

ShinEtsu

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June 29, 1995

TSCA Document Processing Center (TS-790)
Office of Pollution and Toxics
U.S. Environmental Protection Agency
Attention: TSCA Section 8(e) Coordinator
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Washington, D.C. 20460

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95 JUN 30 AM 11:25

Re: SUB-ACUTE ORAL TOXICITY STUDY ON LS-7130 IN RATS

Dear Sir or Madam:

Pursuant to Section 8(e) of the Toxic Substances Control Act ("TSCA"), as interpreted in the Statement of Interpretation and Enforcement Policy, 40 Fed. Reg. 11110, (March 16, 1978), Shin-Etsu Silicones of America, Inc. (Shin-Etsu) submits the enclosed 28 day Sub-Acute Toxicity Study on Hexamethyldisiloxane, CASRN (107-46-0) and Shin-Etsu name LS-7130. As discussed below, it is not clear from the information contained in the translation of the study report from Japanese to English whether or not the study presents "information which reasonably supports the conclusion that such substance... presents a substantial risk" previously unknown to the Administrator. We believe it prudent, however, to submit the study under the circumstances.

This 28-day SubAcute Toxicity Study was conducted by Hita Research Laboratories, Chemical Biotesting Center, Chemicals Inspection and Testing Institute, Japan, on behalf of Shin-Etsu Chemical Co., Ltd., Japan. Shin-Etsu (U.S.) received a copy of this study from our Japanese parent company on June 21, 1995, and prior to such time, did not have any information regarding the study results. Immediately thereafter, Shin-Etsu examined the study for possible TSCA reportability based on the translation of the study from Japanese to English that was provided by our parent company. The translation indicates that "No toxic effect level of LS-7130 was considered to be 8 mg/kg/day in the conditions of this study". It is not clear from the translation, however, that the results support a conclusion of substantial risk.



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Sub-Acute Oral Toxicity Study on
LS-7130 in Rats
Page 2

Shin-Etsu will update this submission in the event that we obtain any further pertinent information as to the study results. In the meantime, if you have any questions regarding this submission or require further information on the toxicology of hexamethyldisiloxane, please do not hesitate to contact Mr. E.J. Hobbs (517)799-8293.

Sincerely,

A handwritten signature in cursive script that reads "Tomio Shibata". The signature is written in black ink and is positioned above the typed name and title.

Tomio Shibata, President
Shin-Etsu Silicones of America, Inc.

Receipt No. D93-1249
Report No. D-3895

STUDY CODE : B11-0232

FINAL REPORT

SUB-ACUTE ORAL TOXICITY STUDY
ON
LS-7130
IN RATS

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95 JUN 30 AM 11:25

August, 1994

Hita Research Laboratories
Chemical Biotesting Center
Chemicals Inspection & Testing Institute
Japan

received
June 21 '95

I, the undersigned, hereby declare that this report provides a correct English translation of the final report (Study code B11-0232, issued on August, 1994).

(date) January 31, 1995

(signature) Nobuya Imatanaka

Nobuya Imatanaka, M.S.
Hita Research Laboratories
Chemical Biotesting Center
Chemicals Inspection &
Testing Institute, Japan

GLP STATEMENT

Hita Research Laboratories, Chemical Biotesting Center
Chemicals Inspection & Testing Institute, Japan

Sponsor: Shin-Etsu Chemical Company, Ltd.
Title: Sub-acute oral toxicity study on LS-7130 in rats
Study Code: B11-0232

I, the undersigned, hereby declare that this study was conducted in compliance with the "Concerning Testing Facilities Stipulated in Article 4 of the Order Prescribing the Items of the Test related to New Chemical Substances and of the Toxicity Investigation related to Designated Chemical Substances" published in Notification No. 39 of Kanpogyo, Notification No. 229 of Yakuhatu and Notification No. 85 of 59 Kikyoku (March 31, 1984), and Notification No. 233 of Kankiken, Notification No. 38 of Eisei and Notification No. 823 of 63 Kikyoku (November 18, 1988), and "OECD Principles of Good Laboratory Practice" (May 12, 1981).

Management: Signed in original August 17, 1994
Shigetaka Yamane, Ph.D.

QUALITY ASSURANCE STATEMENT

Hita Research Laboratories, Chemical Biotesting Center
Chemicals Inspection & Testing Institute, Japan

Sponsor: Shin-Etsu Chemical Co., Ltd.
Title: Sub-acute oral toxicity study on LS-7130 in rats
Study code : B11-0232

This report was audited by the Quality Assurance Section.
I, the undersigned, hereby declare that this report reflects
the original Japanese report.

(Date)

January 31, 1995

(Signature)

Keiji Shiraishi

Section Chief, Quality Assurance

Keiji Shiraishi, B.S.

QUALITY ASSURANCE STATEMENT

Hita Research Laboratories, Chemical Biotesting Center,
Chemicals Inspection & Testing Institute, Japan

Sponsor: Shin-Etsu Chemical Company, Ltd.
Title: Sub-acute oral toxicity study on LS-7130 in rats
Study Code: B11-0232

This study was audited by the Quality Assurance Section and the study procedures were inspected on the following dates.

Dates of Inspections and Audits	Dates of Reports to Study Director	Dates of Reports to Management
February 14, 1994	February 14, 1994	February 14, 1994
March 24, 1994	March 25, 1994	March 25, 1994
March 28, 1994	March 29, 1994	March 30, 1994
April 5, 1994	April 6, 1994	April 6, 1994
April 6, 1994	April 7, 1994	April 7, 1994
April 26, 1994	April 26, 1994	April 26, 1994
May 19, 1994	May 20, 1994	May 20, 1994
June 14, 1994	June 14, 1994	June 14, 1994
July 11, 1994	July 12, 1994	July 12, 1994
August 1, 1994	August 1, 1994	August 1, 1994
August 10, 1994	August 11, 1994	August 15, 1994
August 17, 1994	August 17, 1994	August 17, 1994

I, the undersigned, hereby declare that this report provides an accurate description of the methods and procedures used in this study and that the reported results accurately reflect the raw data obtained.

Section Chief, Quality Assurance: Signed in original August 17, 1994
Keiji Shiraishi, B.S.

Study code: B11-0232

Test substance code: HR 2681

Sponsor code: S-230

TITLE

Sub-acute oral toxicity study on LS-7130 in rats

SPONSOR

Shin-Etsu Chemical Company, Ltd.

6-1, Ohtemachi 2-chome, Chiyoda-ku, Tokyo 100, Japan

TESTING FACILITY

Hita Research Laboratories, Chemical Biotesting Center

Chemical Inspection & Testing Institute, Japan

822, 3-chome, Ishii-machi, Hita, Oita 877, Japan

PURPOSE OF STUDY

The purpose of this study is to define the type, the severity and the reversibility of the toxicity signs induced by the substance and determine the no toxic effect level of the substance by observing the functional and morphological changes in animals receiving repeated doses for 28 days.

TESTING METHOD

This study was conducted in accordance with the "28-day repeated dose toxicity study in mammalian species" prescribed in "The notification on partial revision of testing methods relating to new chemical substances" (Notification No. 700 of Kanpogyo, No. 1039 of Yakuhatu, and No. 1014 of 61 Kikyoku, Dec. 5, 1986), and with the "407, Repeated dose oral toxicity-rodent: 28-day or 14-day study" prescribed in the OECD guidelines for testing of chemicals" (May 12, 1981).

GLP COMPLIANCE

This study was conducted in conformity with the "Concerning testing facilities stipulated in article 4 of the order prescribing the items of the test related to new chemical substances and of the toxicity investigation related to designated chemical substances" published in notification No. 39 of Kanpogyo, Notification No. 229 of Yakuhatsu and Notification No. 85 of 59 Kikyoku (March 31, 1984), and Notification No. 233 of Kankiken, Notification No. 38 of Eisei and Notification No. 823 of 63 Kikyoku (November 18, 1988), and the "OECD principles of good laboratory practice" (May 12, 1981).

PERIODS OF STUDY

Commencement of study:	February	14, 1994
Animal receipt:	March	22, 1994
Start of dosing:	March	29, 1994
Necropsy at the end of the dosing period:	April	26, 1994
Start of the recovery period:	April	26, 1994
Necropsy at the end of the recovery period:	May	10, 1994
Completion of study:	August	17, 1994

LOCATION AND PERIOD FOR RETENTION OF RAW DATA AND SPECIMENS

Data and specimens, and each remaining lot of the test substance will be retained in the archives and the test substance storage room, respectively, of Hita Research Laboratories for 10 years following the date of completion of the study.

After the termination of the retention period, they will be destroyed with the sponsor's approval or returned back to the sponsor upon request.

Some samples and specimens may be destroyed even before the termination in case of deterioration with the sponsor's consent.

AUTHOR AND PERSONS CONCERNED WITH STUDY

Study Director: Signed in original August 17, 1994
 Nobuya Imatanaka, M.S.,
 Mutagenicity & General Toxicology Section
 Hita Research Laboratories

Person in Charge of Pathological Examination:
 Signed in original August 17, 1994
 Kanji Yamasaki, Ph.D. D.V.M.

Person in charge of Clinical Chemistry:
 Signed in original August 17, 1994
 Keiji Shiraishi, B.S.

Study staff: Takayuki Koga, S.L.A.T.

Person in charge of Chemical Analysis:
 Ritsuko Furuta, B.S.

DEVIATIONS FROM THE PROTOCOL

Unforeseeable circumstances and deviations from the protocol which might have affected the quality of this study were noted.

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SUMMARY

A sub-acute oral toxicity study on LS-7130 was conducted by dosing the substance daily for 28 consecutive days (main test) and by feeding without dosing for 14 consecutive days thereafter (recovery test) in male and female Crj:CD(SD)rats (6/sex/group) of 5 weeks of age at the start of dosing.

The main test was composed of 4 dosing groups of 640, 160, 40 and 8 mg/kg/day and a control group, and the recovery test was composed of 2 another dosing groups of 640 and 160 mg/kg/day and another control group.

No death occurred throughout the study period and no abnormalities in relation with the administration of the substance were observed in general conditions.

The following changes were observed during or at the end of the dosing period:

Food consumption was decreased and body weight gain was suppressed in male of 640 mg/kg/day group.

In hematological examinations, white blood cell count was increased and mean corpuscular volume and mean corpuscular hemoglobin were decreased in male of 640 mg/kg/day group.

In blood chemical examinations, GOT, GPT, cholinesterase, γ -GTP, total cholesterol, total protein, total bilirubin and Ca were increased and A/G ratio was decreased in male of 640 mg/kg/day group.

In urinalysis, turbid urine and acidized urine in pH were observed in male of 640 mg/kg/day group.

Organ weights of the spleen and the liver were increased in male of 640 mg/kg/day group.

In necropsy, apparent spotty pattern of surface in the kidney in males of 160 mg/kg/day and higher groups, dark brownish change and enlargement in the liver and enlargement of the hepatic lymph node in male of 640 mg/kg/day group were observed.

In histopathological examinations, bile stasis, cell infiltration around bile stasis and swelling of hepatocytes in the liver in both sexes of 640 mg/kg/day group, increase of eosinophilic bodies in the kidney in males of 40 mg/kg/day and higher groups, bile duct proliferation, single cell necrosis and increase of mitoses of hepatocytes in the liver and deposition of brown pigment and increase of histiocytic cells in the hepatic lymph node in male of 640 mg/kg/day group were observed.

The following changes were observed at the end of the recovery period:

Increases of platelet count and IP, and decreases of hemoglobin concentration and hematocrit value were observed in male of 640 mg/kg/day group. Increases of white blood cell count, total cholesterol, spleen weight and liver weight were still observed in male of 640 mg/kg/day group.

In histopathological examinations, swelling of hepatocytes, increase of mitoses of hepatocytes, single cell necrosis in the liver and increase of eosinophilic bodies in the kidney were convalescing but the some changes corresponding to bile stasis in the liver were still observed.

No toxic effect level of LS-7130 was considered to be 8 mg/kg/day in the conditions of this study.

MATERIALS AND METHODS

1. TEST SUBSTANCE (INFORMATION PROVIDED BY THE SPONSOR)

1.1 Name

Hexamethyldisiloxane

Other name: LS-7130

CAS No.: 107-46-0

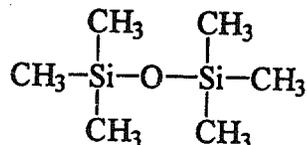
1.2 Lot No.

312077

1.3 Supplier

Shin-Etsu Chemical Co., Ltd.

1.4 Structural formula or rational formula (or outline of manufacturing method, in case both are unknown)

(Molecular formula: C₆H₁₈OSi₂)

1.5 Purity

100 %

1.6 Name and concentration of impurities

—

1.7 Physicochemical properties

Appearance at ordinary temperature: Colorless transparent liquid

Molecular weight: 162.38

Stability: The stability test of the substance was conducted by our laboratory and confirmed that it was stable enough in the formulations for 28 days.

Melting point: No information submitted

Boiling point: 100.5 °C

Vapor pressure: No information submitted

Partition coefficient: No information submitted

Solubility: oil-soluble

Degree of solubility:

in water: insoluble

in DMSO: insoluble

in acetone: soluble

in other solvents:

soluble in Tetrahydrofuran

1.8 Storage conditions

stored in a cold place.

1.9 Care on handling

Gloves, a mask, a head cap and a lab coat were worn when handling.

The test substance is volatile.

2. ANIMALS

Rats (SPF) of the Crj:CD(SD) strain were obtained from Charles River Japan, Inc. (Hino Breeding Center; 735, Shimokomatsuki, Hino-cho, Gamo-gun, Shiga 529-16, Japan). The animals were quarantined and acclimatized, and healthy animals with favorable body weight gain were allocated to groups to ensure homogeneity of mean body weight using body weight-stratified randomization for the study. The animals were 5 weeks old, and weighed 126.9 - 144.8 g for males and 110.0 - 127.5 g for females at the start of dosing. The animals were identified by ear-tagging.

3. HOUSING CONDITIONS

The barrier-system animal room was maintained at a temperature of 23 ± 2 °C and a relative humidity of $55 \pm 10\%$ with 10 to 15 air changes per hour and artificial light for 12 hours (between 7:00 and 19:00). The animals were housed individually in a hanging stainless steel cage with wire-mesh floor (165 w × 300 d × 150 h mm, Tokiwa Kagaku Kikai). The trays were changed twice a week, and racks once 2 weeks. The racks and cages were identified by cards. The animals had free access to an MF pelleted diet (Oriental Yeast Co., Ltd.) and water (chlorinated) from the Hita City supply via sipper tubes from automatic waterer. The diet and housing materials were autoclaved at 121°C for 30 minutes prior to use. Contaminants in both of the diet and drinking water have been regularly analyzed by our laboratory, so they could not have affected this study.

Within reason, there have been no circumstances which might have affected the quality and integrity of the results obtained.

4. GROUPING

The grouping was as follows:

Group	Dose (mg/kg/day)	Volume (ml/kg)	Concen- tration (%)	No. of animals (Animal No.)	
				Male	Female
Vehicle control	0	10	0	6(1- 6)	6(49-54)
Vehicle control (recovery)	0	10	0	6(7-12)	6(55-60)
Low dose	8	10	0.08	6(13-18)	6(61-66)
Intermediate dose (1)	40	10	0.4	6(19-24)	6(67-72)
Intermediate dose (2)	160	10	1.6	6(25-30)	6(73-78)
Intermediate dose (2) (recovery)	160	10	1.6	6(31-36)	6(79-84)
High dose	640	10	6.4	6(37-42)	6(85-90)
High dose (recovery)	640	10	6.4	6(43-48)	6(91-96)

Reason for dosage selection:

A preliminary oral toxicity test was conducted by dosing the substance by oral gavage daily for 14 days at 3 doses of 1,000, 250 and 50 mg/kg/day. As the result, abnormal changes were noted in body weight and blood chemical examinations in 1,000 mg/kg/day group, in organ weights and necropsy in 250 mg/kg/day and higher groups, and in general condition, hematological examinations and histopathological examinations in 50 mg/kg/day and higher groups.

Based on the results of the preliminary test, the main test was to be conducted by oral gavage at the doses of 640, 160, 40, 8 and 0(vehicle control group) mg/kg/day of the substance and the recovery test at the doses of 640, 160 and 0(vehicle control group) mg/kg/day of the substance.

5. PREPARATION OF THE TEST SUBSTANCE

5.1 Preparation of the substance

The dosing of the substance to the test animals was to be conducted by giving a formulation of the substance, which was a solution of the substance in olive oil.

For preparation of the substance, it was weighed accurately and dissolved in olive oil (Lot No. 146RSP, Fujisawa-Astra) in stirring to make the highest formulation of the concentration of 6.4 w/v % (0.064 g/mL). Then the formulation was diluted with olive oil to make 3 lower formulations of 1.6, 0.4 and 0.08 w/v %. These formulations were prepared once a week for dosing.

5.2 Stability of LS-7130

The stability test of the substance was conducted by our laboratory and confirmed that it was stable enough in the formulations for 8 days.

6. EXPERIMENTAL DESIGN

The main test comprised 4 dosing groups and a control group was intended to provide information on the sub-acute toxicity of the substance. The recovery test comprised another two dosing groups and another control group was intended to investigate the reversibility of the effects induced by the substance in the main test.

In the main test, the rats of dosing groups were dosed with the substance in a formulation for 28 consecutive days and the rats of the vehicle control group were dosed with olive oil for the same period.

In the recovery test, the rats of the test groups and the vehicle control group were also dosed with the substance in a formulation and olive oil respectively for 28 consecutive days, and thereafter were fed on the usual basal diet for 14 consecutive days without dosing of the substance.

The dosing by oral gavage of the formulations was conducted using a Nelaton catheter (Terumo Corporation) and a syringe (Terumo Corporation) in the morning.

7. OBSERVATION AND MEASUREMENT

The day of the first dosing was defined as the day 1, and the day before as the day -1. The week of starting from the day 1 was defined as the week 1. Also, the next day of the final dosing was defined as the recovery day 1, and the week starting from the recovery day 1 was defined as the recovery week 1.

7.1 General conditions

All animals were observed at least once per day.

7.2 Body weight

All animals were weighed as follows:

Before dosing: day -2 (at the time of grouping)

During the dosing period: days 1, 3, 5, 8, 10, 12, 15, 17, 19, 22, 24, 26 and 28

During the recovery period: recovery days 1, 3, 5, 8, 10, 12 and 14
in addition, just before the necropsy at the end of the dosing period or recovery period, body weights were measured for calculation of relative organ weights.

7.3 Food consumption

Food consumption was measured as follows:

Before dosing: Once

During the dosing and recovery periods: Twice a week

7.4 Hematological examinations

All animals of the main test groups at the end of the dosing period and of the recovery test groups at the end of the recovery period were fasted overnight (16 - 20 hours), and blood samples were obtained via abdominal aorta from them under ether anesthesia. Sodium citrate was used for examinations of prothrombin time and EDTA-2K was used for another parameters as an anti-coagulant. The blood and plasma samples were examined for the following parameters.

Parameter		Method
1) Red blood cell count (RBC)	($\times 10^4/\text{mm}^3$)	System for detecting change in electrical resistance
2) White blood cell count (WBC)	($\times 10^2/\text{mm}^3$)	System for detecting change in electrical resistance
3) Hemoglobin concentration (Hb)	(g/dl)	Oxyhemoglobin method
4) Hematocrit value (Ht)	(%)	System for detecting pulse
5) Mean corpuscular volume (MCV)	(μm^3)	$\frac{\text{Ht}}{\text{RBC}} \times 10^3$
6) Mean corpuscular hemoglobin (MCH)	(pg)	$\frac{\text{Hb}}{\text{RBC}} \times 10^3$
7) Mean corpuscular hemoglobin concentration (MCHC)	(%)	$\frac{\text{Hb}}{\text{Ht}} \times 10^2$
8) Platelet count	($\times 10^4/\text{mm}^3$)	System for detecting change in electrical resistance
9) Reticulocytes count	(%)	New methylen blue staining
10) Prothrombin time (PT)	(sec)	Magnetic sensor system
11) Activated partial thromboplastin time (APTT)	(sec)	Magnetic sensor system
12) Differentiation of leukocytes	(%)	Wright Staining
Band form neutrophils (N-Band)		
Segmental neutrophils (N-Seg)		
Eosinophils (Eosino)		
Basophils (Baso)		
Lymphocytes (Lymph)		
Monocytes (Mono)		
1) - 8)	Microcell counter M-2000, Toa Medical Electronics	
9), 12)	MICROX HEG-120A, Omron	
10), 11)	KC-10A, Amelung	

7.5 Blood chemical examinations

Sera were separated from the blood samples, used for hematology, and examined following parameters.

Parameter		Method
1) GOT	(IU/l)	UV method
2) GPT	(IU/l)	UV method
3) Alkaline phosphatase (ALP)	(IU/l)	p-Nitrophenyl phosphate method
4) Cholinesterase (ChE)	(IU/l)	Butyrylthiocholine iodide method
5) γ -GTP	(IU/l)	L- γ -Glutamyl-p-nitroanilide method
6) Total cholesterol (T-Cho)	(mg/dl)	COD-DAOS method
7) Triglyceride (TG)	(mg/dl)	GPD-DAOS method
8) Glucose	(mg/dl)	Glucokinase-G-6-PDH method
9) Total protein (T-Protein)	(g/dl)	Biuret method
10) Albumin	(g/dl)	Bromocresol green method
11) A/G ratio		$\frac{\text{Albumin}}{\text{T-Protein} - \text{Albumin}}$
12) Blood urea nitrogen (BUN)	(mg/dl)	Urease indophenol method
13) Creatinine	(mg/dl)	Jaffé's method
14) Total bilirubin (T-Bil)	(mg/dl)	Azo bilirubin method
15) Ca	(mg/dl)	OCPC method
16) IP	(mg/dl)	Fiske-Subbarow method
17) Na	(mEq/l)	Crown-Ether membrane electrode method
18) K	(mEq/l)	Crown-Ether membrane electrode method
19) Cl	(mEq/l)	Coulometric titration method

1) - 10), 12) - 16) 7150 Automatic Analyzer, Hitachi

17) - 19) PVA- α III, A & T

7.6 Urinalysis

All of urine samples, produced during 16 hours period from all animals in the main test at the day 28 and in the recovery test groups at the recovery day 14 in each individual metabolic cage were collected. They were examined for volume, color and additional items of protein, ketones, bilirubin, occult blood, glucose and urobilinogen, all of which were tested using a test paper (N-Multistix[®], Miles-Sankyo).

7.7 Necropsy

All animals in the main test at the end of the dosing period and in the recovery test at the end of the recovery period, were subject to necropsy.

7.8 Organ weights

The following organs obtained at the necropsy were weighed wet of all rats.

Brain, liver, spleen, kidneys, adrenal glands and testes (or ovaries).

7.9 Histopathological examinations

- 1) The organs and tissues described below obtained at the necropsy from all animals were preserved in 10 % formalin.

Brain (cerebrum, cerebellum), hypophysis, eyeball, thyroid glands (with parathyroid glands), heart, lung, liver, kidneys, spleen, adrenal glands, stomach, intestine (duodenum to rectum), testes (or ovaries), urinary bladder, bone marrow (femur), and gross lesions.

- 2) The organs and tissues described below, after paraffin embedding, sectioning and hematoxylin and eosin staining, were examined by light microscopic observation.

The main test groups at the end of the dosing period:

Liver, spleen, kidney, heart, stomach, intestine(duodenum, jejunum, ileum, cecum, colon, rectum) and adrenal gland of vehicle control and 640 mg/kg/day groups

Liver of both sexes and kidney of male of 160 mg/kg/day group

Kidney of male rats of 40 and 8 mg/kg/day groups

The gross lesions at the end of the dosing period:

Hepatic lymph node of males(No.39, 40, 41, 42) of 640 mg/kg/day group

Glandular stomach of a female(No.74) and ovary of a female (No.76) of 160 mg/kg/day group

Kidney of a female(No.68) of 40 mg/kg/day group

Glandular stomach of 2 females(No.63, 66) of 8 mg/kg/day group

Spleen of a female(No.65) of 8 mg/kg/day group

The recovery test groups at the end of the recovery period:

Liver and kidney of male rats of vehicle control and 640 mg/kg/day groups

The gross lesions at the end of the recovery period:

Hepatic lymph node of males(No.43, 45, 46, 47) and glandular stomach of

2 males(No.44, 46) of 640 mg/kg/day group

Heart of a male(No.34) and testis of a male(No.36) of 160 mg/kg/day group

Glandular stomach of a male(No.9) of vehicle control group

Kidney and adrenal gland of a female(No.93) and glandular stomach of a female(No.94) of 640 mg/kg/day group

8. STATISTICAL ANALYSIS

Each of data regarding body weight, food consumption, hematological examination, blood chemical examination, urine volume and organ weights was analyzed using Bartlett's test for homogeneity of variance at a significance level of 5%.

The data of homogeneous variance was analyzed for significant difference by one way analysis of variance. Then if there were a significant difference in it by the analysis, it was analyzed for significant difference in comparison with that of the control group by Dunnett's test in case of equal number of data, otherwise, i.e., in case of unequal number, by Scheffé's test.

The data of not homogenous variance was analyzed by Kruskal-Wallis's test. Then if there were a significant difference in it by the test, it was analyzed for difference in comparison with the control group by nonparametric Dunnet's test in case of equal number of data, otherwise, i.e., in case of unequal number, by nonparametric Scheffé's test.

RESULTS

1. GENERAL CONDITIONS (TABLE 1, ADDENDUM 1)

No death occurred in the test animals throughout the study period.

1.1 During the dosing period, salivation was observed in males (6/12) of 160 mg/kg/day group and in males (4/12) of 640 mg/kg/day group. These phenomenon sporadically occurred just after dosing from the day 11 to the day 22 in male of 160 mg/kg/day group and from the day 8 to the day 21 in male of 640 mg/kg/day group. Scab formation was observed at left cervical region of a male (1/12) of 160 mg/kg/day group.

Salivation was observed in females (4/12) of 160 mg/kg/day group, in females (5/12) of 640 mg/kg/day group and in a female (1/12) of the vehicle control group. The phenomenon sporadically occurred too just after dosing from the day 17 to the day 26 in females of 160 mg/kg/day group, from the day 10 to 25 in females of 640 mg/kg/day group and on the days 13 and 22 in females of the vehicle control group. Scab formation (2/12) and loss of hair at left cervical region (1/12) were observed in females of 160 mg/kg/day group.

1.2 During the recovery period, scab formation (1/6) at left cervical region was observed in a male of the vehicle control group.

Scab formation (2/6) and loss of hair (1/6) at left cervical region in female of 160 mg/kg/day group and scab formation (1/6) at left cervical region in a female of 640 mg/kg/day group, which had been observed in the dosing period, were still present.

2. BODY WEIGHT (FIG.1, TABLE 2, ADDENDUM 2)

2.1 Body weight gain was suppressed from the day 19 to 28 in male of 640 mg/kg/day group while no change was observed in female in the main test.

2.2 No abnormalities were noted in either sexes of the animals during the recovery period.

3. FOOD CONSUMPTION (FIG.2, TABLE 3, ADDENDUM 3)
 - 3.1 Food consumption was decreased from the day 8 to 28 in male of 640 mg/kg/day group, while it was increased at the day 11 in female of 640 mg/kg/day group.
 - 3.2 Food consumption was increased from the recovery day 8 to 14 in male of 640 mg/kg/day group, while no change was noted in female in the recovery period.

4. HEMATOLOGICAL EXAMINATIONS (TABLE 4, ADDENDUM 4)

Values of platelet count in one male (No.4) and one female (No.53) of the vehicle control group were omitted from mean calculation because deviating from the background data in our laboratories.

 - 4.1 At the end of the main test

White blood cell count was increased and mean corpuscular volume and mean corpuscular hemoglobin were decreased in males of 640 mg/kg/day group. Increase of segmental neutrophils and decrease of lymphocytes, which were not significantly different from the vehicle control, were observed in males of the same group.

No abnormalities were observed in females in the main test.
 - 4.2 At the end of the recovery test

White blood cell count and platelet count were increased, and hemoglobin concentration and hematocrit value were decreased in males of 640 mg/kg/day group. Activated partial thromboplastin time was delayed in males of 160 mg/kg/day group. No abnormalities were noted in females in the recovery test.

5. BLOOD CHEMICAL EXAMINATIONS (TABLE 5, ADDENDUM 5)

Values of platelet count in one male (No.4) and one female (No.53) of the vehicle control group were omitted from mean calculation because deviating from the background data in our laboratories.

 - 5.1 At the end of the dosing period

γ -GTP, total cholesterol, total protein and Ca were increased, A/G ratio was decreased and GOT, GTP, cholinesterase and total bilirubin were increased in males of 640 mg/kg/day group.

No abnormalities were observed in females in the main test.
 - 5.2 At the end of the recovery period

IP was increased in males of 160 mg/kg/day and higher groups, and total cholesterol was increased in males of 640 mg/kg/day group.

No abnormalities were observed in females in the main test.

6. URINALYSIS (TABLE 6, ADDENDUM 6)

6.1 At the end of the dosing period

Turbid urine (3/6) and acidized urine in pH were observed in males of 640 mg/kg/day group, and turbid urine (1/6) was observed in a female of the vehicle control group.

6.2 At the end of the recovery period

No abnormalities were observed in either sex in the recovery test.

7. ORGAN WEIGHTS (TABLES 7,8, ADDENDA 7,8)

The data of following organs, in which abnormalities were observed in necropsy, were omitted from the calculation of the mean value of organ weights:

the left testis of one male (No.36; enlarged) of 160 mg/kg/day group, the spleen of one female (No.65; enlarged) of 8 mg/kg/day group and ovaries of one female (No.76; small) of 160 mg/kg/day group and the right kidney (No.93; deformation) and the right adrenal gland (No.93; deformation) of 640 mg/kg/day group.

7.1 At the end of the dosing period

Absolute and relative liver weights, relative spleen weight and relative brain weight were increased in male of 640 mg/kg/day group.

No abnormalities were observed in female in the main test.

7.2 At the end of the recovery period

Absolute and relative spleen weights and relative liver weight were increased in male of 640 mg/kg/day group, and relative adrenal weight was increased in male of 160 mg/kg/day group.

No abnormalities were observed in female in the recovery test.

8. NECROPSY (TABLE 9, ADDENDUM 9)

8.1 At the end of the dosing period

Dark brownish change (6/6) and enlargement (6/6) of the liver, enlargement (4/6) of the hepatic lymph node, apparent spotty pattern of surface (2/6) and peivic dilatation (1/6) in the kidney were observed in males of 640 mg/kg/day group.

Apparent spotty pattern of surface was observed in the kidney of males (6/6) of 160 mg/kg/day group.

Blackish region of mucosa (1/6) in the glandular stomach and small (1/6) of the ovary were observed in a female of 160 mg/kg/day group.

Peivic dilatation (1/6) was observed in the kidney of 40 mg/kg/day group.

Enlargement (1/6) of the spleen and blackish region of mucosa (2/6) in the

glandular stomach were observed in females of 8 mg/kg/day group.

8.2 At the end of the recovery period

Dark brownish change (5/6) in the liver, enlargement (4/6) of the hepatic lymph node and blackish spot (1/6) and blackish region (1/6) of mucosa in the glandular stomach were observed in males of 640 mg/kg/day group.

Whitish spot (1/6) in the heart and enlargement (1/6) of the testis in males of 160 mg/kg/day group and blackish region (1/6) of mucosa in the glandular stomach in a male of the vehicle control group were observed.

Small (1/6) of the kidney, blackish region (1/6) of mucosa in the glandular stomach and small (1/6) of the adrenal gland were observed in females of 640 mg/kg/day group.

9. HISTOPATHOLOGICAL EXAMINATIONS (TABLE 10, ADDENDUM 9)

9.1 At the end of the dosing period

Bile duct proliferation (++,6/6), bile stasis (++,6/6), single cell necrosis (+,6/6), swelling of hepatocytes (+,6/6), cell infiltration around bile stasis (+,5/6), increase of mitoses of hepatocytes in the liver (+,2/6), increase of eosinophilic bodies (+,2/6) and pelvic dilatation (1/6) in the kidney, deposition of brown pigment (+,4/4) and increase of histiocytic cells (+,4/4) in the hepatic lymph node were observed in males of 640 mg/kg/day group.

Increase of eosinophilic bodies (+,6/6) in the kidney and microgranuloma (+,1/6) in the liver were observed in males of 160 mg/kg/day group.

Increase of eosinophilic bodies (\pm ,5/6) in the kidney were observed in males (\pm ,5/6) of 40 mg/kg/day group.

Increase of eosinophilic bodies (\pm ,2/6) and degeneration of tubules with fibrosis (1/6) were observed in the kidney in males of 8 mg/kg/day group.

Degeneration of tubules with fibrosis (1/6) were observed in the kidney in a males of the vehicle control group.

Bile stasis (+,1/6), cell infiltration around bile stasis (+,1/6) and swelling of hepatocytes (+,1/6) in the liver were observed in females of 640 mg/kg/day group.

Microgranuloma (+,1/6) in the liver, necrosis of mucosa in the glandular stomach (1/1) and atrophy of the ovary (1/1) were observed in females of 160 mg/kg/day group.

Pelvic dilatation in the kidney (1/1) were observed in females of 40 mg/kg/day group.

Increase of extramedullary hematopoiesis in the spleen (1/1) and necrosis of

mucosa in the glandular stomach (+,2/2) were observed in females of 8 mg/kg/day group.

Microgranuloma (+,3/6) and perilobular lipid droplets (+,3/6) in the liver were observed in females of the vehicle control group.

9.2 At the end of the recovery period

Bile duct proliferation (++,5/6;+,1/6), bile stasis (++,5/6;+,1/6), cell infiltration around bile stasis (+,6/6) in the liver, deposition of brown pigment (+,4/4) and increase of histiocytic cells (+,4/4) in the hepatic lymph node and necrosis of mucosa in the glandular stomach (+,2/2) were observed in females of 640 mg/kg/day group.

Fibrosis and calcification (1/1) in the heart and decrease of spermatogenesis in the testis (+,1/1) were observed in males of 160 mg/kg/day group.

Microgranuloma in the liver (+,2/6), increase of eosinophilic bodies in the kidney (\pm ,1/6) and necrosis of mucosa in the glandular stomach (+,1/1) were observed in males of the vehicle control group.

Bile stasis (+,2/6), cell infiltration around bile stasis (+,2/6) and perilobular lipid droplets (+,1/6) in the liver, deformation (1/1) and fibrosis and calcification (1/1) in the kidney, necrosis of mucosa in the glandular stomach (+,1/1), deformation (1/1) and fibrosis and calcification (1/1) in the adrenal gland were observed in females of 640 mg/kg/day group.

DISCUSSION AND CONCLUSION

Salivation which were observed in both sexes of 160 and 640 mg/kg/day groups during the dosing period were considered not to be toxicologically significant since the phenomenon only occurred just after dosing and no other abnormalities corresponding to them were accompanied with.

Decrease of food consumption in male of 640 mg/kg/day group during the dosing period, accompanied with suppression of the body weight gain, should be considered to be dose-related.

Increase of white blood cell count observed in male of 640 mg/kg/day group at the end of dosing period was considered to be dose-related.

Decreases of mean corpuscular volume and mean corpuscular hemoglobin observed in males of 640 mg/kg/day group were considered to be less important because no other abnormalities were observed at all in red blood cell count, hemoglobin concentration or hematocrit volume.

Increases of GOT, GPT, cholinesterase, γ -GTP, total cholesterol, total protein, total bilirubin and Ca and decrease of A/G ratio detected in blood chemical examinations in males of 640 mg/kg/day group at the end of the dosing period were dose-related.

Turbid urine, acidized urine in pH and increases of spleen weight and liver weight detected in males of 640 mg/kg/day group at the end of dosing period were dose-related.

The changes of apparent spotty pattern of surface in the kidney in male of 160 mg/kg/day and higher groups, and dark brownish change and enlargement of the liver and enlargement of the hepatic lymph node in male of 640 mg/kg/day group at the end of dosing period, which were found in macroscopic observation in necropsy, were considered to be in relation with the administration of the substance.

Histopathological effects of bile stasis, cell infiltration around bile stasis and swelling of hepatocytes in the liver in both sexes, bile duct proliferation, single cell necrosis and increase of mitoses of hepatocytes in the liver and deposition of brown pigment and increase of histiocytic cells in the hepatic lymph node in male of 640 mg/kg/day group at the end of dosing period were considered to be in relation of the administration of the substance.

Increase of eosinophilic bodies in the kidney was observed in males of 8 mg/kg/day and higher groups at the end of dosing period and of the control group at the end of the recovery period. It was considered that this change was only in relation with the administration of the substance in 40 mg/kg/day and higher groups because of its frequency and degree.

Abnormalities in spleen weight and in urinalysis were considered not to be important because no changes in histopathological examinations were observed in the spleen nor in the kidney.

From the above, it could be said that the main effects of the substance were to the liver in both sexes and to the kidney in males. The most abnormalities in blood chemical examinations, which appeared to be in connection with the pathological changes noted in the liver, should also be considered to be effects of the substance.

The changes of increases of platelet count and IP and decreases of hemoglobin concentration and hematocrit value, which had not been observed at the end of the main test, were noted in males of 640 mg/kg/day group at the end of the recovery test. The changes of increases of white blood cell count, total cholesterol, spleen weight and liver weight, which had been observed at the end of the main test, were still noted at the end of the recovery test. In histopathology, the changes of swelling of hepatocytes, increase of mitoses of hepatocytes and single cell necrosis in the liver and increase of eosinophilic bodies in the kidney were recovered, but the changes corresponding to bile stasis in the liver were still noted in both sexes of 640 mg/kg/day group at the end of the recovery test.

Other statistically significant changes in general conditions, necropsy and histopathological examinations, with no dose-dependence nor difference from the control group, were considered not to be dose-related.

No toxic effect level of the substance was concluded to be 8 mg/kg/day because of increase of eosinophilic bodies observed in the kidney in males of 40 mg/kg/day group in the main test.

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