	0.15	2.85	0.65	0.34	0.51	0.83	15.7	-	369.9
		8	0.18	2.76	0.74	0.34	0.55	0.85	18.0	-	389.5
		9	0.18	3.16	0.76	0.35	0.48	0.80	15.1	-	399.3
		10	0.17	2.70	0.65	0.38	0.51	0.76	18.0	-	385.3
		11	0.16	3.00	0.75	0.37	0.53	0.72	15.5	-	396.8
		12	0.17	2.02	0.73	0.40	0.62	0.83	20.5	-	273.0
	6	13	0.17	3.25	0.56	0.34	0.34	0.85	19.9	-	341.5
		14	0.17	3.49	0.72	0.36	0.57	0.81	18.4	-	336.2
		15	0.21	3.21	0.76	0.35	0.60	0.85	18.5	-	328.5
		16	0.17	3.11	0.76	0.34	0.61	0.84	16.4	-	338.8
		17	0.16	3.28	0.72	0.32	0.53	0.83	16.4	-	369.0
		18	0.13	2.84	0.69	0.38	0.69	1.08	19.7	-	251.1
	36	19	0.17	3.15	0.82	0.34	0.65	1.00	15.8	-	295.5
		20	0.19	3.33	0.81	0.37	0.52	0.83	15.9	-	362.8
		21	0.19	4.01	0.87	0.30	0.52	0.84	14.4	-	345.8
		22	0.18	3.34	0.73	0.31	0.58	0.80	19.3	-	336.5
		23	0.18	3.37	0.74	0.38	0.58	0.87	15.5	-	335.2
		24	0.19	3.14	0.78	0.32	0.54	0.88	13.3	-	369.8
	60	25	0.18	3.88	0.85	0.34	0.49	0.89	15.1	-	354.7
		26	0.21	3.44	0.73	0.38	0.62	0.99	19.4	-	317.0
		27 b)	0.33	6.85	1.23	-	1.01	1.03	34.1	-	163.2
		28	0.23	4.15	0.79	0.42	0.58	0.99	20.0	-	325.0
		29 b)	0.24	6.48	1.32	-	0.73	1.03	28.0	-	256.5
		30	0.16	4.15	0.85	0.40	0.67	1.05	18.2	-	278.1
		Recovery									
		31	0.19	3.25	0.78	0.41	0.52	0.85	20.2	-	385.3
		32 b)	0.22	5.61	0.85	-	0.70	0.93	19.2	-	280.1
		33	0.11	2.85	0.68	0.38	0.57	0.79	24.1	-	339.0
		34 b)	0.12	5.48	1.04	0.35	0.64	0.10	32.4	-	312.1
		35 b)	0.14	5.49	0.97	-	0.83	0.86	27.0	-	215.8
		36	0.20	3.33	0.73	0.35	0.49	0.86	17.2	-	406.2

b) : Dead animal.

Addendum 2 -Continued
Relative organ weights

311-0153

Sex	Exp.group (mg/kg/day)	Animal No.	Spleen (g/100g)	Liver (g/100g)	Kidney (g/100g)	Heart (g/100g)	Brain (g/100g)	Testis (g/100g)	Adrenal gland (mg/100g)	Ovary (mg/100g)	Body weight (g)
Female	Vehicle control	49	0.20	2.94	0.73	0.37	0.88	-	27.8	51.7	203.8
		50	0.20	3.35	0.72	0.42	0.93	-	27.7	63.3	189.8
		51	0.20	3.20	0.78	0.36	0.88	-	30.3	47.4	204.3
		52	0.20	3.10	0.85	0.45	1.06	-	32.5	55.5	167.3
		53	0.23	3.48	0.73	0.41	0.86	-	31.6	39.6	213.9
		54	0.22	3.38	0.85	0.41	1.02	-	30.0	49.9	181.4
		Recovery									
		55	0.20	2.79	0.73	0.34	0.75	-	30.1	50.5	234.7
		56	0.19	2.79	0.68	0.36	0.72	-	28.9	35.7	253.8
		57	0.19	2.94	0.75	0.38	0.81	-	26.2	39.4	228.2
		58	0.19	2.85	0.80	0.39	0.79	-	36.2	47.5	231.4
		59	0.20	2.78	0.82	0.39	0.85	-	36.0	49.8	224.4
		60	0.19	3.22	0.81	0.42	0.76	-	33.2	44.3	250.2
	8	61	0.18	3.47	0.80	0.43	0.88	-	29.4	49.6	207.5
		62	0.23	3.27	0.77	0.45	0.82	-	33.1	51.9	209.6
		63	0.27	3.61	0.78	0.43	0.82	-	33.2	50.9	222.7
		64	0.23	3.24	0.73	0.43	0.88	-	32.3	43.5	199.1
		65	0.22	3.25	0.80	0.42	0.94	-	32.8	58.2	193.4
		66	0.16	3.32	0.80	0.42	0.91	-	36.1	48.3	189.5
	30	67	0.26	3.30	0.77	0.37	0.88	-	29.3	48.9	197.7
		68	0.18	3.19	0.79	0.45	0.82	-	29.5	41.7	215.1
		69	0.18	3.45	0.82	0.38	1.00	-	43.6	48.0	190.0
		70	0.18	3.20	0.76	0.38	0.82	-	27.5	45.8	218.1
		71	0.23	3.18	0.77	0.39	1.01	-	29.9	49.7	179.4
		72	0.20	2.98	0.78	0.46	0.89	-	32.9	48.1	196.0
	60	73	0.22	3.35	0.88	0.41	0.87	-	32.3	51.2	213.3
		74 b)	0.18	6.89	1.09	-	0.97	-	40.0	44.7	186.3
		75 b)	0.17	6.99	1.29	-	1.21	-	32.4	64.5	143.4
		76 b)	0.24	6.46	1.12	-	1.15	-	31.7	40.9	154.1
		77 b)	0.20	6.72	1.30	-	1.09	-	41.2	64.4	169.5
		78 b)	0.16	5.83	0.85	0.59	0.82	-	39.9	50.0	211.6
		Recovery									
		79 b)	0.19	7.15	1.01	-	1.00	-	37.4	29.9	181.8
		80 b)	0.19	7.72	1.05	-	1.12	-	42.7	43.8	166.4
		81 b)	0.19	5.59	0.98	0.64	0.87	-	39.9	47.1	218.7
		82 b)	0.15	5.38	0.89	-	0.82	-	31.2	43.1	219.7
		83 b)	0.26	6.67	1.08	-	0.90	-	37.0	36.9	215.9
		84 b)	0.24	6.64	0.90	0.51	0.86	-	37.3	48.2	213.7

b) : Dead animal.

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Addendum 2 -Continued

Relative organ weights : (administration for 14 days)

Sex	Dosage (mg/kg/day)	Animal No.	Spleen (g/100g)	Liver (g/100g)	Kidney (g/100g)	Heart (g/100g)	Brain (g/100g)	Testis (g/100g)	Adrenal gland (mg/100g)	Ovary (mg/100g)	Body weight (g)
Male	120	37 a)	0.27	4.03	0.80	-	0.60	0.93	15.5	-	234.3
		38 b)	0.43	6.61	1.09	-	1.00	1.10	29.1	-	185.8
		40 b)	0.32	7.51	1.27	-	1.49	0.82	31.1	-	147.5
		41 b)	0.45	6.92	1.02	-	1.06	0.83	22.5	-	172.0
		42 b)	0.36	6.84	1.23	-	1.02	0.85	23.4	-	188.3
		43 b)	0.35	7.10	1.28	-	1.21	0.86	34.8	-	142.3
		44 b)	0.36	6.99	1.06	-	0.94	0.90	25.6	-	193.1
		45 b)	0.44	7.61	1.37	-	1.33	0.94	27.4	-	141.8
		46 b)	0.38	6.00	1.02	-	1.09	0.89	21.6	-	187.1
		47 b)	0.54	7.23	1.28	-	1.10	0.96	23.7	-	155.3
		48 b)	0.25	6.59	1.04	-	0.88	0.87	23.8	-	232.7
		Recovery									
		33 b)	0.68	4.02	0.96	-	0.90	1.17	28.8	-	213.3

a) : Sacrificed animal.

b) : Dead animal.

(50)

Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
	1	ta	NAD	Liver Lipid droplets in hepatocytes++
	2	ta	NAD	Liver Lipid droplets in hepatocytes++
	3	ta	NAD	NAD
	4	ta	Glandular stomach Blackish region of mucosa (φ 1mm, 2 regions)	Liver Lipid droplets in hepatocytes++ Glandular stomach Necrosis of mucosa+
	5	ta	NAD	NAD
Vehicle control	6	fd	Lung Reddish change	Kidney Cyst formation (unilateral)
	Recovery group 7	ta	NAD	NAD
	8	ta	NAD	NAD
	9	ta	NAD	NAD
	10	ta	NAD	Liver Lipid droplets in hepatocytes+
	11	ta	Kidney Dilatation of pelvis (R)	NAD
	12	ta	NAD	Liver Lipid droplets in hepatocytes+
	13	ta	NAD	Liver Lipid droplets in hepatocytes+
	14	ta	NAD	Liver Lipid droplets in hepatocytes+
8	15	ta	NAD	NAD
	16	ta	NAD	Liver Lipid droplets in hepatocytes+
	17	ta	NAD	NAD
	18	ta	NAD	NAD

ta : terminal autopsy, an : autopsied for moribund state, fd : found dead
NAD:no abnormalities detected
+ : slight, ++ : moderate, +++ : severe
(B): bilateral, (L): left, (R): right

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Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
	19	ta	Glandular stomach Blackish region of mucosa (0.5×2mm)	Glandular stomach Necrosis of mucosa+
	20	ta	Heart Whitish region (1mm)	Liver Lipid droplets in hepatocytes+++ Heart Necrosis of muscular fibers with cell reaction+
30	21	ta	N.D	Heart Necrosis of muscular fibers with cell reaction+
	22	ta	N.D	Liver Lipid droplets in hepatocytes++ Heart Necrosis of muscular fibers with cell reaction+
	23	ta	N.D	Liver Necrosis of hepatocytes+
	24	ta	N.D	Liver Lipid droplets in hepatocytes++
	25	ta	Liver Enlargement Interlobar adhesion Whitish region (quadrate lobe, right lobe, ø 10mm)	Liver Granulation tissue with calcification++ Lipid droplets in hepatocytes+++ Swelling of hepatocytes+ Heart Vacuolization of muscular fibers++
	26	ta	Liver Enlargement	Liver Lipid droplets in hepatocytes++ Heart Necrosis of muscular fibers with cell reaction+
60	27	dd	Transparent fluid in thoracic cavity Liver Enlargement Induration Interlobar adhesion Whitish region	Liver Congestion+ Granulation tissue with calcification++, Hemorrhage+ Necrosis of hepatocytes+++ Proliferation of connective tissue and exudation of fibrin on capsule+ Spleen Extramedullary hematopoiesis+ Heart Necrosis of muscular fibers with cell reaction+++

Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
	28	ta	Liver Disseminated whitish region (quadrate lobe, right lobe, caudate process, ϕ 1-5mm) Enlargement Interlobar adhesion Heart Discoloration	Liver Extramedullary hematopoiesis+ Lipid droplets in hepatocytes++ Necrosis of hepatocytes++ Swelling of hepatocytes++ Spleen Extramedullary hematopoiesis+ Heart Necrosis of muscular fibers with cell reaction++
	29	fd	Stains around nose Transparent fluid in thoracic cavity Liver Enlargement Induration Interlobar adhesion Whitish region (quadrate lobe ϕ 6mm, right lobe ϕ 12mm) Kidney Enlargement (B)	Liver Congestion++ Granulation tissue with calcification++ Hemorrhage++ Necrosis of hepatocytes+ Spleen Extramedullary hematopoiesis+ Heart Necrosis of muscular fibers with cell reaction++ Thrombus+ Vacuolization of muscular fibers+
60			Heart Discoloration Lung Dark reddish change Glandular stomach Blackish substance on mucosa Testis Dark reddish change (B)	Lung Congestion+ Testis Hemorrhage (B)+
	30	ta	Anemia Transparent fluid in thoracic cavity Liver Disseminated whitish region (ϕ 1-15mm) Induration Interlobar adhesion Heart Discoloration Glandular stomach Blackish region of mucosa (ϕ 1mm) Celiac lymph nodes Enlargement	Liver Congestion+ Granulation tissue with calcification++ Hemorrhage++ Necrosis of hepatocytes+++ Swelling of hepatocytes++ Heart Vacuolization of muscular fibers++ Glandular stomach Necrosis of mucosa++ Celiac lymph nodes Erythrocytes in sinus+

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Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
	32	fd	Liver Enlargement Interlobar adhesion Whitish region (quadrate lobe 5×10mm, right lobe φ6mm) Heart Discoloration	Liver Congestion* Granulation tissue with calcification** Hemorrhage* Lipid droplets in hepatocytes** Necrosis of hepatocytes** Proliferation of connective tissue and exudation of fibrin on capsule* Heart Necrosis of muscular fibers with cell reaction**
	33	fd	Transparent fluid in thoracic cavity Liver Enlargement Induration Spleen Blackish region (3×5mm)	Liver Congestion* Hemorrhage* Necrosis of hepatocytes** Heart Necrosis of muscular fibers with cell reaction** Lung Congestion* Edema* Adrenal Swelling of cortical cells*
60	Recovery group			
	31	ta	Transparent fluid in thoracic cavity Liver Fibrous substance on surface (left lateral lobe) Interlobar adhesion Heart Discoloration	Liver Congestion* Lipid droplets in hepatocytes* Necrosis of hepatocytes* Proliferation of connective tissue and exudation of fibrin on capsule** Heart Necrosis of muscular fibers with cell reaction** Vacuolization of muscular fibers**
	34	ta	Anemia Stained around nose Transparent fluid in thoracic cavity Liver Disseminated whitish region Induration Interlobar adhesion Heart Discoloration Pancreas Edema Thymus Edema	Liver Congestion* Hemorrhage* Necrosis of hepatocytes** Proliferation of connective tissue and exudation of fibrin on capsule* Swelling of hepatocytes* Heart Vacuolization of muscular fibers** Pancreas NAD Thymus NAD

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Exp group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
60	34	fd	Transparent fluid in abdomnal cavity Transparent fluid in thoracic cavity Liver Disseminated whitish spots Enlargement Induration Heart Disseminated whitish spots Lung Dark reddish change Trachea Whitish foamy substance in lumen Testis Small (B) Adrenal Enlargement (B) Thymus Small	Liver Congestion** Hemorrhage* Necrosis of hepatocytes** Proliferation of connective tissue and exudation of fibrin on capsule* Heart Necrosis of muscular fibers with cell reaction** Vacuolization of muscular fibers* Lung Congestion** Edema* Testis Decreased spermatogenesis (B)** Adrenal Swelling of cortical cells* Thymus Decreased lymphocytes*
	36	ta	Liver Interlobar adhesion	Liver Lipid droplets in hepatocytes* Swelling of hepatocytes*
120	37	aa	Liver Interlobar adhesion Whitish region (right lobe, ø 5mm)	Liver Granulation tissue with calcification** Proliferation of connective tissue and exudation of fibrin on capsule* Swelling of hepatocytes* Heart Necrosis of muscular fibers with cell reaction** Vacuolization of muscular fibers*
	38	fd	Transparent fluid in thoracic cavity Liver Enlargement* Induration Interlobar adhesion	Liver Congestion* Proliferation of connective tissue and exudation of fibrin on capsule* Heart Necrosis of muscular fibers with cell reaction*** Lung Congestion** Edema**

Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
	40	fd	Anemia Reddish fluid in abdominal cavity Reddish fluid in thoracic cavity Stained around nose and mouth Liver Disseminated whitish region Enlargement Interlobar adhesion	Liver Congestion** Hemorrhage** Necrosis of hepatocytes*** Proliferation of connective tissue and exudation of fibrin on capsule* Adrenal Swelling of cortical cells*
	41	fd	Transparent fluid in thoracic cavity Liver Enlargement Induration Interlobar adhesion Whitish region (quadrate lobe, right lobe, ϕ 13mm) Spleen Enlargement Glandular stomach Reddish region of mucosa	Liver Congestion* Extramedullary hematopoiesis* Hemorrhage* Necrosis of hepatocytes*** Proliferation of connective tissue and exudation of fibrin on capsule* Heart Necrosis of muscular fibers with cell reaction*
120	42	fd	Transparent fluid in thoracic cavity Liver Enlargement Induration Interlobar adhesion Whitish region (quadrate lobe, left lateral lobe, right lobe, ϕ 10mm) Spleen Enlargement Heart Discoloration Lung Dark reddish change Trachea Whitish foamy substance in lumen Glandular stomach Pale reddish region of mucosa (ϕ 30mm)	Liver Congestion* Extramedullary hematopoiesis* Granulation tissue with calcification* Hemorrhage* Necrosis of hepatocytes*** Proliferation of connective tissue and exudation of fibrin on capsule* Spleen Extramedullary hematopoiesis* Heart Necrosis of muscular fibers with cell reaction*** Lung Congestion** Edema**

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Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
	43	fd	Reddish fluid in abdominal cavity Reddish fluid in thoracic cavity Liver Brownish change Enlargement Induration Interlobar adhesion Lung Reddish change	Liver Congestion** Hemorrhage** Necrosis of hepatocytes*** Proliferation of connective tissue and exudation of fibrin on capsule* Spleen Extramedullary hematopoesis* Increased histiocytic cells* Heart Necrosis of muscular fibers with cell reaction* Lung Congestion* Glandular stomach Necrosis of mucosa**
120	44	fd	Transparent fluid in abdominal cavity Transparent fluid in thoracic cavity Liver Enlargement Induration Interlobar adhesion Whitish region (quadrate lobe, right lobe, ø 10mm) Spleen Enlargement Lung Dark reddish change Glandular stomach Dark reddish region of mucosa (ø 3mm)	Liver Congestion** Extramedullary hematopoesis* Granulation tissue with calcification* Hemorrhage** Necrosis of hepatocytes*** Proliferation of connective tissue and exudation of fibrin on capsule* Spleen Extramedullary hematopoesis* Heart Necrosis of muscular fibers with cell reaction*** Lung Congestion* Glandular stomach Necrosis of mucosa*

Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
	45	fd	Transparent fluid in thoracic cavity Liver Enlargement Induration Interlobar adhesion Whitish region (quadrate lobe, right lobe, ϕ 15mm) Lung Dark reddish change Trachea Whitish foamy substance in lumen	Liver Congestion+ Extramedullary hematopoiesis+ Hemorrhage++ Necrosis of hepatocytes+++ Proliferation of connective tissue and exudation of fibrin on capsule+ Heart Necrosis of muscular fibers with cell reaction+ Lung Congestion++ Edema++
120	46	fd	Anemia Reddish fluid in abdominal cavity Liver Fibrous substance on surface Induration Interlobar adhesion Whitish region (quadrate lobe, ϕ 12mm) Spleen Enlargement	Liver Congestion+ Hemorrhage+ Necrosis of hepatocytes++ Proliferation of connective tissue and exudation of fibrin on capsule+ Spleen Extramedullary hematopoiesis+
	47	fd	Transparent fluid in thoracic cavity Liver Enlargement Induration Interlobar adhesion Whitish region (quadrate lobe, right lobe, ϕ 13mm) Spleen Enlargement Lung Dark reddish change Glandular stomach Blackish substance on mucosa	Liver Congestion+ Extramedullary hematopoiesis+ Hemorrhage+ Necrosis of hepatocytes+++ Proliferation of connective tissue and exudation of fibrin on capsule+ Swelling of hepatocytes+ Spleen Extramedullary hematopoiesis+ Heart Necrosis of muscular fibers with cell reaction++

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Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
	48	fd	Liver Enlargement Interlobar adhesion Whitish region (quadrate lobe 10mm, left lateral lobe, φ10mm) Heart Whitish region (φ5mm) Glandular stomach Reddish region of mucosa (φ1-10mm)	Liver Congestion++ Hemorrhage++ Necrosis of hepatocytes+++ Proliferation of connective tissue and exudation of fibrin on capsule+ Heart Necrosis of muscular fibers with cell reaction+++ Lung Congestion++ Edema+ Adrenal Swelling of cortical cells+
120				
	Recovery group			
	39	fd	Nasal hemorrhage Reddish fluid in thoracic cavity Reddish tear Transparent fluid in abdominal cavity Liver Induration Interlobar adhesion Spleen Small Lung Dark reddish change	Liver Congestion+ Hemorrhage++ Necrosis of hepatocytes++ Proliferation of connective tissue and exudation of fibrin on capsule+ Heart Vacuolization of muscular fibers++ Lung Congestion++

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Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
	49	ta	N.A.D	Liver Lipid droplets in hepatocytes*
	50	ta	N.A.D	Liver Lipid droplets in hepatocytes*
	51	ta	Loss of hair at left cervical region(φ5mm)	Liver Lipid droplets in hepatocytes** Skin Scab formation
	52	ta	N.A.D	N.A.D
	53	ta	N.A.D	Liver Lipid droplets in hepatocytes* Lung Cell infiltration* Hemorrhage*
Vehicle control	54	ta	N.A.D	N.A.D
	Recovery group 55	ta	N.A.D	N.A.D
	56	ta	N.A.D	Liver Lipid droplets in hepatocytes*
	57	ta	N.A.D	N.A.D
	58	ta	N.A.D	Liver Microgranuloma*
	59	ta	N.A.D	N.A.D
	60	ta	N.A.D	Liver Lipid droplets in hepatocytes*
	61	ta	Glandular stomach Depressed region of mucosa (0.5 × 1mm)	Liver Lipid droplets in hepatocytes* Glandular stomach N.A.D
	62	ta	N.A.D	Liver Lipid droplets in hepatocytes*
8	63	ta	N.A.D	Liver Lipid droplets in hepatocytes**
	64	ta	N.A.D	N.A.D
	65	ta	N.A.D	Liver Lipid droplets in hepatocytes*

Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
8	66	ta	NAD	Liver Lipid droplets in hepatocytes*
	67	ta	NAD	Liver Lipid droplets in hepatocytes*
	68	ta	NAD	Liver Lipid droplets in hepatocytes** Microgranuloma*
	69	ta	NAD	Liver Lipid droplets in hepatocytes*
30	70	ta	NAD	Heart Necrosis of muscular fibers with cell reaction*
	71	ta	NAD	NAD
	72	ta	NAD	Liver Lipid droplets in hepatocytes*
	73	ta	NAD	Liver Lipid droplets in hepatocytes*
60	74	fd	Transparent fluid in abdominal cavity Transparent fluid in thoracic cavity Liver Enlargement Induration Heart Discoloration Lung Dark reddish change Trachea Whitish foamy substance in lumen	Liver Congestion* Hemorrhage* Lipid droplets in hepatocytes* Necrosis of hepatocytes*** Heart Necrosis of muscular fibers with cell reaction*** Lung Congestion** Edema**
	75	fd	Transparent fluid in thoracic cavity Liver Enlargement Interlobar adhesion Whitish region (quadrate lobe, right lobe, ø 10mm)	Liver Congestion* Extramedullary hematopoiesis* Hemorrhage* Lipid droplets in hepatocytes* Necrosis of hepatocytes** Proliferation of corrective tissue and exudation of fibrin on capsule* Heart Necrosis of muscular fibers with cell reaction**

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Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
	76	fd	Liver Enlargement Whitish region (quadrate lobe, right lobe, φ 10mm) Heart Discoloration Glandular stomach Blackish substance on mucosa	Liver Congestion+ Hemorrhage+ Lipid droplets in hepatocytes++ Necrosis of hepatocytes+ Proliferation of connective tissue and exudation of fibrin on capsule+ Spleen Extramedullary hematopoiesis+ Heart Necrosis of muscular fibers with cell reaction++
	77	fd	Lung Dark reddish change Ovary Dark reddish change (B)	Liver Congestion+ Hemorrhage+ Lipid droplets in hepatocytes+ Necrosis of hepatocytes++ Proliferation of connective tissue and exudation of fibrin on capsule+ Heart Necrosis of muscular fibers with cell reaction++ Lung Congestion++ Edema+ Ovary Congestion (B)+
60				
	78	fd	Liver Enlargement Granular surface Heart Discoloration	Liver Congestion+ Hemorrhage+ Lipid droplets in hepatocytes++ Necrosis of hepatocytes++ Proliferation of connective tissue and exudation of fibrin on capsule+ Heart Necrosis of muscular fibers with cell reaction++
	79	fd	Liver Enlargement Heart Discoloration Jejunum Reddish brownish mucoid substance in lumen Ileum Reddish brownish mucoid substance in lumen	Liver Congestion+ Hemorrhage+ Lipid droplets in hepatocytes++ Necrosis of hepatocytes+ Proliferation of connective tissue and exudation of fibrin on capsule+ Heart Necrosis of muscular fibers with cell reaction++

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Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
	80	fd	Liver Enlargement Heart Discoloration	Liver Congestion* Hemorrhage* Lipid droplets in hepatocytes* Necrosis of hepatocytes* Heart Necrosis of muscular fibers with cell reaction** Lung Hemorrhage*
	82	fd	Stained around nose Transparent fluid in abdominal cavity Transparent fluid in thoracic cavity Liver Enlargement Induration Heart Discoloration	Liver Congestion** Hemorrhage* Lipid droplets in hepatocytes** Necrosis of hepatocytes** Heart Necrosis of muscular fibers with cell reaction**
	83	fd	Liver Enlargement Kidney Dilatation of pelvis (L) Heart Discoloration	Liver Congestion* Lipid droplets in hepatocytes* Heart Necrosis of muscular fibers with cell reaction**
60				
	Recovery group			
	81	fd	Liver Disseminated whitish spots Enlargement Heart Discoloration Lung Foamy substance on cut surface Ovary Dark reddish change (B)	Liver Congestion** Hemorrhage** Lipid droplets in hepatocytes** Necrosis of hepatocytes* Heart Necrosis of muscular fibers with cell reaction** Lung Congestion** Edema** Ovary Congestion (B)*
	81	fd	Liver Enlargement Heart Discoloration Ovary Dark reddish change (B)	Liver Congestion* Lipid droplets in hepatocytes* Heart Necrosis of muscular fibers with cell reaction** Ovary Congestion (B)*

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Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
	85	fd	Liver Enlargement Induration Interlobar adhesion Lung Dark reddish change Glandular stomach Reddish region of mucosa (φ 10mm)	Liver Congestion** Hemorrhage** Lipid droplets in hepatocytes** Proliferation of connective tissue and exudation of fibrin on capsule* Heart Necrosis of muscular fibers with cell reaction*** Lung Congestion** Edema**
	86	am	Liver Interlobar adhesion Heart Whitish spots	Liver Lipid droplets in hepatocytes* Proliferation of connective tissue and exudation of fibrin on capsule* Heart Necrosis of muscular fibers with cell reaction*
120				
	87	fd	Anemia Liver Interlobar adhesion Lung Dark reddish change	Liver Congestion* Hemorrhage* Lipid droplets in hepatocytes* Necrosis of hepatocytes* Proliferation of connective tissue and exudation of fibrin on capsule* Heart Necrosis of muscular fibers with cell reaction** Lung Congestion*
	88	fd	Liver Enlargement Heart Discoloration Glandular stomach Reddish region of mucosa (φ 5mm, 2 regions)	Liver Congestion** Hemorrhage* Lipid droplets in hepatocytes** Necrosis of hepatocytes** Heart Necrosis of muscular fibers with cell reaction**

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Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
	89	fd	Transparent fluid in thoracic cavity Liver Enlargement Induration Interlobar adhesion	Liver Congestion+ Extramedullary hematopoiesis+ Hemorrhage+ Lipid droplets in hepatocytes+ Necrosis of hepatocytes+ Proliferation of connective tissue and exudation of fibrin on capsule+ Heart Necrosis of muscular fibers with cell reaction++
	90	am	Liver Interlobar adhesion	Liver Lipid droplets in hepatocytes+
	91	fd	Transparent fluid in thoracic cavity Liver Induration Interlobar adhesion Whitish region (right lobe, ø 10mm) Glandular stomach Reddish change of mucosa	Liver Congestion+ Extramedullary hematopoiesis+ Necrosis of hepatocytes+ Proliferation of connective tissue and exudation of fibrin on capsule+ Spleen Extramedullary hematopoiesis+ Heart Necrosis of muscular fibers with cell reaction+ Lung Congestion+ Edema+
120				
	95	fd	Transparent fluid in thoracic cavity Liver Enlargement Lung Dark reddish change Glandular stomach Blackish region of mucosa (ø 1-2mm, 2 regions)	Liver Congestion+ Hemorrhage+ Lipid droplets in hepatocytes+ Necrosis of hepatocytes+ Spleen Extramedullary hematopoiesis+ Heart Necrosis of muscular fibers with cell reaction++ Lung Congestion++ Glandular stomach Necrosis of mucosa+

0 3 0 0

Exp. group (mg/kg/day)	Animal No.	Fate	Gross findings	Histopathological findings
	96	fd	Liver Enlargement Induration Interlobar adhesion Lung Dark reddish change Trachea Whitish foamy substance in lumen	Liver Congestion** Hemorrhage* Lipid droplets in hepatocytes** Necrosis of hepatocytes* Proliferation of connective tissue and exudation of fibrin on capsule* Spleen Extramedullary hematopoiesis* Heart Necrosis of muscular fibers with cell reaction** Lung Congestion** Edema**
	Recovery group			
	92	fd	Transparent fluid in thoracic cavity Liver Enlargement Induration	Liver Congestion** Hemorrhage* Lipid droplets in hepatocytes* Necrosis of hepatocytes** Proliferation of connective tissue and exudation of fibrin on capsule* Heart Necrosis of muscular fibers with cell reaction** Lung Congestion*
120				
	93	ta	N.D	N.D
	94	ta	Anemia Transparent fluid in thoracic cavity Liver Disseminated whitish spots Enlargement Heart Discoloration	Liver Congestion* Lipid droplets in hepatocytes* Necrosis of hepatocytes* Proliferation of connective tissue and exudation of fibrin on capsule* Heart Necrosis of muscular fibers with cell reaction* Vacuolization of muscular fibers*

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Appendix data

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Report on physicochemical test

Person in charge of analysis: T. Matsuyuki (seal)
Date: September 21, 1992

Test requester: Shin Etsu Chemical Industry Co., Ltd.
Test facility: Hita Research Lab., Chemical Safety Center, Chemical Inspection Association Test substance: X-12-990S (Lot No. 105027)

Test contents: 1) Stability test of test substance
2) Safety test of test substance in prepared solution

Test time: 1) February 26 and July 21, 1992
2) February 21, 24 and 28, 1992

Test method:

1) Safety method of test substance

Before start of administration and after end of administration, sampling was performed from the test substance raw solution (n = 1), and qualitative analysis was performed using IR spectrophotometer (IR-470 model, product of Shimadzu Mfg. Co., Ltd.) During the test period, the test substance was stored in a desiccator kept in a dark and cold place.

2) Stability test of test substance in prepared solution

Before start of administration, the solution was prepared, and the following test was implemented.

The stability test of the test substance in the prepared solution was performed right after preparation, 3 days later and

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7 days later, respectively. Each time, a sample was collected from the middle layer of the solution prepared (n = 3). After pretreatment, quantitative analysis (n = 1) was performed using the gas chromatography method (GC). The preparation method and pretreatment method of the prepared solution are described on p. 7, and the preparation method, analytical conditions, and quantitative calculation method are described on p. 8. During the test period, the prepared solution was stored in a cold and dark place.

Test results:

1) Stability test of test substance

The IR spectrum of the test substance (see Figure 1) is in agreement with the IR spectrum provided by the test requester (see Figure 1), and the test substance was thus identified. Since no change was observed for the IR spectrum after the administration as compared with that before the administration (see Figures 1, 2), it was confirmed that the test substance was stable during the test period.

2) Safety test of the test substance in prepared solution

The test results are listed in Table 1. The test substance in the HR 2074 prepared solution was stable over 7 days. Figure 4 shows a typical chromatogram, and Figure 5 shows the calibration line.

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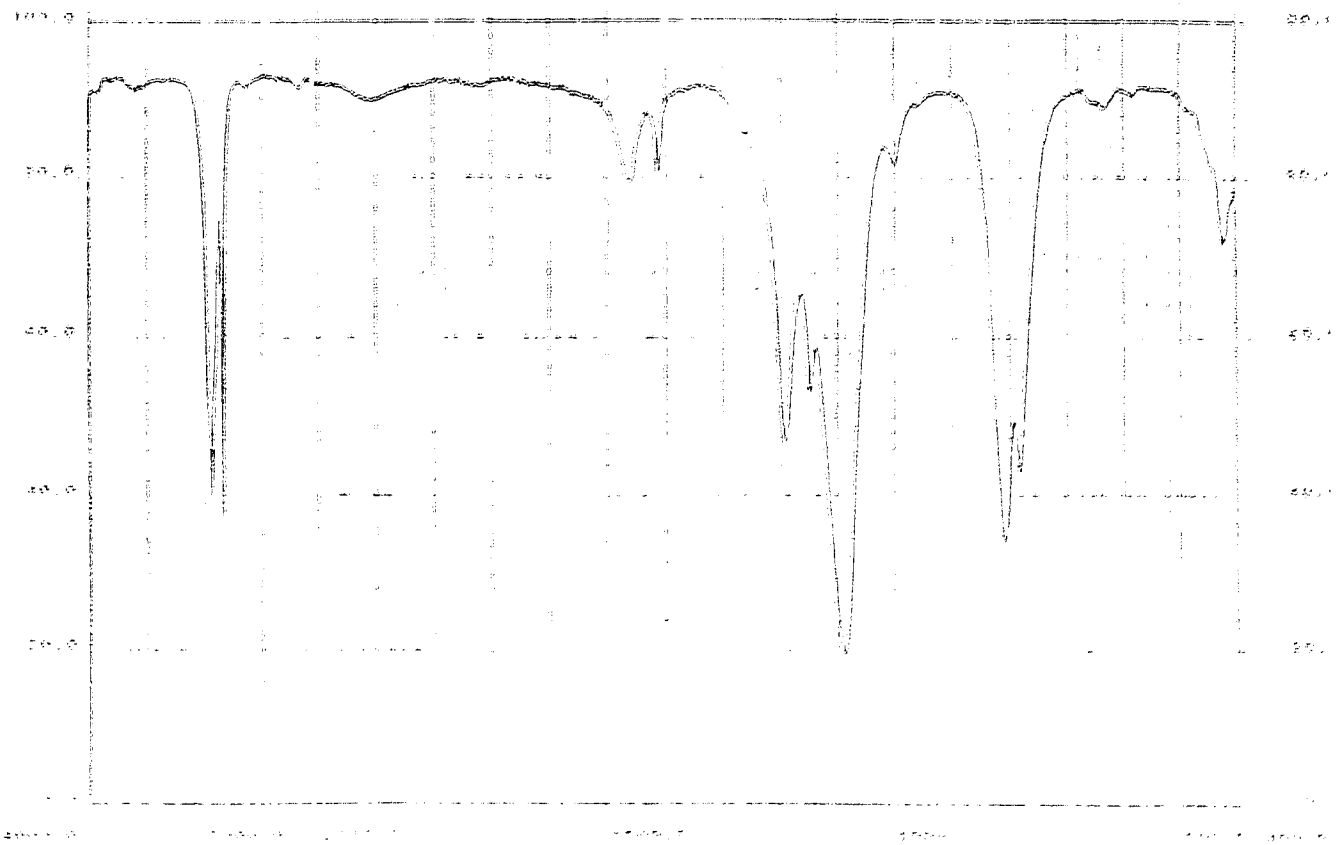
Fig. 1 IR spectrum prior to the start of the administration

(投与開始前) ①

92.2.25 古田 ②

B11-0153 (HR2074)

原液安定性試験 ③



Key to previous page

1 Before start of administration
2 Furuda
3 Stability test of feed solution

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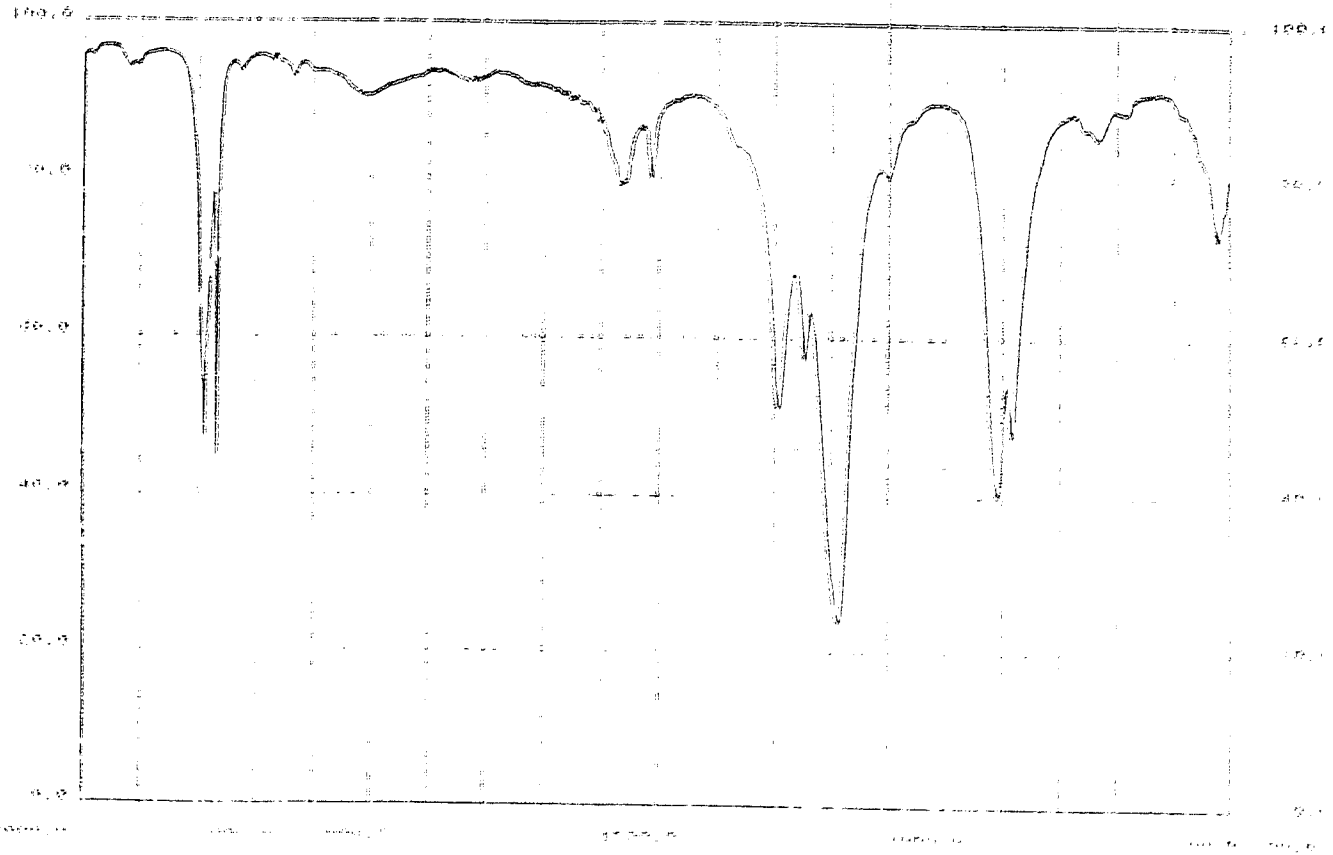
Fig.2 IR spectrum after the end of the administration

(投与終了後) ①

2.7.21 [illegible] ②

[illegible]-0153 (HR2074)

[illegible]定性試験 ③



Key to previous page

1 After end of administration
2 Shimadzu
3 Stability test of feed solution

Fig. 3 IR spectrum provided by the sensor

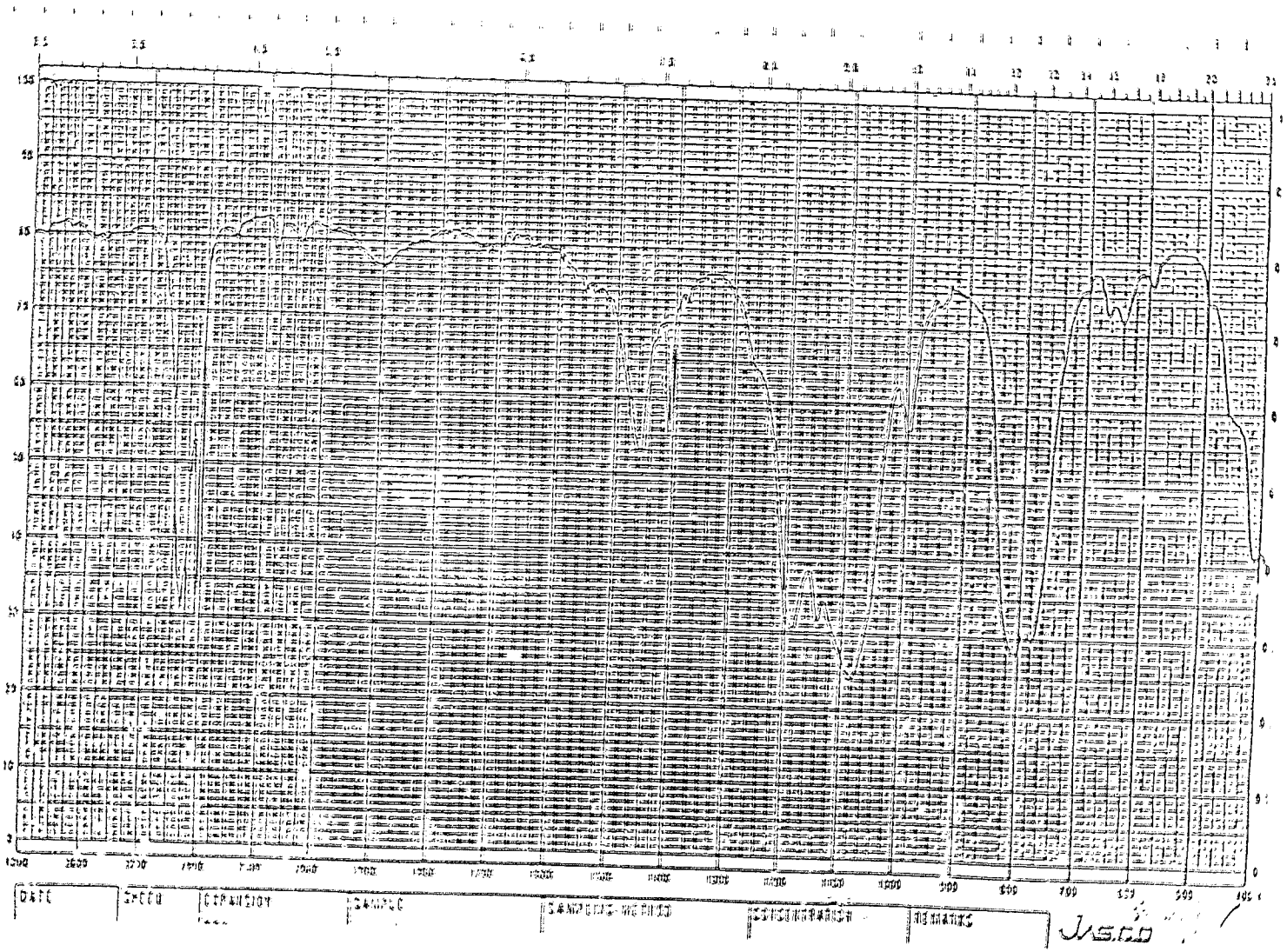


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Table 1 Chemical stability of the test substance in dose formulations

Time point of measurement	Nominated conc. (%,w/v)	Analyzed conc. (μg/ml)	Dilution rate	Measured conc. (%,w/v)	Mean conc. (%,w/v)	R.S.D. (%)	R.P. (%)
Immediately after preparation	10.0	53.4355	2000	10.69			
		52.9624	2000	10.59	10.64	0.45	-
		53.1565	2000	10.63			
	0.05	21.1676	20	0.01234			
		21.2861	20	0.01258	0.01260	0.66	-
		21.4461	20	0.01290			
3 days after	10.0	51.3072	2000	10.26			
		52.0909	2000	10.42	10.39	0.27	97.69
		51.9697	2000	10.39			
	0.05	21.1219	20	0.01224			
		21.0629	20	0.01213	0.01160	2.45	97.64
		20.2099	20	0.01042			
7 days after	10.0	52.7422	2000	10.55			
		52.3578	2000	10.47	10.51	0.37	98.83
		52.5978	2000	10.52			
	0.05	21.2129	20	0.01243			
		21.0350	20	0.01207	0.01257	1.39	99.93
		21.6140	20	0.01322			

R.S.D.(%) : Relative standard deviation
R.P.(%) : Rate to the concentration at immediately after preparation

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Preparation method and pretreatment method of prepared solution

Preparation method of solution

1) Preparation method of solution-1 (10% (w/v) solution)

10 g of test substance were measured and dissolved in olive oil to a volume of 100 mL.

2) Preparation method of solution-2 (0.05% (w/v) solution)

1.5 mL of solution-1 were measured and with olive oil to a volume of 100 mL were added.

Pretreatment method of solution

1) Pretreatment method of solution-1 (10% (w/v) solution)

1 mL of the solution was correctly measured and dissolved in acetone to 50 mL accurately. From the solution, 2.5 mL were accurately measured, and acetone was added to 100 mL accurately as the GC sample.

In this case, the dilution rate of the sample was 2000.

2) Pretreatment method of solution-2 (0.05% (w/v) solution)

1 mL of the solution was accurately measured and dissolved in acetone to 20 mL accurately.

In this case, the dilution rate of the sample was 20.

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Preparation method, analysis conditions, and quantitative calculation method of standard solution

Preparation method of standard solution

About 100 mg of the test substance were measured accurately and were dissolved in acetone to 100 mL accurately. From the solution, 2.5 mL were accurately measured, and added with acetone to 50 mL accurately was added, forming the standard solution.

Analysis conditions

Equipment: Gas chromatograph (GC-9A model, product of Shimadzu Co., Ltd.)

Column: 20 m x 1.2 mm i.d. made of glass

Filler: G-100 (thickness of film: 1 μm)

Temperature: 130°C

Mobile phase: Helium

Flow rate: 20 mL/min

Detector: FID (hydrogen = air = 0.6 kg/cm^2)

Temperature: 180°C

Sensitivity: X10

Injection amount: 2 μL

Recorder: Chromatopack (C-R5A model, product of Shimadzu Co., Ltd.)

Output: 4 mV

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Under the aforementioned analytical conditions, the quantitative detection property was checked in the range of test substance concentration of 0-52.6 μg/mL. As the test substance is a mixture, two peaks are formed on the chromatogram. In the quantitative calculation, the area of the peak near the retention time of 6.9 min was used.

Quantitative calculation method

The peak area was measured using the GC method for the various GC samples and the standard solution. The concentration of the test substance in each prepared solution (Cr: in units of %, w/v) was calculated using the following formula:

$$Cr = \frac{Cs \times Ar \times D}{As \times 10000}$$

where,

Cs: concentration of test substance in the standard solution (μg/mL)

As: Peak area of test substance in the standard solution

Ar: Peak area of the test substance in each GC sample

D: Dilution rate of each prepared solution

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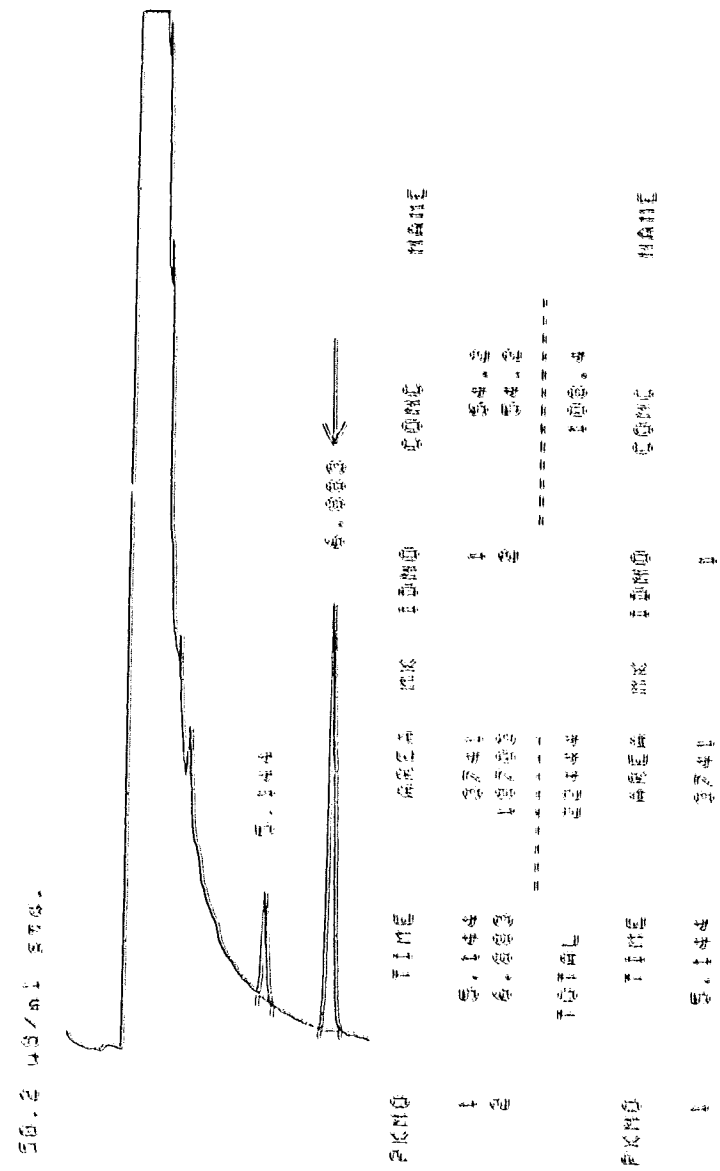

Fig. 4 Typical chromatogram

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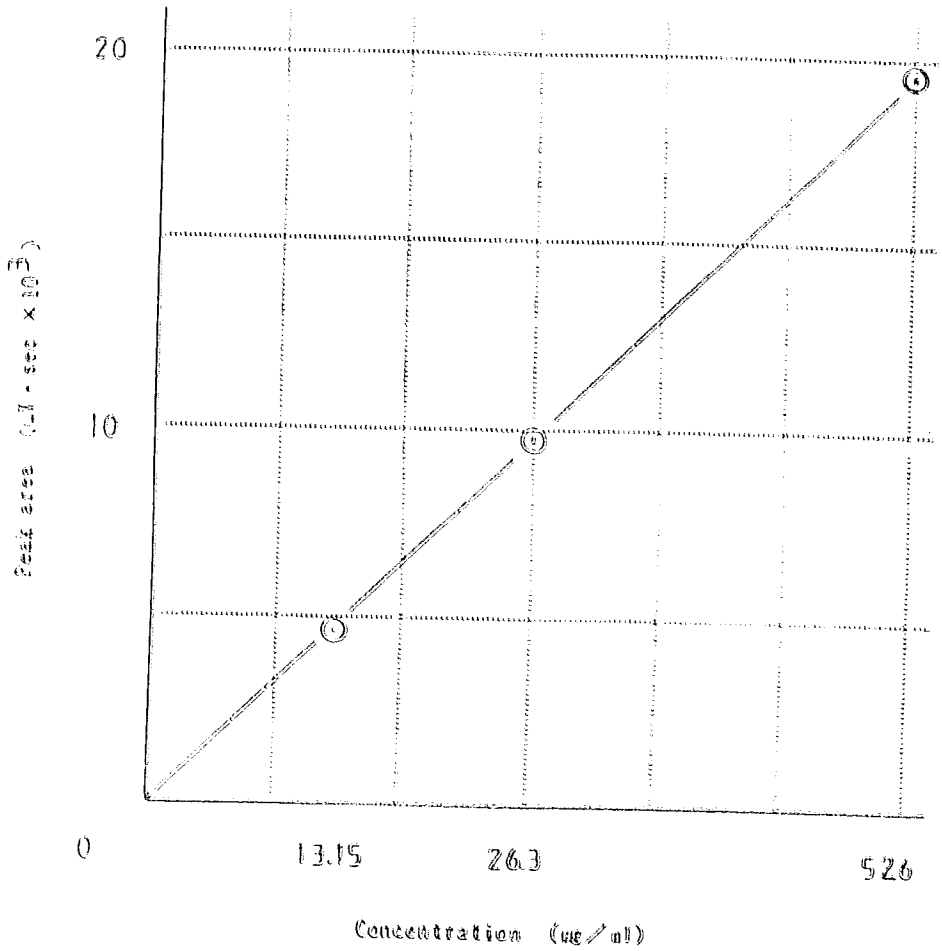

Fig. 5 Calibration curve