Receipt No. 827-06-D-3207

STUDY CODE: B11-0837

FINAL REPORT

TWENTY-EIGHT-DAY REPEATED-DOSE
ORAL TOXICITY STUDY OF
13F-SFMA IN RATS

July 2007

Hita Laboratory
Chemicals Evaluation and Research Institute
Japan

STATEMENT

I, the undersigned, hereby declare that this report provides correct English translation of the final report (Study Code B11-0837, issued on July 10, 2007).

October 6, 2009

Date

Hita Laboratory

Chemicals Evaluation and Research Institute, Japan

GLP STATEMENT

Hita Laboratory

Chemicals Evaluation and Research Institute, Japan

Sponsor: DAIKIN INDUSTRIES, LTD.

Title: Twenty-Eight-Day Repeated-Dose Oral Toxicity Study of 13F-SFMA in Rats

Study Code: B11-0837

I, the undersigned, hereby declare that this study was conducted in compliance with "Concerning Standard of the Testing Facilities Conducting the Test Relating to the New Chemical Substances" on Japanese GLP [Notification No. 1121003 of the Pharmaceutical and Food Safety Bureau, MHLW, No. 3 (November 17, 2003) of the Manufacturing Industries Bureau, METI & No. 031121004 of the Environmental Health Department, MOE (November 21, 2003)].

And, I confirmed that this report accurately reflects the raw data obtained and that data of the study has reliability.

Study Director: Signed in original July 10, 2007

QUALITY ASSURANCE STATEMENT

Hita Laboratory

Chemicals Evaluation and Research Institute, Japan

Sponsor: DAIKIN INDUSTRIES, LTD.

Title: Twenty-Eight-Day Repeated-Dose Oral Toxicity Study of 13F-SFMA in Rats

Study Code: B11-0837

This study was audited and inspected by Quality Assurance Section of Hita Laboratory, Chemicals Evaluation and Research Institute, Japan. The dates audited and/or inspected and the dates reported of these results to the study director and management are as follows.

Items of Inspections and Audits	Dates of Inspections and Audits	Dates of Inspections and Audits Reports
Protocol	February 19, 2007	February 19, 2007
Amendment to protocol	February 27, 2007	February 27, 2007
Re-inspection of protocol	March 1, 2007	March 1, 2007
Administration and clinical observation	March 1, 2007	March 1, 2007
Preparation of test substance	March 6, 2007	March 7, 2007
Detailed clinical observation and sensorimotor function	March 23, 2007	March 23, 2007
Urine sampling	March 28, 2007	March 28, 2007
Amendment to protocol (2nd)	March 29, 2007	March 29, 2007
Amendment to protocol (3rd)	May 7, 2007	May 7, 2007
Pathological data	June 22, 2007	June 22, 2007
Animal data	June 25, 2007	June 25, 2007
Detailed clinical observation data and sensorimotor function data	June 25, 2007	June 25, 2007
Documents of test substance and housing conditions	June 25, 2007	June 25, 2007
Re-inspection of animal data	June 26, 2007	June 26, 2007
Re-inspection of documents of test substance and housing conditions	June 26, 2007	June 26, 2007
Clinical chemistry data	June 28, 2007	June 28, 2007
Draft of final report	July 4, 2007	July 4, 2007
Re-inspection of draft final report	July 5, 2007	July 5, 2007
Draft of final report (2nd)	July 9, 2007	July 9, 2007
Re-inspection of draft final report (2nd)	July 10, 2007	July 10, 2007
Final report	July 10, 2007	July 10, 2007

Following items were reported to the study director and management on the basis of the audit of facility or audit results in other study.

Items of Audits	Dates of Audits	Dates of Audits Reports
Animal receipt	January 16, 2007	July 10, 2007
Body weight measurements	February 23, 2007	July 10, 2007
Food intake measurements	February 23, 2007	July 10, 2007
Blood sampling	January 16, 2007	July 10, 2007
Trimming, necropsy and organ weight measurements	January 16, 2007	July 10, 2007
Hematology	January 16, 2007	July 10, 2007
Blood chemistry	January 16, 2007	July 10, 2007
Urinalysis	January 16, 2007	July 10, 2007
Pathological preparation	February 6, 9 and 15, 2007	July 10, 2007

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 July 10, 2007

TABLE OF CONTENTS

Study Code: B11-0837
Test Substance Code: HR6852
Sponsor Code: D-0060

TITLE

Twenty-Eight-Day Repeated-Dose Oral Toxicity Study of 13F-SFMA in Rats

SPONSOR

DAIKIN INDUSTRIES, LTD.
1-1, Nishihitotsuya, Settsu, Osaka 566-8585, Japan

TESTING FACILITY

Hita Laboratory
Chemicals Evaluation and Research Institute, Japan
822, 3-chome, Ishii-machi, Hita, Oita 877-0061, Japan

PURPOSE OF STUDY

The purpose of this study is to define the type, severity and reversibility of toxicological signs of the test substance by observing the functional and morphological changes in rats receiving repeated doses orally for 28 days.

TESTING METHOD

This study was conducted in accordance with "28-day Repeated Dose Toxicity Study in Mammalian Species" prescribed in "Concerning Testing Methods Relating to the New Chemical Substances" [Notification No. 1121002 of the Pharmaceutical and Food Safety Bureau, MHLW, No. 2 (November 13, 2003) of the Manufacturing Industries Bureau, METI & No. 031121002 of the Environmental Health Department, MOE (November 21, 2003)].

GLP COMPLIANCE

This study was conducted in compliance with "Concerning Standard of the Testing Facilities Conducting the Test Relating to the New Chemical Substances" on Japanese GLP [Notification No. 1121003 of the Pharmaceutical and Food Safety Bureau, MHLW, No. 3 (November 17, 2003) of the Manufacturing Industries Bureau, METI & No. 031121004 of the Environmental Health Department, MOE (November 21, 2003)].

PERIOD OF THE STUDY

Commencement of Study:	February 16, 2007
Animal Receipt:	February 20, 2007
Initiation of Examination (Initiation of Dosing):	March 1, 2007
Terminal Necropsy of Dosing Period:	March 29, 2007
Initiation of Recovery Period:	March 29, 2007
Terminal Necropsy of Recovery Period:	April 12, 2007
Termination of Examination (Termination of Histology):	June 7, 2007
Completion of Study:	July 10, 2007

LOCATION AND PERIOD FOR RETENTION OF RAW DATA AND SPECIMENS

The raw data, protocol and amendment, study contract documents, test substance information, final report, other record documents and specimens will be stored in the archive of the Hita Laboratory of our organization, and samples of every lot of the test substance in the test substance storage room, for a period of 10 years from the date of receipt of the notification that they are applicable to Article 4, Paragraphs 1 or 2, Article 4-2, Paragraphs 2, 3 or 8, Article 5-4, Paragraph 2, Article 24, Paragraph 2 or Article 25-3, Paragraph 2 of the Japanese Chemical Substances Control Law No. 117 (1973). The sponsor will inform the Hita Laboratory of the date of receipt of the notification. After termination of the retention period, any measures taken will be done so with the approval of the sponsor. Samples and specimens that are liable to deteriorate markedly will be retained for 10 years after receipt of the notification or only for as long as the quality of the preparation permits evaluation, and they will be disposed with approval of the sponsor.

RETENTION OF THE ORIGINAL PROTOCOL AND FINAL REPORT

An original protocol, original protocol amendments and an original final report will be retained at Hita Laboratory. The copies of their originals that the study director will have been recognized to be accurate copy will be sent to the sponsor.

AUTHOR AND PERSONNEL CONCERNED WITH THE STUDY

Study Director: Katsumi Miyata, M.S., J.A.C.L.C.T.
(Planning and management of the study, evaluation of the results, report creation, and over all responsible for the technical conduct of the study)

Study Staff: Takashi Anai, B.S.
(Quarantine, acclimation and housing management of animals, preparation and administration of the test substance, clinical observation, detailed clinical observation, sensorimotor function, body weights and food intakes measurements, and responsible for the animal examination)

Person in charge of Pathologic Examination:
Satsuki Hoshuyama, Ph.D., D.V.M.
(Necropsy, collection of tissues, organ weight measurements, histopathological examinations, and responsible for the histopathology)

Person in charge of Clinical Chemistry:
Keiji Shiraishi, B.S., C.T. (until March 29, 2007)
Nobuhiko Higasihara, B.S. (from March 30, 2007)
(Hematological and blood chemical examinations, urinalysis, and responsible for the biochemistry of the specimens)

AUTHOR APPROVAL

Study Director: Signed in original July 10, 2007
Katsumi Miyata, M.S., J.A.C.L.C.T.
Section 2 (Toxicology area)
Hita Laboratory

SUMMARY

A 28-day repeated-dose oral toxicity study of 13F-SFMA followed by a 14-day recovery study was performed in groups of five male and five female Crl:CD(SD) rats at 5 weeks of age. The high dose was set at 25 mg/kg/day, and altogether three doses including 5 and 1 mg/kg/day were employed. Recovery groups were also set for the 25 mg/kg and vehicle control groups to investigate the reversibility of the effects.

No death occurred during the dosing or recovery periods.
No effects of the test substance on the clinical signs, detailed clinical observation, sensorimotor function, body weight or food intakes were observed. At the end of the dosing period, increased relative kidney weight in males and increased absolute and relative liver weights in females of the 25 mg/kg group were observed in the organ weights. In the histopathological examinations, decreased iron pigment of the ameloblasts at maturation stage in the incisors was observed in both sexes of the 25 mg/kg group. No abnormalities were observed in the hematology, blood chemical examinations or urinalysis.

In the recovery group, among the treatment related changes, while the effect on the incisors was observed as mottled teeth in the necropsy, no histopathological abnormalities were observed in the ameloblasts. In addition, increased liver and kidney weights were not observed. Therefore, the changes of the incisors, kidneys and liver were considered to be reversible.

The above-mentioned results showed that the major effect of 13F-SFMA was observed in the incisors. In addition, increased weights of the liver and kidney were observed. These changes were considered to be reversible. Therefore, the No-Observed-Adverse-Effect Level (NOAEL) of 13F-SFMA were considered to be 5 mg/kg/day based on decreased iron pigment of the ameloblasts at maturation stage in the incisors in both sexes, increased relative kidney weight in males and increased absolute and relative liver weights in females of the 25 mg/kg group.

MATERIALS AND METHODS

1. TEST SUBSTANCE (Information provided by the sponsor)

1.1 Name

3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl methacrylate

Other Name: 13F-SFMA

CAS No.: 2144-53-8

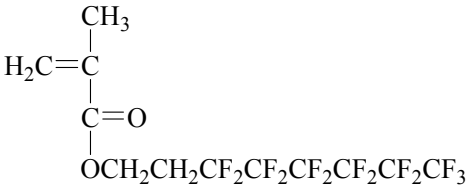
1.2 Lot No.

6Y002

1.3 Supplier

DAIKIN INDUSTRIES, LTD.

1.4 Structural Formula



(Molecular formula: $C_{12}H_9F_{13}O_2$)

1.5 Purity

99.8%

1.6 Names and Concentration of Impurities

Unknown component 0.2%

1.7 Physicochemical Properties

Appearance at Ordinary Temperature: clear colorless liquid

Molecular Weight: 432.18

Stability: –

Melting Point: –

Boiling Point: 92ºC (8 mmHg)

Vapor presser: –

Partition Coefficient (1-octanol/water partition coefficient): –

Hydrolyzability: Unknown

$CH_2=C(CH_3)COOCH_2CH_2C_6F_{13} \rightarrow C_6F_{13}CH_2CH_2OH+CH_2=C(CH_3)COOH$

Degree of Solubility: Water; insoluble

DMSO; soluble (arbitrary mixable)

Acetone; soluble (arbitrary mixable)

1.8 Storage Conditions

The test substance was stored at room temperature (cabinet No. 1 in the test substance storage room, permissible temperature range: 10-30ºC) under light screening.

1.9 Handling Precaution

Glove, mask, cap and lab coat were put on.

2. ANIMALS

Crl:CD(SD) rats (SPF) of thirty three males and thirty three females were obtained from Charles River Japan, Inc. Hino Breeding Center, 735, Shimokomatsuki, Hino-cho, Gamo-gun, Shiga 529-1633, Japan. Animals were acclimatized for 9 days including 6 days quarantine. One male showed wound of the lip during the acclimation period, and all animals excluded this male were allocated to groups to ensure homogeneity of mean body weights using body weight-stratified randomization on two days before the start of the administration. The animals not treated were excluded from the study and euthanized under ether anesthesia. At the onset of the treatment, the animals were five weeks old with body weight ranges of 140.4-159.9 g and 116.3-141.3 g for males and females, respectively. Animals were identified by means of a marker on the tail before grouping, and ear-tags after grouping.

3. HOUSING CONDITIONS

All animals were bred at the barrier-system animal rooms (room No. 4 during the quarantine period, room No. 8 after the quarantine), which were maintained at a stable temperature (21-25°C) and relative humidity (40-70%) with 10-15 air changes per hour and artificial light-dark cycle of 12-12 hours (light on: 7:00 and light off: 19:00), in the biotron (1) throughout the whole feeding period including the quarantine and acclimation periods. The actual temperature and humidity were 22.5-23.8°C and 49.9-58.8%, respectively.

The rats were housed in hanging stainless steel cages with wire-mesh floor at three or five animals/cage (260 W×380 D×180 H mm, TOKIWA KAGAKU KIKAI) for quarantine and acclimation, and at one animal/cage (165 W×300 D×150 H mm, TOKIWA KAGAKU KIKAI) after grouping. Undertrays were changed once a week before grouping, and twice a week after grouping. Feeders, cages and racks were changed once at the grouping, and once at the termination of the dosing period for the recovery group. Racks and cages were identified by individual cards.

The animals had free access to an MF pelleted diet (Lot No. 061204, Oriental Yeast) and chlorinated water from the Hita City supply via automatic watering system with sipper tubes. The diet and housing materials were autoclaved at 121°C for 30 min prior to use. Analysis of the diet was performed in Japan Food Research laboratories, and the analytical data were provided by the manufacturer. The tested parameters met the requirements in our laboratories according to the “Toxic Substances Control Act of US-EPA”. Contaminants in drinking water were analyzed

twice yearly in Oita Prefecture Pharmaceutical Association according to the water regulations of the "Notification No. 101 of Environmental Health Bureau, MHLW" except for test of the taste in our Hita laboratory. Contaminants in the water were in the stated ranges in our laboratory.

4. GROUPING

Grouping was as follows.

Group	Dose	Volume	Concentration of dosing formulation	Number of Animals (Animal No.)	
	(mg/kg/day)	(mL/kg)	(w/v%)	Male	Female
Vehicle control	0	10	0	5 (1 - 5)	5 (31 - 35)
Vehicle control (recovery)	0	10	0	5 (6 - 10)	5 (36 - 40)
Low dose	1	10	0.01	5 (11 - 15)	5 (41 - 45)
Intermediate dose	5	10	0.05	5 (16 - 20)	5 (46 - 50)
High dose	25	10	0.25	5 (21 - 25)	5 (51 - 55)
High dose (recovery)	25	10	0.25	5 (26 - 30)	5 (56 - 60)

Rationale for dosage selection: A range finding study of a 7-day repeated oral treatment was performed at 0, 25, 250, 500 and 1,000 mg/kg/day in our Hita laboratory. As a result, an increase in relative liver weight was noted at 25 mg/kg or more. Increases in absolute liver and relative kidney weights, and enlargement of the liver were noted at 250 mg/kg and more. Death occurred at 1,000 mg/kg. Accordingly, the high dose was set at 25 mg/kg/day and lower doses of 5 and 1 mg/kg were set for the present study. Recovery groups were also set for the 25 mg/kg and vehicle control groups.

5. STABILITY OF THE TEST SUBSTANCE

Stability of the test substance during the dosing period was confirmed with infrared spectrophotometer (IR) in our Hita laboratory (See APPENDIX 2, Study code: X18-0837). IR spectrum of the test substance within 4000 cm^{-1}-400 cm^{-1} was compared with that provided by the sponsor before dosing to determine the identity. The test substance was also analyzed to confirm the stability before and after the dosing period to confirm the stability.

6. PREPARATION OF FORMULATIONS

6.1 Vehicle

Hydrolyzability of the test substance had been unknown. Therefore, olive oil (Lot No. 040OHY, Fujimi Pharmaceutical) was selected as the vehicle.

6.2 Preparation and Storage

The test substance was accurately weighed and mixed with olive oil to prepare the 0.25 w/v% formulation under light shielding. The 0.01 and 0.05 w/v% formulations were prepared from the

0.25 w/v% formulation by dilution with olive oil. These preparations were performed once a day, once five days or once a week. The formulations were stored at a dark and cold place.

6.3 Homogeneity and Stability Tests

The homogeneity and stability analyses were performed in our Hita laboratory (See APPENDIX 2, Study code: X18-0837). In the homogeneity analysis, top, middle and bottom layers of the 1.0 and 0.01 w/v% formulations were taken (n=1) immediately after preparation, and quantitatively analyzed by Gas chromatography (n=1) after sample pretreatment. In the stability test, the formulations for homogeneity samples were stored at the dark and cold place and analyzed after 5 and 9 days. The test substance in formulations was confirmed to be stable for 8 days.

6.4 Concentration Conformation

The concentration analysis was performed in our Hita laboratory (See APPENDIX 2, Study code: X18-0837). The concentrations of 0.25, 0.05 and 0.01 w/v% formulations were confirmed to be within 100±10% of each nominal concentration at the first preparation of the dosing period. In addition, concentration of the 0.01 w/v% formulation was lower than the range of the nominal concentration at first preparation; therefore, this formulation was diluted from the 0.25 w/v% formulation again, and confirmed to be within 100±10%.

7. ADMINISTRATION

The formulations were repeatedly administered daily in the morning by oral gavage using a syringe (Terumo) connected to a Nelaton catheter (Terumo) for 28 days. Thereafter, a 14-day recovery period was set.

8. OBSERVATIONS

Concerning the numbering of day and week, the day of the initiation of dosing was regarded as day 1, the day before initiation of dosing as day −1 and the week of initiation of dosing as week 1. The day after the last dosing was regarded as day 1 (recovery) and the week of the initiation of recovery as week 1 (recovery).

8.1 Clinical Signs

During the dosing period, all animals were observed three times a day, i.e., before dosing, during and after dosing, and in the afternoon, daily from day 1 to day 28. During the recovery period, observation was performed twice daily.

8.2 Detailed Clinical Observations

The detailed examinations in all animals were performed once before dosing. Thereafter, the examinations were performed once weekly during the dosing and recovery periods on a blind test basis. The blind test was performed using the random numbers and observation labels without

identifying the dosing group.

1) Observations at removal from cage

Animal reactions such as excitement from external stimuli (holding animals or bringing a hand close to animals to hold, etc.) were observed.

Observation items: Ease of removal, Vocalization

2) Handling observations

Observation items: Muscle tone, Hypothermia, Piloerection, Hair appearance (staining and unkempt hair), Skin and mucous color (paleness, reddening and cyanosis), Eyes (lacrimation, exophthalmos and pupillary size), Salivation, Secretion

3) Observation in arena

Animals were placed in a standard arena (on an observation platform) and observed for 1 min or more, and the frequencies of defecation (number of feces) and urination (number of pools) were recorded for 1 min.

Observation items: Posture, Motor activity level, Respiration, Lid closure, Gait characteristics, Tremor, Twitch, Convulsion, Stereotypical behavior, Abnormal behavior

8.3 Sensorimotor Function

All animals were examined in week 4 of the dosing period, but not in the recovery period since no abnormalities were noted in week 4 of the dosing period.

1) Reflex

Reactions of animals were observed and made a score when proper stimuli were given their test subjected sensory organs. The examinations were also performed on a blind test basis.

(1) Approach contact/touch response

The animal's response when a blunt probe was brought approximately 3 cm from the animal's nose for 4 seconds was assessed.

(2) Pinna response

The animal's response when a sudden sound of a finger snap was produced was assessed.

(3) Pain response

The animal's response when the animal's tail was pinched with a clothespin between one-third and base of the tail was assessed.

(4) Pupillary reflex

Following darkness adaptation of the animal's eyes, pupil constriction in response to a bright beam of a penlight was observed.

(5) Air righting reflex

The animal's response when the animal was held with ventral surface uppermost approx. 30 cm height from the flat surface and released was assessed.

2) Grip strength

The forelimbs and hindlimbs grip strengths were measured with the automated grip strength meter (COLUMBUS) on a blind test basis. Two trials were performed, and the mean values of the forelimbs and hindlimbs were calculated for each animal.

3) Locomotor activity counts

Locomotor activity level of each animal was counted with an activity monitoring system (SCANET: MV-10, MAYTES) by the number of crossing IR beam for 1 hour at 10 min intervals.

8.4 Body Weights

Body weights were measured at allocation to groups, and on days 1, 3, 8, 12, 17, 21, 26 and 28 during the dosing period and on days 1, 5, 10 and 14 (recovery) during the recovery period. In addition, immediately before necropsy, body weights were measured for calculation of the relative organ weights.

8.5 Food Intakes

Food intakes were measured at allocation to groups, and on days 1, 3, 8, 15, 22 and 28 during the dosing period and on days 1, 4, 8 and 14 (recovery) during the recovery period. Mean food intakes per day were calculated from their remainders for each period.

8.6 Hematological Examinations

Blood or plasma samples were obtained by blood sampling from the abdominal aorta under ether anesthesia after overnight fasting (16 to 20 hr) at completion of the dosing period (excluding the recovery groups) and at completion of the recovery period. The samples were determined for the following items. The blood smears were also made for the microscopic examination in case of the measurement by the following instruments could not be done. As an anticoagulant, 3.2% sodium citrate aqueous solution (Lot No. LTR3558, Wako Pure Chemical Industries) was used for the determination of prothrombin time and activated partial thromboplastin time, and EDTA-2K (Lot No. G5071, Sysmex) for other measurements.

	Parameters		Method
1)	Red blood cell count (RBC)	($\times 10^4/\mu L$)	Electrical resistance detection
2)	White blood cell count (WBC)	($\times 10^2/\mu L$)	Electrical resistance detection
3)	Hemoglobin conc. (Hb)	(g/dL)	Noncyanhemoglobin method
4)	Hematocrit value (Ht)	(%)	$\frac{RBC \times MCV}{10^3}$
5)	Mean corpuscular volume (MCV)	(fL)	Electrical resistance detection
6)	Mean corpuscular hemoglobin (MCH)	(pg)	$\frac{Hb}{RBC} \times 10^3$
7)	Mean corpuscular hemoglobin conc. (MCHC)	(g/dL)	$\frac{Hb}{Ht} \times 10^2$

8)	Platelet count (Platelet)	($\times 10^4/\mu L$)	Electrical resistance detection
9)	Reticulocyte count (Reticulo)	(%)	RNA staining
10)	Prothrombin time (PT)	(sec)	Magnetic sensor
11)	Activated partial thromboplastin time (APTT)	(sec)	Magnetic sensor
12)	Differentiation of leukocytes	(%)	Flow cytometry technique
	Neutrophils (Neutro)		
	Eosinophils (Eosino)		
	Basophils (Baso)		
	Lymphocytes (Lymph)		
	Monocytes (Mono)		
	Large unstained cells (LUC)		

1) - 8) CELL-DYN3500, Abbott Laboratories

9), 12) ADVIA 120, Bayer Medical

10), 11) KC-10A, AMELUNG

8.7 Blood Chemical Examinations

Serum samples were separated from blood samples collected at the same times as those described in section 8.6, and the following items were determined in the obtained serum samples.

	Parameters		Method
1)	Aspartate aminotransferase (AST)	(IU/L)	UV method (method based on JSCC)
2)	Alanine aminotransferase (ALT)	(IU/L)	UV method (method based on JSCC)
3)	Alkaline phosphatase (ALP)	(IU/L)	*p*-Nitrophenyl phosphate method
4)	Cholinesterase (ChE)	(IU/L)	Butyrylthiocholine iodide method
5)	γ-Glutamyl transpeptidase (γ-GTP)	(IU/L)	L-γ-glutamyl-3-carboxy-4-nitroanilide method
6)	Total cholesterol (T-Cho)	(mg/dL)	COD·ADPS method
7)	Triglyceride (TG)	(mg/dL)	GPO·ADPS glycerol blocking method
8)	Glucose	(mg/dL)	Hexokinase·G-6-PDH method
9)	Total protein (T-Protein)	(g/dL)	Biuret method
10)	Albumin	(g/dL)	Bromocresol green method
11)	A/G ratio		$\frac{\text{Albumin}}{\text{T - Protein} - \text{Albumin}}$ (calculated value)
12)	Blood urea nitrogen (BUN)	(mg/dL)	Urease·GlDH method
13)	Creatinine	(mg/dL)	Creatininase·F-DAOS method
14)	Total bilirubin (T-Bil)	(mg/dL)	Enzyme method
15)	Calcium (Ca)	(mg/dL)	OCPC method
16)	Inorganic phosphorus (IP)	(mg/dL)	Fiske-Subbarow method

17)	Sodium (Na)	(mEq/L)	Crown-Ether membrane electrode method
18)	Potassium (K)	(mEq/L)	Crown-Ether membrane electrode method
19)	Chloride (Cl)	(mEq/L)	Coulometric titration method

1), 2), 4), 9), 10), 14)	7150 Automatic Analyzer, Hitachi
3), 5)-8), 12), 13), 15), 16)	7170 Automatic Analyzer, Hitachi
17)-19)	PVA-αIII, A & T

8.8 Urinalyses

Urinalysis was performed once (day 28) during the dosing period (excluding the recovery groups) and once (day 14) during the recovery period. Urine samples (accumulated for 15-17 hr) collected in individual metabolic cages (150 W×200 D×263 H mm) were determined with drinking water *ad libitum*. The urine sediments were stained and examined in males and females of the vehicle control and 25 mg/kg groups at the end of the dosing period.

	Parameters		Method
1)	Urine volume	(m/L)	Volumetric method
2)	Color		Macroscopy
3)	Turbidity		Macroscopy
4)	Urine specific gravity (Sp.Gr.)		Refractive index
5)	pH		Test paper
6)	Protein		Test paper
7)	Glucose		Test paper
8)	Occult blood		Test paper
9)	Urinary sediments		Sternheimer modified

1)	Measuring cylinder
4)	SPR-N, ATAGO
5)-8)	Hema-Combistix, Bayer Medical
9)	Biological microscope, BH2, OLYMPUS

8.9 Pathological Examinations

1) Necropsy

All animals were subjected to the detailed gross necropsy including body surface, all orifices, subcutaneous tissue, cranial, thoracic, abdominal and pelvic cavities, and these contents.

2) Organ weights

The weights of the following organs were measured in all animals. The relative organ weight was calculated based on the body weight at the time of necropsy.

Liver(g), heart(g), kidneys*(g), testes*(g), epididymides*(g), ovaries*(mg), brain(g), spleen(g), thymus(mg) and adrenals*(mg)

* Left and right organs were measured totally.

3) Histopathological examinations

(1) The following organs and tissues were taken in all animals.

Category	Organs and Tissues
Respiratory system	Trachea, lungs
Digestive system	Incisors, stomach, intestine (duodenum to rectum, with Peyer's patches), liver
Cardiovascular system	Heart
Urinary system	Kidneys, urinary bladder
Reproductive system	Testes, epididymides, prostate, seminal vesicle, ovaries, uterus, vagina
Nervous system	Brain (cerebrum, cerebellum and pons), spinal cord, sciatic nerve
Hematopoietic and lymphatic systems	Bone marrow (femur), axillar and mesenteric lymph nodes, spleen, thymus
Endocrine system	Pituitary gland, thyroid (with parathyroids), adrenals
Special sense organ	Eye balls

The trachea, lungs and urinary bladder were filled with 10% neutralized buffered formalin before taken. The stomach and intestines were filled and fixed with 10% neutralized buffered formalin and were washed with water. All organs/tissues were preserved in 10% neutralized buffered formalin. However, testes and epididymides were fixed in Bouin's solution.

(2) The following organs and tissues were taken as macroscopic lesions.

Group (Animal No.)	Organs and Tissues
5 mg/kg group (No. 18)	Skin
25 mg/kg group (No. 53)	Skin

(3) Light microscopic examinations were performed for the organs and tissues of the following groups after embedding in paraffin, sectioning and hematoxylin and eosin (HE) staining. Decalcification was done for incisors and bone marrow (femur) with 10% formic acid formalin before cutting.

In the table, parentheses show examinations which were not done although HE specimen was prepared since no abnormality was noted histopathologically in the 25 mg/kg group.

Organ and tissue	Vehicle control group	Vehicle control recovery group	1 mg/kg group	5 mg/kg group	25 mg/kg group	25 mg/kg recovery group
Trachea	♂♀	-	-	-	♂♀	-
Lungs	♂♀	-	-	-	♂♀	-
Incisors[a)]	♂♀	♂♀	♂♀	♂♀	♂♀	♂♀
Forestomach	♂♀	-	-	-	♂♀	-
Glandular stomach	♂♀	-	-	-	♂♀	-
Duodenum-ileum	♂♀	-	-	-	♂♀	-
Cecum- rectum	♂♀	-	-	-	♂♀	-
Liver[b)]	♂♀	(♀)	(♀)	(♀)	♂♀	(♀)
Heart	♂♀	-	-	-	♂♀	-
Kidneys[b)]	♂♀	(♂)	(♂)	(♂)	♂♀	(♂)
Urinary bladder	♂♀	-	-	-	♂♀	-
Testes	♂	-	-	-	♂	-
Epididymides	♂	-	-	-	♂	-
Prostate	♂	-	-	-	♂	-
Seminal vesicles	♂	-	-	-	♂	-
Ovaries	♀	-	-	-	♀	-
Uterus	♀	-	-	-	♀	-
Vagina	♀	-	-	-	♀	-
Cerebrum, cerebellum, pons	♂♀	-	-	-	♂♀	-
Spinal cord	♂♀	-	-	-	♂♀	-
Sciatic nerve	♂♀	-	-	-	♂♀	-
Bone marrow	♂♀	-	-	-	♂♀	-
Axillar lymph nodes	♂♀	-	-	-	♂♀	-
Mesenteric lymph nodes	♂♀	-	-	-	♂♀	-
Spleen	♂♀	-	-	-	♂♀	-
Thymus	♂♀	-	-	-	♂♀	-
Pituitary gland	♂♀	-	-	-	♂♀	-
Thyroids	♂♀	-	-	-	♂♀	-
Parathyroids	♂♀	-	-	-	♂♀	-

Adrenals	♂♀	-	-	-	♂♀	-
Eye balls	♂♀	-	-	-	♂♀	-

a) Since changes suspected to be effects of the test substance were noted in males and females of the 25 mg/kg recovery group, histopathological examinations for both sexes of all groups including the recovery groups were done.

b) Since changes suspected to be effects of the test substance were noted in the organ weights in males or females of the 25 mg/kg group, HE staining samples were prepared for both sexes of all groups including the recovery groups.

(4) The following organs/tissues were examined as macroscopic lesions.

Group (Animal No.)	Organs and tissues
1 mg/kg group (No. 12)	Spleen
5 mg/kg group (No. 18)	Skin
5 mg/kg group (No. 47)	Thyroids
25 mg/kg group (No. 53)	Skin

9. STATISTICAL ANALYSIS

Data regarding body weights (excluding those at the time of necropsy), food intakes, hematological examinations, blood chemical examinations, urine volume, urine specific gravity, organ weights, grip strength and locomotor activity counts were analyzed by using the Bartlett's test for homogeneity of variance. If the variances were homogeneous at a significance level of 5%, one way analysis of variance was performed. If there was a significant difference in this analysis, the difference between the vehicle control group and each of the treatment group was analyzed by the Dunnett's test. If the variances were not homogeneous, the Kruskal-Wallis's test was used. If there was a significant difference in this analysis, the difference between the vehicle control group and each of the treatment group was analyzed by the nonparametric Dunnett's test.

The frequencies of defecation (number of feces) and urination (number of pools) were analyzed by using the Kruskal-Wallis's test. If there was a significant difference in this analysis, the difference between the vehicle control group and each of the treatment group was analyzed by the nonparametric Dunnett's test.

ENVIRONMENTAL FACTORS THAT MIGHT HAVE AFFECTED THE RELIABILITY OF THE STUDY RESULTS

Food intakes were increased in the vehicle and 25 mg/kg recovery groups on day 14 of the recovery period. There was no large quantity of spilled feed. In addition, there were no abnormalities in an electric balance, row data or calculating formula. Therefore, the cause of this event was unclear. However, since no abnormal body weight or food intakes were observed during the dosing or recovery periods except for day 14 of recovery. Therefore, these changes were not considered to be the effect attributable to the test substance and the evaluation of the test results was considered to be valid.

RESULTS

1. CLINICAL SIGNS (Table 1, Addendum 1)

1.1 During Dosing Period

Males: Post dose salivation was noted in two animals of the vehicle control group, two animals of the 1 mg/kg group, one animal of the 5 mg/kg group and nine animals of the 25 mg/kg group. Exudate, scab formation and loss of hair were noted in one animal of the 5 mg/kg group.

Females: Post dose salivation was noted in four animals of the vehicle control group and seven animals of the 25 mg/kg group. Loss of hair was noted in one animal of the 25 mg/kg group.

1.2 During Recovery Period

Males: Mottled teeth were noted in all animals of the 25 mg/kg group.

Females: Mottled teeth were noted in one animal of the 25 mg/kg group.

2. DETAILED CLINICAL OBSERVATIONS (Table 2, Addendum 2)

2.1 During Dosing Period

Males: Continuous vocalization was noted in each one animal of the vehicle control group in week 2 and 25 mg/kg group in week 3 when the animals were taken out form their cages.

Females: No abnormalities were observed in any treatment groups.

2.2 During Recovery Period

No abnormalities were noted in either sex of the 25 mg/kg recovery group.

3. SENSORIMOTOR FUNCTION (Tables 3, 4 and 5, Addenda 3, 4 and 5)

3.1 During Dosing Period

No abnormalities were observed in either sex of any treatment groups.

3.2 During Recovery Period

Males and females were not examined since no abnormalities attributable to the test substance were noted during the dosing period.

4. BODY WEIGHTS (Fig.1, Table 6, Addendum 6)

4.1 During Dosing Period

No statistically significant changes of body weights were noted in males or females of any treatment groups.

4.2 During Recovery Period

No statistically significant changes of body weights were noted in males or females of the 25 mg/kg recovery group.

5. FOOD INTAKES (Fig.2, Table 7, Addendum 7)

5.1 During Dosing Period

No significant changes were noted in males or females of any treatment groups.

5.2 During Recovery Period

Food intakes were increased in males and females of the vehicle and 25 mg/kg recovery groups on day 14 of recovery.

6. HEMATOLOGICAL EXAMINATIONS (Table 8, Addendum 8)

6.1 At Termination of Dosing Period

Males: A statistically significant prolonged APTT was noted in the 25 mg/kg group.

Females: No statistically significant changes were noted in any treatment groups.

6.2 At Termination of Recovery Period

Males: No statistically significant changes were noted in the 25 mg/kg recovery group.

Females: Hb was statistically decreased in the 25 mg/kg recovery group.

7. BLOOD CHEMICAL EXAMINATIONS (Table 9, Addendum 9)

7.1 At Termination of Dosing Period

Males: No statistically significant changes were noted in any treatment groups.

Females: ChE was significantly increased in the 1 mg/kg group.

7.2 At Termination of Recovery Period

Males: A statistically significant decrease in IP was noted in the 25 mg/kg recovery group.

Females: No statistically significant changes were noted in the 25 mg/kg recovery group.

8. URINALYSES (Table 10, Addendum 10)

8.1 At Termination of Dosing Period

No abnormalities were noted in either sex of any treatment groups.

8.2 At Termination of Recovery Period

No abnormalities were noted in either sex of the 25 mg/kg recovery group.

9. ORGAN WEIGHTS (Tables 11 and 12, Addenda 11 and 12)

9.1 At Termination of Dosing Period

Males: Relative kidney weight was significantly increased in the 25 mg/kg group.

Females: Increased absolute and relative liver weights were noted in the 25 mg/kg group.

9.2 At Termination of Recovery Period

No statistically significant changes were noted in males or females of the 25 mg/kg recovery group.

10. NECROPSY (Table 13, Addendum 13)

10.1 At Termination of Dosing Period

Males: Whitish region on the capsule of the spleen was noted in one animal (No. 12) of the 1 mg/kg group. Loss of hair was noted in one animal (No. 18) of the 5 mg/kg group.

Females: Whitish region on the capsule of the spleen was noted in one animal (No. 34) of the vehicle control group. Decrease in size of the left lobe of the thyroid was noted in one animal (No. 47) of the 5 mg/kg group. Sparsed fur of the neck was noted in one animal (No. 53) of the 25 mg/kg group.

10.2 At Termination of Recovery Period

Males: Mottled teeth were noted in three animals (Nos. 27, 29 and 30) of the 25 mg/kg recovery group.

Females: Mottled teeth were noted in one animal (No. 56) of the 25 mg/kg recovery group.

11. HISTOPATHOLOGICAL EXAMINATIONS (Table 14, Addendum 13)

11.1 At Termination of Dosing Period

Males: Solitary cyst in the medulla of the kidney in two animals (Nos. 1 and 2) and degeneration of the spermatocytes in the testis in one animal (No. 3) were noted in the vehicle control group. Capsulitis of the spleen was noted in one animal (No. 12) of the 1 mg/kg group. Cell infiltration in the subcutis and scab formation of the skin were noted in one animal (No. 18) of the 5 mg/kg group. Decreased iron pigments of the ameloblasts at maturation stage in the incisors was noted in two animals (Nos. 24 and 25) of the 25 mg/kg group.

Females: Mineralization in the cortico-medullary junction of the kidney in one animal (No. 32) and capsulitis of the spleen in one animal (No. 34) were noted in the vehicle control group. Hypoplasia of the left lobe of the thyroid was noted in one animal (No. 47) of the 5 mg/kg group. Decreased iron pigments of the ameloblasts at maturation stage in the incisor in one animal (No. 51) and decreased hair follicles in one anmal (No. 53) of the 25 mg/kg group were noted.

11.2 At Termination of Recovery Period

No abnormalities were noted in either sex of the 25 mg/kg recovery group.

DISCUSSION

An oral toxicity of 13F-SFMA was examined in male and female Crl: CD (SD) rats treated with a daily dose of 0, 1, 5 and 25 mg/kg/day for 28 days followed by a 14-day recovery period.

No death occurred during the dosing or recovery periods.

Effects of 13F-SFMA were observed in the incisors, liver and kidneys.

As an effect on the incisors, decreased iron pigment of the ameloblasts at maturation stage in the incisors was observed in the histopathological examinations in both sexes of the 25 mg/kg group at the end of the dosing period. Since the test substance possesses fluorine in its structure, and it has been reported that treatment with fluoride compounds induces decreased intracellular iron pigment in the ameloblasts, degeneration and necrosis of the ameloblasts, etc. in animals with a brown enamel surface layer such as the rat[1) 2)]; therefore, this change was considered to be an effect of the test substance.

As effects on the liver, increased absolute and relative weights were observed in females of the 25 mg/kg group, and as an effect on the kidney, increased relative weight was observed in males of the 25 mg/kg group. In the 7-day repeated dose toxicity study of 13F-SFMA (Study code: P11-0837) conducted as a range finding study for the present study, increased liver and kidney weights and enlargement of the liver, and discoloration of the kidney in dead animals were also observed. Therefore, all these changes were considered to be effects of the test substance.

In the clinical signs, salivation was observed in males of all groups including the vehicle control group, and in females of the vehicle control group and the 25 mg/kg group. However, since there were no neurological abnormalities, and it was only observed after administration in all cases, this change was not considered to be of toxicological significance. Also, exudate, scab formation and loss of hair were observed in one male in the 5 mg/kg group. However, since these findings are commonly observed as the spontaneous lesions, they were considered to be incidental changes.

In the detailed clinical observations, continuous vocalization was observed in one male of the 25 mg/kg group in week 3 when the animals were taken out from their cages. However, since this was single occurrence and also observed in the vehicle control group, it was not considered to be treatment related.

In the hematology, APTT was prolonged in males of the 25 mg/kg group. However, since the degree of prolongation was slight, and the change was within the range of our historical control data[3)], this change was not considered to be treatment related.

In the blood chemical examinations, ChE was increased in females of the 1 mg/kg group. However, this change was not dose-dependent.

In the histopathological examinations, decreased hair follicles were observed in one female of the 25 mg/kg group. While this change was macroscopically observed as sparsed fur of the neck, since it is commonly observed as a spontaneous lesion, it was considered to be an incidental change.

In the recovery group, among the treatment related changes, the effect on the incisors was observed as mottled teeth in the necropsy. Since the surface enamel is dissolved during the decalcification, changes corresponding with the macroscopic finding could not be observed histopathologically. However, since no abnormalities were observed in the ameloblasts, it was considered that the macroscopic abnormality is also gradually recovered with extension of the incisors. Also, since no abnormalities were observed in the weight of the liver or kidney either, it was considered that both changes in the liver and kidney are reversible. In addition, increased IP in males and decreased Hb in females were observed in the 25 mg/kg group. However, since similar changes were not observed at the end of the dosing period, and no findings associated with these changes were observed in the kidney or the RBC, etc. at the end of the recovery period, they were considered to be incidental changes.

The above-mentioned results showed that the major effect of 13F-SFMA was decreased iron pigment of the ameloblasts at maturation stage in the incisors. Also, increased weights of the kidney and liver were observed. Therefore, the No-Observed-Adverse-Effect Level (NOAEL) of 13F-SFMA were considered to be 5 mg/kg/day based on decreased iron pigment of the ameloblasts at maturation stage in the incisors in both sexes, increased relative kidney weight in males and increased absolute and relative liver weights in females of the 25 mg/kg group.

1) The Japanese Society of Toxicological Pathology, 2000, Toxicological Histopathology, p.137-152, Secretariat of the Japanese Society of Toxicological Pathology, Tokyo.

2) Hideaki Ogura and Keiichi Ohya, 1995, Study on Physiology and Pharmacology in Hard Tissue－Effects of chemicals on formation and absorption mechanism of teeth and bone－, *Folia Pharmacol*

Jpn, 105, p.305-318.

3) Historical data of hematological examinations of Crl:CD(SD) rats in Hita Laboratory (8-10 weeks old)

Vehicle: olive oil

Items	Sex	n	Mean	Mean-2S.D.	Mean+2S.D.
APTT (sec)	male	201	23.6	14.8	32.4

n: number of animals examined.