FINAL REPORT

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats by Oral Administration

Study Period: December 19, 2016-August 1, 2017

Sponsor: Daikin Industries, Ltd.
1-1 Nishi-Hitotsuya, Settsu-shi, Osaka 566-8585, Japan

Testing Facility: Ina Research Inc.
2148-188 Nishiminowa, Ina-shi, Nagano 399-4501, Japan

Study Director: August 1, 2017
Date

Research Administration Dept.
Safety Research Center
Ina Research Inc.

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1. Abstract

13F-OLE was administered orally to 10 Crl:CD(SD) rats/sex/group at 5, 25 and 200 mg/kg/day to evaluate the potential adverse effects of 13F-OLE on mating, fertility, pregnant/lactating female rats and offspring. The dosing periods set for males were from 14 days prior to mating, throughout the mating period, until the last day of the mating period and for females were from 14 days prior to mating, throughout the mating and gestation periods, until Day 13 of lactation. Animals in the control group received the vehicle, olive oil containing 1.0 w/v% Tween 80, in the same manner for comparison.

In parental animals (F_0), a decrease in the thyroid hormone (T4) levels, large liver and increases in the absolute and relative weights of the liver were noted in males in the 200 mg/kg/day group. No treatment-related effects were noted in the clinical observations, body weights, food consumption or gross pathology.

For the reproductive function of parental animals (F_0), no treatment-related effects were noted on the estrous cycle, copulation index, fertility index, delivery, duration of gestation, gestation index, number of implantation sites, delivery index, birth index, nursing behavior or histopathology of reproductive organs.

In the subsequent generation (F_1), no treatment-related effects were noted in the clinical observations, body weights, number of newborns, number of live newborns, sex ratio, external morphology, viability index on Day 4 or 14 of lactation, anogenital distance, number of nipples/areolas or thyroid hormone (T4) levels.

Based on these results, the no observed adverse effect levels (NOAELs) of 13F-OLE were considered to be 25 mg/kg/day for parental males and 200 mg/kg/day for parental females, parental reproductive function and offspring under the conditions of this study.

2. Tabulated summary

Study Title: Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats by Oral Administration

Test Article	13F-OLE			
Species/Strain	Rat/Crl:CD(SD)			
Initial Age of First Dose	Male: 11 weeks, Female: 10 weeks			
Duration of Dosing	Male: 14 days prior to mating until the day before sacrifice Female: 14 days prior to mating until day 13 of lactation			
Daily Dose (mg/kg/day)	0	5	25	200
F_0 Male				
No. Animals	10	10	10	10
No. Died or Sacrificed Moribund	0	0	0	0
Clinical Observations	–	–	–	–
Body Weight	–	–	–	–
Body Weight Gain	–	–	–	–
Food Consumption	–	–	–	–
Copulation Index (%)	100.0	100.0	90.0	100.0
Fertility Index (%)	100.0	100.0	100.0	100.0
Gross Pathology Liver: Large	0	0	0	7
Organ Weights (mean) Liver Absolute (g)	15.9	16.9	17.4	23.4**
Relative (%)	3.304	3.519	3.669*	4.932**
Thyroid Hormone (T4) Level (ng/mL, mean)	541.0	529.8	506.3	474.8**
Histopathology (testes and epididymides)	–	NE	NE	–
Mean No. Cells in 1 Seminiferous Tubule	–	NE	NE	–
No. Leydig Cells	–	NE	NE	–
Sertoli Cell Ratio	–	NE	NE	–
F_0 Female				
No. Animals	10	10	10	10
No. Died or Sacrificed Moribund	1 (L1)	0	0	0
No. Dams that Delivered Live Newborns	10	10	9	9
No. Total Litter Loss	0	0	1 (L2)	1 (L2)
Until Copulation				
Clinical Observations	–	–	–	–
Body Weight	–	–	–	–
Body Weight Gain	–	–	–	–
Food Consumption	–	–	–	–

Until Copulation (continued)				
Estrous Cycle (mean)	4.2	4.2	4.1	4.2
Copulation Index (%)	100.0	100.0	90.0	100.0
Fertility Index (%)	100.0	100.0	100.0	100.0
Pre-coital Period (mean)	2.1	3.4	2.8	2.7
Gestation Period				
Clinical Observations	–	–	–	–
Body Weight	–	–	–	–
Body Weight Gain	–	–	–	–
Food Consumption	–	–	–	–
Duration of Gestation (mean)	22.0	22.0	22.1	22.0
Implantation Sites (mean)	16.4	16.1	16.4	16.0
Gestation Index (%)	100.0	100.0	100.0	100.0
Delivery Index (%)	96.4	88.9	95.1	93.9
Abnormal Parturition	–	–	–	–
Birth Index (%)	89.3	87.1	84.5	91.2
Lactation Period				
Clinical Observation				
Decrease in Movement	1 [a]	0	1 [b]	0
Soiled Fur	1 [a]	0	0	0
Emaciation	0	0	0	1
Scattering of all Offspring in the Cage	1 [a]	0	1 [b]	2 [c]
Body Weight	–	–	–	–
Body Weight Gain	–	–	–	–
Food Consumption	–	–	–	–
Gross Pathology				
Thymus: Small	1 [a]	0	1 [b]	1 [b]
Spleen: Discoloration, pale	1 [a]	0	0	0
Spleen: Small	0	0	1 [b]	0
Organ Weights (mean)				
Liver Absolute (g)	15.7	16.9	16.9	17.4
Relative (%)	4.130	4.402	4.410	4.672**
Histopathology (ovary)	–	–	–	–
Thyroid Hormone (T4) Level (ng/mL, mean)	374.2	379.2	362.4	382.6

Offspring (F_1)					
No. Litters		10	10	9	9
Mean No. Newborn/Litter		15.8	14.2	15.7	15.0
Mean No. Live Newborn/Litter		14.6	13.9	13.9	14.6
Sex Ratio (Male/Total)		0.50	0.47	0.37	0.55
External Malformation		0	0	0	0
No. Dead Newborn		12	3	16	4
Clinical Observation					
No milk in the stomach		1	0	1	3
Subnormal body surface temperature		1	0	1	1
Death of some offspring		5	3	0	4
Body Weight (mean, g)					
Male	0 days of age	6.37	6.64	6.47	6.17
	4 days of age	10.38	10.86	10.25	8.54
	13 days of age	34.6	35.7	35.4	31.6
Female	0 days of age	6.04	6.33	6.21	5.92
	4 days of age	9.72	10.46	9.96	8.28
	13 days of age	33.4	34.8	34.7	30.5
Viability Index on Day 4 of Lactation (0-4, %)		86.9	98.0	88.9	74.1
Thyroid Hormone (T4) Level (ng/mL, mean)	4 days of age	113.5	130.8	130.3	131.2
AGD (mean)					
Male	Absolute (mm)	6.05	6.31	6.06	5.75
	Relative (mm%)	0.278	0.285	0.281	0.284
Female	Absolute (mm)	3.67	3.77	3.65	3.49
	Relative (mm%)	0.172	0.173	0.171	0.174
No. Nipples/Areolas (mean)		0	0	0	0
Viability Index on Day 14 of Lactation (4-14, %)		100.0	100.0	100.0	100.0
Gross Pathology					
Spleen: Discoloration, pale		0	0	0	1
Stomach: Dilation		0	0	0	1
Stomach: Retention, contents		0	0	0	1
Liver: Focus, greenish brown		0	0	0	1
Thyroid Hormone (T4) Level (ng/mL, mean)	14 days of age	327.2	332.7	328.3	323.0

−: No change or none appreciable * - $p < 0.05$, ** - $p < 0.01$ NE: Not examined
L: Lactation period AGD: Anogenital distance
a: Dead animal, b: Total litter loss, c: Total litter loss in 1 dam

3. Purpose

The purpose of this study was to evaluate the potential adverse effects of 13F-OLE on mating, fertility, pregnant/lactating female rats and offspring when 13F-OLE was administered orally to males from 14 days prior to mating until the last day of the mating period and to females from 14 days prior to mating, throughout the mating and gestation periods, until 13 days after delivery.

4. Sponsor

Name: Daikin Industries, Ltd.

Address: 1-1 Nishi-Hitotsuya, Settsu-shi, Osaka 566-8585, Japan

5. Testing facility

Name: Ina Research Inc.

Address: 2148-188 Nishiminowa, Ina-shi, Nagano 399-4501, Japan

6. Facility for verification of concentrations of dosing formulations

Name: Chemicals Evaluation and Research Institute, Japan
Hita Laboratory

Address: 3-822 Ishii-machi, Hita-shi, Oita 877-0061, Japan

Delegated phases of the study:
Verification of concentrations of dosing formulations and storage of related documents

Chief analyst:

Study No.: X02-0302

7. Facility for determination of thyroid hormone levels

Name: Sekisui Medical Co., Ltd.
Drug Development Solutions Business
Drug Development Solutions Center

Address: 2117 Muramatsu, Tokai-mura, Naka-gun, Ibaraki 319-1182, Japan

Delegated phases of the study:
Determination of thyroid hormone (T4) levels in serum and storage of related documents

Chief analyst:

Study No.: TK-1318-G

8. Study Director and study personnel

Study Director: , Ph.D.

Study personnel:

Quarantine: , D.V.M., DJCLAM

Animal care and use:

Preparation of the dosing formulations:

Sample collection for determination of thyroid hormone (T4) levels:

Necropsy and organ weights:

Histotechnology:

Histopathology:

9. Study schedule

Initiation of the study: December 19, 2016

Initiation of experiments (initiation of dosing): January 10, 2017

Animal receipt: December 20, 2016

Initiation of dosing: January 10, 2017

Initiation of mating: January 24, 2017

Necropsy of males: February 8, 2017

Terminal necropsy: March 2 to 10, 2017

Termination of experiments (day of determination of thyroid hormone (T4) levels): April 21, 2017

Completion of the study: August 1, 2017

10. Regulatory compliance

GLP standards: Standard Concerning Testing Laboratories Implementing Tests for New Chemical Substances etc. (Notification No. 0331-8, Ministry of Health, Labour and Welfare, Notification No. Heisei 23.03.29-6, Ministry of Economy, Trade and Industry and Notification No. 110331010, Ministry of the Environment of Japan, Mar. 31, 2011), with the exception that analyses of the characteristics and stability of the test article, stipulated in Article 22 of the Ordinance, were conducted under non-GLP conditions

Guidelines: OECD GUIDELINE FOR THE TESTING OF CHEMICALS 421 (2016), Reproduction/Developmental Toxicity Screening Test

11. Animal welfare

This study was conducted in compliance with the "Act on Welfare and Management of Animals" in Japan and the "Guidance for Animal Care and Use" of Ina Research Inc. and in accordance with the protocol reviewed by the Institutional Animal Care and Use Committee (IACUC) of Ina Research Inc., which is fully accredited by AAALAC International (Accredited Unit No. 001107).

12. Materials and methods

12.1 Test article

Name: 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluoro-octa-1-ene (hereafter referred to as "13F-OLE")

Supplier: Sponsor

CAS No.: 25291-17-2

Lot No.: C2160215

Characteristics (Attachment 1, non-GLP):

Structural formula: $H_2C{=}C(H)\text{-}CF_2CF_2CF_2CF_2CF_2CF_3$

Purity: 99.95%

Description: Transparent, colorless liquid

Density:	1.560 g/cm^3
Stability (non-GLP):	The test article (the same lot as used in this study) stored at the facility of the sponsor was analyzed in a separately conducted quality test after termination of the dosing period. The stability of the test article during the period of use was confirmed based on the results obtained from the sponsor.
Storage conditions:	Light-resistant container at room temperature (acceptable range 10.0-30.0°C, actual temperature 16.3-23.4°C) The actual temperature represents the range recorded from the day of receipt at Ina Research Inc. to the last day of use in this study (Nov. 21, 2016 to Mar. 7, 2017).
Storage area:	Test Article Storage Room of Ina Research Inc.
Archival sample:	10 mL
Disposition:	The unused portion of the test article was discarded in an exclusive container for industrial waste incineration.

12.2 Control article/vehicle

12.2.1 Tween 80

Name:	Tween 80
Manufacturer:	Kanto Chemical Co., Inc.
Lot No.:	808H1833
Storage conditions:	Tight, light-resistant container at room temperature (acceptable range 1.0-30.0°C, actual temperature 19.1-21.2°C) The actual temperature represents the range recorded from the first day of management to the last day of use in this study (Dec. 29, 2016 to Mar. 7, 2017).
Storage area:	Test Article Storage Room of Ina Research Inc.
Archival sample:	10 mL

12.2.2 Olive oil

Name:	Olive Oil, JP (hereafter referred to as “olive oil”)
Manufacturer:	Kozakai Pharmaceutical Co., Ltd.
Lot Nos.:	UJ-08, VI-09 and VH-18

Storage conditions:	Room temperature (acceptable range 1.0-30.0°C, actual temperature 19.1-21.2°C)
	The actual temperature represents the range recorded from the first day of management to the last day of use in this study (Dec. 29, 2016 to Mar. 7, 2017).
Storage area:	Test Article Storage Room of Ina Research Inc.
Archival samples:	10 mL/lot

12.3 Formulations

12.3.1 Preparation of the control formulation/vehicle

Method of preparation:	At the first preparation, the control formulation/vehicle was prepared as follows:
	Olive oil was added to Tween 80 (2.00 g, equivalent to 1.0 w/v% of the final volume) to a volume of 200 mL to yield olive oil containing 1.0 w/v% Tween 80.
	Subsequent preparations were made in the same proportion. The volumes prepared at each preparation during the study period were 100-1200 mL.
Storage conditions:	Tight, light-resistant container under refrigeration (acceptable range 1.0-8.0°C, actual temperature 1.8-7.4°C)
	The control formulation/vehicle was used within 8 days, including the day of preparation, as prescribed in the protocol.
Storage area:	Refrigerator in the Test Article Preparation Room of Ina Research Inc.

12.3.2 Preparation of the test article dosing formulations

Methods of preparation:	At the first preparation, the test article dosing formulations were prepared as follows:
40 mg/mL formulation:	Tween 80 (1.01 g, equivalent to 1.0 w/v% of the final volume) was added to the test article (2.56 mL). While this mixture was being stirred thoroughly, olive oil was added to a volume of 100 mL to yield a 40 mg/mL formulation. Since the test article is a liquid, the concentration of the test article was regarded as 1560 mg/mL, which was calculated from its density.

5 mg/mL formulation:	A thoroughly stirred 40 mg/mL formulation (7.50 mL) was taken and diluted with olive oil containing 1.0 w/v% Tween 80 to a volume of 60 mL.
1 mg/mL formulation:	A thoroughly stirred 40 mg/mL formulation (1.50 mL) was taken and diluted with olive oil containing 1.0 w/v% Tween 80 to a volume of 60 mL.
	Subsequent preparations were made in the same proportions. The volumes prepared at each preparation during the study period were 80-420 mL for the 40 mg/mL formulation, 50-360 mL for the 5 mg/mL formulation and 20-360 mL for the 1 mg/mL formulation.
Storage conditions:	Tight, light-resistant containers under refrigeration (acceptable range 1.0-8.0°C, actual temperature 1.8-7.4°C)
	The test article dosing formulations were used within 8 days, including the day of preparation, as prescribed in the protocol.
Storage area:	Refrigerator in the Test Article Preparation Room of Ina Research Inc.
Disposition:	The unused portions of the test article dosing formulations were discarded in an exclusive container for industrial waste incineration.

12.3.3 Analyses of the dosing formulations

12.3.3.1 Stability and homogeneity of the test article in the vehicle

Analytical facility:	Chemicals Evaluation and Research Institute, Japan Hita Laboratory
Analytical report:	Verification of the stability of 13F-OLE and the homogeneity, stability and concentrations of formulations (Study Code No. X18-0838)
Summary results:	It has been confirmed that 0.04 and 10.0 w/v% 13F-OLE formulations in the vehicle (olive oil containing 1.0 w/v% Tween 80) are homogeneous and stable for 8 days after preparation when stored in a cold dark place.

12.3.3.2 Verification of the concentrations

Analytical facility:	Chemicals Evaluation and Research Institute, Japan Hita Laboratory
Timing:	At the first preparation At the last preparation of all dosing formulations
Samples:	1 mL samples from all dosing formulations, including the control formulation
Shipment of samples:	After preparation of the dosing formulations, samples were promptly taken from each formulation while being stirred using a magnetic stirrer and put into light-resistant containers. The samples were labeled with the sample information (the Study No., name of the test article, concentration, sample size, date of preparation and a color-coded sticker indicating the individual groups) and sent under refrigeration to the facility for verification of concentrations of the dosing formulations.
Dates of shipment:	January 3 and February 28, 2017
Analytical method:	Concentrations were determined using gas chromatography.
Criteria for acceptability:	
Test article dosing formulations:	Within 90.0-110.0% of the nominal concentrations
Control formulation:	No peaks derived from 13F-OLE are present in the chromatogram.
Results (Attachment 2):	Conformed (No peaks derived from 13F-OLE were present in the chromatogram of the control formulation and the measured concentrations of the test article dosing formulations were 91.6-93.8% of the nominal concentrations.) A copy of the analysis report prepared by the facility for verification of concentrations of the dosing formulations was obtained and the analytical results were reflected in the final report.

12.4 Test system

Species:	Rat (SPF)
Strain:	Crl:CD(SD)

Supplier: Charles River Laboratories Japan, Inc. (Atsugi Breeding Center)

Justification for selection of the species:
The rat, for which the OECD Guideline 421 is designed, was selected for this study. In addition, reproductive and teratological background data for this strain of rats are well established and historical data are available.

Number and sex ordered (received):
44 males and 52 females

Age at receipt: Males: 8 weeks
Females: 7 weeks

Quarantine/acclimation: The animals were quarantined for 6 days from receipt. During this period, the animals were observed for clinical signs daily and were weighed on the first (at receipt), third and seventh (last) days of quarantine.

The animals were acclimated from receipt until the day of group assignment and were also observed for clinical signs daily after the quarantine period until 1 day prior to initiation of dosing. Body weights were measured on the day of group assignment. Sampling and examinations of vaginal smears were conducted for females from 13 days prior to group assignment.

Age at initiation of dosing: Males: 11 weeks
Females: 10 weeks

Body weights at group assignment:
Males: 368-437 g
Females: 244-280 g

12.5 Husbandry

Animal room: Room No. 112

Temperature: Acceptable range: 21.0-25.0°C
Actual temperature: 22.5-23.2°C

Humidity: Acceptable range: 40.0-70.0%
Actual humidity: 52.0-70.8%

See section 13, "Deviations from the protocol and unforeseeable circumstances".

Ventilation: Acceptable range: 15-17 clean, fresh air changes per hour

Lighting: 12-hour light-dark cycle (fluorescent lighting from 7:00 to 19:00)

Feeding method: The animals were allowed free access to the feed in stainless steel feeders.

Watering method: Drinking water was provided to the animals *ad libitum* via an automatic watering system. Glass water bottles fitted with sipper tubes were used during housing in polycarbonate cages.

Cages: The animals were housed in the following cages depending on the housing period:

Housing period	Cage type	No. of animals/cage	Frequency of cage changes
Acclimation period and from group assignment until initiation of mating	Stainless steel wire mesh cages [a)]	1 animal	At least once every 2 weeks
During co-housing	Stainless steel wire mesh cages [a)]	1 animal/sex	
Days 0-18 of gestation	Stainless steel wire mesh cages [a)]	1 animal	
From Day 18 of gestation to the end of the lactation period	Polycarbonate cages [b)]	1 dam with its offspring	At least once every 4 days Cages were not changed from Day 21 of gestation to Day 3 of lactation.

[a)] 29W × 22D × 21H cm [b)] 28W × 44D × 18H cm

Stainless steel chip trays (changed at least twice weekly) and bedding material were used for stainless steel wire mesh cages. Bedding material was used for polycarbonate cages.

Enrichment: Wood blocks for gnawing were placed in the cages during the housing period.

12.6 Animal rearing materials and analyses for contaminants

12.6.1 Feed

Name: NMF (pelleted feed)

Supplier: Oriental Yeast Co., Ltd. (Chiba)

Lot Nos.: 160927, 161128 and 170121

Analysis of feed: Individual lot analysis of the feed was conducted by the supplier for nutritional content and microorganisms and by Eurofins Scientific (Germany) for contaminants such as heavy metals.

Analytical results: Analytical results conformed to the criteria of Ina Research Inc.

12.6.2 Drinking water

Source: Municipal drinking water

Analysis of drinking water: Monthly analysis of the water was conducted by the Ina Municipal Waterworks Bureau (analyzed by the Water Quality Control Center, Kamiina District Tap Water Quality Control Association). Water collected from the animal facilities at Ina Research Inc. was analyzed quarterly by the Kamiina Pharmaceutical Association.

Analytical results: Analytical results conformed to the criteria of Ina Research Inc.

12.6.3 Wood blocks for enrichment

Name: Wood blocks (approx. 5 × 2 × 2 cm, autoclaved at 121°C for 20 min)

Supplier: Chiba Animal Materials Co., Ltd.

Material: Fir tree

Frequency of wood block changes:
Wood blocks were changed once weekly.

Analysis of wood blocks: Annual analysis of the wood blocks was conducted by the supplier (analyzed by the Tokyo Kenbikyoin Foundation) for traces of hazardous substances and bacteria.

Analytical results: Analytical results conformed to the criteria of Ina Research Inc.

12.6.4 Bedding material

Name: Sunflake® (autoclaved at 121°C for 20 min)

Supplier: Charles River Laboratories Japan, Inc.

Material: Fir tree

Analysis of bedding material:
Analysis of the bedding material was conducted by Eurofins Scientific (Germany) for traces of hazardous substances and bacteria.

Analytical results: Analytical results conformed to the criteria of Ina Research Inc.

12.7 Identification

Animals:

Parental animals (F_0):

At receipt: The animals were identified with individual ID Nos. (201-244 for males and 201-252 for females) by ear-punch codes.

Offspring (F_1):

At 4 days of age: The offspring in each litter were identified by tattooing individual ID Nos. (serial numbers from 1 for males and from 5 for females for each dam in principle).

At 10 days of age: The offspring were identified by staining on the fur (Animal Color).

Cages: Each cage was identified with a cage card denoting the following information:

At animal receipt: Study No., strain, sex and ID No.

At group assignment: The group (dose level) and Animal No. were also recorded on each cage card. A color-coded sticker [a)] indicating the individual groups was attached to each cage card.

At copulation: The date of copulation was also recorded for females.

At delivery: The date of delivery was also recorded for females.

[a)] Control: White
Low dose: Blue
Mid dose: Yellow
High dose: Red

12.8 Group assignment

Timing: 1 day prior to initiation of dosing

Animal selection and method of group assignment:

Group assignment was conducted using a computer system (Provantis). Males with no testicular abnormalities (confirmed by palpation on the day of group assignment) and females with regular estrous cycles (confirmed by vaginal smear examinations for 14 days, including the day of group assignment), both with no clinical signs until group assignment, were selected for group assignment.
Of these animals, 40 animals/sex closest to the respective mean body weights were stratified based on the body

weights measured on the day of group assignment. Then, animals were selected from each stratum and randomly distributed into groups. Animal Nos. were randomly assigned to the animals in each group.

Group	Males		Females	
	No. of animals	Animal Nos.	No. of animals	Animal Nos.
Control	10	DK1M01-DK1M10	10	DK1F01-DK1F10
Low dose	10	DK2M01-DK2M10	10	DK2F01-DK2F10
Mid dose	10	DK3M01-DK3M10	10	DK3F01-DK3F10
High dose	10	DK4M01-DK4M10	10	DK4F01-DK4F10

Disposition of animals not used in the study:

The extra 4 males and 12 females at group assignment were euthanized by inhalation of carbon dioxide 1 day after group assignment.

12.9 Dose levels

Group	Dosing article	Dose level (mg/kg/day)	Concentration (mg/mL)	Dose volume (mL/kg/day)
Control	Olive oil containing 1.0 w/v% Tween 80	0	0	5
Low dose	13F-OLE	5	1	5
Mid dose	13F-OLE	25	5	5
High dose	13F-OLE	200	40	5

12.10 Justification for selection of the dose levels

In a previously conducted 28-day repeated oral dose toxicity study in rats[1)] at dose levels of 5, 25 and 200 mg/kg/day, findings noted in males were centrilobular lipid droplets in the hepatocytes and microgranuloma in the liver at 25 and 200 mg/kg/day and a large liver and increased liver weights (absolute and relative) at 200 mg/kg/day. However, no appreciable treatment-related toxicity was noted in any female. Based on these results as well as the dosing period of up to approximately 9 weeks for females in the current study, 200 mg/kg/day was selected as the high dose level. Then, 25 and 5 mg/kg/day were selected as the mid and low dose levels, respectively.

12.11 Dosing

12.11.1 Route

Route: Oral

Justification for selection of the route:

The oral route is a possible route of exposure for humans and is commonly used in toxicity studies.

12.11.2 Method

Method: The dosing formulations were drawn into sterile, disposable 3 mL polypropylene syringes and promptly administered via oral gavage using sterile, disposable gastric intubation tubes. The test article dosing formulations were drawn into syringes while being thoroughly stirred using magnetic stirrers. The control formulation was not stirred. The dosing instruments were changed for each group.

Calculation of the dosing volumes:

The dosing volume for each animal was calculated based on the most recent body weight measurement.

Justification for selection of the method:

This method is commonly used and is suitable for rats.

12.11.3 Frequency

Frequency: Once daily

Time: Between 9:00 and 13:00

Since the following dams were in the course of delivery during the above time period, dosing to these dams was conducted after completion of delivery (after 13:00):

Animal Nos. DK1F02, DK1F08, DK2F02, DK3F02, DK3F10, DK4F05 and DK4F09

Justification for selection of the frequency:

Once-daily administration is commonly used.

12.11.4 Duration

Dosing period:

Males:	From 14 days prior to mating, throughout the mating period, until the last day of the mating period
Females:	From 14 days prior to mating, throughout the mating and gestation periods, until Day 13 of lactation

Justification for selection of the duration:

Males:	The selected duration is appropriate to evaluate the effects on the reproductive function of males that cannot be detected by histopathological examinations of the male reproductive organs.
Females:	The selected duration is appropriate to evaluate the effects on the estrous cycle, mating, fertility, tubal transport, implantation, pre-implantation development of embryos, pregnant/lactating female rats and development of the conceptuses and offspring.

12.12 Observations and examinations

The first day of the dosing period was designated as Day 1 of dosing. The day of copulation was designated as Day 0 of gestation. The day on which completion of delivery was confirmed was designated as Day 0 of lactation.

For the offspring, the day of birth was designated as 0 days of age and 0-6 days of age were designated as 0 weeks of age.

12.12.1 Clinical observations of parental animals (F_0)

Number of animals:	All parental animals
Duration:	
Males:	From Day 1 of dosing to the day of necropsy
Females:	From Day 1 of dosing to Day 14 of lactation
Frequency:	Twice daily (pre-dosing and up to 1 hour post-dosing) during the dosing period and once daily (in the morning) during the other periods
Observation method:	Cage-side observations were conducted for each animal. When some abnormalities were suspected, such animals were removed from the cages and observed.
Note:	Clinical signs observed at other times were also recorded.

12.12.2 Body weights of parental animals (F_0)

Number of animals: All parental animals

Duration:

Males: From Day 1 of dosing to the day of necropsy

Females: From Day 1 of dosing to Day 13 of lactation

Frequency:

Males: Once weekly (every Tuesday)

Females: Once weekly (every Tuesday) from Day 1 of dosing to the day of copulation, Days 0, 7, 14 and 20 of gestation and Days 0, 4, 7, 11 and 13 of lactation

Equipment: Electronic balance (UX4200H, Shimadzu Corporation)

12.12.3 Food consumption of parental animals (F_0)

Number of animals: All parental animals

Duration:

Males: From Day 1 of dosing to the day of initiation of mating

Females: From Day 1 of dosing to Day 13 of lactation (excluding the mating period)

Frequency: Provided amounts of feed were recorded on Day 1 of dosing, provided and residual amounts were recorded on the subsequent body weight measurement days and residual amounts were recorded on the day of initiation of the mating period.

During gestation, provided amounts of feed were recorded on Day 0 of gestation, provided and residual amounts were recorded on the subsequent body weight measurement days and residual amounts were recorded on Day 20 of gestation.

During lactation, provided amounts of feed were recorded on Day 0 of lactation, provided and residual amounts were recorded on the subsequent body weight measurement days and residual amounts were recorded on Day 13 of lactation.

Daily food consumption was calculated and reported as the food consumption from the day of feeding to the day of residual amount measurement.

Equipment: Electronic balance (UX4200H, Shimadzu Corporation)

12.12.4 Evaluation of vaginal smears of females (F_0) until copulation

Number of animals: All females

Duration: From Day 1 of dosing until the day of copulation (until the last day of confirmation of copulation if the evidence of copulation was not confirmed)

Method: Vaginal smears were taken using cotton swabs at nearly the same time every morning, dried, stained with Giemsa and examined by microscopy to determine the phases of the estrous cycle. From 1 day after initiation of mating, the vaginal smears were examined by microscopy for the presence/absence of sperm prior to staining with Giemsa.

Calculation of the mean length of the estrous cycle in days:

Duration: For 15 days from Day 1 of dosing until initiation of the mating period

Method: The number of days from the beginning of one estrous cycle to the next (from estrous to the next estrous) was counted and the mean length of the estrous cycle in days was calculated.

Preservation of the specimens:
Vaginal smears were preserved after evaluation.

12.12.5 Mating ability of animals (F_0)

Number of animals: All males and females

Duration: From Day 15 of dosing until the day of copulation (up to 14 days)

Mating period and pairing: Males and females in the same group were co-housed on a one-to-one basis for a maximum of 14 days.

Co-housing: Each pair was co-housed throughout the mating period from 16:00 on the first day of the mating period.

Confirmation of copulation: The presence of sperm in the vaginal smear or a vaginal plug in the vagina was regarded as evidence of copulation.

12.12.6 Determination of thyroid hormone (T4) levels in males (F_0)

Number of animals: All males

Analytical facility: Sekisui Medical Co., Ltd.
Drug Development Solutions Business
Drug Development Solutions Center

Timing:	At necropsy
Sample size:	Approximately 1 mL (at least 300 µL as serum)
Sampling method:	Blood samples were collected from the jugular vein of unanesthetized animals using sterile, disposable polypropylene syringes fitted with sterile, disposable 25 G needles.
Sample handling:	Untreated blood samples were placed in polyester resin tubes with procoagulant film, allowed to stand for 30-60 minutes at room temperature and centrifuged (1600 × g, 10 min, 4°C) to obtain serum.
Storage conditions of serum:	Frozen (acceptable range −95°C to −65°C, actual temperature −83.9°C to −81.4°C, storage period Feb. 8 to 9, 2017)
Storage area of serum:	Deep freezer in the Freezer Room of Ina Research Inc.
Shipment of serum samples:	All serum samples were labeled with the sample information (Study No., Animal No., generation and date of sampling) and sent frozen (packed on dry ice) to the facility for determination of T4 levels.
Date of shipment:	February 9, 2017
Method:	T4 levels were determined using a Rat Thyroid Hormone Magnetic Bead Panel.
Results:	A copy of the analysis report prepared by the facility for determination of T4 levels was obtained and the analytical results were reflected in the final report.

12.12.7 Gross pathology of males (F_0)

Number of animals:	All males
Timing:	1 day after the last day of the mating period
Euthanasia:	After completion of blood collection from the jugular vein (see section 12.12.6, "Determination of thyroid hormone (T4) levels in males (F0)"), the males were anesthetized by inhalation of isoflurane, JP (Mylan Seiyaku, Ltd., hereafter referred to as "isoflurane"). The abdomen was incised and the animals were euthanized by exsanguination from the posterior vena cava and abdominal aorta for complete macroscopic postmortem examinations.

Organs weighed: The liver, testes (separately for the right and left), epididymides (separately for the right and left), the levator ani plus bulbocavernosus muscle complex, Cowper's gland, glans penis and body weights on the day of necropsy (for calculation of organ-to-terminal body weight ratios)

Equipment: Electronic balance (AUX220, Shimadzu Corporation)

Preservation of the specimens:

The liver, seminal vesicles (including coagulating glands), prostate (ventral lobe), thyroids, organs/tissues with gross lesions and the ear with ID No. were fixed and preserved in 10 vol% neutral buffered formalin. The testes and epididymides were fixed and preserved in Bouin's fixative. Prior to fixation, the tunica albuginea was punctured gradually and shallowly at both poles of the testis with needles.

12.12.8 Histopathology of males (F_0)

Number of animals: All males

Histotechnology and light microscopy:

The testes and epididymides from the control and high dose groups were routinely processed and stained with hematoxylin and eosin (HE).

All histopathological specimens were examined by light microscopy.

For the testes, the number of cells (Sertoli cells, spermatogonium, pachytene spermatocytes and spermatids) in stages VII and VIII were counted for 3 seminiferous tubules/animal. In addition, the number of Leydig cells/clump was counted for 3 cell clumps/animal. Then, the mean number of cells and Sertoli cell ratio were calculated according to the following formulae:

Mean number of cells in 1 seminiferous tubule =

Total number of each cell type / Number of target seminiferous tubules

Sertoli cell ratio (%) =

100 × Mean number of each cell type[a)] / Mean number of Sertoli cells

[a)] Cells other than Sertoli cells in the seminiferous tubules

12.12.9 Dosing period and gross pathology of females which failed to mate (F_0)

Number of animals: 2 animals (Animal Nos. DK3F04 and DK4F07)

Dosing period: Until 1 day prior to necropsy

Timing of gross pathology: 10 days after termination of the mating period

Method: The animals were anesthetized by inhalation of isoflurane. The abdomen was incised and the animals were euthanized by exsanguination from the posterior vena cava and abdominal aorta for complete macroscopic postmortem examinations.

Preservation of the specimens:
The ovaries, uterus and ear with ID No. were fixed and preserved in 10 vol% neutral buffered formalin.

Note: Gross pathology of Animal No. DK4F07 revealed fetuses in the uterus. Live fetuses were euthanized by decapitation. The uterus with the fetuses and placentae were fixed and preserved and the number of implantation sites was examined.

12.12.10 Observations for delivery and nursing behavior of dams (F_0)

Number of animals: All dams

Timing: After Day 20 of gestation

Frequency:

Observation for delivery: At least twice daily (in the morning and afternoon)

Observation for nursing behavior:
Twice daily (pre-dosing and up to 1 hour post-dosing) during the dosing period and once (in the morning) on the day of necropsy

Parameters:

Delivery: The presence/absence of abnormal delivery and live newborns

Duration of gestation: The number of gestational days on the day of completion of delivery

Poor nursing during the lactation period:
Scattering of offspring in the cages, total litter loss, cannibalization, etc.

12.12.11 Evaluation of vaginal smears of dams (F_0) after delivery

Number of animals: All dams

Timing: In the morning on Day 14 of lactation

Method: Vaginal smears were taken using cotton swabs, dried, stained with Giemsa and examined by microscopy to determine the phases of the estrous cycle.

Preservation of the specimens:
Vaginal smears after observations

12.12.12 Determination of T4 levels in dams (F_0)

Number of animals: All dams

Analytical facility: Sekisui Medical Co., Ltd.
Drug Development Solutions Business
Drug Development Solutions Center

Timing: At necropsy

Sample size: Approximately 1 mL (at least 300 µL as serum)

Sampling method: Blood samples were collected from the jugular vein of unanesthetized animals using sterile, disposable polypropylene syringes fitted with sterile, disposable 25 G needles.

Sample handling: Untreated blood samples were placed in polyester resin tubes with procoagulant film, allowed to stand for 30-60 minutes at room temperature and centrifuged (1600 × g, 10 min, 4°C) to obtain serum.

Storage conditions of serum: Frozen (acceptable range −95°C to −65°C, actual temperature −84.9°C to −80.7°C, storage period Mar. 2 to Apr. 19, 2017)

Storage area of serum: Deep freezer in the Freezer Room of Ina Research Inc.

Shipment of serum samples: All serum samples were labeled with the sample information (Study No., Animal No., generation and date of sampling) and sent frozen (packed on dry ice) to the facility for determination of T4 levels.

Date of shipment: April 19, 2017

Method: T4 levels were determined using a Rat Thyroid Hormone Magnetic Bead Panel.

Results: A copy of the analysis report prepared by the facility for determination of T4 levels was obtained and the analytical results were reflected in the final report.

12.12.13 Gross pathology of dams (F_0)

Number of animals: All dams

Timing: Day 14 of lactation

Euthanasia: After completion of blood collection from the jugular vein (see section 12.12.12, "Determination of T4 levels in dams (F0)"), the dams were anesthetized by inhalation of isoflurane. The abdomen was incised and the animals were euthanized by exsanguination from the posterior vena cava and abdominal aorta for complete macroscopic postmortem examinations.

Parameters: Gross pathology and the number of implantation sites

Organs weighed: The liver and body weights on the day of necropsy (for calculation of organ-to-terminal body weight ratios)

Preservation of the specimens:

The liver, ovaries, uterus, mammary glands and the ear with ID No. from all dams were fixed and preserved in 10 vol% neutral buffered formalin.

12.12.14 Histopathology of dams (F_0)

Number of animals: All dams

Histotechnology and light microscopy:

The ovaries from the control and high dose groups were routinely processed and stained with hematoxylin and eosin (HE).

All histopathological specimens were examined by light microscopy.

12.12.15 Observations of newborns (F_1) at birth

Number of animals: All newborns

Parameters:

Newborns: Total number of newborns and viability

Live newborns: Total number, sex and body weights of live newborns and the number of newborns with external morphology (including the oral cavity)

Dead newborns: Number of dead newborns

Preservation of the specimens:
Dead newborns were fixed and preserved in 10 vol% neutral buffered formalin.

12.12.16 Clinical observations of offspring (F_1)

Number of animals: All offspring

Duration: Daily from the day of birth until Day 14 of lactation

Preservation of the specimens:
Dead offspring were fixed and preserved in 10 vol% neutral buffered formalin.

Note: For the dead offspring, external macroscopic abnormalities (especially external reproductive organs) were observed and recorded.

12.12.17 Body weights of offspring (F_1)

Number of animals: All offspring

Duration: From the day of birth until Day 13 of lactation

Intervals: At 4, 7, 11 and 13 days of age

Equipment: Electronic balance (UX4200H, Shimadzu Corporation)

12.12.18 Adjustment of litter size of offspring (F_1)

Adjustment of litter size: All litters of greater than 8 offspring were culled to 8 animals (4 males and 4 females in principle) at 4 days of age. Culling was not conducted when the litter size was less than 8.

Euthanasia of culled offspring:
For 2 offspring/litter of the culled offspring, blood samples were collected according to section 12.12.21, "Determination of T4 levels in offspring (F1, at 4 days of age)". Remaining culled offspring were anesthetized by inhalation of isoflurane. The abdomen was incised and the offspring were euthanized by exsanguination from the posterior vena cava and abdominal aorta.

Preservation of the specimens:
The culled offspring were fixed and preserved in 10 vol% neutral buffered formalin.

12.12.19 Measurements of the anogenital distance in offspring (F_1)

Timing and number of animals:
For all offspring after adjustment of litter size at 4 days of age, the anogenital distance (AGD) was measured using a digital caliper.

Note: The AGD values divided by the cube root of body weights on the day of AGD measurements were regarded as the measurement values.

12.12.20 Count of the number of nipples/areolas in male offspring (F_1)

Number of animals: All male offspring

Timing: At 13 days of age

12.12.21 Determination of T4 levels in offspring (F_1, at 4 days of age)

Timing and number of animals for blood sampling:
2 offspring/litter of the culled offspring at adjustment of litter size at 4 days of age
(Basically, blood samples were collected from 1 offspring/sex/litter. However, if only 1 sex of culled offspring was available, blood sampling was conducted for 2 offspring of the same sex. If there were no culled offspring available, blood sampling was not conducted.)

Analytical facility: Sekisui Medical Co., Ltd.
Drug Development Solutions Business
Drug Development Solutions Center

Sample size: Approximately 0.15 mL/animal (at least 50 μL as serum)

Sampling method: Blood samples were collected from the jugular vein of unanesthetized animals using sterile, disposable polypropylene syringes fitted with sterile, disposable 30 G needles.

Sample handling: Untreated blood samples were pooled by litter, placed in polypropylene tubes with procoagulant film, allowed to stand for 30-60 minutes at room temperature and centrifuged (1600 × g, 10 min, 4°C) to obtain serum.

Storage conditions of serum: Frozen (acceptable range −95°C to −65°C, actual temperature −85.9°C to −80.7°C, storage period Feb. 20 to Apr. 19, 2017)

Storage area of serum: Deep freezer in the Freezer Room of Ina Research Inc.

Shipment of serum samples: All serum samples were labeled with the sample information (Study No., Animal No., generation and date of sampling) and sent frozen (packed on dry ice) to the facility for determination of T4 levels.

Date of shipment: April 19, 2017

Method: T4 levels were determined using a Rat Thyroid Hormone Magnetic Bead Panel.

Results: A copy of the analysis report prepared by the facility for determination of T4 levels was obtained and the analytical results were reflected in the final report.

Handling of animals: After completion of blood sampling, the animals were anesthetized by inhalation of isoflurane. The abdomen was incised and the animals were euthanized by exsanguination from the posterior vena cava and abdominal aorta.

Preservation of the specimens:
The culled offspring used for blood sampling were fixed and preserved in 10 vol% neutral buffered formalin.

12.12.22 Determination of T4 levels in offspring (F_1, at 14 days of age)

Number of animals: 2 offspring/litter
(Basically, blood samples were collected from 1 offspring/sex/litter. However, if only 1 sex of offspring was available, blood sampling was conducted for 2 offspring of the same sex.)

Analytical facility: Sekisui Medical Co., Ltd.
Drug Development Solutions Business
Drug Development Solutions Center

Timing: At necropsy

Sample size: Approximately 0.2 mL/animal (at least 60 μL as serum)

Sampling method: Blood samples were collected from the jugular vein of unanesthetized animals using sterile, disposable polypropylene syringes fitted with sterile, disposable 30 G needles.

Sample handling: Untreated blood samples were pooled by litter, placed in polypropylene tubes with procoagulant film, allowed to stand for 30-60 minutes at room temperature and centrifuged (1600 × g, 10 min, 4°C) to obtain serum.

Storage conditions of serum: Frozen (acceptable range −95°C to −65°C, actual temperature −84.5°C to −81.1°C, storage period Mar. 2 to 14, 2017)

Storage area of serum: Deep freezer in the Freezer Room of Ina Research Inc.

Shipment of serum samples: All serum samples were labeled with the sample information (Study No., Animal No., generation and date of sampling) and sent frozen (packed on dry ice) to the facility for determination of T4 levels.

Date of shipment: March 14, 2017

Method: T4 levels were determined using a Rat Thyroid Hormone Magnetic Bead Panel.

Results: A copy of the analysis report prepared by the facility for determination of T4 levels was obtained and the analytical results were reflected in the final report.

12.12.23 Gross pathology of offspring (F_1)

Number of animals: All offspring

Timing: At 14 days of age

Method: The animals were anesthetized by inhalation of isoflurane. The abdomen was incised and the animals were euthanized by exsanguination from the posterior vena cava and abdominal aorta for complete macroscopic postmortem examinations. For the animals used for determination of T4 levels, these procedures were conducted after blood sampling.

Preservation of the specimens: The thyroids of the animals used for determination of T4 levels (1 offspring/sex/litter or 2 offspring/litter of the same sex) were fixed and preserved in 10 vol% neutral buffered formalin.

12.12.24 Observations of dams (F_0) with total litter loss

Number of animals: 2 animals (Animal Nos. DK3F08 and DK4F04)

Timing: The day on which total litter loss (deaths of all offspring) occurred

Euthanasia: The dams were anesthetized by inhalation of isoflurane. The abdomen was incised and the animals were euthanized by exsanguination from the posterior vena cava and abdominal aorta.

Parameters: Gross pathology and the number of implantation sites

Preservation of the specimens:
The ovaries, uterus, mammary glands, the ear with ID No. and organs/tissues with gross lesions were fixed and preserved in 10 vol% neutral buffered formalin.

12.12.25 Observations of dead dams (F_0)

Number of animals: 1 animal (Animal No. DK1F02)

Timing: Promptly after being found

Euthanasia: Live offspring at 3 days of age or younger were euthanized by decapitation.

Parameters: Gross pathology and the number of implantation sites

Preservation of the specimens:
The brain, pituitary, thymus, lungs, heart, liver, spleen, adrenals, kidneys, ovaries, uterus and the ear with ID No. were fixed and preserved in 10 vol% neutral buffered formalin.
In addition, the mammary glands and offspring were also fixed and preserved in 10 vol% neutral buffered formalin.

12.13 Statistical analysis

The following statistical analyses were conducted between the control and treated groups. Differences from the control group were evaluated at the 5% level of significance and presented as $p < 0.05$ or $p < 0.01$ in the tables. Data for offspring were pooled by litter. The litter served as the unit of analysis. Body weights, AGD data and the AGD values divided by the cube root of body weights at 4 days of age were analyzed separately for males and females. For the paired organs that were weighed separately, the sum of the right and left weights were used for statistical analysis.

Body weight data for females (F_0) during the mating period were excluded from calculations.

The mean number of cells in 1 seminiferous tubule and the Sertoli cell ratio were expressed to the first decimal place. The AGD values divided by the cube root of body weights at 4 days of age were expressed to the third decimal place.

Test 1: χ^2 test

Parameters:

- Copulation index = (Number of pairs with successful copulation / Number of pairs mated) × 100
- Fertility index = (Number of pregnant females / Number of pairs with successful copulation) × 100
- Gestation index = (Number of dams with live newborns / Number of pregnant dams) × 100

Test 2: For comparison between 3 groups or more, Bartlett's test was conducted to determine homogeneity of variance (5% level of significance). When the variance was homogeneous between the groups, the mean values were compared between the control and each treated group using Dunnett's test. When the variance was heterogeneous based on Bartlett's test, the data were transformed to natural logarithms and reanalyzed for homogeneity of variance using Bartlett's test (5% level of significance). When the variance was homogeneous between the groups, transformed data were compared between the control and each treated group using Dunnett's test. When the variance was still heterogeneous based on Bartlett's test, untransformed quantitative data were analyzed for differences in mean ranks using Steel's test between the control and each treated group.

For comparison between 2 groups, data were analyzed for equality of variance using the F-test (5% level of significance) and Student's t-test or Aspin-Welch's t-test was conducted depending on whether the variance was equal or not.

Parameters:

- Body weights
- Body weight gain (calculated based on body weights on Day 1 of dosing prior to mating, on Day 0 of gestation during the gestation period and on Day 0 of lactation during the lactation period)
- Food consumption
- Organ weights (absolute and relative weights)
- Mean number of cells in 1 seminiferous tubule

- Estrous cycle (the mean length of the estrous cycle in days)
- Number of days until copulation
- Number of implantation sites
- Number of newborns
- Number of live newborns
- Anogenital distance (AGD)
- AGD values divided by the cube root of body weights at 4 days of age
- Number of nipples/areolas
- T4 levels

Test 3: For comparison between 3 groups or more, data were analyzed for differences in mean ranks using Steel's test.
For comparison between 2 groups, data were analyzed using Wilcoxon's rank-sum test.

Parameters:

- Sertoli cell ratio
- Duration of gestation
- Delivery index = (Number of newborns / Number of implantation sites) × 100
- Birth index = (Number of live newborns / Number of implantation sites) × 100
- Sex ratio of live newborns
- Incidence of external malformations of live newborns
- Viability index on Day 4 of lactation = (Number of live offspring on Day 4 of lactation / Number of live newborns) × 100
- Viability index on Day 14 of lactation = (Number of live offspring on Day 14 of lactation / Number of live offspring after adjustment of litter size at 4 days of age) × 100

12.14 Computer systems

Provantis system:

- For group assignment and dosing
- For data acquisition of body weights, food consumption, estrous cycles, mating ability, organ weights (excluding the levator ani plus bulbocavernosus muscle complex, Cowper's gland and glans penis), observations for delivery and viability of newborns
- For statistical analysis and tabulation of data

INATOX-DP (SAS) system: For statistical analysis and tabulation of data

EXSAS - Statistical analysis system for biological experiment data:
For statistical analysis

13. Deviations from the protocol and unforeseeable circumstances

The following instances occurred; however, these were considered not to have affected the evaluation of the study results:

1) Gross pathology of 1 female (Animal No. DK4F07) in the high dose group which was judged to have failed to mate revealed fetuses in the uterus, therefore the vaginal smears of this animal were re-examined. The presence of sperm was confirmed in the vaginal smear on January 25, 2017 and therefore it was surmised that the sperm on the smear was overlooked. Consequently, the data after the gestation period were unavailable for this animal, but the data on mating ability was employed since successful copulation and pregnancy were confirmed by re-examination of vaginal smears and gross pathology. This instance was considered not to have affected the study evaluation since the other 9 females in this group were pregnant and it is stipulated in OECD Guideline 421 that the acceptable minimum number of pregnant females per group is 8.
2) The humidity in the animal room exceeded the upper limit of the acceptable range (70.0%), up to 70.8%, on 1 day during the study period. However, the increase was due to routine cleaning procedures and the humidity returned to the acceptable range within 1 hour of termination of cleaning procedures.

14. Archives

Storage materials:

- Protocol
- Draft and final reports
- All raw data
- Other study-related documents
- Test article archival sample
- Control article archival samples
- Specimens
  - Vaginal smears
  - Organs/tissues fixed in 10 vol% neutral buffered formalin
  - Histopathological specimens (paraffin blocks and slides)

Storage period: 10 years from submission of the final report

Further storage will be determined after consultation with the sponsor prior to the expiration of the storage period.

Storage area: Archives in the main facility of Ina Research Inc.

15. Results

15.1 Effects on males (F_0)

15.1.1 Mortality

(Table 1, Appendix 1)

No death occurred in any male in any group.

15.1.2 Clinical observations

(Table 1, Appendix 1)

No clinical signs were observed in any male in any group.

15.1.3 Body weights

(Figures 1 and 2, Tables 2 and 3, Appendices 2 and 3)

No statistically significant differences were noted in body weights or body weight gain between the control and any treated group.

15.1.4 Food consumption

(Figure 3, Table 4, Appendix 4)

No statistically significant differences were noted in food consumption between the control and any treated group.

15.1.5 Thyroid hormone (T4) levels

(Table 5, Appendix 5)

A statistically significant decrease in T4 levels was noted in the 200 mg/kg/day group as compared to the control group. No statistically significant differences from the control group were noted in the 25 or 5 mg/kg/day group.

15.1.6 Gross pathology

(Table 6, Appendix 6)

Large liver was observed in 7 of the 10 males in the 200 mg/kg/day group.

No gross pathological lesions were observed in any male in the 25 or 5 mg/kg/day group.

15.1.7 Organ weights

(Table 7, Appendix 7)

Statistically significant increases were noted in the absolute and relative weights of the liver in the 200 mg/kg/day group and in the relative weight of the liver in the 25 mg/kg/day group as compared to the control group. No statistically significant differences from the control group were noted in the absolute or relative weights of the testes, epididymides, levator ani muscle and bulbocavernosus muscle, Cowper's gland or glans penis in either group.

No statistically significant differences from the control group were noted in the 5 mg/kg/day group.

15.2 Effects on females (F_0)

15.2.1 Mortality

(Tables 9 and 22, Appendices 9 and 22)

No death occurred in any female in any treated group.

One dam (Animal No. DK1F02) in the control group died after exhibiting a decrease in movement, soiled fur and scattering of all offspring in the cage on Day 1 of lactation. Since death occurred 1 day after delivery, the cause of death was judged to be a deteriorated physical condition due to poor recovery from delivery. Gross pathology of this animal revealed a small thymus and discoloration of the spleen.

15.2.2 Clinical observations

(Table 9, Appendix 9)

In 1 dam (Animal No. DK4F05) in the 200 mg/kg/day group, emaciation attributable to decreased food consumption was observed on Days 4 to 6 of lactation. In another dam (Animal No. DK3F08) in the 25 mg/kg/day group with total litter loss, a decrease in movement was observed on Day 2 of lactation. These signs were considered not to be treatment-related since 1) they were limited to 1 dam in each group; 2) no treatment-related effects on food consumption were noted in the 200 mg/kg/day group as described later; and 3) the sign observed in the 25 mg/kg/day group was also observed in the dam that died in the control group.

No clinical signs were observed in the 5 mg/kg/day group.

15.2.3 Body weights

(Figures 4 and 5, Tables 10 to 15, Appendices 10 to 15)

No statistically significant differences were noted in body weights or body weight gain between the control and any treated group up to copulation or throughout the gestation period.

During the lactation period, statistically significant decreases in body weight gain were noted in the 5 mg/kg/day group on Days 11 and 13 of lactation as compared to the control group. However, these decreases were not dose-related and were therefore considered to be incidental changes unrelated to treatment with the test article.

15.2.4 Food consumption

(Figure 6, Tables 16 to 18, Appendices 16 to 18)

No statistically significant differences were noted in food consumption between the control and any treated group up to copulation or throughout the gestation or lactation period.

15.2.5 Thyroid hormone (T4) levels

(Table 21, Appendix 21)

No statistically significant differences from the control group were noted in T4 levels in any treated group.

15.2.6 Gross pathology

(Table 22, Appendix 22)

No gross pathological lesions were observed in any dam in any group on Day 14 of lactation.

In 1 dam each (Animal Nos. DK3F08 and DK4F04) in the 25 and 200 mg/kg/day groups with total litter loss, small thymus and/or small spleen were observed. Both lesions were considered to be stress-related.

15.2.7 Organ weights

(Table 23, Appendix 23)

A statistically significant increase was noted in the relative weight of the liver in the 200 mg/kg/day group as compared to the control group.

No statistically significant differences from the control group were noted in the 25 or 5 mg/kg/day group.

15.3 Effects on the reproductive function of parental animals (F_0)

15.3.1 Evaluation of vaginal smears

(Table 19, Appendix 19)

No statistically significant differences were noted in the mean length of the estrous cycle in days between the control and any treated group.

15.3.2 Mating ability and fertility

(Table 20, Appendix 20)

No statistically significant differences were noted in the mating index, fertility index or the number of days until copulation between the control and any treated group. Only 1 pair in the 25 mg/kg/day group failed to mate.

15.3.3 Delivery, duration of gestation, gestation index, number of implantation sites, delivery index and birth index

(Table 25, Appendix 24)

No abnormal delivery was observed for any dam in any group. No statistically significant differences were noted in the duration of gestation, gestation index, number of implantation sites, delivery index or birth index between the control and any treated group.

15.3.4 Nursing behavior

(Table 9, Appendix 9)

Scattering of all offspring in the cage was observed in 2 dams (Animal Nos. DK4F04 and DK4F10) in the 200 mg/kg/day group and in 1 dam (Animal No. DK3F08) in the 25 mg/kg/day group on the day of and/or 1 day after delivery, all of which were assumed to be due to the poor nursing behavior of the dams. Of these dams, total litter loss occurred for 2 dams (Animal Nos. DK4F04 and DK3F08) on Day 2 of lactation.

Poor nursing behavior was not observed in the 5 mg/kg/day group.

For 1 dam (Animal No. DK1F02) in the control group that died, scattering of all offspring in the cage was observed on Day 1 of lactation.

15.3.5 Histopathology

(Tables 8 and 24, Appendix 8)

In males, no histopathological lesions were observed in the testes or epididymides in the 200 mg/kg/day group. No statistically significant differences were noted in the mean number of Sertoli cells, spermatogonium, pachytene spermatocytes, spermatids or Leydig cells or the Sertoli cell ratio between the control and 200 mg/kg/day groups.

In females, no histopathological lesions were observed in the ovaries in the 200 mg/kg/day group. In addition, vaginal smears on the day of gross pathology indicated the anestrous cycle for all dams.

15.4 Effects on offspring (F_1)

15.4.1 Observations of newborns

(Tables 25, 27 and 28, Appendices 24, 26 and 27)

No statistically significant differences were noted in the number of live newborns or body weights or sex ratio of live newborns between the control and any treated group.

No external malformations were observed in any live newborn.

15.4.2 Clinical observations

(Table 26, Appendix 25)

No milk in the stomach of offspring was observed for 3 dams (Animal Nos. DK4F02, DK4F04 and DK4F10) in the 200 mg/kg/day group and subnormal body surface temperature of offspring was also observed in 1 (Animal No. DK4F04) of these dams on Day 1 of lactation. In addition, both signs were observed in 1 dam (Animal No. DK3F08) in the 25 mg/kg/day group on the day of delivery and/or Day 1 of lactation. These signs were assumed to be due to poor nursing behavior of the dams and total litter loss occurred on Day 2 of lactation in 1 dam each (Animal Nos. DK4F04 and DK3F08) in the 200 and 25 mg/kg/day groups.

Death of some offspring/litter occurred in 5, 3, 0 and 4 dams in the control and 5, 25 and 200 mg/kg/day groups, respectively. The incidence of death was not dose-related and therefore the deaths were considered to be unrelated to treatment with the test article.

15.4.3 Body weights

(Figures 7 and 8, Tables 27 and 28, Appendices 26 and 27)

No statistically significant differences were noted in body weights between the control and any treated group.

15.4.4 Viability index on Days 4 and 14 of lactation

(Table 29, Appendix 28)

No statistically significant differences were noted in the viability index on Day 4 of lactation between the control and any treated group.

The viability index on Day 14 of lactation was 100% in all groups.

15.4.5 Anogenital distance (AGD)

(Tables 30 and 31, Appendices 29 and 30)

No statistically significant differences between the control and any treated group were noted in the absolute or relative AGD values in any male or female offspring at 4 days of age.

15.4.6 Number of nipples/areolas in male offspring

(Table 32, Appendix 31)

No nipples or areolas were observed in any male offspring in any group at 13 days of age.

15.4.7 Thyroid hormone (T4) levels

(Table 33, Appendix 32)

No statistically significant differences from the control group were noted in T4 levels in any treated group at 4 or 14 days of age.

15.4.8 Gross pathology

(Table 34, Appendix 33)

Gross pathology at 14 days of age revealed discoloration of the spleen, dilation and retention of contents in the stomach and a greenish brown focus in the liver in 1 female offspring (DK4F01-2F) in the 200 mg/kg/day group. However, these lesions were limited to 1 offspring and no gross pathological lesions were observed in any other offspring in this group. Therefore, these were considered to be incidental lesions unrelated to treatment with the test article.

16. Discussion and conclusion

In parental animals (F_0), a decrease in thyroid hormone (T4) levels was noted in males in the 200 mg/kg/day group. In the gross pathology and organ weights, an increase in the relative weight of the liver in males in the 25 mg/kg/day group and a large liver and increases in the absolute and relative weights of the liver in males and an increase in the relative weight of the liver in females in the 200 mg/kg/day group were noted. The changes in the liver in males in the 25 mg/kg/day group and in females in the 200 mg/kg/day group were judged not to be toxic changes since they were noted only in the relative weights and no macroscopic lesions were observed in the liver, indicating the changes were slight. No treatment-related effects were noted on the clinical observations, body weights or food consumption.

For the reproductive function of parental animals (F_0), poor nursing behavior was observed in 1 and 2 dams in the 25 and 200 mg/kg/day groups, respectively, and total litter loss occurred for 1 dam each in these groups. However, poor nursing behavior and total litter loss observed in these groups were considered to be incidental findings unrelated to treatment with the test article since 1) these were limited to 1 to 2 dams in each group; 2) poor nursing behavior was also observed in 1 dam in the control group that died; and 3) no treatment-related effects were noted on the viability index on Day 4 of lactation. No treatment-related effects were noted on the estrous cycle, copulation index, fertility index, duration of gestation, delivery, gestation index, number of implantation sites, delivery index, birth index or histopathology of reproductive organs.

In the subsequent generation (F_1), no treatment-related effects were noted in the clinical observations, body weights, number of newborns, number of live newborns, sex ratio, external morphology, viability index on Day 4 or 14 of lactation, anogenital distance, number of nipples/areolas or thyroid hormone (T4) levels.

Based on these results, the no observed adverse effect levels (NOAELs) of 13F-OLE were considered to be 25 mg/kg/day for parental males and 200 mg/kg/day for parental females, parental reproductive function and offspring under the conditions of this study.

17. References

1) Shiraishi K. Twenty-eight-day repeated-dose oral toxicity study in rats. Chemicals Evaluation and Research Institute, Japan; 2007. Study No. B11-0838.

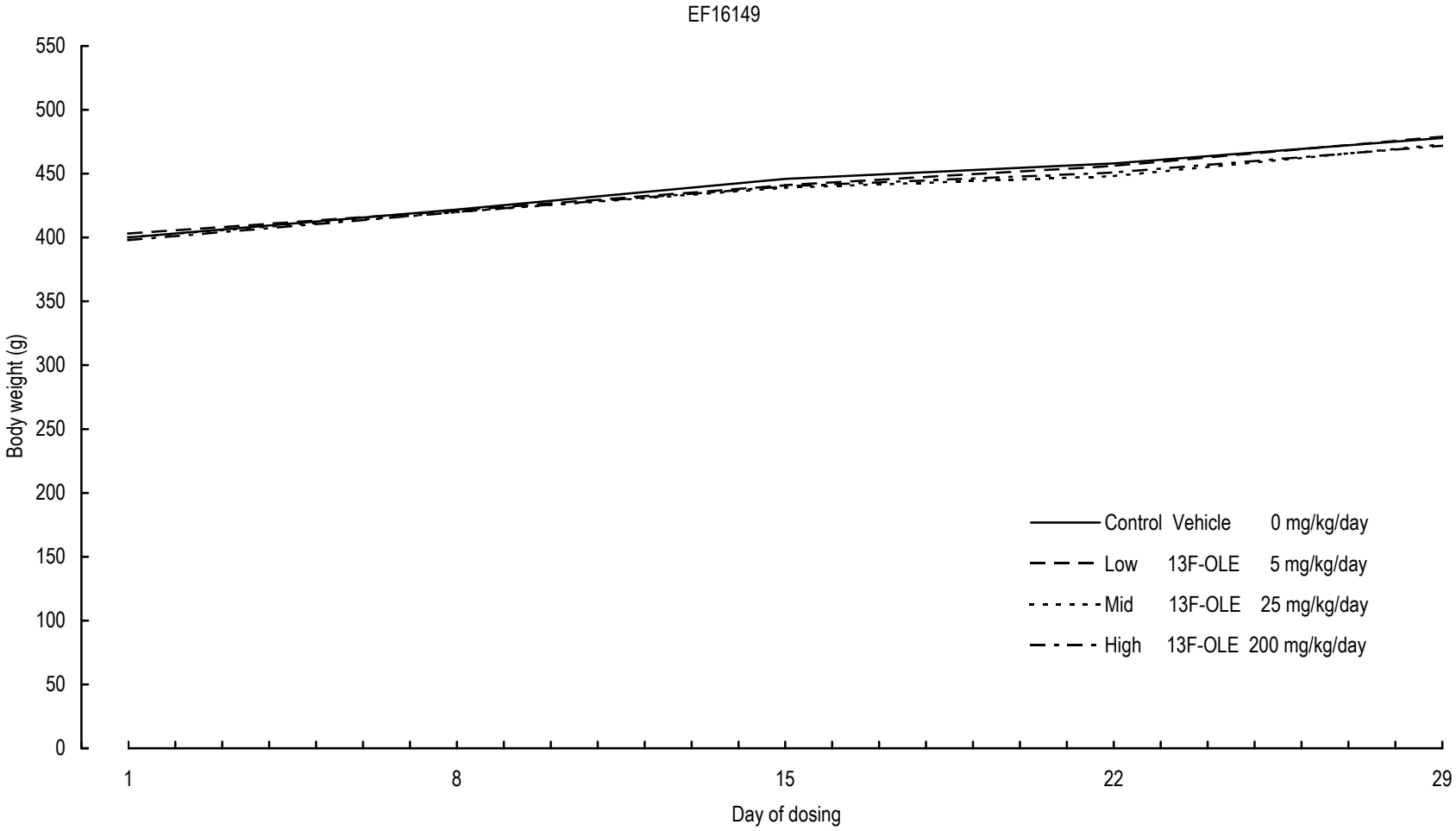


Fig. 1 Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats by Oral Administration
Body weight (males)

Vehicle: 1.0 w/v% olive oil containing Tween 80

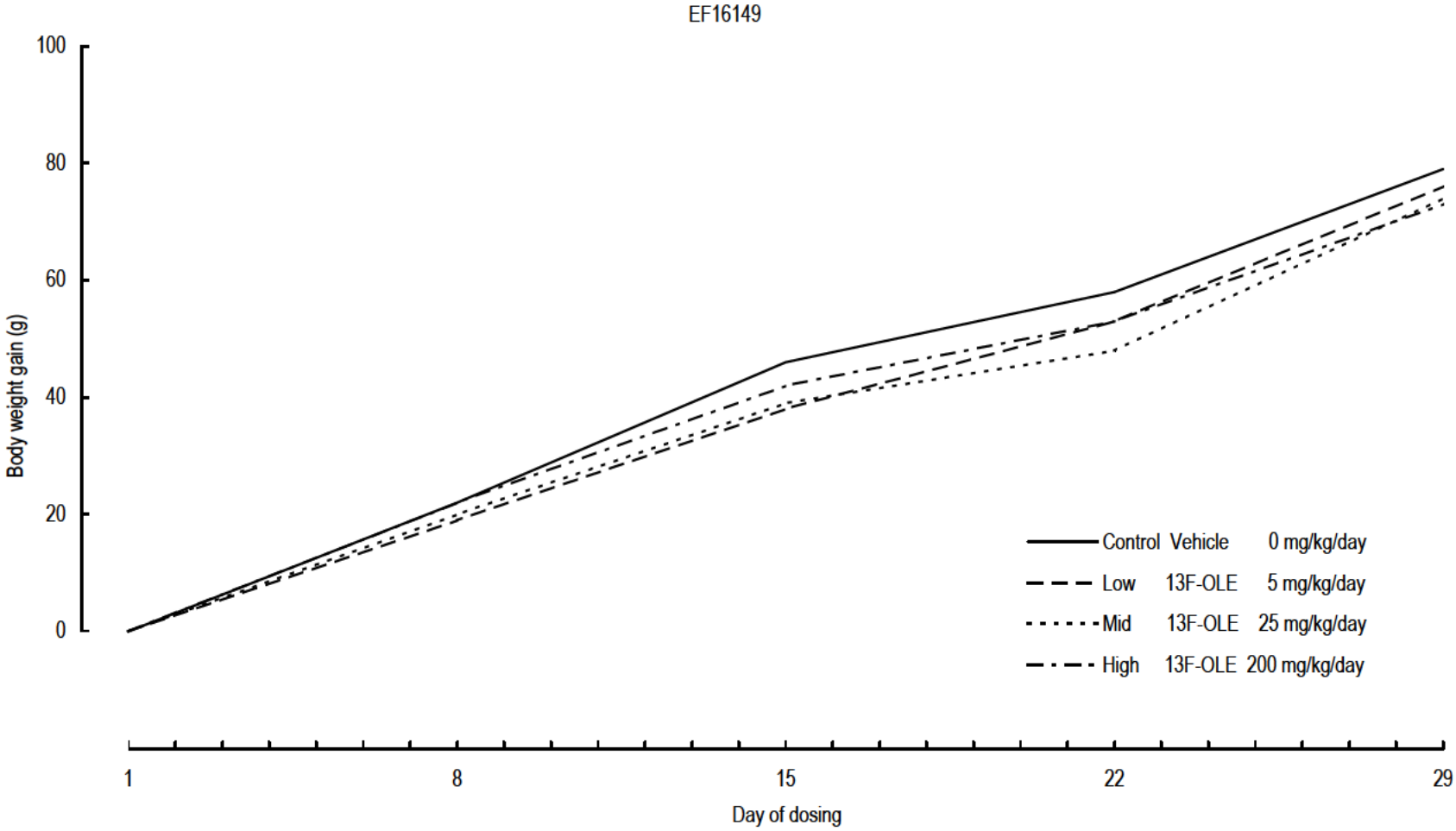


Fig. 2 Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats by Oral Administration
Body weight gain (males)
Vehicle: 1.0 w/v% olive oil containing Tween 80

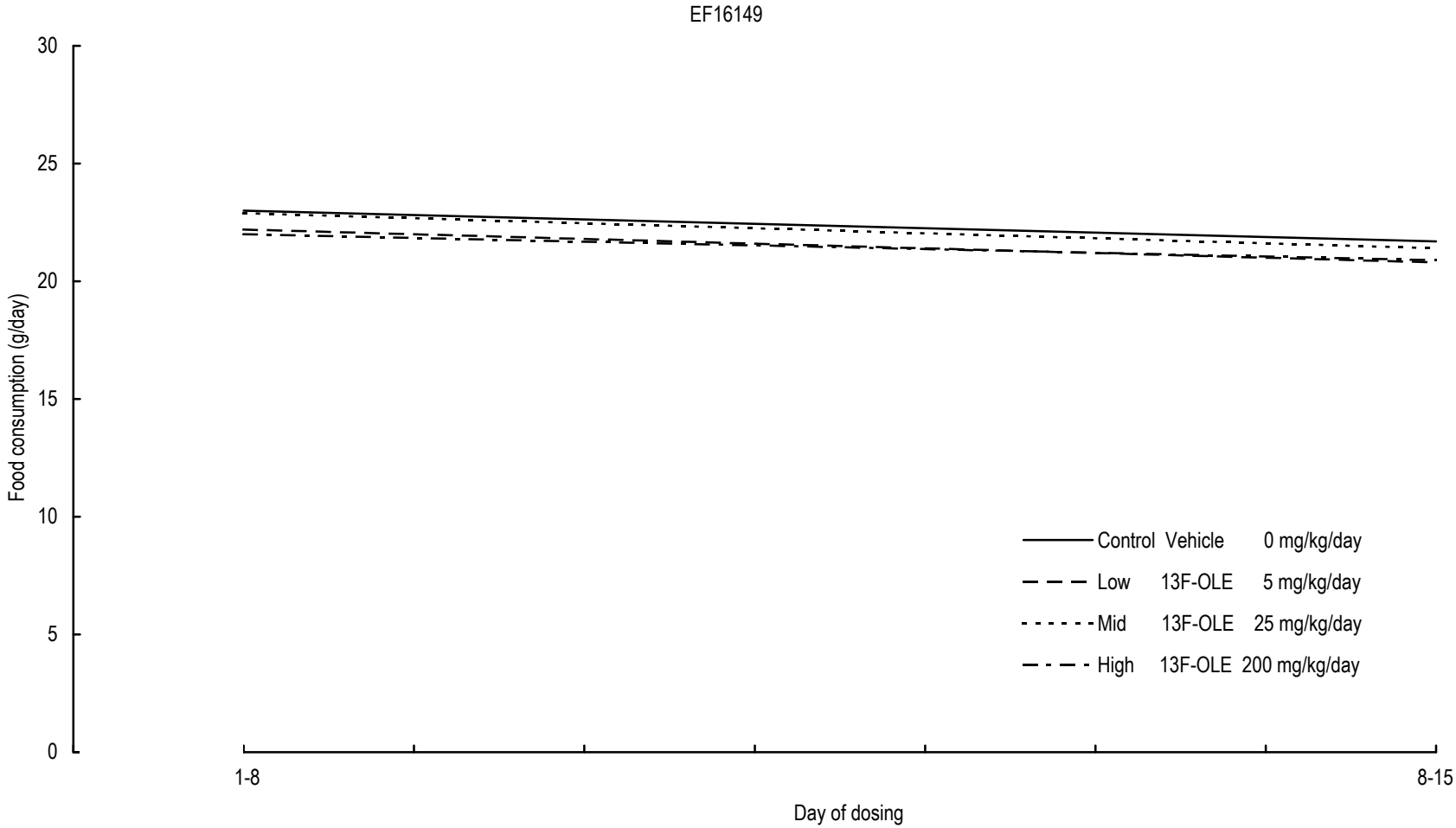


Fig. 3 Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats by Oral Administration
Food consumption (males)

Vehicle: 1.0 w/v% olive oil containing Tween 80

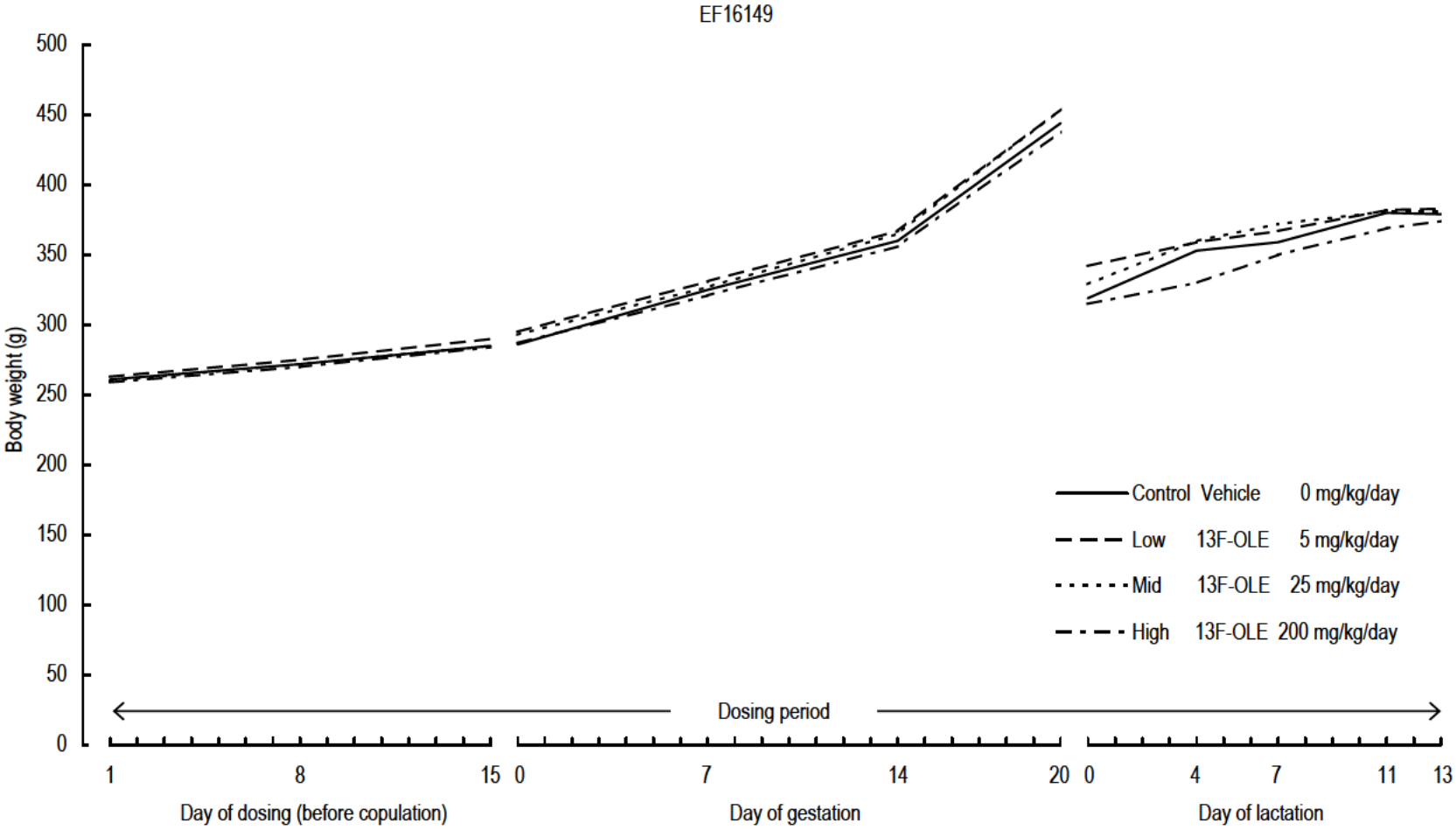


Fig. 4 Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats by Oral Administration
Body weight (females)
Vehicle: 1.0 w/v% olive oil containing Tween 80

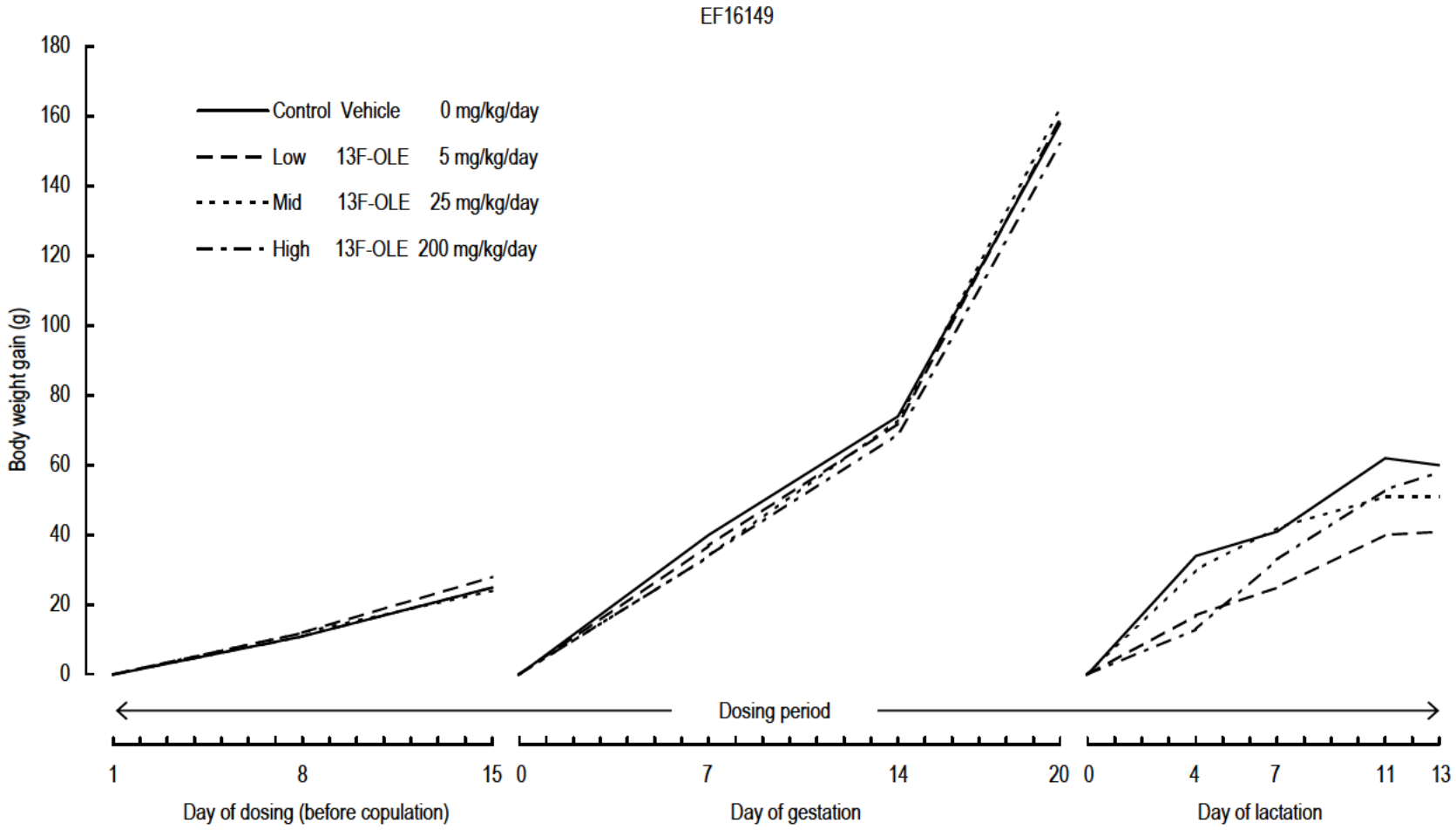


Fig. 5 Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats by Oral Administration
Body weight gain (females)
Vehicle: 1.0 w/v% olive oil containing Tween 80

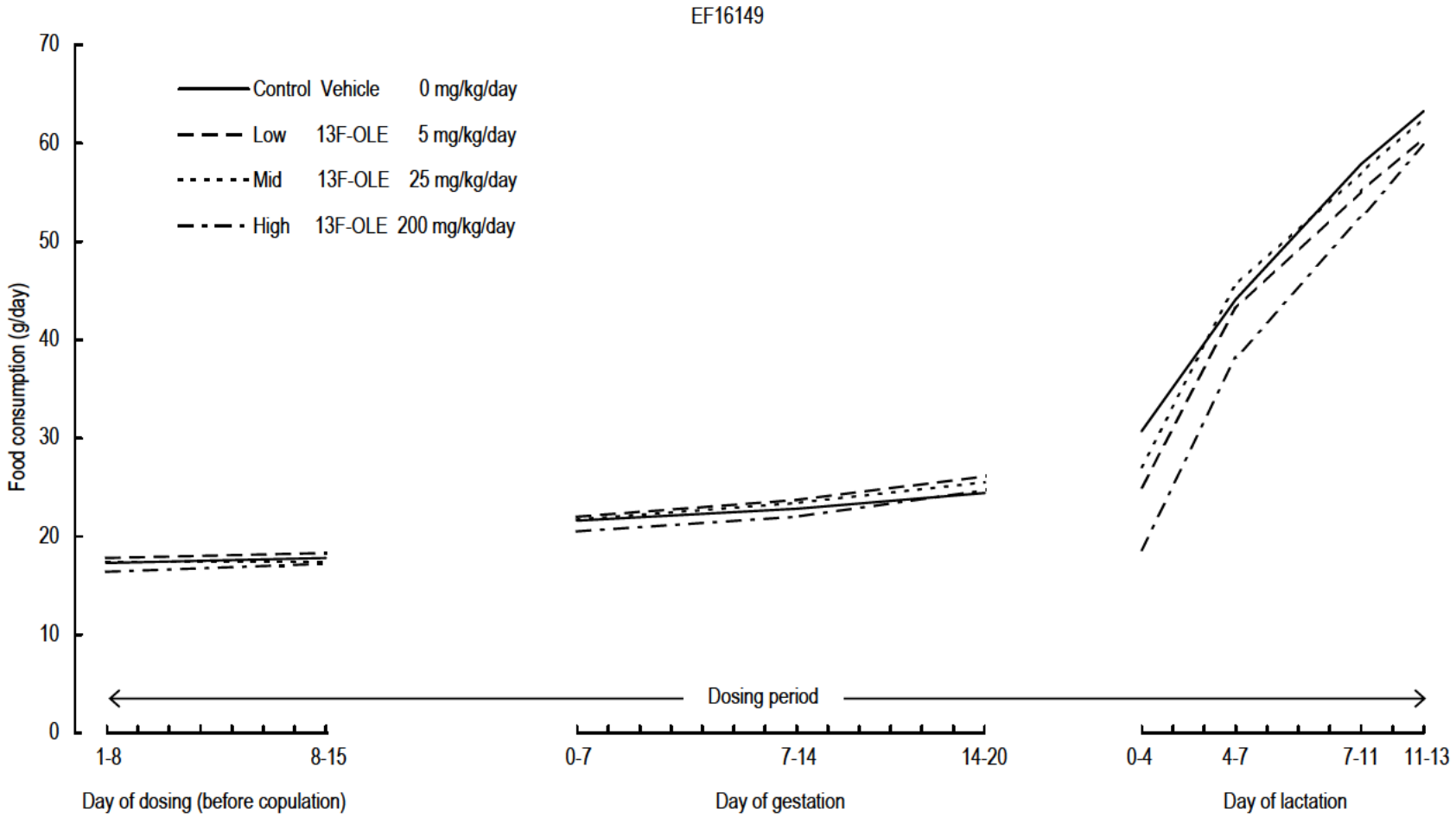
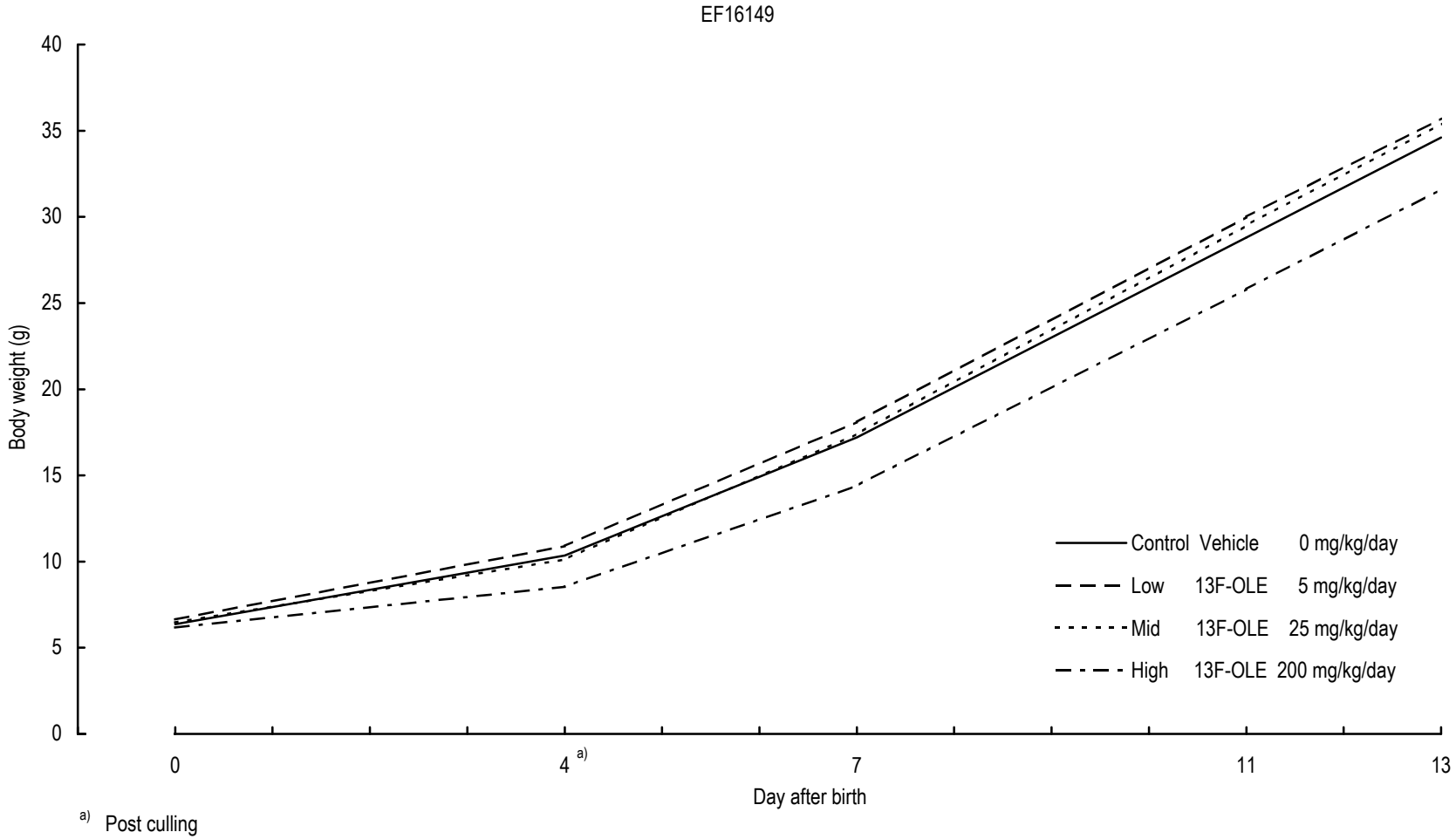


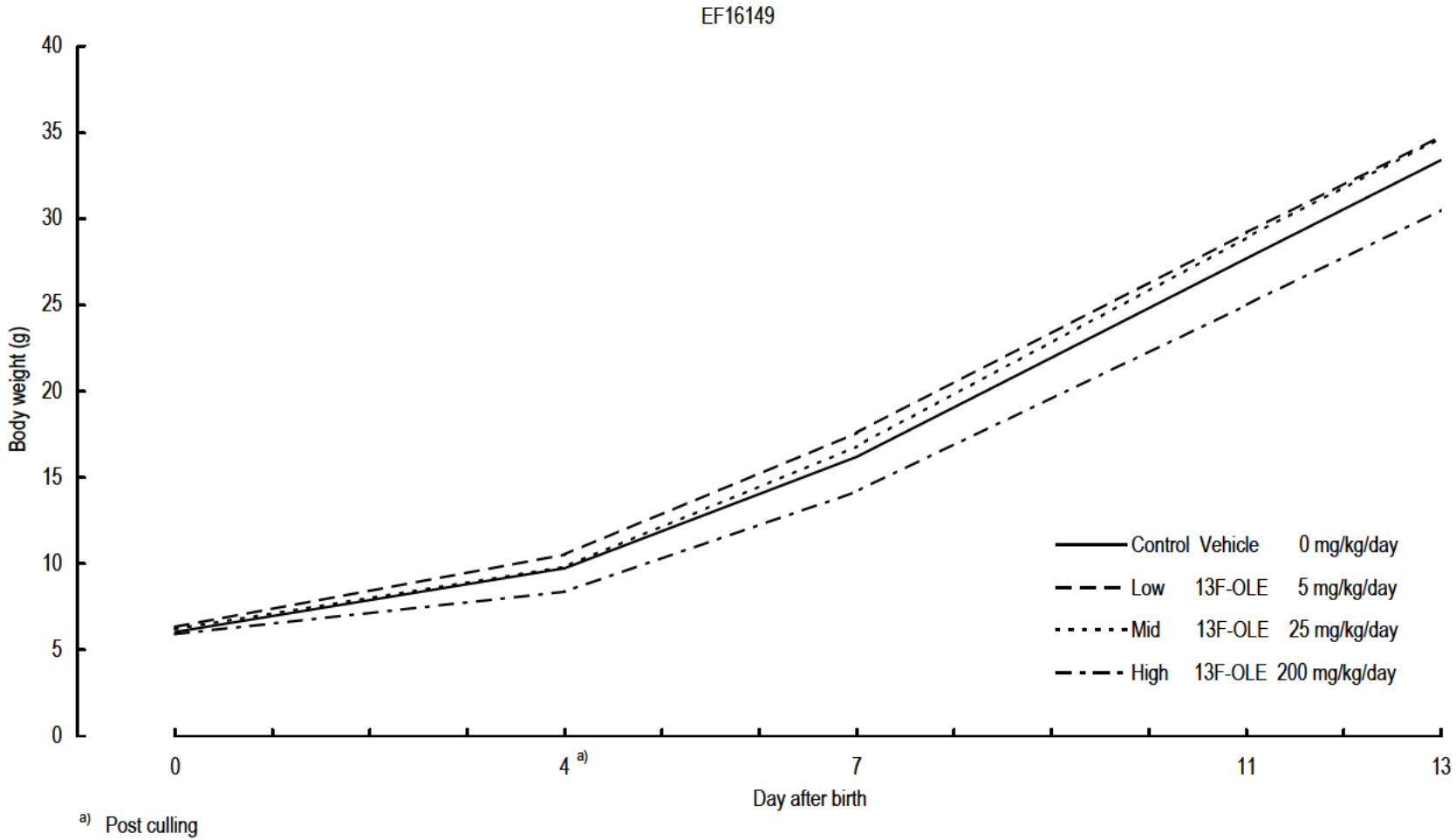
Fig. 6 Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats by Oral Administration
Food consumption (females)
Vehicle: 1.0 w/v% olive oil containing Tween 80



a) Post culling

Fig. 7 Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats by Oral Administration
Body weight of male offspring (F1) after birth

Vehicle: 1.0 w/v% olive oil containing Tween 80



a) Post culling

Fig. 8 Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats by Oral Administration
Body weight of female offspring (F1) after birth
Vehicle: 1.0 w/v% olive oil containing Tween 80

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 1 Clinical observations (males)

Dosing period

Sex: Male	Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Number of animals	10	10	10	10
Number of animals with abnormal signs	0	0	0	0

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 2 Body weight (males)

Body weight (g)

Sex: Male Day(s) Relative to Start Date		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
1	Mean	400 [1]	403	400	398
	S.D.	21	21	24	21
	N	10	10	10	10
8	Mean	422 [1]	421	420	420
	S.D.	26	27	26	24
	N	10	10	10	10
15	Mean	446 [1]	441	439	440
	S.D.	29	27	30	26
	N	10	10	10	10
22	Mean	458 [1]	456	448	451
	S.D.	30	31	28	27
	N	10	10	10	10
29	Mean	478 [1]	479	473	472
	S.D.	28	35	26	30
	N	10	10	10	10

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 3 Body weight gain (males)

Body weight gain from Day 1 (g)

Sex: Male Day(s) Relative to Start Date		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
1	Mean	0 R[1]	0	0	0
	S.D.	0	0	0	0
	N	10	10	10	10
8	Mean	22 I[2]	19	20	22
	S.D.	7	8	6	6
	N	10	10	10	10
15	Mean	46 I[2]	38	39	42
	S.D.	11	11	13	9
	N	10	10	10	10
22	Mean	58 I[2]	53	48	53
	S.D.	11	13	11	11
	N	10	10	10	10
29	Mean	79 I[2]	76	74	73
	S.D.	13	16	9	15
	N	10	10	10	10

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

1 [R - Automatic Transformation Selected: Rank]

2 [I - Automatic Transformation Selected: Identity (No Transformation)]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 4 Food consumption (males)

Food consumption (g/day)

Sex: Male Day(s) Relative to Start Date		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
1 → 8	Mean	23.0 [1]	22.2	22.9	22.0
	S.D.	2.0	1.9	2.0	1.4
	N	10	10	10	10
8 → 15	Mean	21.7 [1]	20.8	21.4	20.9
	S.D.	1.3	1.7	2.1	1.7
	N	10	10	10	10

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 5 Determination of the thyroid hormone (T4) levels (males)

Day(s): 30 Relative to Start Date

Sex: Male		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Total T4	Mean	541.0 I[1]	529.8	506.3	474.8 dd[2]
(ng/mL)	S.D.	29.5	33.5	50.3	42.9
	N	10	10	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80 || T4: Thyroxine]

1 [I - Automatic Transformation Selected: Identity (No Transformation)]

2 [dd - Test: Dunnett 2 Sided p < 0.01]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 6 Gross pathology (males)

Day(s): 30 Relative to Start Date

Sex: Male	Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Number of animals	10	10	10	10
Number of animals with abnormalities	0	0	0	7
Number of animals with the following abnormalities				
Liver: Large	0	0	0	7

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 7 Absolute and relative organ weights (males)

Day(s): 30 Relative to Start Date

Sex: Male		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Final Bodyweight (g)	Mean	482 I[1]	480	472	473
	S.D.	29	34	26	31
	N	10	10	10	10
Liver (g)	Mean	15.9 L[2]	16.9	17.4	23.4 dd[3]
	S.D.	1.8	1.7	1.7	3.7
	N	10	10	10	10
Liver (%)	Mean	3.304 L[2]	3.519	3.669 d[4]	4.932 dd[3]
	S.D.	0.275	0.303	0.243	0.680
	N	10	10	10	10
Testis Total (g)	Mean	3.29 I[1]	3.20	3.39	3.28
	S.D.	0.25	0.32	0.27	0.19
	N	10	10	10	10
Testis Total (%)	Mean	0.683 I[1]	0.669	0.719	0.694
	S.D.	0.056	0.072	0.039	0.041
	N	10	10	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]
2 [L - Automatic Transformation Selected: Log]
3 [dd - Test: Dunnett 2 Sided p < 0.01]
4 [d - Test: Dunnett 2 Sided p < 0.05]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 7 Absolute and relative organ weights (males)

Day(s): 30 Relative to Start Date

Sex: Male		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Epididymis	Mean	1.31 [1]	1.26	1.29	1.31
Total	S.D.	0.11	0.10	0.06	0.09
(g)	N	10	10	10	10
Epididymis	Mean	0.272 [1]	0.265	0.274	0.278
Total	S.D.	0.019	0.029	0.019	0.025
(%)	N	10	10	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 7 Absolute and relative organ weights (males)

Day(s): 30 Relative to Start Date

Sex: Male		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Levator ani muscle and bulbocavernosus muscle (g)	Mean	1.536 I[1]	1.501	1.547	1.530
	S.D.	0.150	0.135	0.141	0.174
	N	10	10	10	10
Levator ani muscle and bulbocavernosus muscle (%)	Mean	0.319 I[1]	0.313	0.328	0.324
	S.D.	0.028	0.022	0.029	0.032
	N	10	10	10	10
Cowper's gland (g)	Mean	0.139 I[1]	0.147	0.130	0.140
	S.D.	0.013	0.018	0.016	0.020
	N	10	10	10	10
Cowper's gland (%)	Mean	0.0290 I[1]	0.0308	0.0275	0.0296
	S.D.	0.0027	0.0043	0.0034	0.0040
	N	10	10	10	10
Glans penis (g)	Mean	0.114 I[1]	0.110	0.109	0.116
	S.D.	0.015	0.012	0.011	0.010
	N	10	10	10	10
Glans penis (%)	Mean	0.0236 I[1]	0.0230	0.0231	0.0247
	S.D.	0.0025	0.0031	0.0029	0.0028
	N	10	10	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]

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Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 8-1 Histopathology (males)

Sex: Male Day(s): 30 Relative to Start Date

		Group	Control Vehicle 0 mg/kg/day									
Organ and tissue	Findings	\ Animal No.	DK1M01	DK1M02	DK1M03	DK1M04	DK1M05	DK1M06	DK1M07	DK1M08	DK1M09	DK1M10
Testis	Pathological change		-	-	-	-	-	-	-	-	-	-
Epididymis	Pathological change		-	-	-	-	-	-	-	-	-	-

		Group	High 13F-OLE 200 mg/kg/day									
Organ and tissue	Findings	\ Animal No.	DK4M01	DK4M02	DK4M03	DK4M04	DK4M05	DK4M06	DK4M07	DK4M08	DK4M09	DK4M10
Testis	Pathological change		-	-	-	-	-	-	-	-	-	-
Epididymis	Pathological change		-	-	-	-	-	-	-	-	-	-

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80
-: None/Negative 1: Minimal 2: Mild 3: Moderate 4: Marked 5: Severe P: Present

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 8-2 Histopathology (males) -Cell numbers in seminiferous tubules-

Sex: Male Day(s): 30 Relative to Start Date

				Control Vehicle 0 mg/kg/day	High 13F-OLE 200 mg/kg/day
Stage VII to VIII	Sertoli cells	Mean of cells	Mean	11.1	12.2
			S.D.	3.7	2.4
			N	10	10
	Spermatogonia	Mean of cells	Mean	4.7	5.4
			S.D.	0.9	1.6
			N	10	10
		%	Mean	51.1	44.9
			S.D.	34.1	13.3
			N	10	10
	Pachytene spermatocytes	Mean of cells	Mean	58.6	58.3
			S.D.	6.5	7.4
			N	10	10
		%	Mean	623.3	498.6
			S.D.	386.3	142.1
			N	10	10
	Spermatids	Mean of cells	Mean	141.9	150.9
			S.D.	11.6	14.9
			N	10	10
		%	Mean	1498.6	1279.3
			S.D.	837.7	280.4
			N	10	10
	Leydig cells	Mean of cells	Mean	13.5	14.9
			S.D.	3.2	4.5
			N	10	10

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80
%: Sertoli cell ratio: 100 × mean of each cell type / mean number of sertoli cells

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 9 Clinical observations (females)

Sex: Female	Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Before copulation				
Number of animals	10	10	10	10
Number of animals which were successfully mated	10	10	9	9
Number of animals with abnormal signs	0	0	0	0
Number of animals which were not successfully mated	0	0	1	0
Number of animals with abnormal signs	.	.	0	.
Number of animals misjudged not successfully mated	0	0	0	1 [a)]
Number of animals with abnormal signs	.	.	.	0
Gestation period				
Number of dams	10	10	9	9
Number of dams with abnormal signs	0	0	0	0
Lactation period				
Number of dams	10	10	9	9
Number of dams which delivered	10	10	9	9
Number of surviving dams	9	10	9	9
Number of dams with abnormal signs	0	0	1	3
Number of dams with the following signs				
Decrease in movement	0	0	1	0
Emaciation	0	0	0	1
Scattering of all offspring in the cage	0	0	1	2
Death of all offspring	0	0	1	1
Number of dead dams	1	0	0	0
Number of dams with abnormal signs	1	.	.	.
Number of dams with the following signs				
Decrease in movement	1	.	.	.
Soiled fur (reddish brown) of perivaginal ostium	1	.	.	.
Scattering of all offspring in the cage	1	.	.	.

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80

[a)] This animal was judged not successfully mated and was necropsied, but was found to be pregnant.

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 10 Body weight (females) -Pre-mating period-

Body weight (g)

Sex: Female Day(s) Relative to Start Date		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
1	Mean	261 [1]	263	260	259
	S.D.	14	11	9	11
	N	10	10	10	10
8	Mean	272 [1]	275	272	270
	S.D.	11	12	13	14
	N	10	10	10	10
15	Mean	285 [1]	290	285	284
	S.D.	21	14	14	13
	N	10	10	10	10

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 11 Body weight gain (females) -Pre-mating period-

Body weight gain from Day 1 (g)

Sex: Female Day(s) Relative to Start Date		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
1	Mean	0 R[1]	0	0	0
	S.D.	0	0	0	0
	N	10	10	10	10
8	Mean	11 I[2]	12	12	11
	S.D.	8	5	7	10
	N	10	10	10	10
15	Mean	25 I[2]	28	24	25
	S.D.	13	12	10	9
	N	10	10	10	10

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

1 [R - Automatic Transformation Selected: Rank]

2 [I - Automatic Transformation Selected: Identity (No Transformation)]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 12 Body weight (females) -Gestation period-

Body weight (g)

Sex: Female Day(s) Relative to Mating Litter:		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
0	Mean	286 [1]	295	293	287
	S.D.	20	16	11	13
	N	10	10	9	9
7	Mean	325 [1]	331	327	321
	S.D.	20	17	18	16
	N	10	10	9	9
14	Mean	360 [1]	367	365	356
	S.D.	21	19	20	15
	N	10	10	9	9
20	Mean	444 [1]	454	454	438
	S.D.	23	19	27	20
	N	10	10	9	9

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 13 Body weight gain (females) -Gestation period-

Body weight gain from Day 0 (g)

Sex: Female Day(s) Relative to Mating Litter:		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
0	Mean	0 R[1]	0	0	0
	S.D.	0	0	0	0
	N	10	10	9	9
7	Mean	40 I[2]	37	34	34
	S.D.	5	9	11	6
	N	10	10	9	9
14	Mean	74 I[2]	72	73	69
	S.D.	7	11	15	6
	N	10	10	9	9
20	Mean	158 I[2]	159	162	152
	S.D.	11	16	21	13
	N	10	10	9	9

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

1 [R - Automatic Transformation Selected: Rank]

2 [I - Automatic Transformation Selected: Identity (No Transformation)]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 14 Body weight (females) -Lactation period-

Body weight (g)

Sex: Female Day(s) Relative to Littering (A)		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
0	Mean	319 I[1]	342	329	315
	S.D.	24	33	17	28
	N	10	10	9	9
4	Mean	353 R[2]	359	360	330
	S.D.	20	22	17	47
	N	9	10	8	8
7	Mean	359 I[1]	367	372	350
	S.D.	19	23	23	33
	N	9	10	8	8
11	Mean	380 I[1]	382	381	369
	S.D.	26	19	21	27
	N	9	10	8	8
13	Mean	379 I[1]	383	381	374
	S.D.	17	19	21	20
	N	9	10	8	8

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

1 [I - Automatic Transformation Selected: Identity (No Transformation)]

2 [R - Automatic Transformation Selected: Rank]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 15 Body weight gain (females) -Lactation period-

Body weight gain from Day 0 (g)

Sex: Female Day(s) Relative to Littering (A)		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
0	Mean	0 R[1]	0	0	0
	S.D.	0	0	0	0
	N	10	10	9	9
4	Mean	34 I[2]	17	30	13
	S.D.	18	15	13	29
	N	9	10	8	8
7	Mean	41 I[2]	25	42	33
	S.D.	16	14	16	23
	N	9	10	8	8
11	Mean	62 I[2]	40 d[3]	51	53
	S.D.	18	18	14	23
	N	9	10	8	8
13	Mean	60 I[2]	41 d[3]	51	58
	S.D.	16	18	15	17
	N	9	10	8	8

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

1 [R - Automatic Transformation Selected: Rank]

2 [I - Automatic Transformation Selected: Identity (No Transformation)]

3 [d - Test: Dunnett 2 Sided p < 0.05]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 16 Food consumption (females) -Pre-mating period-

Food consumption (g/day)

Sex: Female		Control	Low	Mid	High
		Vehicle	13F-OLE	13F-OLE	13F-OLE
		0	5	25	200
Day(s) Relative to Start Date		mg/kg/day	mg/kg/day	mg/kg/day	mg/kg/day
1-8	Mean	17.3 I[1]	17.8	17.4	16.4
	S.D.	1.4	0.9	1.7	1.7
	N	10	10	10	10
8-15	Mean	17.8 I[1]	18.3	17.4	17.2
	S.D.	2.5	1.4	1.2	1.3
	N	10	10	10	10

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 17 Food consumption (females) -Gestation period-

Food consumption (g/day)

Sex: Female Day(s) Relative to Mating Litter:		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
0 → 7	Mean	21.6 I'	22.0	21.7	20.5
	S.D.	2.3	1.6	2.9	2.2
	N	10	10	9	9
7 → 14	Mean	22.8 I'	23.7	23.4	22.0
	S.D.	1.7	2.5	3.3	1.7
	N	10	10	9	9
14 → 20	Mean	24.4 I'	26.1	25.5	24.7
	S.D.	1.8	2.1	3.4	2.1
	N	10	10	9	9

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 18 Food consumption (females) -Lactation period-

Food consumption (g/day)

Sex: Female Day(s) Relative to Littering (A)		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
0 → 4	Mean	30.7 R[1]	25.0	27.1	18.6
	S.D.	5.7	4.9	4.8	12.6
	N	9	10	8	8
4 → 7	Mean	44.1 R[1]	43.2	45.6	38.2
	S.D.	3.4	3.2	5.8	12.0
	N	9	10	8	8
7 → 11	Mean	57.9 R[1]	55.1	57.0	52.5
	S.D.	3.4	3.5	4.1	8.2
	N	9	10	8	8
11 → 13	Mean	63.3 I[2]	60.6	62.6	59.8
	S.D.	4.1	6.2	4.1	7.2
	N	9	10	8	8

Statistical Test: Generalised Anova/Ancova Test Transformation: Automatic

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

1 [R - Automatic Transformation Selected: Rank]

2 [I - Automatic Transformation Selected: Identity (No Transformation)]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 19 Vaginal smears

Sex: Female	Day(s) Relative to Start Date		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Estrous cycle length	(Days)	Mean	4.2 I[1]	4.2	4.1	4.2
		S.D.	0.4	0.5	0.3	0.3
		N	10	10	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 20 Mating ability and fertility

Sex: Female	Day(s) Relative to Pairing (L)		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Mating ability	Copulation	N	10	10	10	10
		+	10	10	9	10
		%	100.0	100.0	90.0	100.0
Fertility	Pregnancy	N	10	10	9	10
		+	10	10	9	10
		%	100.0	100.0	100.0	100.0
Pre-coital period	(Days)	Mean	2.1 I[1]	3.4	2.8	2.7
		S.D.	1.2	2.3	1.2	1.8
		N	10	10	9	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 21 Determination of the thyroid hormone (T4) levels (females)

Day(s): 14 Relative to Littering (A)

Sex: Female		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Total T4	Mean	374.2 I[1]	379.2	362.4	382.6
(ng/mL)	S.D.	48.5	35.0	31.9	34.7
	N	9	10	8	8

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80 || T4: Thyroxine]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]

Table 22 Gross pathology (females)

Day(s): 14 Relative to Littering (A)

Sex: Female	Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Number of dams	10	10	9	10
Number of dams which nursed their offspring for 14 days	9	10	8	8
Number of dams with abnormalities	0	0	0	0
Number of dams necropsied due to death	1	0	0	0
Number of dams with abnormalities	1	·	·	·
Number of dams with the following abnormalities				
Thymus: Small	1	·	·	·
Spleen: Discoloration, pale	1	·	·	·
Number of dams with total litter loss	0	0	1	1
Number of dams with abnormalities	·	·	1	1
Number of dams with the following abnormalities				
Thymus: Small	·	·	1	1
Spleen: Small	·	·	1	0
Number of animals misjudged not successfully mated	0	0	0	1 [a)]
Number of dams with abnormalities	·	·	·	1
Number of dams with the following abnormalities				
Uterus: Remain, fetuses	·	·	·	1
Number of animals which were not successfully mated	0	0	1	0
Number of animals with abnormalities	·	·	0	·

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80

[a)] This animal was judged not successfully mated and was necropsied, but was found to be pregnant.

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 23 Absolute and relative organ weights (females)

Day(s): 14 Relative to Littering (A)

Sex: Female		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Final Bodyweight (g)	Mean	380 I[1]	384	383	372
	S.D.	21	19	24	21
	N	9	10	8	8
Liver (g)	Mean	15.7 I[1]	16.9	16.9	17.4
	S.D.	1.2	1.3	1.9	1.5
	N	9	10	8	8
Liver (%)	Mean	4.130 I[1]	4.402	4.410	4.672 dd[2]
	S.D.	0.208	0.393	0.416	0.349
	N	9	10	8	8

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]
2 [dd - Test: Dunnett 2 Sided p < 0.01]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 24-1 Histopathology (females)

Sex: Female Day(s): 14 Relative to Littering (A)

		Group	Control Vehicle 0 mg/kg/day								
Organ and tissue	Findings	\ Animal No.	DK1F01	DK1F03	DK1F04	DK1F05	DK1F06	DK1F07	DK1F08	DK1F09	DK1F10
Ovary	Pathological change		-	-	-	-	-	-	-	-	-

		Group	High 13F-OLE 200 mg/kg/day							
Organ and tissue	Findings	\ Animal No.	DK4F01	DK4F02	DK4F03	DK4F05	DK4F06	DK4F08	DK4F09	DK4F10
Ovary	Pathological change		-	-	-	-	-	-	-	-

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80
-: None/Negative 1: Minimal 2: Mild 3: Moderate 4: Marked 5: Severe P: Present

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 24-2 Histopathology (females) -Vaginal smears-

Sex: Female Day(s): 14 Relative to Littering (A)

Control Vehicle 0 mg/kg/day	Vaginal smears
DK1F01	D
DK1F03	D
DK1F04	D
DK1F05	D
DK1F06	D
DK1F07	D
DK1F08	D
DK1F09	D
DK1F10	D

Low 13F-OLE 5 mg/kg/day	Vaginal smears
DK2F01	D
DK2F02	D
DK2F03	D
DK2F04	D
DK2F05	D
DK2F06	D
DK2F07	D
DK2F08	D
DK2F09	D
DK2F10	D

Mid 13F-OLE 25 mg/kg/day	Vaginal smears
DK3F01	D
DK3F02	D
DK3F03	D
DK3F05	D
DK3F06	D
DK3F07	D
DK3F09	D
DK3F10	D

High 13F-OLE 200 mg/kg/day	Vaginal smears
DK4F01	D
DK4F02	D
DK4F03	D
DK4F05	D
DK4F06	D
DK4F08	D
DK4F09	D
DK4F10	D

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80
D: Diestrus

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 25 Reproductive observations (females) at delivery

Sex: Female	Day(s) Relative to Littering (A)		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Gestation index	0	N	10	10	9	9
		+	10	10	9	9
		%	100.0	100.0	100.0	100.0
Duration of gestation (Days)	0	Mean	22.0	22.0	22.1	22.0
		S.D.	0.0	0.0	0.3	0.0
		N	10	10	9	9
Implantation sites (Number)	0-14	Mean	16.4 [1]	16.1	16.4	16.0
		S.D.	1.3	2.0	1.1	1.4
		Total	164	161	148	144
		N	10	10	9	9
Newborns (Number)	0	Mean	15.8 [2]	14.2	15.7	15.0
		S.D.	1.5	3.6	1.7	1.4
		Total	158	142	141	135
		N	10	10	9	9
Live newborns (Number)	0	Mean	14.6 [1]	13.9	13.9	14.6
		S.D.	3.0	3.4	4.1	1.7
		Total	146	139	125	131
		N	10	10	9	9

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]
2 [R - Automatic Transformation Selected: Rank]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 25 Reproductive observations (females) at delivery

Sex: Female	Day(s) Relative to Littering (A)		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Male live newborns (Number)	0	Mean	7.3	6.4	5.3	7.9
		S.D.	2.5	2.5	2.7	2.0
		Total	73	64	48	71
Female live newborns (Number)	0	Mean	7.3	7.5	8.6	6.7
		S.D.	2.3	3.1	3.2	2.4
		Total	73	75	77	60
Dead newborns (Number)	0	Mean	1.2	0.3	1.8	0.4
		S.D.	3.1	0.5	4.3	0.9
		Total	12	3	16	4
		N	10	10	9	9
External anomalies (Number)	0	Mean	0.0	0.0	0.0	0.0
		S.D.	0.0	0.0	0.0	0.0
		Total	0	0	0	0
		N	10	10	9	9
Delivery index (%)	0	Mean	96.4	88.9	95.1	93.9
		S.D.	4.2	21.7	5.2	5.9
		N	10	10	9	9

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 25 Reproductive observations (females) at delivery

Sex: Female	Day(s) Relative to Littering (A)		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Birth index (%)	0	Mean	89.3	87.1	84.5	91.2
		S.D.	17.5	21.1	23.7	9.0
		N	10	10	9	9
Sex ratio (Male/Total)	0	Mean	0.50	0.47	0.37	0.55
		S.D.	0.13	0.16	0.15	0.14
		N	10	10	9	9
External anomalies (%)	0	Mean	0.0	0.0	0.0	0.0
		S.D.	0.0	0.0	0.0	0.0
		N	10	10	9	9

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 26 Clinical observations of offspring (F1) after birth

	Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Number of dams	10	10	9	9
Number of dams whose offspring had abnormal signs	5	3	1	5
Number of dams whose offspring had the following signs				
No milk in the stomach of all/some offspring	1	0	1	3
Subnormal body surface temperature of all offspring	1	0	1	1
Death of some offspring	5	3	0	4
Death of all offspring	0	0	1	1

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 27 Body weight of male offspring (F1) after birth

Sex: Female	Day(s) Relative to Littering (A)		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Male F1 weight (g)	0	Mean	6.37 [1]	6.64	6.47	6.17
		S.D.	0.40	0.49	0.36	0.57
		N	10	10	9	9
	4	Mean	10.38 [2]	10.86	10.25	8.54
		S.D.	0.74	1.82	1.27	2.42
		N	9	10	8	8
Post culling	4	Mean	10.35 [2]	10.90	10.13	8.54
		S.D.	0.76	1.77	1.38	2.47
		N	9	10	8	8
Male F1 wt.	7	Mean	17.2 [2]	18.1	17.4	14.4
		S.D.	1.3	2.4	2.3	4.5
		N	9	10	8	8
	11	Mean	28.8 [2]	30.0	29.5	25.8
		S.D.	1.9	2.8	3.5	5.6
		N	9	10	8	8
	13	Mean	34.6 [1]	35.7	35.4	31.6
		S.D.	2.3	2.7	4.1	5.4
		N	9	10	8	8

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]
2 [R - Automatic Transformation Selected: Rank]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 28 Body weight of female offspring (F1) after birth

Sex: Female	Day(s) Relative to Littering (A)		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Female F1 weight (g)	0	Mean	6.04 [1]	6.33	6.21	5.92
		S.D.	0.38	0.53	0.48	0.48
		N	10	10	9	9
	4	Mean	9.72 [1]	10.46	9.96	8.28
		S.D.	1.04	1.82	1.25	2.15
		N	9	10	8	7
Post culling	4	Mean	9.73 [1]	10.53	9.82	8.37
		S.D.	1.11	1.79	1.18	2.27
		N	9	10	8	7
Female F1 wt.	7	Mean	16.2 [1]	17.6	16.8	14.2
		S.D.	1.7	2.4	1.9	4.1
		N	9	10	8	7
	11	Mean	27.7 [1]	29.2	28.9	25.0
		S.D.	2.3	3.0	2.6	5.2
		N	9	10	8	7
	13	Mean	33.4 [1]	34.8	34.7	30.5
		S.D.	2.6	2.8	3.3	5.2
		N	9	10	8	7

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 29 Viability of offspring (F1)

Sex: Female	Day(s) Relative to Littering (A)		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Live newborns (Number)	0	Mean	14.6	13.9	13.9	14.6
		S.D.	3.0	3.4	4.1	1.7
		Total	146	139	125	131
		N	10	10	9	9
Live F1 pre culling (Number)	4	Mean	13.4	13.6	13.4	10.9
		S.D.	4.8	3.3	5.4	5.5
		Total	134	136	121	98
		N	10	10	9	9
Viability index (%)	0-4	Mean	86.9	98.0	88.9	74.1
		S.D.	30.9	3.2	33.3	37.6
		N	10	10	9	9
Live F1 post culling (Number)	4	Mean	8.0	7.7	8.0	7.4
		S.D.	0.0	0.9	0.0	1.8
		Total	72	77	64	59
		N	9	10	8	8
Live F1 (Number)	14	Mean	8.0	7.7	8.0	7.4
		S.D.	0.0	0.9	0.0	1.8
		Total	72	77	64	59
		N	9	10	8	8

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 29 Viability of offspring (F1)

Sex: Female	Day(s) Relative to Littering (A)		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Viability index (%)	4-14	Mean	100.0	100.0	100.0	100.0
		S.D.	0.0	0.0	0.0	0.0
		N	9	10	8	8

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 30 Anogenital distance (AGD) of male offspring (F1) at 4 days of age

		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
AGD	Mean	6.05 R[1]	6.31	6.06	5.75
(mm)	S.D.	0.19	0.46	0.31	0.65
	N	9	10	8	8
Relative AGD	Mean	0.278 I[2]	0.285	0.281	0.284
	S.D.	0.008	0.012	0.014	0.007
	N	9	10	8	8

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80 || Relative AGD: Values calculated by dividing AGD values by the cube root of body weights at 4 days of age]
1 [R - Automatic Transformation Selected: Rank] 2 [I - Automatic Transformation Selected: Identity (No Transformation)]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Table 31 Anogenital distance (AGD) of female offspring (F1) at 4 days of age

		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
AGD (mm)	Mean	3.67 I[1]	3.77	3.65	3.49
	S.D.	0.13	0.19	0.14	0.25
	N	9	10	8	7
Relative AGD	Mean	0.172 I[1]	0.173	0.171	0.174
	S.D.	0.008	0.005	0.009	0.006
	N	9	10	8	7

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80 || Relative AGD: Values calculated by dividing AGD values by the cube root of body weights at 4 days of age]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]

Table 32 Number of nipples and number of areolas of male offspring (F1) at 13 days of age

		Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Number of nipples	Mean	0	0	0	0
	S.D.	0	0	0	0
	N	9	10	8	8
Number of areolas	Mean	0	0	0	0
	S.D.	0	0	0	0
	N	9	10	8	8

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Table 33 Determination of the thyroid hormone (T4) levels of offspring (F1)

			Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Total T4 (ng/mL)	At 4 days of age	Mean	113.5 I[1]	130.8	130.3	131.2
		S.D.	20.5	31.1	26.5	27.9
		N	9	9	8	7
	At 14 days of age	Mean	327.2 I[1]	332.7	328.3	323.0
		S.D.	26.5	28.1	25.8	13.9
		N	9	10	8	8

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80 || T4: Thyroxine]
1 [I - Automatic Transformation Selected: Identity (No Transformation)]

Table 34 Gross pathology of offspring (F1) at 14 days of age

	Control Vehicle 0 mg/kg/day	Low 13F-OLE 5 mg/kg/day	Mid 13F-OLE 25 mg/kg/day	High 13F-OLE 200 mg/kg/day
Male				
Number of offspring	36	40	31	31
Number of offspring with abnormalities	0	0	0	0
Female				
Number of offspring	36	37	33	28
Number of offspring with abnormalities	0	0	0	1
Number of offspring with the following abnormalities				
Spleen: Discoloration, pale	0	0	0	1
Stomach: Dilation	0	0	0	1
Retention, contents	0	0	0	1
Liver: Focus, greenish brown	0	0	0	1

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 1 Clinical observations (males)

Sex: Male Dosing period

Group	Animal No.	Findings
Control	DK1M01	No abnormalities
Vehicle	DK1M02	No abnormalities
0	DK1M03	No abnormalities
mg/kg/day	DK1M04	No abnormalities
	DK1M05	No abnormalities
	DK1M06	No abnormalities
	DK1M07	No abnormalities
	DK1M08	No abnormalities
	DK1M09	No abnormalities
	DK1M10	No abnormalities
Low	DK2M01	No abnormalities
13F-OLE	DK2M02	No abnormalities
5	DK2M03	No abnormalities
mg/kg/day	DK2M04	No abnormalities
	DK2M05	No abnormalities
	DK2M06	No abnormalities
	DK2M07	No abnormalities
	DK2M08	No abnormalities
	DK2M09	No abnormalities
	DK2M10	No abnormalities

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 1 Clinical observations (males)

Sex: Male Dosing period

Group	Animal No.	Findings
Mid	DK3M01	No abnormalities
13F-OLE	DK3M02	No abnormalities
25	DK3M03	No abnormalities
mg/kg/day	# DK3M04	No abnormalities
	DK3M05	No abnormalities
	DK3M06	No abnormalities
	DK3M07	No abnormalities
	DK3M08	No abnormalities
	DK3M09	No abnormalities
	DK3M10	No abnormalities
High	DK4M01	No abnormalities
13F-OLE	DK4M02	No abnormalities
200	DK4M03	No abnormalities
mg/kg/day	DK4M04	No abnormalities
	DK4M05	No abnormalities
	DK4M06	No abnormalities
	DK4M07	No abnormalities
	DK4M08	No abnormalities
	DK4M09	No abnormalities
	DK4M10	No abnormalities

General Footnote: #: Not successfully mated

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 2 Body weight (males)

Sex: Male Body weight (g)

Control Vehicle 0 mg/kg/day	Day(s) Relative to Start Date				
	1	8	15	22	29
DK1M01	406	432	455	466	496
DK1M02	417	440	459	478	489
DK1M03	373	398	413	423	446
DK1M04	424	453	481	493	512
DK1M05	371	377	394	402	422
DK1M06	380	401	431	440	462
DK1M07	421	453	484	481	498
DK1M08	399	419	445	458	478
DK1M09	422	442	468	487	500
DK1M10	382	404	427	447	480
Mean	400	422	446	458	478
S.D.	21	26	29	30	28
N	10	10	10	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 2 Body weight (males)

Sex: Male Body weight (g)

Low 13F-OLE 5 mg/kg/day	Day(s) Relative to Start Date				
	1	8	15	22	29
DK2M01	395	413	443	463	483
DK2M02	408	426	447	459	489
DK2M03	384	403	421	434	453
DK2M04	418	436	452	470	496
DK2M05	373	374	394	410	421
DK2M06	437	465	493	517	542
DK2M07	379	392	411	420	438
DK2M08	394	423	447	450	475
DK2M09	423	442	450	478	504
DK2M10	417	439	450	459	484
Mean	403	421	441	456	479
S.D.	21	27	27	31	35
N	10	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 2 Body weight (males)

Sex: Male Body weight (g)

Mid 13F-OLE 25 mg/kg/day	Day(s) Relative to Start Date				
	1	8	15	22	29
DK3M01	361	371	377	392	431
DK3M02	377	395	412	433	454
DK3M03	426	438	444	464	493
DK3M04	413	440	464	453	477
DK3M05	433	457	479	498	522
DK3M06	410	434	458	458	483
DK3M07	372	394	417	423	445
DK3M08	398	418	439	444	470
DK3M09	389	415	442	451	477
DK3M10	418	437	457	464	480
Mean	400	420	439	448	473
S.D.	24	26	30	28	26
N	10	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 2 Body weight (males)

Sex: Male Body weight (g)

High 13F-OLE 200 mg/kg/day	Day(s) Relative to Start Date				
	1	8	15	22	29
DK4M01	381	402	419	441	465
DK4M02	415	440	461	479	497
DK4M03	411	436	457	460	482
DK4M04	404	426	447	455	481
DK4M05	397	413	436	458	484
DK4M06	380	409	431	443	460
DK4M07	367	380	398	400	411
DK4M08	432	463	488	494	521
DK4M09	377	398	415	422	440
DK4M10	419	432	447	459	474
Mean	398	420	440	451	472
S.D.	21	24	26	27	30
N	10	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 3 Body weight gain (males)

Sex: Male Body weight gain from Day 1 (g)

Control Vehicle 0 mg/kg/day	Day(s) Relative to Start Date				
	1	8	15	22	29
DK1M01	0	26	49	60	90
DK1M02	0	23	42	61	72
DK1M03	0	25	40	50	73
DK1M04	0	29	57	69	88
DK1M05	0	6	23	31	51
DK1M06	0	21	51	60	82
DK1M07	0	32	63	60	77
DK1M08	0	20	46	59	79
DK1M09	0	20	46	65	78
DK1M10	0	22	45	65	98
Mean	0	22	46	58	79
S.D.	0	7	11	11	13
N	10	10	10	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 3 Body weight gain (males)

Sex: Male Body weight gain from Day 1 (g)

Low 13F-OLE 5 mg/kg/day	Day(s) Relative to Start Date				
	1	8	15	22	29
DK2M01	0	18	48	68	88
DK2M02	0	18	39	51	81
DK2M03	0	19	37	50	69
DK2M04	0	18	34	52	78
DK2M05	0	1	21	37	48
DK2M06	0	28	56	80	105
DK2M07	0	13	32	41	59
DK2M08	0	29	53	56	81
DK2M09	0	19	27	55	81
DK2M10	0	22	33	42	67
Mean	0	19	38	53	76
S.D.	0	8	11	13	16
N	10	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 3 Body weight gain (males)

Sex: Male Body weight gain from Day 1 (g)

Mid 13F-OLE 25 mg/kg/day	Day(s) Relative to Start Date				
	1	8	15	22	29
DK3M01	0	10	16	31	70
DK3M02	0	18	35	56	77
DK3M03	0	12	18	38	67
DK3M04	0	27	51	40	64
DK3M05	0	24	46	65	89
DK3M06	0	24	48	48	73
DK3M07	0	22	45	51	73
DK3M08	0	20	41	46	72
DK3M09	0	26	53	62	88
DK3M10	0	19	39	46	62
Mean	0	20	39	48	74
S.D.	0	6	13	11	9
N	10	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 3 Body weight gain (males)

Sex: Male Body weight gain from Day 1 (g)

High 13F-OLE 200 mg/kg/day	Day(s) Relative to Start Date				
	1	8	15	22	29
DK4M01	0	21	38	60	84
DK4M02	0	25	46	64	82
DK4M03	0	25	46	49	71
DK4M04	0	22	43	51	77
DK4M05	0	16	39	61	87
DK4M06	0	29	51	63	80
DK4M07	0	13	31	33	44
DK4M08	0	31	56	62	89
DK4M09	0	21	38	45	63
DK4M10	0	13	28	40	55
Mean	0	22	42	53	73
S.D.	0	6	9	11	15
N	10	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 4 Food consumption (males)

Sex: Male Food consumption (g/day)

Control Vehicle 0 mg/kg/day	Day(s) Relative to Start Date	
	1 → 8	8 → 15
DK1M01	24.6	21.7
DK1M02	24.8	23.4
DK1M03	21.3	20.4
DK1M04	23.9	22.3
DK1M05	18.8	19.2
DK1M06	22.2	21.8
DK1M07	25.2	23.8
DK1M08	22.0	21.3
DK1M09	24.4	22.2
DK1M10	22.4	21.4
Mean	23.0	21.7
S.D.	2.0	1.3
N	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 4 Food consumption (males)

Sex: Male Food consumption (g/day)

Low 13F-OLE 5 mg/kg/day	Day(s) Relative to Start Date	
	1 → 8	8 → 15
DK2M01	21.6	21.4
DK2M02	22.4	21.6
DK2M03	21.5	21.4
DK2M04	24.6	22.5
DK2M05	18.0	18.3
DK2M06	24.4	23.0
DK2M07	21.2	20.8
DK2M08	22.6	20.9
DK2M09	22.7	17.4
DK2M10	23.0	21.2
Mean	22.2	20.8
S.D.	1.9	1.7
N	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 4 Food consumption (males)

Sex: Male Food consumption (g/day)

Mid 13F-OLE 25 mg/kg/day	Day(s) Relative to Start Date	
	1 → 8	8 → 15
DK3M01	20.9	17.4
DK3M02	19.9	19.1
DK3M03	22.7	21.2
DK3M04	23.8	23.4
DK3M05	24.7	22.5
DK3M06	23.3	23.3
DK3M07	21.0	20.2
DK3M08	22.2	20.7
DK3M09	25.9	23.9
DK3M10	25.1	22.2
Mean	22.9	21.4
S.D.	2.0	2.1
N	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 4 Food consumption (males)

Sex: Male Food consumption (g/day)

High 13F-OLE 200 mg/kg/day	Day(s) Relative to Start Date	
	1 → 8	8 → 15
DK4M01	22.1	20.7
DK4M02	22.7	21.9
DK4M03	24.4	23.9
DK4M04	22.1	20.9
DK4M05	21.3	21.6
DK4M06	21.1	20.5
DK4M07	20.0	19.1
DK4M08	23.9	22.4
DK4M09	20.2	18.7
DK4M10	22.4	19.0
Mean	22.0	20.9
S.D.	1.4	1.7
N	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 5 Determination of the thyroid hormone (T4) levels (males)

Sex: Male Day(s): 30 Relative to Start Date

Control Vehicle 0 mg/kg/day	Total T4 (ng/mL)
DK1M01	542.4
DK1M02	575.9
DK1M03	501.7
DK1M04	538.3
DK1M05	557.0
DK1M06	577.3
DK1M07	555.8
DK1M08	556.0
DK1M09	494.8
DK1M10	510.7
Mean	541.0
S.D.	29.5
N	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80 || T4: Thyroxine]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 5 Determination of the thyroid hormone (T4) levels (males)

Sex: Male Day(s): 30 Relative to Start Date

Low 13F-OLE 5 mg/kg/day	Total T4 (ng/mL)
DK2M01	497.4
DK2M02	529.0
DK2M03	539.3
DK2M04	548.9
DK2M05	526.6
DK2M06	573.0
DK2M07	527.2
DK2M08	462.8
DK2M09	575.5
DK2M10	518.1
Mean	529.8
S.D.	33.5
N	10

General Footnote: [T4: Thyroxine]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 5 Determination of the thyroid hormone (T4) levels (males)

Sex: Male Day(s): 30 Relative to Start Date

Mid 13F-OLE 25 mg/kg/day	Total T4 (ng/mL)
DK3M01	431.1
DK3M02	496.4
DK3M03	479.8
DK3M04	495.6
DK3M05	444.0
DK3M06	499.6
DK3M07	572.9
DK3M08	570.2
DK3M09	567.7
DK3M10	505.4
Mean	506.3
S.D.	50.3
N	10

General Footnote: [T4: Thyroxine]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 5 Determination of the thyroid hormone (T4) levels (males)

Sex: Male Day(s): 30 Relative to Start Date

High 13F-OLE 200 mg/kg/day	Total T4 (ng/mL)
DK4M01	456.2
DK4M02	499.9
DK4M03	412.2
DK4M04	417.8
DK4M05	515.9
DK4M06	488.4
DK4M07	480.0
DK4M08	553.5
DK4M09	461.0
DK4M10	462.7
Mean	474.8
S.D.	42.9
N	10

General Footnote: [T4: Thyroxine]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 6 Gross pathology (males)

Sex: Male Day(s): 30 Relative to Start Date

Group	Animal No.	Findings
Control	DK1M01	No pathological changes
Vehicle	DK1M02	No pathological changes
0	DK1M03	No pathological changes
mg/kg/day	DK1M04	No pathological changes
	DK1M05	No pathological changes
	DK1M06	No pathological changes
	DK1M07	No pathological changes
	DK1M08	No pathological changes
	DK1M09	No pathological changes
	DK1M10	No pathological changes
Low	DK2M01	No pathological changes
13F-OLE	DK2M02	No pathological changes
5	DK2M03	No pathological changes
mg/kg/day	DK2M04	No pathological changes
	DK2M05	No pathological changes
	DK2M06	No pathological changes
	DK2M07	No pathological changes
	DK2M08	No pathological changes
	DK2M09	No pathological changes
	DK2M10	No pathological changes

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 6 Gross pathology (males)

Sex: Male Day(s): 30 Relative to Start Date

Group		Animal No.	Findings
Mid		DK3M01	No pathological changes
13F-OLE		DK3M02	No pathological changes
25		DK3M03	No pathological changes
mg/kg/day	#	DK3M04	No pathological changes
		DK3M05	No pathological changes
		DK3M06	No pathological changes
		DK3M07	No pathological changes
		DK3M08	No pathological changes
		DK3M09	No pathological changes
		DK3M10	No pathological changes
High		DK4M01	Liver: Large
13F-OLE		DK4M02	Liver: Large
200		DK4M03	Liver: Large
mg/kg/day		DK4M04	Liver: Large
		DK4M05	Liver: Large
		DK4M06	Liver: Large
		DK4M07	No pathological changes
		DK4M08	Liver: Large
		DK4M09	No pathological changes
		DK4M10	No pathological changes

General Footnote: #: Not successfully mated

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 7 Absolute and relative organ weights (males)

Sex: Male Day(s): 30 Relative to Start Date

Control Vehicle 0 mg/kg/day	Final Bodyweight (g)	Liver (g)	Liver (%)	Testis Right (g)	Testis Right (%)	Testis Left (g)	Testis Left (%)	Testis Total (g)	Testis Total (%)
DK1M01	498	16.2	3.253	1.76	0.353	1.81	0.363	3.57	0.717
DK1M02	496	18.5	3.730	1.74	0.351	1.76	0.355	3.50	0.706
DK1M03	447	14.0	3.132	1.39	0.311	1.46	0.327	2.85	0.638
DK1M04	515	15.2	2.951	1.64	0.318	1.66	0.322	3.30	0.641
DK1M05	424	12.9	3.042	1.64	0.387	1.68	0.396	3.32	0.783
DK1M06	467	17.2	3.683	1.50	0.321	1.52	0.325	3.02	0.647
DK1M07	507	17.2	3.393	1.75	0.345	1.73	0.341	3.48	0.686
DK1M08	482	16.1	3.340	1.75	0.363	1.80	0.373	3.55	0.737
DK1M09	509	17.8	3.497	1.48	0.291	1.52	0.299	3.00	0.589
DK1M10	474	14.3	3.017	1.64	0.346	1.63	0.344	3.27	0.690
Mean	482	15.9	3.304	.	.	.	.	3.29	0.683
S.D.	29	1.8	0.275	.	.	.	.	0.25	0.056
N	10	10	10	.	.	.	.	10	10

General Footnote: [Relative organ weights: Organ to final body weight ratios || Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 7 Absolute and relative organ weights (males)

Sex: Male Day(s): 30 Relative to Start Date

Control Vehicle 0 mg/kg/day	Epididymis Right (g)	Epididymis Right (%)	Epididymis Left (g)	Epididymis Left (%)	Epididymis Total (g)	Epididymis Total (%)
DK1M01	0.69	0.139	0.75	0.151	1.44	0.289
DK1M02	0.70	0.141	0.64	0.129	1.34	0.270
DK1M03	0.57	0.128	0.57	0.128	1.14	0.255
DK1M04	0.69	0.134	0.62	0.120	1.31	0.254
DK1M05	0.60	0.142	0.60	0.142	1.20	0.283
DK1M06	0.64	0.137	0.55	0.118	1.19	0.255
DK1M07	0.72	0.142	0.72	0.142	1.44	0.284
DK1M08	0.65	0.135	0.65	0.135	1.30	0.270
DK1M09	0.64	0.126	0.65	0.128	1.29	0.253
DK1M10	0.74	0.156	0.73	0.154	1.47	0.310
Mean	.	.	.	.	1.31	0.272
S.D.	.	.	.	.	0.11	0.019
N	.	.	.	.	10	10

General Footnote: [Relative organ weights: Organ to final body weight ratios || Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 7 Absolute and relative organ weights (males)

Sex: Male Day(s): 30 Relative to Start Date

Control Vehicle 0 mg/kg/day	Levator ani muscle and bulbocavernosus muscle (g)	Levator ani muscle and bulbocavernosus muscle (%)	Cowper's gland (g)	Cowper's gland (%)	Glans penis (g)	Glans penis (%)
DK1M01	1.741	0.350	0.133	0.0267	0.120	0.0241
DK1M02	1.689	0.341	0.124	0.0250	0.108	0.0218
DK1M03	1.388	0.311	0.138	0.0309	0.095	0.0213
DK1M04	1.456	0.283	0.157	0.0305	0.125	0.0243
DK1M05	1.480	0.349	0.136	0.0321	0.102	0.0241
DK1M06	1.319	0.282	0.130	0.0278	0.096	0.0206
DK1M07	1.714	0.338	0.165	0.0325	0.138	0.0272
DK1M08	1.395	0.289	0.143	0.0297	0.101	0.0210
DK1M09	1.552	0.305	0.129	0.0253	0.121	0.0238
DK1M10	1.621	0.342	0.139	0.0293	0.132	0.0278
Mean	1.536	0.319	0.139	0.0290	0.114	0.0236
S.D.	0.150	0.028	0.013	0.0027	0.015	0.0025
N	10	10	10	10	10	10

General Footnote: [Relative organ weights: Organ to final body weight ratios || Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 7 Absolute and relative organ weights (males)

Sex: Male Day(s): 30 Relative to Start Date

Low 13F-OLE 5 mg/kg/day	Final Bodyweight (g)	Liver (g)	Liver (%)	Testis Right (g)	Testis Right (%)	Testis Left (g)	Testis Left (%)	Testis Total (g)	Testis Total (%)
DK2M01	488	16.9	3.463	1.43	0.293	1.43	0.293	2.86	0.586
DK2M02	488	18.3	3.750	1.66	0.340	1.60	0.328	3.26	0.668
DK2M03	456	17.3	3.794	1.83	0.401	1.86	0.408	3.69	0.809
DK2M04	491	18.7	3.809	1.39	0.283	1.38	0.281	2.77	0.564
DK2M05	425	13.1	3.082	1.40	0.329	1.40	0.329	2.80	0.659
DK2M06	543	17.7	3.260	1.75	0.322	1.68	0.309	3.43	0.632
DK2M07	440	16.1	3.659	1.53	0.348	1.54	0.350	3.07	0.698
DK2M08	477	18.6	3.899	1.79	0.375	1.70	0.356	3.49	0.732
DK2M09	506	15.8	3.123	1.59	0.314	1.63	0.322	3.22	0.636
DK2M10	484	16.2	3.347	1.71	0.353	1.73	0.357	3.44	0.711
Mean	480	16.9	3.519	.	.	.	.	3.20	0.669
S.D.	34	1.7	0.303	.	.	.	.	0.32	0.072
N	10	10	10	.	.	.	.	10	10

General Footnote: [Relative organ weights: Organ to final body weight ratios]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 7 Absolute and relative organ weights (males)

Sex: Male Day(s): 30 Relative to Start Date

Low 13F-OLE 5 mg/kg/day	Epididymis Right (g)	Epididymis Right (%)	Epididymis Left (g)	Epididymis Left (%)	Epididymis Total (g)	Epididymis Total (%)
DK2M01	0.60	0.123	0.60	0.123	1.20	0.246
DK2M02	0.64	0.131	0.59	0.121	1.23	0.252
DK2M03	0.73	0.160	0.69	0.151	1.42	0.311
DK2M04	0.65	0.132	0.57	0.116	1.22	0.248
DK2M05	0.56	0.132	0.56	0.132	1.12	0.264
DK2M06	0.57	0.105	0.56	0.103	1.13	0.208
DK2M07	0.60	0.136	0.69	0.157	1.29	0.293
DK2M08	0.69	0.145	0.68	0.143	1.37	0.287
DK2M09	0.72	0.142	0.66	0.130	1.38	0.273
DK2M10	0.65	0.134	0.63	0.130	1.28	0.264
Mean	.	.	.	.	1.26	0.265
S.D.	.	.	.	.	0.10	0.029
N	.	.	.	.	10	10

General Footnote: [Relative organ weights: Organ to final body weight ratios]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 7 Absolute and relative organ weights (males)

Sex: Male Day(s): 30 Relative to Start Date

Low 13F-OLE 5 mg/kg/day	Levator ani muscle and bulbocavernosus muscle (g)	Levator ani muscle and bulbocavernosus muscle (%)	Cowper's gland (g)	Cowper's gland (%)	Glans penis (g)	Glans penis (%)
DK2M01	1.565	0.321	0.154	0.0316	0.097	0.0199
DK2M02	1.615	0.331	0.171	0.0350	0.120	0.0246
DK2M03	1.420	0.311	0.156	0.0342	0.126	0.0276
DK2M04	1.293	0.263	0.161	0.0328	0.117	0.0238
DK2M05	1.352	0.318	0.129	0.0304	0.103	0.0242
DK2M06	1.671	0.308	0.120	0.0221	0.112	0.0206
DK2M07	1.442	0.328	0.144	0.0327	0.121	0.0275
DK2M08	1.393	0.292	0.170	0.0356	0.089	0.0187
DK2M09	1.626	0.321	0.138	0.0273	0.110	0.0217
DK2M10	1.629	0.337	0.128	0.0264	0.104	0.0215
Mean	1.501	0.313	0.147	0.0308	0.110	0.0230
S.D.	0.135	0.022	0.018	0.0043	0.012	0.0031
N	10	10	10	10	10	10

General Footnote: [Relative organ weights: Organ to final body weight ratios]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 7 Absolute and relative organ weights (males)

Sex: Male Day(s): 30 Relative to Start Date

Mid 13F-OLE 25 mg/kg/day	Final Bodyweight (g)	Liver (g)	Liver (%)	Testis Right (g)	Testis Right (%)	Testis Left (g)	Testis Left (%)	Testis Total (g)	Testis Total (%)
DK3M01	431	14.4	3.341	1.55	0.360	1.53	0.355	3.08	0.715
DK3M02	450	17.3	3.844	1.72	0.382	1.70	0.378	3.42	0.760
DK3M03	495	19.0	3.838	1.90	0.384	1.88	0.380	3.78	0.764
DK3M04	476	19.0	3.992	1.54	0.324	1.59	0.334	3.13	0.658
DK3M05	519	18.5	3.565	1.90	0.366	1.92	0.370	3.82	0.736
DK3M06	481	17.9	3.721	1.55	0.322	1.60	0.333	3.15	0.655
DK3M07	445	14.6	3.281	1.63	0.366	1.64	0.369	3.27	0.735
DK3M08	465	17.1	3.677	1.60	0.344	1.67	0.359	3.27	0.703
DK3M09	481	18.9	3.929	1.69	0.351	1.71	0.356	3.40	0.707
DK3M10	480	16.8	3.500	1.84	0.383	1.78	0.371	3.62	0.754
Mean	472	17.4	3.669	.	.	.	.	3.39	0.719
S.D.	26	1.7	0.243	.	.	.	.	0.27	0.039
N	10	10	10	.	.	.	.	10	10

General Footnote: [Relative organ weights: Organ to final body weight ratios]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 7 Absolute and relative organ weights (males)

Sex: Male Day(s): 30 Relative to Start Date

Mid 13F-OLE 25 mg/kg/day	Epididymis Right (g)	Epididymis Right (%)	Epididymis Left (g)	Epididymis Left (%)	Epididymis Total (g)	Epididymis Total (%)
DK3M01	0.62	0.144	0.65	0.151	1.27	0.295
DK3M02	0.71	0.158	0.62	0.138	1.33	0.296
DK3M03	0.65	0.131	0.62	0.125	1.27	0.257
DK3M04	0.62	0.130	0.61	0.128	1.23	0.258
DK3M05	0.63	0.121	0.64	0.123	1.27	0.245
DK3M06	0.67	0.139	0.64	0.133	1.31	0.272
DK3M07	0.62	0.139	0.64	0.144	1.26	0.283
DK3M08	0.63	0.135	0.62	0.133	1.25	0.269
DK3M09	0.65	0.135	0.64	0.133	1.29	0.268
DK3M10	0.69	0.144	0.76	0.158	1.45	0.302
Mean	.	.	.	.	1.29	0.274
S.D.	.	.	.	.	0.06	0.019
N	.	.	.	.	10	10

General Footnote: [Relative organ weights: Organ to final body weight ratios]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 7 Absolute and relative organ weights (males)

Sex: Male Day(s): 30 Relative to Start Date

Mid 13F-OLE 25 mg/kg/day	Levator ani muscle and bulbocavernosus muscle (g)	Levator ani muscle and bulbocavernosus muscle (%)	Cowper's gland (g)	Cowper's gland (%)	Glans penis (g)	Glans penis (%)
DK3M01	1.650	0.383	0.114	0.0265	0.131	0.0304
DK3M02	1.340	0.298	0.124	0.0276	0.106	0.0236
DK3M03	1.604	0.324	0.110	0.0222	0.109	0.0220
DK3M04	1.688	0.355	0.154	0.0324	0.097	0.0204
DK3M05	1.610	0.310	0.147	0.0283	0.122	0.0235
DK3M06	1.557	0.324	0.136	0.0283	0.103	0.0214
DK3M07	1.280	0.288	0.141	0.0317	0.100	0.0225
DK3M08	1.565	0.337	0.142	0.0305	0.114	0.0245
DK3M09	1.479	0.307	0.112	0.0233	0.098	0.0204
DK3M10	1.692	0.353	0.118	0.0246	0.108	0.0225
Mean	1.547	0.328	0.130	0.0275	0.109	0.0231
S.D.	0.141	0.029	0.016	0.0034	0.011	0.0029
N	10	10	10	10	10	10

General Footnote: [Relative organ weights: Organ to final body weight ratios]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 7 Absolute and relative organ weights (males)

Sex: Male Day(s): 30 Relative to Start Date

High 13F-OLE 200 mg/kg/day	Final Bodyweight (g)	Liver (g)	Liver (%)	Testis Right (g)	Testis Right (%)	Testis Left (g)	Testis Left (%)	Testis Total (g)	Testis Total (%)
DK4M01	465	22.4	4.817	1.46	0.314	1.46	0.314	2.92	0.628
DK4M02	497	24.6	4.950	1.72	0.346	1.73	0.348	3.45	0.694
DK4M03	485	27.6	5.691	1.71	0.353	1.74	0.359	3.45	0.711
DK4M04	479	31.0	6.472	1.74	0.363	1.69	0.353	3.43	0.716
DK4M05	488	23.5	4.816	1.67	0.342	1.67	0.342	3.34	0.684
DK4M06	466	22.3	4.785	1.51	0.324	1.53	0.328	3.04	0.652
DK4M07	413	19.3	4.673	1.55	0.375	1.60	0.387	3.15	0.763
DK4M08	522	23.6	4.521	1.74	0.333	1.68	0.322	3.42	0.655
DK4M09	437	19.9	4.554	1.62	0.371	1.61	0.368	3.23	0.739
DK4M10	477	19.3	4.046	1.67	0.350	1.65	0.346	3.32	0.696
Mean	473	23.4	4.932	.	.	.	.	3.28	0.694
S.D.	31	3.7	0.680	.	.	.	.	0.19	0.041
N	10	10	10	.	.	.	.	10	10

General Footnote: [Relative organ weights: Organ to final body weight ratios]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 7 Absolute and relative organ weights (males)

Sex: Male Day(s): 30 Relative to Start Date

High 13F-OLE 200 mg/kg/day	Epididymis Right (g)	Epididymis Right (%)	Epididymis Left (g)	Epididymis Left (%)	Epididymis Total (g)	Epididymis Total (%)
DK4M01	0.65	0.140	0.61	0.131	1.26	0.271
DK4M02	0.67	0.135	0.72	0.145	1.39	0.280
DK4M03	0.64	0.132	0.66	0.136	1.30	0.268
DK4M04	0.65	0.136	0.63	0.132	1.28	0.267
DK4M05	0.67	0.137	0.76	0.156	1.43	0.293
DK4M06	0.63	0.135	0.65	0.139	1.28	0.275
DK4M07	0.63	0.153	0.59	0.143	1.22	0.295
DK4M08	0.65	0.125	0.65	0.125	1.30	0.249
DK4M09	0.72	0.165	0.74	0.169	1.46	0.334
DK4M10	0.59	0.124	0.61	0.128	1.20	0.252
Mean	.	.	.	.	1.31	0.278
S.D.	.	.	.	.	0.09	0.025
N	.	.	.	.	10	10

General Footnote: [Relative organ weights: Organ to final body weight ratios]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 7 Absolute and relative organ weights (males)

Sex: Male Day(s): 30 Relative to Start Date

High 13F-OLE 200 mg/kg/day	Levator ani muscle and bulbocavernosus muscle (g)	Levator ani muscle and bulbocavernosus muscle (%)	Cowper's gland (g)	Cowper's gland (%)	Glans penis (g)	Glans penis (%)
DK4M01	1.566	0.337	0.128	0.0275	0.141	0.0303
DK4M02	1.526	0.307	0.130	0.0262	0.113	0.0227
DK4M03	1.889	0.389	0.190	0.0392	0.118	0.0243
DK4M04	1.594	0.333	0.132	0.0276	0.114	0.0238
DK4M05	1.555	0.319	0.140	0.0287	0.119	0.0244
DK4M06	1.342	0.288	0.127	0.0273	0.109	0.0234
DK4M07	1.224	0.296	0.120	0.0291	0.112	0.0271
DK4M08	1.550	0.297	0.137	0.0262	0.116	0.0222
DK4M09	1.586	0.363	0.139	0.0318	0.120	0.0275
DK4M10	1.464	0.307	0.153	0.0321	0.101	0.0212
Mean	1.530	0.324	0.140	0.0296	0.116	0.0247
S.D.	0.174	0.032	0.020	0.0040	0.010	0.0028
N	10	10	10	10	10	10

General Footnote: [Relative organ weights: Organ to final body weight ratios]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 8 Histopathology (males) -Cell numbers in seminiferous tubules-

Sex: Male Day(s): 30 Relative to Start Date

Control	Stage VII to VIII																					
Vehicle	Sertoli cells				Spermatogonia					Pachytene spermatocytes					Spermatids					Leydig cells			
0	Number of cells			Mean	Number of cells			Mean		Number of cells			Mean		Number of cells			Mean		Number of cells			Mean
mg/kg/day	1	2	3	of cells	1	2	3	of cells	%	1	2	3	of cells	%	1	2	3	of cells	%	1	2	3	of cells
DK1M01	4	5	3	4.0	7	6	3	5.3	132.5	59	60	82	67.0	1675.0	128	117	195	146.7	3667.5	20	22	19	20.3
DK1M02	6	12	8	8.7	6	3	3	4.0	46.0	43	59	56	52.7	605.7	100	125	148	124.3	1428.7	15	13	12	13.3
DK1M03	10	5	13	9.3	4	3	5	4.0	43.0	54	59	51	54.7	588.2	140	162	139	147.0	1580.6	14	17	15	15.3
DK1M04	10	15	11	12.0	4	4	5	4.3	35.8	35	52	56	47.7	397.5	133	123	138	131.3	1094.2	10	18	13	13.7
DK1M05	16	12	16	14.7	3	4	4	3.7	25.2	58	73	57	62.7	426.5	144	164	168	158.7	1079.6	10	12	14	12.0
DK1M06	12	13	15	13.3	7	4	5	5.3	39.8	56	63	57	58.7	441.4	138	151	138	142.3	1069.9	14	19	8	13.7
DK1M07	15	19	14	16.0	4	5	7	5.3	33.1	65	72	63	66.7	416.9	174	141	130	148.3	926.9	14	13	12	13.0
DK1M08	15	11	13	13.0	4	3	6	4.3	33.1	63	54	51	56.0	430.8	116	132	124	124.0	953.8	11	8	7	8.7
DK1M09	12	13	14	13.0	4	3	5	4.0	30.8	67	63	64	64.7	497.7	137	154	148	146.3	1125.4	12	9	8	9.7
DK1M10	11	4	7	7.3	4	6	10	6.7	91.8	60	48	57	55.0	753.4	158	164	129	150.3	2058.9	15	17	13	15.0
Mean				11.1				4.7	51.1				58.6	623.3				141.9	1498.6				13.5
S.D.				3.7				0.9	34.1				6.5	386.3				11.6	837.7				3.2
N				10				10	10				10	10				10	10				10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80 || %: Sertoli cell ratio: 100 × mean of each cell type / mean number of sertoli cells]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 8 Histopathology (males) -Cell numbers in seminiferous tubules-

Sex: Male Day(s): 30 Relative to Start Date

High	Stage VII to VIII																					
13F-OLE	Sertoli cells				Spermatogonia					Pachytene spermatocytes					Spermatids					Leydig cells			
200	Number of cells			Mean	Number of cells			Mean		Number of cells			Mean		Number of cells			Mean		Number of cells			Mean
mg/kg/day	1	2	3	of cells	1	2	3	of cells	%	1	2	3	of cells	%	1	2	3	of cells	%	1	2	3	of cells
DK4M01	9	6	11	8.7	6	7	4	5.7	65.5	40	54	45	46.3	532.2	124	115	125	121.3	1394.3	15	11	12	12.7
DK4M02	6	9	10	8.3	5	5	3	4.3	51.8	68	70	69	69.0	831.3	149	150	172	157.0	1891.6	15	19	22	18.7
DK4M03	13	20	8	13.7	7	11	10	9.3	67.9	57	50	51	52.7	384.7	164	130	158	150.7	1100.0	20	26	31	25.7
DK4M04	14	15	11	13.3	6	5	5	5.3	39.8	46	58	56	53.3	400.8	148	177	147	157.3	1182.7	14	17	18	16.3
DK4M05	14	10	13	12.3	5	4	4	4.3	35.0	58	62	50	56.7	461.0	128	126	145	133.0	1081.3	12	10	15	12.3
DK4M06	11	14	12	12.3	3	4	3	3.3	26.8	46	60	52	52.7	428.5	142	152	150	148.0	1203.3	9	12	10	10.3
DK4M07	17	15	17	16.3	5	8	5	6.0	36.8	62	46	64	57.3	351.5	139	160	163	154.0	944.8	11	17	13	13.7
DK4M08	16	12	13	13.7	4	6	5	5.0	36.5	74	61	55	63.3	462.0	179	169	153	167.0	1219.0	15	10	13	12.7
DK4M09	14	13	11	12.7	7	5	4	5.3	41.7	59	72	60	63.7	501.6	142	150	154	148.7	1170.9	17	12	15	14.7
DK4M10	17	7	8	10.7	5	4	6	5.0	46.7	74	59	70	67.7	632.7	179	162	174	171.7	1604.7	12	15	10	12.3
Mean				12.2				5.4	44.9				58.3	498.6				150.9	1279.3				14.9
S.D.				2.4				1.6	13.3				7.4	142.1				14.9	280.4				4.5
N				10				10	10				10	10				10	10				10

General Footnote: [%: Sertoli cell ratio: 100 × mean of each cell type / mean number of sertoli cells]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 9 Clinical observations (females)

Sex: Female

Group	Animal No.	Findings		
		Before copulation	Gestation period	Lactation period
Control	DK1F01	No abnormalities	No abnormalities	No abnormalities
Vehicle	DK1F02	No abnormalities	No abnormalities	Decrease in movement on Day 1
0				Soiled fur (reddish brown) of perivaginal ostium on Day 1
mg/kg/day				Scattering of all offspring in the cage on Day 1
				Found dead on Day 1
	DK1F03	No abnormalities	No abnormalities	No abnormalities
	DK1F04	No abnormalities	No abnormalities	No abnormalities
	DK1F05	No abnormalities	No abnormalities	No abnormalities
	DK1F06	No abnormalities	No abnormalities	No abnormalities
	DK1F07	No abnormalities	No abnormalities	No abnormalities
	DK1F08	No abnormalities	No abnormalities	No abnormalities
	DK1F09	No abnormalities	No abnormalities	No abnormalities
	DK1F10	No abnormalities	No abnormalities	No abnormalities
Low	DK2F01	No abnormalities	No abnormalities	No abnormalities
13F-OLE	DK2F02	No abnormalities	No abnormalities	No abnormalities
5	DK2F03	No abnormalities	No abnormalities	No abnormalities
mg/kg/day	DK2F04	No abnormalities	No abnormalities	No abnormalities
	DK2F05	No abnormalities	No abnormalities	No abnormalities
	DK2F06	No abnormalities	No abnormalities	No abnormalities
	DK2F07	No abnormalities	No abnormalities	No abnormalities
	DK2F08	No abnormalities	No abnormalities	No abnormalities
	DK2F09	No abnormalities	No abnormalities	No abnormalities
	DK2F10	No abnormalities	No abnormalities	No abnormalities

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 9 Clinical observations (females)

Sex: Female

Group	Animal No.	Findings		
		Before copulation	Gestation period	Lactation period
Mid	DK3F01	No abnormalities	No abnormalities	No abnormalities
13F-OLE	DK3F02	No abnormalities	No abnormalities	No abnormalities
25	DK3F03	No abnormalities	No abnormalities	No abnormalities
mg/kg/day	# DK3F04	No abnormalities	–	–
	DK3F05	No abnormalities	No abnormalities	No abnormalities
	DK3F06	No abnormalities	No abnormalities	No abnormalities
	DK3F07	No abnormalities	No abnormalities	No abnormalities
	DK3F08 [a)]	No abnormalities	No abnormalities	Scattering of all offspring in the cage on Days 0 and 1 Decrease in movement on Day 2
	DK3F09	No abnormalities	No abnormalities	No abnormalities
	DK3F10	No abnormalities	No abnormalities	No abnormalities
High	DK4F01	No abnormalities	No abnormalities	No abnormalities
13F-OLE	DK4F02	No abnormalities	No abnormalities	No abnormalities
200	DK4F03	No abnormalities	No abnormalities	No abnormalities
mg/kg/day	DK4F04 [a)]	No abnormalities	No abnormalities	Scattering of all offspring in the cage on Days 0 and 1
	DK4F05	No abnormalities	No abnormalities	Emaciation on Days 4-6
	DK4F06	No abnormalities	No abnormalities	No abnormalities
	DK4F07 [b)]	No abnormalities	–	–
	DK4F08	No abnormalities	No abnormalities	No abnormalities
	DK4F09	No abnormalities	No abnormalities	No abnormalities
	DK4F10	No abnormalities	No abnormalities	Scattering of all offspring in the cage on Day 1

General Footnote: #: Not successfully mated

[a)] Necropsied on Day 2 of lactation due to total litter loss

[b)] This animal was judged not successfully mated and was necropsied, but was found to be pregnant.

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 10 Body weight (females) -Pre-mating period-

Sex: Female Body weight (g)

Control Vehicle 0 mg/kg/day	Day(s) Relative to Start Date		
	1	8	15
DK1F01	241	254	252
DK1F02	268	280	309
DK1F03	270	271	273
DK1F04	256	269	283
DK1F05	251	273	285
DK1F06	278	275	300
DK1F07	262	268	276
DK1F08	284	297	325
DK1F09	250	265	279
DK1F10	246	263	270
Mean	261	272	285
S.D.	14	11	21
N	10	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 10 Body weight (females) -Pre-mating period-

Sex: Female Body weight (g)

Low 13F-OLE 5 mg/kg/day	Day(s) Relative to Start Date					
	1	8	15	22	29	36
DK2F01	259	280	316	.	.	.
DK2F02	246	261	270	.	.	.
DK2F03	250	262	290	.	.	.
DK2F04	262	265	283	.	.	.
DK2F05	264	271	289	.	.	.
DK2F06	274	284	291	318	.	.
DK2F07	252	268	277	.	.	.
DK2F08	266	274	281	.	.	.
DK2F09	282	295	306	.	.	.
DK2F10	273	288	300	.	.	.
Mean	263	275	290	318	.	.
S.D.	11	12	14	.	.	.
N	10	10	10	1	.	.

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 10 Body weight (females) -Pre-mating period-

Sex: Female Body weight (g)

Mid 13F-OLE 25 mg/kg/day	Day(s) Relative to Start Date					
	1	8	15	22	29	36
DK3F01	270	276	275	.	.	.
DK3F02	261	270	289	.	.	.
DK3F03	272	293	307	.	.	.
DK3F04	247	257	271	297	327	311
DK3F05	264	267	286	.	.	.
DK3F06	257	264	272	.	.	.
DK3F07	260	268	278	.	.	.
DK3F08	270	294	308	.	.	.
DK3F09	258	272	288	.	.	.
DK3F10	245	259	274	.	.	.
Mean	260	272	285	297	327	311
S.D.	9	13	14	.	.	.
N	10	10	10	1	1	1

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 10 Body weight (females) -Pre-mating period-

Sex: Female Body weight (g)

High 13F-OLE 200 mg/kg/day	Day(s) Relative to Start Date					
	1	8	15	22	29	36
DK4F01	266	259	282	.	.	.
DK4F02	266	270	293	.	.	.
DK4F03	247	262	281	.	.	.
DK4F04	276	291	297	.	.	.
DK4F05	246	255	279	.	.	.
DK4F06	271	294	308	.	.	.
DK4F07	260	264	274	305	336	362
DK4F08	246	260	259	.	.	.
DK4F09	253	279	289	.	.	.
DK4F10	257	264	279	.	.	.
Mean	259	270	284	305	336	362
S.D.	11	14	13	.	.	.
N	10	10	10	1	1	1

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 11 Body weight gain (females) -Pre-mating period-

Sex: Female Body weight gain from Day 1 (g)

Control Vehicle 0 mg/kg/day	Day(s) Relative to Start Date		
	1	8	15
DK1F01	0	13	11
DK1F02	0	12	41
DK1F03	0	1	3
DK1F04	0	13	27
DK1F05	0	22	34
DK1F06	0	-3	22
DK1F07	0	6	14
DK1F08	0	13	41
DK1F09	0	15	29
DK1F10	0	17	24
Mean	0	11	25
S.D.	0	8	13
N	10	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 11 Body weight gain (females) -Pre-mating period-

Sex: Female Body weight gain from Day 1 (g)

Low 13F-OLE 5 mg/kg/day	Day(s) Relative to Start Date					
	1	8	15	22	29	36
DK2F01	0	21	57	.	.	.
DK2F02	0	15	24	.	.	.
DK2F03	0	12	40	.	.	.
DK2F04	0	3	21	.	.	.
DK2F05	0	7	25	.	.	.
DK2F06	0	10	17	44	.	.
DK2F07	0	16	25	.	.	.
DK2F08	0	8	15	.	.	.
DK2F09	0	13	24	.	.	.
DK2F10	0	15	27	.	.	.
Mean	0	12	28	44	.	.
S.D.	0	5	12	.	.	.
N	10	10	10	1	.	.

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 11 Body weight gain (females) -Pre-mating period-

Sex: Female Body weight gain from Day 1 (g)

Mid 13F-OLE 25 mg/kg/day	Day(s) Relative to Start Date					
	1	8	15	22	29	36
DK3F01	0	6	5	.	.	.
DK3F02	0	9	28	.	.	.
DK3F03	0	21	35	.	.	.
DK3F04	0	10	24	50	80	64
DK3F05	0	3	22	.	.	.
DK3F06	0	7	15	.	.	.
DK3F07	0	8	18	.	.	.
DK3F08	0	24	38	.	.	.
DK3F09	0	14	30	.	.	.
DK3F10	0	14	29	.	.	.
Mean	0	12	24	50	80	64
S.D.	0	7	10	.	.	.
N	10	10	10	1	1	1

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 11 Body weight gain (females) -Pre-mating period-

Sex: Female Body weight gain from Day 1 (g)

High 13F-OLE 200 mg/kg/day	Day(s) Relative to Start Date					
	1	8	15	22	29	36
DK4F01	0	-7	16	.	.	.
DK4F02	0	4	27	.	.	.
DK4F03	0	15	34	.	.	.
DK4F04	0	15	21	.	.	.
DK4F05	0	9	33	.	.	.
DK4F06	0	23	37	.	.	.
DK4F07	0	4	14	45	76	102
DK4F08	0	14	13	.	.	.
DK4F09	0	26	36	.	.	.
DK4F10	0	7	22	.	.	.
Mean	0	11	25	45	76	102
S.D.	0	10	9	.	.	.
N	10	10	10	1	1	1

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 12 Body weight (females) -Gestation period-

Sex: Female Body weight (g)

Control Vehicle 0 mg/kg/day	Day(s) Relative to Mating (L)			
	0	7	14	20
DK1F01	251	291	336	429
DK1F02	308	351	390	476
DK1F03	284	316	354	432
DK1F04	279	327	359	434
DK1F05	285	324	352	440
DK1F06	297	331	363	456
DK1F07	279	322	355	420
DK1F08	324	364	402	491
DK1F09	279	314	342	424
DK1F10	271	313	346	433
Mean	286	325	360	444
S.D.	20	20	21	23
N	10	10	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 12 Body weight (females) -Gestation period-

Sex: Female Body weight (g)

Low 13F-OLE 5 mg/kg/day	Day(s) Relative to Mating (L)			
	0	7	14	20
DK2F01	315	336	369	455
DK2F02	273	308	344	430
DK2F03	290	334	371	480
DK2F04	279	314	350	435
DK2F05	292	319	358	459
DK2F06	312	365	407	451
DK2F07	277	317	346	430
DK2F08	295	333	368	444
DK2F09	314	346	380	473
DK2F10	301	342	379	479
Mean	295	331	367	454
S.D.	16	17	19	19
N	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 12 Body weight (females) -Gestation period-

Sex: Female Body weight (g)

Mid 13F-OLE 25 mg/kg/day	Day(s) Relative to Mating (L)			
	0	7	14	20
DK3F01	292	327	376	472
DK3F02	287	313	351	441
DK3F03	316	351	385	484
DK3F05	287	333	374	459
DK3F06	287	310	339	419
DK3F07	287	304	344	426
DK3F08	304	356	401	501
DK3F09	292	323	357	438
DK3F10	281	322	362	448
Mean	293	327	365	454
S.D.	11	18	20	27
N	9	9	9	9

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 12 Body weight (females) -Gestation period-

Sex: Female Body weight (g)

High 13F-OLE 200 mg/kg/day	Day(s) Relative to Mating (L)			
	0	7	14	20
DK4F01	281	315	350	445
DK4F02	289	335	369	447
DK4F03	277	308	342	411
DK4F04	298	329	363	438
DK4F05	276	313	349	440
DK4F06	313	351	385	471
DK4F08	273	299	333	404
DK4F09	291	322	357	448
DK4F10	282	313	357	441
Mean	287	321	356	438
S.D.	13	16	15	20
N	9	9	9	9

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 13 Body weight gain (females) -Gestation period-

Sex: Female Body weight gain from Day 0 (g)

Control Vehicle 0 mg/kg/day	Day(s) Relative to Mating (L)			
	0	7	14	20
DK1F01	0	40	85	178
DK1F02	0	43	82	168
DK1F03	0	32	70	148
DK1F04	0	48	80	155
DK1F05	0	39	67	155
DK1F06	0	34	66	159
DK1F07	0	43	76	141
DK1F08	0	40	78	167
DK1F09	0	35	63	145
DK1F10	0	42	75	162
Mean	0	40	74	158
S.D.	0	5	7	11
N	10	10	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 13 Body weight gain (females) -Gestation period-

Sex: Female Body weight gain from Day 0 (g)

Low 13F-OLE 5 mg/kg/day	Day(s) Relative to Mating (L)			
	0	7	14	20
DK2F01	0	21	54	140
DK2F02	0	35	71	157
DK2F03	0	44	81	190
DK2F04	0	35	71	156
DK2F05	0	27	66	167
DK2F06	0	53	95	139
DK2F07	0	40	69	153
DK2F08	0	38	73	149
DK2F09	0	32	66	159
DK2F10	0	41	78	178
Mean	0	37	72	159
S.D.	0	9	11	16
N	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 13 Body weight gain (females) -Gestation period-

Sex: Female Body weight gain from Day 0 (g)

Mid 13F-OLE 25 mg/kg/day	Day(s) Relative to Mating (L)			
	0	7	14	20
DK3F01	0	35	84	180
DK3F02	0	26	64	154
DK3F03	0	35	69	168
DK3F05	0	46	87	172
DK3F06	0	23	52	132
DK3F07	0	17	57	139
DK3F08	0	52	97	197
DK3F09	0	31	65	146
DK3F10	0	41	81	167
Mean	0	34	73	162
S.D.	0	11	15	21
N	9	9	9	9

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 13 Body weight gain (females) -Gestation period-

Sex: Female Body weight gain from Day 0 (g)

High 13F-OLE 200 mg/kg/day	Day(s) Relative to Mating (L)			
	0	7	14	20
DK4F01	0	34	69	164
DK4F02	0	46	80	158
DK4F03	0	31	65	134
DK4F04	0	31	65	140
DK4F05	0	37	73	164
DK4F06	0	38	72	158
DK4F08	0	26	60	131
DK4F09	0	31	66	157
DK4F10	0	31	75	159
Mean	0	34	69	152
S.D.	0	6	6	13
N	9	9	9	9

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 14 Body weight (females) -Lactation period-

Sex: Female Body weight (g)

Control Vehicle 0 mg/kg/day	Day(s) Relative to Littering (A)				
	0	4	7	11	13
DK1F01	297	345	346	379	376
DK1F02	319	.	.	.	.
DK1F03	310	329	343	348	367
DK1F04	322	359	357	382	378
DK1F05	343	340	352	376	378
DK1F06	316	353	366	399	383
DK1F07	306	348	353	369	365
DK1F08	373	398	403	436	414
DK1F09	292	339	344	350	354
DK1F10	307	365	370	383	393
Mean	319	353	359	380	379
S.D.	24	20	19	26	17
N	10	9	9	9	9

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 14 Body weight (females) -Lactation period-

Sex: Female Body weight (g)

Low 13F-OLE 5 mg/kg/day	Day(s) Relative to Littering (A)				
	0	4	7	11	13
DK2F01	329	358	365	372	374
DK2F02	308	335	348	363	366
DK2F03	351	354	357	371	368
DK2F04	326	349	350	379	375
DK2F05	319	352	354	380	382
DK2F06	417	404	413	422	425
DK2F07	307	338	341	359	366
DK2F08	345	365	369	378	380
DK2F09	372	391	398	400	405
DK2F10	346	346	379	397	386
Mean	342	359	367	382	383
S.D.	33	22	23	19	19
N	10	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 14 Body weight (females) -Lactation period-

Sex: Female Body weight (g)

Mid 13F-OLE 25 mg/kg/day	Day(s) Relative to Littering (A)				
	0	4	7	11	13
DK3F01	334	374	380	394	407
DK3F02	323	330	336	362	354
DK3F03	364	374	395	406	403
DK3F05	336	376	382	370	374
DK3F06	307	345	344	347	352
DK3F07	315	350	369	382	379
DK3F08	318	.	.	.	.
DK3F09	326	361	367	379	380
DK3F10	339	372	404	409	399
Mean	329	360	372	381	381
S.D.	17	17	23	21	21
N	9	8	8	8	8

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 14 Body weight (females) -Lactation period-

Sex: Female Body weight (g)

High 13F-OLE 200 mg/kg/day	Day(s) Relative to Littering (A)				
	0	4	7	11	13
DK4F01	305	349	347	366	364
DK4F02	329	344	358	377	390
DK4F03	317	337	356	357	366
DK4F04	304	.	.	.	.
DK4F05	294	248	281	322	344
DK4F06	369	391	390	398	397
DK4F08	282	272	334	346	355
DK4F09	345	362	377	396	399
DK4F10	291	335	353	390	377
Mean	315	330	350	369	374
S.D.	28	47	33	27	20
N	9	8	8	8	8

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 15 Body weight gain (females) -Lactation period-

Sex: Female Body weight gain from Day 0 (g)

Control Vehicle 0 mg/kg/day	Day(s) Relative to Littering (A)				
	0	4	7	11	13
DK1F01	0	48	49	82	79
DK1F02	0	.	.	.	.
DK1F03	0	19	33	38	57
DK1F04	0	37	35	60	56
DK1F05	0	-3	9	33	35
DK1F06	0	37	50	83	67
DK1F07	0	42	47	63	59
DK1F08	0	25	30	63	41
DK1F09	0	47	52	58	62
DK1F10	0	58	63	76	86
Mean	0	34	41	62	60
S.D.	0	18	16	18	16
N	10	9	9	9	9

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 15 Body weight gain (females) -Lactation period-

Sex: Female Body weight gain from Day 0 (g)

Low 13F-OLE 5 mg/kg/day	Day(s) Relative to Littering (A)				
	0	4	7	11	13
DK2F01	0	29	36	43	45
DK2F02	0	27	40	55	58
DK2F03	0	3	6	20	17
DK2F04	0	23	24	53	49
DK2F05	0	33	35	61	63
DK2F06	0	-13	-4	5	8
DK2F07	0	31	34	52	59
DK2F08	0	20	24	33	35
DK2F09	0	19	26	28	33
DK2F10	0	0	33	51	40
Mean	0	17	25	40	41
S.D.	0	15	14	18	18
N	10	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 15 Body weight gain (females) -Lactation period-

Sex: Female Body weight gain from Day 0 (g)

Mid 13F-OLE 25 mg/kg/day	Day(s) Relative to Littering (A)				
	0	4	7	11	13
DK3F01	0	40	46	60	73
DK3F02	0	7	13	39	31
DK3F03	0	10	31	42	39
DK3F05	0	40	46	34	38
DK3F06	0	38	37	40	45
DK3F07	0	35	54	67	64
DK3F08	0	.	.	.	.
DK3F09	0	35	41	53	54
DK3F10	0	33	65	70	60
Mean	0	30	42	51	51
S.D.	0	13	16	14	15
N	9	8	8	8	8

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 15 Body weight gain (females) -Lactation period-

Sex: Female Body weight gain from Day 0 (g)

High 13F-OLE 200 mg/kg/day	Day(s) Relative to Littering (A)				
	0	4	7	11	13
DK4F01	0	44	42	61	59
DK4F02	0	15	29	48	61
DK4F03	0	20	39	40	49
DK4F04	0	.	.	.	.
DK4F05	0	-46	-13	28	50
DK4F06	0	22	21	29	28
DK4F08	0	-10	52	64	73
DK4F09	0	17	32	51	54
DK4F10	0	44	62	99	86
Mean	0	13	33	53	58
S.D.	0	29	23	23	17
N	9	8	8	8	8

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 16 Food consumption (females) -Pre-mating period-

Sex: Female Food consumption (g/day)

Control Vehicle 0 mg/kg/day	Day(s) Relative to Start Date	
	1-8	8-15
DK1F01	14.8	14.3
DK1F02	19.1	21.8
DK1F03	17.0	17.3
DK1F04	17.9	18.5
DK1F05	17.5	16.9
DK1F06	17.4	18.6
DK1F07	15.6	15.7
DK1F08	18.3	21.1
DK1F09	18.9	18.9
DK1F10	16.8	14.8
Mean	17.3	17.8
S.D.	1.4	2.5
N	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 16 Food consumption (females) -Pre-mating period-

Sex: Female Food consumption (g/day)

Low 13F-OLE 5 mg/kg/day	Day(s) Relative to Start Date	
	1-8	8-15
DK2F01	18.7	20.8
DK2F02	16.8	16.2
DK2F03	16.5	19.6
DK2F04	17.4	19.2
DK2F05	19.1	18.9
DK2F06	18.7	17.9
DK2F07	17.2	16.6
DK2F08	17.8	18.5
DK2F09	18.2	17.8
DK2F10	17.5	17.7
Mean	17.8	18.3
S.D.	0.9	1.4
N	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 16 Food consumption (females) -Pre-mating period-

Sex: Female Food consumption (g/day)

Mid 13F-OLE 25 mg/kg/day	Day(s) Relative to Start Date	
	1-8	8-15
DK3F01	14.9	15.6
DK3F02	18.0	18.0
DK3F03	19.0	18.2
DK3F04	17.0	18.3
DK3F05	17.1	18.1
DK3F06	15.3	15.0
DK3F07	17.2	17.0
DK3F08	20.6	18.9
DK3F09	16.8	17.1
DK3F10	18.5	17.5
Mean	17.4	17.4
S.D.	1.7	1.2
N	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 16 Food consumption (females) -Pre-mating period-

Sex: Female Food consumption (g/day)

High 13F-OLE 200 mg/kg/day	Day(s) Relative to Start Date	
	1-8	8-15
DK4F01	14.2	18.3
DK4F02	16.4	17.7
DK4F03	15.0	16.4
DK4F04	15.6	14.6
DK4F05	15.5	16.8
DK4F06	19.4	18.8
DK4F07	16.3	16.6
DK4F08	15.9	16.1
DK4F09	19.1	18.1
DK4F10	16.2	18.5
Mean	16.4	17.2
S.D.	1.7	1.3
N	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 17 Food consumption (females) -Gestation period-

Sex: Female Food consumption (g/day)

Control Vehicle 0 mg/kg/day	Day(s) Relative to Mating (L)		
	0 → 7	7 → 14	14 → 20
DK1F01	18.3	21.7	26.7
DK1F02	26.1	25.2	22.4
DK1F03	21.6	23.3	22.5
DK1F04	23.9	23.7	24.3
DK1F05	21.6	22.6	26.6
DK1F06	20.7	21.9	24.6
DK1F07	19.7	20.6	21.7
DK1F08	22.8	25.7	26.1
DK1F09	21.8	21.8	24.6
DK1F10	19.4	21.3	25.0
Mean	21.6	22.8	24.4
S.D.	2.3	1.7	1.8
N	10	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 17 Food consumption (females) -Gestation period-

Sex: Female Food consumption (g/day)

Low 13F-OLE 5 mg/kg/day	Day(s) Relative to Mating (L)		
	0 → 7	7 → 14	14 → 20
DK2F01	20.8	23.2	26.1
DK2F02	20.9	22.2	22.5
DK2F03	24.1	24.7	27.9
DK2F04	22.4	24.0	26.4
DK2F05	20.4	22.5	27.6
DK2F06	24.1	29.3	29.5
DK2F07	20.8	19.7	23.3
DK2F08	23.1	25.2	25.3
DK2F09	20.3	22.2	25.2
DK2F10	23.6	23.9	27.2
Mean	22.0	23.7	26.1
S.D.	1.6	2.5	2.1
N	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 17 Food consumption (females) -Gestation period-

Sex: Female Food consumption (g/day)

Mid 13F-OLE 25 mg/kg/day	Day(s) Relative to Mating (L)		
	0 → 7	7 → 14	14 → 20
DK3F01	20.3	22.5	24.7
DK3F02	20.4	21.1	24.4
DK3F03	23.2	23.8	27.0
DK3F05	23.8	25.9	29.2
DK3F06	17.9	18.4	21.2
DK3F07	18.6	20.4	21.1
DK3F08	24.9	27.8	31.0
DK3F09	20.3	22.4	23.7
DK3F10	26.0	28.0	27.7
Mean	21.7	23.4	25.5
S.D.	2.9	3.3	3.4
N	9	9	9

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 17 Food consumption (females) -Gestation period-

Sex: Female Food consumption (g/day)

High 13F-OLE 200 mg/kg/day	Day(s) Relative to Mating (L)		
	0 → 7	7 → 14	14 → 20
DK4F01	21.2	22.4	25.9
DK4F02	21.5	22.4	24.4
DK4F03	18.7	20.8	21.9
DK4F04	16.5	18.7	22.4
DK4F05	21.5	22.6	25.6
DK4F06	23.9	23.9	27.5
DK4F08	18.7	20.6	22.0
DK4F09	20.9	23.5	25.8
DK4F10	21.4	23.3	26.9
Mean	20.5	22.0	24.7
S.D.	2.2	1.7	2.1
N	9	9	9

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 18 Food consumption (females) -Lactation period-

Sex: Female Food consumption (g/day)

Control Vehicle 0 mg/kg/day	Day(s) Relative to Littering (A)			
	0 → 4	4 → 7	7 → 11	11 → 13
DK1F01	35.0	45.6	60.4	66.6
DK1F03	26.8	43.6	55.7	64.6
DK1F04	33.0	40.1	55.4	59.2
DK1F05	24.6	42.2	58.2	60.9
DK1F06	33.5	47.1	63.7	66.7
DK1F07	33.0	42.8	53.4	58.2
DK1F08	20.3	39.6	54.8	58.7
DK1F09	31.5	45.3	58.4	65.5
DK1F10	38.4	50.4	61.1	69.3
Mean	30.7	44.1	57.9	63.3
S.D.	5.7	3.4	3.4	4.1
N	9	9	9	9

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 18 Food consumption (females) -Lactation period-

Sex: Female Food consumption (g/day)

Low 13F-OLE 5 mg/kg/day	Day(s) Relative to Littering (A)			
	0 → 4	4 → 7	7 → 11	11 → 13
DK2F01	25.1	46.1	55.1	60.1
DK2F02	24.2	46.3	54.5	64.0
DK2F03	24.7	37.7	54.2	61.1
DK2F04	30.7	43.3	54.7	61.2
DK2F05	24.8	42.8	63.1	67.8
DK2F06	20.9	37.6	50.0	45.7
DK2F07	28.8	44.4	57.7	67.0
DK2F08	28.5	46.1	55.2	62.7
DK2F09	29.1	43.4	52.0	58.3
DK2F10	13.8	44.5	54.9	58.2
Mean	25.0	43.2	55.1	60.6
S.D.	4.9	3.2	3.5	6.2
N	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 18 Food consumption (females) -Lactation period-

Sex: Female Food consumption (g/day)

Mid 13F-OLE 25 mg/kg/day	Day(s) Relative to Littering (A)			
	0 → 4	4 → 7	7 → 11	11 → 13
DK3F01	28.5	44.3	54.5	64.0
DK3F02	17.6	37.5	54.0	59.9
DK3F03	24.4	48.4	60.2	67.0
DK3F05	31.7	46.2	58.4	66.1
DK3F06	25.3	43.8	53.5	56.7
DK3F07	31.3	46.8	55.4	57.7
DK3F09	26.8	40.6	54.7	62.4
DK3F10	31.5	56.9	65.3	66.9
Mean	27.1	45.6	57.0	62.6
S.D.	4.8	5.8	4.1	4.1
N	8	8	8	8

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 18 Food consumption (females) -Lactation period-

Sex: Female Food consumption (g/day)

High 13F-OLE 200 mg/kg/day	Day(s) Relative to Littering (A)			
	0 → 4	4 → 7	7 → 11	11 → 13
DK4F01	34.0	44.1	58.0	63.7
DK4F02	10.2	27.8	35.6	42.4
DK4F03	23.3	45.0	52.4	60.2
DK4F05	0.7	12.5	45.5	62.1
DK4F06	32.6	44.8	57.3	62.8
DK4F08	4.2	40.1	52.7	63.1
DK4F09	26.3	45.4	59.5	64.1
DK4F10	17.4	45.8	58.8	60.0
Mean	18.6	38.2	52.5	59.8
S.D.	12.6	12.0	8.2	7.2
N	8	8	8	8

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears prior to mating period

Sex: Female Day(s) Relative to Start Date

Control Vehicle 0 mg/kg/day	Stage of estrous cycle											
	1	2	3	4	5	6	7	8	9	10	11	12
DK1F01	E	M	D	D	D	E	M	D	D	E	E	M
DK1F02	M	D	P	E	M	D	D	E	M	D	P	E
DK1F03	D	D	E	M	D	D	E	M	D	P	E	M
DK1F04	M	D	P	E	E	M	D	P	E	M	D	D
DK1F05	E	M	D	P	E	M	D	P	E	M	D	P
DK1F06	D	D	E	M	D	D	E	E	M	D	D	P
DK1F07	D	P	E	M	D	P	E	M	D	P	E	M
DK1F08	M	D	P	E	M	D	P	E	M	D	P	E
DK1F09	M	D	P	E	M	D	D	E	M	D	P	E
DK1F10	E	M	D	P	E	M	D	D	E	M	D	D

General Footnote: [P: Proestrus E: Estrus M: Metestrus D: Diestrus || Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears prior to mating period

Sex: Female Day(s) Relative to Start Date

Control Vehicle 0 mg/kg/day	Stage of estrous cycle		
	13	14	15
DK1F01	D	D	E
DK1F02	M	D	P
DK1F03	D	P	E
DK1F04	E	M	D
DK1F05	E	M	D
DK1F06	E	M	D
DK1F07	D	P	E
DK1F08	M	D	P
DK1F09	M	D	P
DK1F10	E	M	D

General Footnote: [P: Proestrus E: Estrus M: Metestrus D: Diestrus || Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears prior to mating period

Sex: Female Day(s) Relative to Start Date

Low 13F-OLE 5 mg/kg/day	Stage of estrous cycle											
	1	2	3	4	5	6	7	8	9	10	11	12
DK2F01	E	M	D	D	E	M	D	D	D	D	D	D
DK2F02	D	E	M	D	P	E	M	D	P	E	M	D
DK2F03	M	D	D	E	M	D	D	E	M	D	D	E
DK2F04	M	D	P	E	M	D	P	E	M	D	P	E
DK2F05	M	D	P	E	M	D	D	E	M	D	P	E
DK2F06	D	D	E	E	M	D	D	E	E	M	D	D
DK2F07	D	E	M	D	D	E	M	D	P	E	M	D
DK2F08	D	P	E	M	D	P	E	M	D	P	E	M
DK2F09	D	D	E	M	D	D	E	M	D	D	E	M
DK2F10	D	E	M	D	D	E	M	D	P	E	M	D

General Footnote: [P: Proestrus E: Estrus M: Metestrus D: Diestrus]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears prior to mating period

Sex: Female Day(s) Relative to Start Date

Low 13F-OLE 5 mg/kg/day	Stage of estrous cycle		
	13	14	15
DK2F01	D	D	D
DK2F02	P	E	M
DK2F03	M	D	D
DK2F04	M	D	P
DK2F05	M	D	P
DK2F06	P	E	M
DK2F07	D	E	M
DK2F08	D	D	E
DK2F09	D	D	E
DK2F10	P	E	M

General Footnote: [P: Proestrus E: Estrus M: Metestrus D: Diestrus]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears prior to mating period

Sex: Female Day(s) Relative to Start Date

Mid 13F-OLE 25 mg/kg/day	Stage of estrous cycle											
	1	2	3	4	5	6	7	8	9	10	11	12
DK3F01	D	D	E	M	D	D	E	M	D	D	E	M
DK3F02	M	D	P	E	M	D	P	E	M	D	P	E
DK3F03	D	E	M	D	D	E	M	D	P	E	M	D
DK3F04	P	E	M	D	D	E	M	D	P	E	M	D
DK3F05	D	E	E	M	D	D	E	E	M	D	P	E
DK3F06	D	P	E	M	D	D	E	M	D	P	E	M
DK3F07	D	P	E	M	D	P	E	M	D	P	E	M
DK3F08	E	M	D	P	E	M	D	P	E	M	D	D
DK3F09	P	E	M	D	P	E	M	D	P	E	M	D
DK3F10	P	E	M	D	D	E	M	D	P	E	M	D

General Footnote: [P: Proestrus E: Estrus M: Metestrus D: Diestrus]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears prior to mating period

Sex: Female Day(s) Relative to Start Date

Mid 13F-OLE 25 mg/kg/day	Stage of estrous cycle		
	13	14	15
DK3F01	D	D	E
DK3F02	M	D	P
DK3F03	P	E	M
DK3F04	P	E	M
DK3F05	M	D	D
DK3F06	D	P	E
DK3F07	D	P	E
DK3F08	E	M	D
DK3F09	P	E	M
DK3F10	P	E	M

General Footnote: [P: Proestrus E: Estrus M: Metestrus D: Diestrus]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears prior to mating period

Sex: Female Day(s) Relative to Start Date

High 13F-OLE 200 mg/kg/day	Stage of estrous cycle											
	1	2	3	4	5	6	7	8	9	10	11	12
DK4F01	M	D	P	E	M	D	P	E	M	D	P	E
DK4F02	D	P	E	M	D	D	P	E	M	D	D	P
DK4F03	E	M	D	P	E	M	D	P	E	M	D	D
DK4F04	D	E	M	D	D	E	M	D	P	E	M	D
DK4F05	M	D	P	E	M	D	D	E	M	D	D	D
DK4F06	P	E	M	D	D	E	M	D	P	E	M	D
DK4F07	D	E	E	M	D	D	E	M	D	P	E	M
DK4F08	D	P	E	M	D	P	E	M	D	P	E	M
DK4F09	E	M	D	D	E	M	D	D	E	M	D	D
DK4F10	M	D	P	E	M	D	D	E	M	D	P	E

General Footnote: [P: Proestrus E: Estrus M: Metestrus D: Diestrus]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears prior to mating period

Sex: Female Day(s) Relative to Start Date

High 13F-OLE 200 mg/kg/day	Stage of estrous cycle		
	13	14	15
DK4F01	M	D	P
DK4F02	E	M	D
DK4F03	E	M	D
DK4F04	D	E	M
DK4F05	E	M	D
DK4F06	P	E	M
DK4F07	D	D	E
DK4F08	D	P	E
DK4F09	E	M	D
DK4F10	M	D	D

General Footnote: [P: Proestrus E: Estrus M: Metestrus D: Diestrus]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears prior to mating period

Sex: Female Day(s) Relative to Start Date

Control Vehicle 0 mg/kg/day	Length of estrous cycle(Days)										
											Mean
	1	3	4	5	6	7	8	9	10	11	1 to 15
DK1F01	5	.	.	.	4	.	.	.	5	.	4.7
DK1F02	.	.	4	.	.	.	4	.	.	.	4.0
DK1F03	.	4	.	.	.	4	.	.	.	4	4.0
DK1F04	.	.	5	.	.	.	.	4	.	.	4.5
DK1F05	4	.	.	4	.	.	.	4	.	.	4.0
DK1F06	.	4	.	.	.	6	.	.	.	.	5.0
DK1F07	.	4	.	.	.	4	.	.	.	4	4.0
DK1F08	.	.	4	.	.	.	4	.	.	.	4.0
DK1F09	.	.	4	.	.	.	4	.	.	.	4.0
DK1F10	4	.	.	4	.	.	.	4	.	.	4.0
Mean	.	.	.	.	.	.	.	.	.	.	4.2
S.D.	.	.	.	.	.	.	.	.	.	.	0.4
N	.	.	.	.	.	.	.	.	.	.	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears prior to mating period

Sex: Female Day(s) Relative to Start Date

Low 13F-OLE 5 mg/kg/day	Length of estrous cycle(Days)											Mean
	1	2	3	4	5	6	7	8	9	10	11	1 to 15
DK2F01	4	.	.	.	.	.	.	.	.	.	.	4.0
DK2F02	.	4	.	.	.	4	.	.	.	4	.	4.0
DK2F03	.	.	.	4	.	.	.	4	.	.	.	4.0
DK2F04	.	.	.	4	.	.	.	4	.	.	.	4.0
DK2F05	.	.	.	4	.	.	.	4	.	.	.	4.0
DK2F06	.	.	5	.	.	.	.	6	.	.	.	5.5
DK2F07	.	4	.	.	.	4	.	.	.	4	.	4.0
DK2F08	.	.	4	.	.	.	4	.	.	.	4	4.0
DK2F09	.	.	4	.	.	.	4	.	.	.	4	4.0
DK2F10	.	4	.	.	.	4	.	.	.	4	.	4.0
Mean	.	.	.	.	.	.	.	.	.	.	.	4.2
S.D.	.	.	.	.	.	.	.	.	.	.	.	0.5
N	.	.	.	.	.	.	.	.	.	.	.	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears prior to mating period

Sex: Female Day(s) Relative to Start Date

Mid 13F-OLE 25 mg/kg/day	Length of estrous cycle(Days)											
												Mean
	1	2	3	4	5	6	7	8	9	10	11	1 to 15
DK3F01	.	.	4	.	.	.	4	.	.	.	4	4.0
DK3F02	.	.	.	4	.	.	.	4	.	.	.	4.0
DK3F03	.	4	.	.	.	4	.	.	.	4	.	4.0
DK3F04	.	4	.	.	.	4	.	.	.	4	.	4.0
DK3F05	.	5	.	.	.	.	5	.	.	.	.	5.0
DK3F06	.	.	4	.	.	.	4	.	.	.	4	4.0
DK3F07	.	.	4	.	.	.	4	.	.	.	4	4.0
DK3F08	4	.	.	.	4	.	.	.	4	.	.	4.0
DK3F09	.	4	.	.	.	4	.	.	.	4	.	4.0
DK3F10	.	4	.	.	.	4	.	.	.	4	.	4.0
Mean	.	.	.	.	.	.	.	.	.	.	.	4.1
S.D.	.	.	.	.	.	.	.	.	.	.	.	0.3
N	.	.	.	.	.	.	.	.	.	.	.	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears prior to mating period

Sex: Female Day(s) Relative to Start Date

High 13F-OLE 200 mg/kg/day	Length of estrous cycle(Days)											
												Mean
	1	2	3	4	5	6	7	8	9	10	11	1 to 15
DK4F01	.	.	.	4	.	.	.	4	.	.	.	4.0
DK4F02	.	.	5	.	.	.	.	5	.	.	.	5.0
DK4F03	4	.	.	.	4	.	.	.	4	.	.	4.0
DK4F04	.	4	.	.	.	4	.	.	.	4	.	4.0
DK4F05	.	.	.	4	.	.	.	5	.	.	.	4.5
DK4F06	.	4	.	.	.	4	.	.	.	4	.	4.0
DK4F07	.	5	.	.	.	.	4	.	.	.	4	4.3
DK4F08	.	.	4	.	.	.	4	.	.	.	4	4.0
DK4F09	4	.	.	.	4	.	.	.	4	.	.	4.0
DK4F10	.	.	.	4	.	.	.	4	.	.	.	4.0
Mean	.	.	.	.	.	.	.	.	.	.	.	4.2
S.D.	.	.	.	.	.	.	.	.	.	.	.	0.3
N	.	.	.	.	.	.	.	.	.	.	.	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears during mating period

Sex: Female Day(s) Relative to Pairing (1)

Control Vehicle 0 mg/kg/day	Stage of estrous cycle			
	1	2	3	4
DK1F01	S	.	.	.
DK1F02	S	.	.	.
DK1F03	M	D	P	S
DK1F04	P	S	.	.
DK1F05	P	S	.	.
DK1F06	D	D	S	.
DK1F07	M	D	P	S
DK1F08	S	.	.	.
DK1F09	S	.	.	.
DK1F10	P	S	.	.

General Footnote: [P: Proestrus E: Estrus M: Metestrus D: Diestrus S: Successful mating || Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears during mating period

Sex: Female Day(s) Relative to Pairing (1)

Low 13F-OLE 5 mg/kg/day	Stage of estrous cycle													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
DK2F01	D	D	D	S	.	.	.	.	.	.	.	.	.	.
DK2F02	D	P	S	.	.	.	.	.	.	.	.	.	.	.
DK2F03	E	S	.	.	.	.	.	.	.	.	.	.	.	.
DK2F04	S	.	.	.	.	.	.	.	.	.	.	.	.	.
DK2F05	S	.	.	.	.	.	.	.	.	.	.	.	.	.
DK2F06	D	E	M	D	D	D	D	D	S	.	.	.	.	.
DK2F07	D	P	S	.	.	.	.	.	.	.	.	.	.	.
DK2F08	M	D	P	S	.	.	.	.	.	.	.	.	.	.
DK2F09	M	D	D	S	.	.	.	.	.	.	.	.	.	.
DK2F10	D	P	S	.	.	.	.	.	.	.	.	.	.	.

General Footnote: [P: Proestrus E: Estrus M: Metestrus D: Diestrus S: Successful mating]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears during mating period

Sex: Female Day(s) Relative to Pairing (1)

Mid 13F-OLE 25 mg/kg/day	Stage of estrous cycle													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
DK3F01	M	D	D	S	.	.	.	.	.	.	.	.	.	.
DK3F02	S	.	.	.	.	.	.	.	.	.	.	.	.	.
DK3F03	D	P	S	.	.	.	.	.	.	.	.	.	.	.
DK3F04	D	P	E	M	D	D	D	D	D	D	D	D	D	D
DK3F05	S	.	.	.	.	.	.	.	.	.	.	.	.	.
DK3F06	M	D	P	S	.	.	.	.	.	.	.	.	.	.
DK3F07	M	D	P	S	.	.	.	.	.	.	.	.	.	.
DK3F08	P	S	.	.	.	.	.	.	.	.	.	.	.	.
DK3F09	D	P	S	.	.	.	.	.	.	.	.	.	.	.
DK3F10	D	P	S	.	.	.	.	.	.	.	.	.	.	.

General Footnote: [P: Proestrus E: Estrus M: Metestrus D: Diestrus S: Successful mating]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 19 Vaginal smears during mating period

Sex: Female Day(s) Relative to Pairing (1)

High 13F-OLE 200 mg/kg/day	Stage of estrous cycle													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
DK4F01	S	.	.	.	.	.	.	.	.	.	.	.	.	.
DK4F02	D	P	S	.	.	.	.	.	.	.	.	.	.	.
DK4F03	D	E	M	D	D	P	S	.	.	.	.	.	.	.
DK4F04	D	P	S	.	.	.	.	.	.	.	.	.	.	.
DK4F05	D	S	.	.	.	.	.	.	.	.	.	.	.	.
DK4F06	D	P	S	.	.	.	.	.	.	.	.	.	.	.
DK4F07	S	.	.	.	.	.	.	.	.	.	.	.	.	.
DK4F08	M	D	P	S	.	.	.	.	.	.	.	.	.	.
DK4F09	D	S	.	.	.	.	.	.	.	.	.	.	.	.
DK4F10	S	.	.	.	.	.	.	.	.	.	.	.	.	.

General Footnote: [P: Proestrus E: Estrus M: Metestrus D: Diestrus S: Successful mating]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 20 Mating ability and fertility

Sex: Female Day(s): 1-14 Relative to Pairing (L)

Control Vehicle 0 mg/kg/day	Male Animal No. (First pair)	Copulation	Pregnancy	Pre-coital period (Days)
DK1F01	DK1M01	Yes	Yes	1
DK1F02	DK1M02	Yes	Yes	1
DK1F03	DK1M03	Yes	Yes	4
DK1F04	DK1M04	Yes	Yes	2
DK1F05	DK1M05	Yes	Yes	2
DK1F06	DK1M06	Yes	Yes	3
DK1F07	DK1M07	Yes	Yes	4
DK1F08	DK1M08	Yes	Yes	1
DK1F09	DK1M09	Yes	Yes	1
DK1F10	DK1M10	Yes	Yes	2
Mean	.	.	.	2.1
S.D.	.	.	.	1.2
N	.	.	.	10
%	.	100.0	100.0	.

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 20 Mating ability and fertility

Sex: Female Day(s): 1-14 Relative to Pairing (L)

Low 13F-OLE 5 mg/kg/day	Male Animal No. (First pair)	Copulation	Pregnancy	Pre-coital period (Days)
DK2F01	DK2M01	Yes	Yes	4
DK2F02	DK2M02	Yes	Yes	3
DK2F03	DK2M03	Yes	Yes	2
DK2F04	DK2M04	Yes	Yes	1
DK2F05	DK2M05	Yes	Yes	1
DK2F06	DK2M06	Yes	Yes	9
DK2F07	DK2M07	Yes	Yes	3
DK2F08	DK2M08	Yes	Yes	4
DK2F09	DK2M09	Yes	Yes	4
DK2F10	DK2M10	Yes	Yes	3
Mean	.	.	.	3.4
S.D.	.	.	.	2.3
N	.	.	.	10
%	.	100.0	100.0	.

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 20 Mating ability and fertility

Sex: Female Day(s): 1-14 Relative to Pairing (L)

Mid 13F-OLE 25 mg/kg/day	Male Animal No. (First pair)	Copulation	Pregnancy	Pre-coital period (Days)
DK3F01	DK3M01	Yes	Yes	4
DK3F02	DK3M02	Yes	Yes	1
DK3F03	DK3M03	Yes	Yes	3
DK3F04	DK3M04	No	.	.
DK3F05	DK3M05	Yes	Yes	1
DK3F06	DK3M06	Yes	Yes	4
DK3F07	DK3M07	Yes	Yes	4
DK3F08	DK3M08	Yes	Yes	2
DK3F09	DK3M09	Yes	Yes	3
DK3F10	DK3M10	Yes	Yes	3
Mean	.	.	.	2.8
S.D.	.	.	.	1.2
N	.	.	.	9
%	.	90.0	100.0	.

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 20 Mating ability and fertility

Sex: Female Day(s): 1-14 Relative to Pairing (L)

High 13F-OLE 200 mg/kg/day	Male Animal No. (First pair)	Copulation	Pregnancy	Pre-coital period (Days)
DK4F01	DK4M01	Yes	Yes	1
DK4F02	DK4M02	Yes	Yes	3
DK4F03	DK4M03	Yes	Yes	7
DK4F04	DK4M04	Yes	Yes	3
DK4F05	DK4M05	Yes	Yes	2
DK4F06	DK4M06	Yes	Yes	3
DK4F07	DK4M07	Yes	Yes	1
DK4F08	DK4M08	Yes	Yes	4
DK4F09	DK4M09	Yes	Yes	2
DK4F10	DK4M10	Yes	Yes	1
Mean	.	.	.	2.7
S.D.	.	.	.	1.8
N	.	.	.	10
%	.	100.0	100.0	.

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 21 Determination of the thyroid hormone (T4) levels (females)

Sex: Female Day(s): 14 Relative to Littering (A)

Control Vehicle 0 mg/kg/day	Total T4 (ng/mL)
DK1F01	388.2
DK1F03	382.2
DK1F04	384.4
DK1F05	343.6
DK1F06	345.7
DK1F07	393.6
DK1F08	284.5
DK1F09	380.4
DK1F10	465.2
Mean	374.2
S.D.	48.5
N	9

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80 || T4: Thyroxine]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 21 Determination of the thyroid hormone (T4) levels (females)

Sex: Female Day(s): 14 Relative to Littering (A)

Low 13F-OLE 5 mg/kg/day	Total T4 (ng/mL)
DK2F01	378.1
DK2F02	349.5
DK2F03	409.7
DK2F04	386.8
DK2F05	404.8
DK2F06	403.3
DK2F07	309.6
DK2F08	418.6
DK2F09	390.1
DK2F10	341.4
Mean	379.2
S.D.	35.0
N	10

General Footnote: [T4: Thyroxine]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 21 Determination of the thyroid hormone (T4) levels (females)

Sex: Female Day(s): 14 Relative to Littering (A)

Mid 13F-OLE 25 mg/kg/day	Total T4 (ng/mL)
DK3F01	396.2
DK3F02	357.7
DK3F03	303.1
DK3F05	391.3
DK3F06	358.6
DK3F07	347.0
DK3F09	348.5
DK3F10	396.5
Mean	362.4
S.D.	31.9
N	8

General Footnote: [T4: Thyroxine]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 21 Determination of the thyroid hormone (T4) levels (females)

Sex: Female Day(s): 14 Relative to Littering (A)

High 13F-OLE 200 mg/kg/day	Total T4 (ng/mL)
DK4F01	346.1
DK4F02	429.2
DK4F03	366.6
DK4F05	442.0
DK4F06	353.3
DK4F08	375.1
DK4F09	367.3
DK4F10	381.0
Mean	382.6
S.D.	34.7
N	8

General Footnote: [T4: Thyroxine]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 22 Gross pathology (females)

Sex: Female Day(s): 14 Relative to Littering (A)

Group	Animal No.	Findings
Control	DK1F01	No pathological changes
Vehicle	DK1F02 [a)]	Thymus: Small
0		Spleen: Discoloration, pale
mg/kg/day	DK1F03	No pathological changes
	DK1F04	No pathological changes
	DK1F05	No pathological changes
	DK1F06	No pathological changes
	DK1F07	No pathological changes
	DK1F08	No pathological changes
	DK1F09	No pathological changes
	DK1F10	No pathological changes
Low	DK2F01	No pathological changes
13F-OLE	DK2F02	No pathological changes
5	DK2F03	No pathological changes
mg/kg/day	DK2F04	No pathological changes
	DK2F05	No pathological changes
	DK2F06	No pathological changes
	DK2F07	No pathological changes
	DK2F08	No pathological changes
	DK2F09	No pathological changes
	DK2F10	No pathological changes

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80
[a)] Necropsied on Day 1 of lactation due to death

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 22 Gross pathology (females)

Sex: Female Day(s): 14 Relative to Littering (A)

Group	Animal No.	Findings
Mid	DK3F01	No pathological changes
13F-OLE	DK3F02	No pathological changes
25	DK3F03	No pathological changes
mg/kg/day	# DK3F04	No pathological changes
	DK3F05	No pathological changes
	DK3F06	No pathological changes
	DK3F07	No pathological changes
	DK3F08 [a)]	Thymus: Small
		Spleen: Small
	DK3F09	No pathological changes
	DK3F10	No pathological changes
High	DK4F01	No pathological changes
13F-OLE	DK4F02	No pathological changes
200	DK4F03	No pathological changes
mg/kg/day	DK4F04 [a)]	Thymus: Small
	DK4F05	No pathological changes
	DK4F06	No pathological changes
	DK4F07 [b)]	Uterus: Remain, fetuses, right horn
	DK4F08	No pathological changes
	DK4F09	No pathological changes
	DK4F10	No pathological changes

General Footnote: #: Not successfully mated

[a)] Necropsied on Day 2 of lactation due to total litter loss

[b)] This animal was judged not successfully mated and was necropsied, but was found to be pregnant.
Intrauterine content: Right horn: 1 living male fetus, 1 dead female fetus and 3 implantation sites were observed.
Left horn: 3 implantation sites were observed.

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 23 Absolute and relative organ weights (females)

Sex: Female Day(s): 14 Relative to Littering (A)

Control Vehicle 0 mg/kg/day	Final Bodyweight (g)	Liver (g)	Liver (%)
DK1F01	377	14.4	3.820
DK1F03	360	13.5	3.750
DK1F04	375	15.8	4.213
DK1F05	371	15.6	4.205
DK1F06	397	17.2	4.332
DK1F07	370	15.6	4.216
DK1F08	426	17.5	4.108
DK1F09	356	15.4	4.326
DK1F10	386	16.2	4.197
Mean	380	15.7	4.130
S.D.	21	1.2	0.208
N	9	9	9

General Footnote: [Relative organ weights: Organ to final body weight ratios || Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 23 Absolute and relative organ weights (females)

Sex: Female Day(s): 14 Relative to Littering (A)

Low 13F-OLE 5 mg/kg/day	Final Bodyweight (g)	Liver (g)	Liver (%)
DK2F01	375	18.6	4.960
DK2F02	366	16.8	4.590
DK2F03	381	16.8	4.409
DK2F04	376	16.2	4.309
DK2F05	384	15.7	4.089
DK2F06	424	15.6	3.679
DK2F07	361	17.0	4.709
DK2F08	376	17.0	4.521
DK2F09	409	19.5	4.768
DK2F10	391	15.6	3.990
Mean	384	16.9	4.402
S.D.	19	1.3	0.393
N	10	10	10

General Footnote: [Relative organ weights: Organ to final body weight ratios]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 23 Absolute and relative organ weights (females)

Sex: Female Day(s): 14 Relative to Littering (A)

Mid 13F-OLE 25 mg/kg/day	Final Bodyweight (g)	Liver (g)	Liver (%)
DK3F01	414	21.3	5.145
DK3F02	360	15.3	4.250
DK3F03	399	16.5	4.135
DK3F05	380	15.1	3.974
DK3F06	345	17.1	4.957
DK3F07	376	16.1	4.282
DK3F09	375	16.4	4.373
DK3F10	411	17.1	4.161
Mean	383	16.9	4.410
S.D.	24	1.9	0.416
N	8	8	8

General Footnote: [Relative organ weights: Organ to final body weight ratios]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 23 Absolute and relative organ weights (females)

Sex: Female Day(s): 14 Relative to Littering (A)

High 13F-OLE 200 mg/kg/day	Final Bodyweight (g)	Liver (g)	Liver (%)
DK4F01	357	15.6	4.370
DK4F02	390	17.4	4.462
DK4F03	368	15.5	4.212
DK4F05	344	16.3	4.738
DK4F06	395	18.8	4.759
DK4F08	350	18.7	5.343
DK4F09	400	19.5	4.875
DK4F10	375	17.3	4.613
Mean	372	17.4	4.672
S.D.	21	1.5	0.349
N	8	8	8

General Footnote: [Relative organ weights: Organ to final body weight ratios]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 24 Reproductive observations (females) at delivery

Sex: Female Day(s) Relative to Littering (A)

Control Vehicle 0 mg/kg/day	Observation items							
	Duration of gestation (Days)	Implantation sites (Number)	Newborns (Number)	Live newborns (Number)	Male live newborns (Number)	Female live newborns (Number)	Dead newborns (Number)	External anomalies (Number)
	Day 0	Days 0-14	Day 0	Day 0	Day 0	Day 0	Day 0	Day 0
DK1F01	22	15	14	14	10	4	0	0
DK1F02	22	17	17	7	3	4	10	0
DK1F03	22	18	18	18	10	8	0	0
DK1F04	22	16	15	15	9	6	0	0
DK1F05	22	15	15	15	6	9	0	0
DK1F06	22	18	18	17	7	10	1	0
DK1F07	22	17	15	15	9	6	0	0
DK1F08	22	14	14	14	4	10	0	0
DK1F09	22	17	16	15	6	9	1	0
DK1F10	22	17	16	16	9	7	0	0
Mean	22.0	16.4	15.8	14.6	7.3	7.3	1.2	0.0
S.D.	0.0	1.3	1.5	3.0	2.5	2.3	3.1	0.0
Total	.	164	158	146	73	73	12	0
N	10	10	10	10	10	10	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 24 Reproductive observations (females) at delivery

Sex: Female Day(s) Relative to Littering (A)

Low 13F-OLE 5 mg/kg/day	Observation items							
	Duration of gestation (Days)	Implantation sites (Number)	Newborns (Number)	Live newborns (Number)	Male live newborns (Number)	Female live newborns (Number)	Dead newborns (Number)	External anomalies (Number)
	Day 0	Days 0-14	Day 0	Day 0	Day 0	Day 0	Day 0	Day 0
DK2F01	22	18	15	15	7	8	0	0
DK2F02	22	16	16	16	4	12	0	0
DK2F03	22	20	18	17	7	10	1	0
DK2F04	22	14	13	13	6	7	0	0
DK2F05	22	15	15	15	7	8	0	0
DK2F06	22	17	5	5	3	2	0	0
DK2F07	22	16	15	15	12	3	0	0
DK2F08	22	14	14	13	4	9	1	0
DK2F09	22	14	14	14	8	6	0	0
DK2F10	22	17	17	16	6	10	1	0
Mean	22.0	16.1	14.2	13.9	6.4	7.5	0.3	0.0
S.D.	0.0	2.0	3.6	3.4	2.5	3.1	0.5	0.0
Total	.	161	142	139	64	75	3	0
N	10	10	10	10	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 24 Reproductive observations (females) at delivery

Sex: Female Day(s) Relative to Littering (A)

Mid 13F-OLE 25 mg/kg/day	Observation items							
	Duration of gestation (Days)	Implantation sites (Number)	Newborns (Number)	Live newborns (Number)	Male live newborns (Number)	Female live newborns (Number)	Dead newborns (Number)	External anomalies (Number)
	Day 0	Days 0-14	Day 0	Day 0	Day 0	Day 0	Day 0	Day 0
DK3F01	22	18	18	18	5	13	0	0
DK3F02	22	16	15	13	3	10	2	0
DK3F03	22	18	18	18	7	11	0	0
DK3F05	22	15	13	13	4	9	0	0
DK3F06	22	15	15	14	7	7	1	0
DK3F07	22	16	15	15	10	5	0	0
DK3F08	23	17	17	4	1	3	13	0
DK3F09	22	16	15	15	7	8	0	0
DK3F10	22	17	15	15	4	11	0	0
Mean	22.1	16.4	15.7	13.9	5.3	8.6	1.8	0.0
S.D.	0.3	1.1	1.7	4.1	2.7	3.2	4.3	0.0
Total	.	148	141	125	48	77	16	0
N	9	9	9	9	9	9	9	9

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 24 Reproductive observations (females) at delivery

Sex: Female Day(s) Relative to Littering (A)

High 13F-OLE 200 mg/kg/day	Observation items							
	Duration of gestation (Days)	Implantation sites (Number)	Newborns (Number)	Live newborns (Number)	Male live newborns (Number)	Female live newborns (Number)	Dead newborns (Number)	External anomalies (Number)
	Day 0	Days 0-14	Day 0	Day 0	Day 0	Day 0	Day 0	Day 0
DK4F01	22	17	16	16	11	5	0	0
DK4F02	22	17	14	14	11	3	0	0
DK4F03	22	14	13	13	5	8	0	0
DK4F04	22	17	15	13	8	5	2	0
DK4F05	22	16	15	13	8	5	2	0
DK4F06	22	14	14	14	7	7	0	0
DK4F08	22	15	15	15	6	9	0	0
DK4F09	22	16	15	15	8	7	0	0
DK4F10	22	18	18	18	7	11	0	0
Mean	22.0	16.0	15.0	14.6	7.9	6.7	0.4	0.0
S.D.	0.0	1.4	1.4	1.7	2.0	2.4	0.9	0.0
Total	.	144	135	131	71	60	4	0
N	9	9	9	9	9	9	9	9

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 24 Reproductive observations (females) at delivery

Sex: Female Day(s) Relative to Littering (A)

Control Vehicle 0 mg/kg/day	Observation items			
	Delivery index (%)	Birth index (%)	Sex ratio (Male/Total)	External anomalies (%)
	Day 0	Day 0	Day 0	Day 0
DK1F01	93.3	93.3	0.71	0.0
DK1F02	100.0	41.2	0.43	0.0
DK1F03	100.0	100.0	0.56	0.0
DK1F04	93.8	93.8	0.60	0.0
DK1F05	100.0	100.0	0.40	0.0
DK1F06	100.0	94.4	0.41	0.0
DK1F07	88.2	88.2	0.60	0.0
DK1F08	100.0	100.0	0.29	0.0
DK1F09	94.1	88.2	0.40	0.0
DK1F10	94.1	94.1	0.56	0.0
Mean	96.4	89.3	0.50	0.0
S.D.	4.2	17.5	0.13	0.0
N	10	10	10	10

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 24 Reproductive observations (females) at delivery

Sex: Female Day(s) Relative to Littering (A)

Low 13F-OLE 5 mg/kg/day	Observation items			
	Delivery index (%)	Birth index (%)	Sex ratio (Male/Total)	External anomalies (%)
	Day 0	Day 0	Day 0	Day 0
DK2F01	83.3	83.3	0.47	0.0
DK2F02	100.0	100.0	0.25	0.0
DK2F03	90.0	85.0	0.41	0.0
DK2F04	92.9	92.9	0.46	0.0
DK2F05	100.0	100.0	0.47	0.0
DK2F06	29.4	29.4	0.60	0.0
DK2F07	93.8	93.8	0.80	0.0
DK2F08	100.0	92.9	0.31	0.0
DK2F09	100.0	100.0	0.57	0.0
DK2F10	100.0	94.1	0.38	0.0
Mean	88.9	87.1	0.47	0.0
S.D.	21.7	21.1	0.16	0.0
N	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 24 Reproductive observations (females) at delivery

Sex: Female Day(s) Relative to Littering (A)

Mid 13F-OLE 25 mg/kg/day	Observation items			
	Delivery index (%)	Birth index (%)	Sex ratio (Male/Total)	External anomalies (%)
	Day 0	Day 0	Day 0	Day 0
DK3F01	100.0	100.0	0.28	0.0
DK3F02	93.8	81.3	0.23	0.0
DK3F03	100.0	100.0	0.39	0.0
DK3F05	86.7	86.7	0.31	0.0
DK3F06	100.0	93.3	0.50	0.0
DK3F07	93.8	93.8	0.67	0.0
DK3F08	100.0	23.5	0.25	0.0
DK3F09	93.8	93.8	0.47	0.0
DK3F10	88.2	88.2	0.27	0.0
Mean	95.1	84.5	0.37	0.0
S.D.	5.2	23.7	0.15	0.0
N	9	9	9	9

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 24 Reproductive observations (females) at delivery

Sex: Female Day(s) Relative to Littering (A)

High 13F-OLE 200 mg/kg/day	Observation items			
	Delivery index (%)	Birth index (%)	Sex ratio (Male/Total)	External anomalies (%)
	Day 0	Day 0	Day 0	Day 0
DK4F01	94.1	94.1	0.69	0.0
DK4F02	82.4	82.4	0.79	0.0
DK4F03	92.9	92.9	0.38	0.0
DK4F04	88.2	76.5	0.62	0.0
DK4F05	93.8	81.3	0.62	0.0
DK4F06	100.0	100.0	0.50	0.0
DK4F08	100.0	100.0	0.40	0.0
DK4F09	93.8	93.8	0.53	0.0
DK4F10	100.0	100.0	0.39	0.0
Mean	93.9	91.2	0.55	0.0
S.D.	5.9	9.0	0.14	0.0
N	9	9	9	9

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 25 Clinical observations of offspring (F1) after birth

Group	Dam No.	Findings
Control	DK1F01	No abnormalities
Vehicle	DK1F02	Death of three offspring on Day 1
0		No milk in the stomach of all offspring on Day 1
mg/kg/day		Subnormal body surface temperature of all offspring on Day 1
	DK1F03	Death of one offspring on Day 1
	DK1F04	No abnormalities
	DK1F05	Death of one offspring on Day 2
		Death of one offspring on Day 4
	DK1F06	Death of one offspring on Day 4
	DK1F07	No abnormalities
	DK1F08	No abnormalities
	DK1F09	No abnormalities
	DK1F10	Death of one offspring on Day 3
Low	DK2F01	No abnormalities
13F-OLE	DK2F02	No abnormalities
5	DK2F03	Death of one offspring on Day 4
mg/kg/day	DK2F04	Death of one offspring on Day 1
	DK2F05	No abnormalities
	DK2F06	No abnormalities
	DK2F07	No abnormalities
	DK2F08	No abnormalities
	DK2F09	No abnormalities
	DK2F10	Death of one offspring on Day 2

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 25 Clinical observations of offspring (F1) after birth

Group	Dam No.	Findings
Mid	DK3F01	No abnormalities
13F-OLE	DK3F02	No abnormalities
25	DK3F03	No abnormalities
mg/kg/day	DK3F05	No abnormalities
	DK3F06	No abnormalities
	DK3F07	No abnormalities
	DK3F08	Subnormal body surface temperature of all offspring on Days 0 and 1
		No milk in the stomach of all offspring on Day 1
		Death of four offspring on Day 2 (death of all offspring)
	DK3F09	No abnormalities
	DK3F10	No abnormalities
High	DK4F01	No abnormalities
13F-OLE	DK4F02	No milk in the stomach of three offspring on Day 1
200		Death of eight offspring on Day 1
mg/kg/day		Death of three offspring on Day 2
	DK4F03	No abnormalities
	DK4F04	No milk in the stomach of all offspring on Day 1
		Subnormal body surface temperature of all offspring on Day 1
		Death of five offspring on Day 1
		Death of eight offspring on Day 2 (death of all offspring)
	DK4F05	No abnormalities
	DK4F06	Death of one offspring on Day 1
	DK4F08	Death of two offspring on Day 2
		Death of one offspring on Day 3
	DK4F09	No abnormalities
	DK4F10	No milk in the stomach of all offspring on Day 1
		Death of five offspring on Day 2

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 26 Body weight of male offspring (F1) after birth

Sex: Female Day(s) Relative to Littering (A)

Control Vehicle 0 mg/kg/day	Male F1 weight (g)					
			Post culling			
	0	4	4	7	11	13
DK1F01	6.47	11.03	10.89	17.7	30.7	37.1
DK1F02	6.01	.	.	.	.	.
DK1F03	5.90	9.56	9.14	15.4	26.1	31.2
DK1F04	6.47	10.56	10.43	17.3	28.0	33.9
DK1F05	6.63	9.15	9.49	15.5	26.1	31.2
DK1F06	5.90	9.55	9.51	16.1	29.0	35.3
DK1F07	6.28	10.86	11.05	18.8	29.5	34.4
DK1F08	7.13	10.95	10.95	17.5	29.1	35.4
DK1F09	6.15	10.87	10.77	17.4	29.3	35.5
DK1F10	6.74	10.90	10.95	19.0	31.7	37.8
Mean	6.37	10.38	10.35	17.2	28.8	34.6
S.D.	0.40	0.74	0.76	1.3	1.9	2.3
N	10	9	9	9	9	9

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 26 Body weight of male offspring (F1) after birth

Sex: Female Day(s) Relative to Littering (A)

Low 13F-OLE 5 mg/kg/day	Male F1 weight (g)					
			Post culling			
	0	4	4	7	11	13
DK2F01	6.47	10.50	10.53	17.7	29.4	35.5
DK2F02	6.04	9.39	9.37	16.5	27.4	33.3
DK2F03	6.02	9.22	9.70	16.7	28.1	33.7
DK2F04	7.30	12.00	11.71	18.2	29.8	35.2
DK2F05	6.59	10.36	10.63	17.0	29.4	35.2
DK2F06	7.59	14.86	14.89	23.2	36.6	41.8
DK2F07	6.66	10.09	10.04	17.6	29.1	35.5
DK2F08	6.74	12.10	12.10	20.0	31.9	37.2
DK2F09	6.56	11.40	11.42	20.0	31.8	37.3
DK2F10	6.46	8.70	8.63	14.6	26.7	32.1
Mean	6.64	10.86	10.90	18.1	30.0	35.7
S.D.	0.49	1.82	1.77	2.4	2.8	2.7
N	10	10	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 26 Body weight of male offspring (F1) after birth

Sex: Female Day(s) Relative to Littering (A)

Mid 13F-OLE 25 mg/kg/day	Male F1 weight (g)					
			Post culling			
	0	4	4	7	11	13
DK3F01	6.28	8.37	8.01	12.8	22.5	27.8
DK3F02	6.28	9.78	9.43	17.5	30.2	35.5
DK3F03	6.54	10.21	10.25	18.1	31.4	37.8
DK3F05	6.82	12.82	12.84	20.9	34.4	41.3
DK3F06	6.30	10.43	10.36	17.7	29.5	35.2
DK3F07	5.89	9.88	9.48	16.5	27.9	32.9
DK3F08	6.72	.	.	.	.	.
DK3F09	6.33	9.64	9.82	17.0	28.5	34.3
DK3F10	7.12	10.87	10.85	18.9	32.0	38.3
Mean	6.47	10.25	10.13	17.4	29.5	35.4
S.D.	0.36	1.27	1.38	2.3	3.5	4.1
N	9	8	8	8	8	8

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 26 Body weight of male offspring (F1) after birth

Sex: Female Day(s) Relative to Littering (A)

High 13F-OLE 200 mg/kg/day	Male F1 weight (g)					
			Post culling			
	0	4	4	7	11	13
DK4F01	6.75	10.87	10.70	18.1	30.9	37.0
DK4F02	5.33	6.43	6.43	11.0	22.8	30.3
DK4F03	6.96	10.19	10.26	17.7	28.4	33.1
DK4F04	5.96	.	.	.	.	.
DK4F05	6.24	5.35	5.41	7.7	16.2	21.7
DK4F06	6.60	11.99	12.06	20.4	33.1	38.1
DK4F08	6.19	6.33	5.96	10.3	21.1	26.9
DK4F09	6.12	9.41	9.71	16.5	28.6	34.4
DK4F10	5.35	7.76	7.83	13.6	25.2	31.1
Mean	6.17	8.54	8.54	14.4	25.8	31.6
S.D.	0.57	2.42	2.47	4.5	5.6	5.4
N	9	8	8	8	8	8

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 27 Body weight of female offspring (F1) after birth

Sex: Female Day(s) Relative to Littering (A)

Control Vehicle 0 mg/kg/day	Female F1 weight (g)					
			Post culling			
	0	4	4	7	11	13
DK1F01	6.18	10.75	10.75	17.4	30.0	36.0
DK1F02	5.81	.	.	.	.	.
DK1F03	5.54	8.78	8.84	14.9	26.2	31.4
DK1F04	6.13	10.15	10.22	16.9	27.5	33.9
DK1F05	6.13	7.82	7.53	12.7	22.5	27.8
DK1F06	5.56	8.63	8.76	15.1	27.5	33.7
DK1F07	6.06	10.52	10.87	17.5	28.7	33.3
DK1F08	6.78	10.11	10.24	16.8	28.1	33.5
DK1F09	5.85	10.24	10.24	16.7	28.6	34.9
DK1F10	6.39	10.50	10.17	17.7	29.9	36.2
Mean	6.04	9.72	9.73	16.2	27.7	33.4
S.D.	0.38	1.04	1.11	1.7	2.3	2.6
N	10	9	9	9	9	9

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 27 Body weight of female offspring (F1) after birth

Sex: Female Day(s) Relative to Littering (A)

Low 13F-OLE 5 mg/kg/day	Female F1 weight (g)					
			Post culling			
	0	4	4	7	11	13
DK2F01	6.11	10.23	10.09	17.0	28.2	33.7
DK2F02	5.84	8.23	8.06	15.0	25.9	31.4
DK2F03	5.52	9.01	9.13	15.9	27.1	32.8
DK2F04	6.99	11.62	11.63	18.0	29.4	34.9
DK2F05	6.56	10.57	10.43	16.7	29.1	35.4
DK2F06	7.27	14.22	14.21	22.3	35.2	39.6
DK2F07	6.41	10.57	10.55	18.4	29.9	35.7
DK2F08	6.44	11.76	11.85	20.0	32.2	37.7
DK2F09	5.92	10.28	10.88	18.7	30.1	36.1
DK2F10	6.21	8.15	8.52	14.4	25.2	30.6
Mean	6.33	10.46	10.53	17.6	29.2	34.8
S.D.	0.53	1.82	1.79	2.4	3.0	2.8
N	10	10	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 27 Body weight of female offspring (F1) after birth

Sex: Female Day(s) Relative to Littering (A)

Mid 13F-OLE 25 mg/kg/day	Female F1 weight (g)					
			Post culling			
	0	4	4	7	11	13
DK3F01	6.04	8.60	8.49	13.6	25.3	30.8
DK3F02	5.81	9.06	8.83	15.7	27.9	32.9
DK3F03	6.05	9.46	9.52	17.0	30.4	37.4
DK3F05	6.70	12.45	12.16	19.6	33.2	40.2
DK3F06	5.90	9.80	9.74	17.1	28.3	33.7
DK3F07	5.58	9.36	9.37	15.9	26.5	31.3
DK3F08	6.43	.	.	.	.	.
DK3F09	6.24	9.82	9.59	16.6	28.1	33.8
DK3F10	7.15	11.12	10.91	19.0	31.3	37.5
Mean	6.21	9.96	9.82	16.8	28.9	34.7
S.D.	0.48	1.25	1.18	1.9	2.6	3.3
N	9	8	8	8	8	8

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 27 Body weight of female offspring (F1) after birth

Sex: Female Day(s) Relative to Littering (A)

High 13F-OLE 200 mg/kg/day	Female F1 weight (g)					
			Post culling			
	0	4	4	7	11	13
DK4F01	6.56	10.71	10.91	18.1	30.6	35.7
DK4F02	5.17	.	.	.	.	.
DK4F03	6.58	9.88	9.53	16.3	26.4	31.5
DK4F04	5.65	.	.	.	.	.
DK4F05	5.80	5.25	5.31	7.5	16.1	21.6
DK4F06	6.09	9.85	10.82	18.7	30.3	36.0
DK4F08	5.90	5.83	6.02	10.6	21.0	26.3
DK4F09	6.17	9.13	8.99	15.4	27.1	32.7
DK4F10	5.38	7.28	7.04	12.5	23.6	29.6
Mean	5.92	8.28	8.37	14.2	25.0	30.5
S.D.	0.48	2.15	2.27	4.1	5.2	5.2
N	9	7	7	7	7	7

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 28 Viability of offspring (F1)

Sex: Female Day(s) Relative to Littering (A)

Control Vehicle 0 mg/kg/day	Live offspring			
	Live newborns (Number)	Live F1 pre culling (Number)	Live F1 post culling (Number)	Live F1 (Number)
	Day 0	Day 4	Day 4	Day 14
DK1F01	14	14	8	8
DK1F02	7	0	.	.
DK1F03	18	17	8	8
DK1F04	15	15	8	8
DK1F05	15	13	8	8
DK1F06	17	16	8	8
DK1F07	15	15	8	8
DK1F08	14	14	8	8
DK1F09	15	15	8	8
DK1F10	16	15	8	8
Mean	14.6	13.4	8.0	8.0
S.D.	3.0	4.8	0.0	0.0
Total	146	134	72	72
N	10	10	9	9

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 28 Viability of offspring (F1)

Sex: Female Day(s) Relative to Littering (A)

Low 13F-OLE 5 mg/kg/day	Live offspring			
	Live newborns (Number)	Live F1 pre culling (Number)	Live F1 post culling (Number)	Live F1 (Number)
	Day 0	Day 4	Day 4	Day 14
DK2F01	15	15	8	8
DK2F02	16	16	8	8
DK2F03	17	16	8	8
DK2F04	13	12	8	8
DK2F05	15	15	8	8
DK2F06	5	5	5	5
DK2F07	15	15	8	8
DK2F08	13	13	8	8
DK2F09	14	14	8	8
DK2F10	16	15	8	8
Mean	13.9	13.6	7.7	7.7
S.D.	3.4	3.3	0.9	0.9
Total	139	136	77	77
N	10	10	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 28 Viability of offspring (F1)

Sex: Female Day(s) Relative to Littering (A)

Mid 13F-OLE 25 mg/kg/day	Live offspring			
	Live newborns (Number)	Live F1 pre culling (Number)	Live F1 post culling (Number)	Live F1 (Number)
	Day 0	Day 4	Day 4	Day 14
DK3F01	18	18	8	8
DK3F02	13	13	8	8
DK3F03	18	18	8	8
DK3F05	13	13	8	8
DK3F06	14	14	8	8
DK3F07	15	15	8	8
DK3F08	4	0	.	.
DK3F09	15	15	8	8
DK3F10	15	15	8	8
Mean	13.9	13.4	8.0	8.0
S.D.	4.1	5.4	0.0	0.0
Total	125	121	64	64
N	9	9	8	8

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 28 Viability of offspring (F1)

Sex: Female Day(s) Relative to Littering (A)

High 13F-OLE 200 mg/kg/day	Live offspring			
	Live newborns (Number)	Live F1 pre culling (Number)	Live F1 post culling (Number)	Live F1 (Number)
	Day 0	Day 4	Day 4	Day 14
DK4F01	16	16	8	8
DK4F02	14	3	3	3
DK4F03	13	13	8	8
DK4F04	13	0	.	.
DK4F05	13	13	8	8
DK4F06	14	13	8	8
DK4F08	15	12	8	8
DK4F09	15	15	8	8
DK4F10	18	13	8	8
Mean	14.6	10.9	7.4	7.4
S.D.	1.7	5.5	1.8	1.8
Total	131	98	59	59
N	9	9	8	8

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 28 Viability of offspring (F1)

Sex: Female Day(s) Relative to Littering (A)

Control Vehicle 0 mg/kg/day	Live offspring	
	Viability index (%)	Viability index (%)
	Days 0-4	Days 4-14
DK1F01	100.0	100.0
DK1F02	0.0	.
DK1F03	94.4	100.0
DK1F04	100.0	100.0
DK1F05	86.7	100.0
DK1F06	94.1	100.0
DK1F07	100.0	100.0
DK1F08	100.0	100.0
DK1F09	100.0	100.0
DK1F10	93.8	100.0
Mean	86.9	100.0
S.D.	30.9	0.0
N	10	9

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 28 Viability of offspring (F1)

Sex: Female Day(s) Relative to Littering (A)

Low 13F-OLE 5 mg/kg/day	Live offspring	
	Viability index (%)	Viability index (%)
	Days 0-4	Days 4-14
DK2F01	100.0	100.0
DK2F02	100.0	100.0
DK2F03	94.1	100.0
DK2F04	92.3	100.0
DK2F05	100.0	100.0
DK2F06	100.0	100.0
DK2F07	100.0	100.0
DK2F08	100.0	100.0
DK2F09	100.0	100.0
DK2F10	93.8	100.0
Mean	98.0	100.0
S.D.	3.2	0.0
N	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 28 Viability of offspring (F1)

Sex: Female Day(s) Relative to Littering (A)

Mid 13F-OLE 25 mg/kg/day	Live offspring	
	Viability index (%)	Viability index (%)
	Days 0-4	Days 4-14
DK3F01	100.0	100.0
DK3F02	100.0	100.0
DK3F03	100.0	100.0
DK3F05	100.0	100.0
DK3F06	100.0	100.0
DK3F07	100.0	100.0
DK3F08	0.0	.
DK3F09	100.0	100.0
DK3F10	100.0	100.0
Mean	88.9	100.0
S.D.	33.3	0.0
N	9	8

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 28 Viability of offspring (F1)

Sex: Female Day(s) Relative to Littering (A)

High 13F-OLE 200 mg/kg/day	Live offspring	
	Viability index (%)	Viability index (%)
	Days 0-4	Days 4-14
DK4F01	100.0	100.0
DK4F02	21.4	100.0
DK4F03	100.0	100.0
DK4F04	0.0	.
DK4F05	100.0	100.0
DK4F06	92.9	100.0
DK4F08	80.0	100.0
DK4F09	100.0	100.0
DK4F10	72.2	100.0
Mean	74.1	100.0
S.D.	37.6	0.0
N	9	8

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 29 Anogenital distance (AGD) of male offspring (F1) at 4 days of age

Control Vehicle 0 mg/kg/day	AGD (mm)	Relative AGD
DK1F01	6.13	0.277
DK1F03	5.87	0.281
DK1F04	6.00	0.275
DK1F05	6.02	0.285
DK1F06	5.95	0.281
DK1F07	6.28	0.282
DK1F08	6.01	0.271
DK1F09	5.81	0.264
DK1F10	6.42	0.289
Mean	6.05	0.278
S.D.	0.19	0.008
N	9	9

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80 || Relative AGD: Values calculated by dividing AGD values by the cube root of body weights at 4 days of age]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 29 Anogenital distance (AGD) of male offspring (F1) at 4 days of age

Low 13F-OLE 5 mg/kg/day	AGD (mm)	Relative AGD
DK2F01	6.28	0.287
DK2F02	6.17	0.293
DK2F03	6.11	0.287
DK2F04	6.42	0.283
DK2F05	5.74	0.262
DK2F06	7.38	0.300
DK2F07	6.24	0.289
DK2F08	6.74	0.294
DK2F09	6.06	0.269
DK2F10	5.95	0.290
Mean	6.31	0.285
S.D.	0.46	0.012
N	10	10

General Footnote: [Relative AGD: Values calculated by dividing AGD values by the cube root of body weights at 4 days of age]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 29 Anogenital distance (AGD) of male offspring (F1) at 4 days of age

Mid 13F-OLE 25 mg/kg/day	AGD (mm)	Relative AGD
DK3F01	5.67	0.284
DK3F02	5.57	0.264
DK3F03	6.18	0.285
DK3F05	6.01	0.256
DK3F06	6.11	0.281
DK3F07	6.15	0.291
DK3F09	6.34	0.296
DK3F10	6.48	0.293
Mean	6.06	0.281
S.D.	0.31	0.014
N	8	8

General Footnote: [Relative AGD: Values calculated by dividing AGD values by the cube root of body weights at 4 days of age]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 29 Anogenital distance (AGD) of male offspring (F1) at 4 days of age

High 13F-OLE 200 mg/kg/day	AGD (mm)	Relative AGD
DK4F01	6.09	0.276
DK4F02	5.04	0.272
DK4F03	6.21	0.286
DK4F05	4.96	0.283
DK4F06	6.76	0.296
DK4F08	5.14	0.284
DK4F09	6.04	0.284
DK4F10	5.74	0.289
Mean	5.75	0.284
S.D.	0.65	0.007
N	8	8

General Footnote: [Relative AGD: Values calculated by dividing AGD values by the cube root of body weights at 4 days of age]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 30 Anogenital distance (AGD) of female offspring (F1) at 4 days of age

Control Vehicle 0 mg/kg/day	AGD (mm)	Relative AGD
DK1F01	3.73	0.169
DK1F03	3.55	0.172
DK1F04	3.54	0.163
DK1F05	3.68	0.188
DK1F06	3.66	0.178
DK1F07	3.87	0.175
DK1F08	3.56	0.164
DK1F09	3.57	0.165
DK1F10	3.86	0.178
Mean	3.67	0.172
S.D.	0.13	0.008
N	9	9

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80 || Relative AGD: Values calculated by dividing AGD values by the cube root of body weights at 4 days of age]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 30 Anogenital distance (AGD) of female offspring (F1) at 4 days of age

Low 13F-OLE 5 mg/kg/day	AGD (mm)	Relative AGD
DK2F01	3.77	0.175
DK2F02	3.58	0.179
DK2F03	3.51	0.168
DK2F04	3.86	0.170
DK2F05	3.75	0.172
DK2F06	4.16	0.172
DK2F07	3.84	0.175
DK2F08	3.97	0.174
DK2F09	3.61	0.163
DK2F10	3.69	0.182
Mean	3.77	0.173
S.D.	0.19	0.005
N	10	10

General Footnote: [Relative AGD: Values calculated by dividing AGD values by the cube root of body weights at 4 days of age]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 30 Anogenital distance (AGD) of female offspring (F1) at 4 days of age

Mid 13F-OLE 25 mg/kg/day	AGD (mm)	Relative AGD
DK3F01	3.64	0.179
DK3F02	3.47	0.168
DK3F03	3.57	0.169
DK3F05	3.49	0.152
DK3F06	3.63	0.170
DK3F07	3.83	0.182
DK3F09	3.76	0.177
DK3F10	3.81	0.172
Mean	3.65	0.171
S.D.	0.14	0.009
N	8	8

General Footnote: [Relative AGD: Values calculated by dividing AGD values by the cube root of body weights at 4 days of age]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 30 Anogenital distance (AGD) of female offspring (F1) at 4 days of age

High 13F-OLE 200 mg/kg/day	AGD (mm)	Relative AGD
DK4F01	3.76	0.170
DK4F03	3.70	0.175
DK4F05	3.08	0.177
DK4F06	3.67	0.166
DK4F08	3.24	0.178
DK4F09	3.47	0.167
DK4F10	3.49	0.183
Mean	3.49	0.174
S.D.	0.25	0.006
N	7	7

General Footnote: [Relative AGD: Values calculated by dividing AGD values by the cube root of body weights at 4 days of age]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 31 Number of nipples and number of areolas of male offspring (F1) at 13 days of age

Control Vehicle 0 mg/kg/day	Number of nipples	Number of areolas
DK1F01	0	0
DK1F03	0	0
DK1F04	0	0
DK1F05	0	0
DK1F06	0	0
DK1F07	0	0
DK1F08	0	0
DK1F09	0	0
DK1F10	0	0
Mean	0	0
S.D.	0	0
N	9	9

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 31 Number of nipples and number of areolas of male offspring (F1) at 13 days of age

Low 13F-OLE 5 mg/kg/day	Number of nipples	Number of areolas
DK2F01	0	0
DK2F02	0	0
DK2F03	0	0
DK2F04	0	0
DK2F05	0	0
DK2F06	0	0
DK2F07	0	0
DK2F08	0	0
DK2F09	0	0
DK2F10	0	0
Mean	0	0
S.D.	0	0
N	10	10

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 31 Number of nipples and number of areolas of male offspring (F1) at 13 days of age

Mid 13F-OLE 25 mg/kg/day	Number of nipples	Number of areolas
DK3F01	0	0
DK3F02	0	0
DK3F03	0	0
DK3F05	0	0
DK3F06	0	0
DK3F07	0	0
DK3F09	0	0
DK3F10	0	0
Mean	0	0
S.D.	0	0
N	8	8

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 31 Number of nipples and number of areolas of male offspring (F1) at 13 days of age

High 13F-OLE 200 mg/kg/day	Number of nipples	Number of areolas
DK4F01	0	0
DK4F02	0	0
DK4F03	0	0
DK4F05	0	0
DK4F06	0	0
DK4F08	0	0
DK4F09	0	0
DK4F10	0	0
Mean	0	0
S.D.	0	0
N	8	8

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 32 Determination of the thyroid hormone (T4) levels of offspring (F1)

Control Vehicle 0 mg/kg/day	Total T4 (ng/mL)	
	At 4 days of age	At 14 days of age
DK1F01	110.1	313.5
DK1F03	138.0	377.5
DK1F04	120.5	307.0
DK1F05	91.0	293.7
DK1F06	108.1	323.9
DK1F07	149.9	349.3
DK1F08	99.4	303.5
DK1F09	115.8	343.2
DK1F10	88.3	333.4
Mean	113.5	327.2
S.D.	20.5	26.5
N	9	9

General Footnote: [Vehicle: 1.0 w/v% olive oil containing Tween 80 || T4: Thyroxine]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 32 Determination of the thyroid hormone (T4) levels of offspring (F1)

Low 13F-OLE 5 mg/kg/day	Total T4 (ng/mL)	
	At 4 days of age	At 14 days of age
DK2F01	121.6	368.5
DK2F02	115.8	302.4
DK2F03	120.1	311.9
DK2F04	128.3	353.1
DK2F05	149.4	337.8
DK2F06	.	363.1
DK2F07	99.9	361.0
DK2F08	102.8	327.8
DK2F09	202.7	310.9
DK2F10	136.7	290.2
Mean	130.8	332.7
S.D.	31.1	28.1
N	9	10

General Footnote: [T4: Thyroxine]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 32 Determination of the thyroid hormone (T4) levels of offspring (F1)

Mid 13F-OLE 25 mg/kg/day	Total T4 (ng/mL)	
	At 4 days of age	At 14 days of age
DK3F01	135.6	340.7
DK3F02	145.4	320.0
DK3F03	145.8	317.8
DK3F05	143.4	356.1
DK3F06	82.6	289.8
DK3F07	98.4	300.9
DK3F09	129.9	363.7
DK3F10	160.9	337.3
Mean	130.3	328.3
S.D.	26.5	25.8
N	8	8

General Footnote: [T4: Thyroxine]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 32 Determination of the thyroid hormone (T4) levels of offspring (F1)

High 13F-OLE 200 mg/kg/day	Total T4 (ng/mL)	
	At 4 days of age	At 14 days of age
DK4F01	129.7	333.8
DK4F02	.	300.5
DK4F03	153.0	342.5
DK4F05	166.9	332.0
DK4F06	151.3	306.9
DK4F08	122.4	322.4
DK4F09	107.9	325.5
DK4F10	87.5	320.1
Mean	131.2	323.0
S.D.	27.9	13.9
N	7	8

General Footnote: [T4: Thyroxine]

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 33 Gross pathology of male offspring (F1) at 14 days of age

Group	F1 No.	Findings
Control	DK1F01-1M	No pathological changes
Vehicle	DK1F01-2M	No pathological changes
0	DK1F01-3M	No pathological changes
mg/kg/day	DK1F01-4M	No pathological changes
	DK1F03-1M	No pathological changes
	DK1F03-2M	No pathological changes
	DK1F03-3M	No pathological changes
	DK1F03-4M	No pathological changes
	DK1F04-1M	No pathological changes
	DK1F04-2M	No pathological changes
	DK1F04-3M	No pathological changes
	DK1F04-4M	No pathological changes
	DK1F05-1M	No pathological changes
	DK1F05-2M	No pathological changes
	DK1F05-3M	No pathological changes
	DK1F05-4M	No pathological changes
	DK1F06-1M	No pathological changes
	DK1F06-2M	No pathological changes
	DK1F06-3M	No pathological changes
	DK1F06-4M	No pathological changes
	DK1F07-1M	No pathological changes
	DK1F07-2M	No pathological changes
	DK1F07-3M	No pathological changes
	DK1F07-4M	No pathological changes
	DK1F08-1M	No pathological changes
	DK1F08-2M	No pathological changes
	DK1F08-3M	No pathological changes
	DK1F08-4M	No pathological changes
	DK1F09-1M	No pathological changes
	DK1F09-2M	No pathological changes
	DK1F09-3M	No pathological changes
	DK1F09-4M	No pathological changes
	DK1F10-1M	No pathological changes
	DK1F10-2M	No pathological changes
	DK1F10-3M	No pathological changes
	DK1F10-4M	No pathological changes

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 33 Gross pathology of male offspring (F1) at 14 days of age

Group	F1 No.	Findings
Low	DK2F01-1M	No pathological changes
13F-OLE	DK2F01-2M	No pathological changes
5	DK2F01-3M	No pathological changes
mg/kg/day	DK2F01-4M	No pathological changes
	DK2F02-1M	No pathological changes
	DK2F02-2M	No pathological changes
	DK2F02-3M	No pathological changes
	DK2F02-4M	No pathological changes
	DK2F03-1M	No pathological changes
	DK2F03-2M	No pathological changes
	DK2F03-3M	No pathological changes
	DK2F03-4M	No pathological changes
	DK2F04-1M	No pathological changes
	DK2F04-2M	No pathological changes
	DK2F04-3M	No pathological changes
	DK2F04-4M	No pathological changes
	DK2F05-1M	No pathological changes
	DK2F05-2M	No pathological changes
	DK2F05-3M	No pathological changes
	DK2F05-4M	No pathological changes
	DK2F06-1M	No pathological changes
	DK2F06-2M	No pathological changes
	DK2F06-3M	No pathological changes
	DK2F07-1M	No pathological changes
	DK2F07-2M	No pathological changes
	DK2F07-3M	No pathological changes
	DK2F07-4M	No pathological changes
	DK2F07-5M	No pathological changes
	DK2F08-1M	No pathological changes
	DK2F08-2M	No pathological changes
	DK2F08-3M	No pathological changes
	DK2F08-4M	No pathological changes
	DK2F09-1M	No pathological changes
	DK2F09-2M	No pathological changes
	DK2F09-3M	No pathological changes
	DK2F09-4M	No pathological changes
	DK2F10-1M	No pathological changes
	DK2F10-2M	No pathological changes
	DK2F10-3M	No pathological changes
	DK2F10-4M	No pathological changes

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 33 Gross pathology of male offspring (F1) at 14 days of age

Group	F1 No.	Findings
Mid	DK3F01-1M	No pathological changes
13F-OLE	DK3F01-2M	No pathological changes
25	DK3F01-3M	No pathological changes
mg/kg/day	DK3F01-4M	No pathological changes
	DK3F02-1M	No pathological changes
	DK3F02-2M	No pathological changes
	DK3F02-3M	No pathological changes
	DK3F03-1M	No pathological changes
	DK3F03-2M	No pathological changes
	DK3F03-3M	No pathological changes
	DK3F03-4M	No pathological changes
	DK3F05-1M	No pathological changes
	DK3F05-2M	No pathological changes
	DK3F05-3M	No pathological changes
	DK3F05-4M	No pathological changes
	DK3F06-1M	No pathological changes
	DK3F06-2M	No pathological changes
	DK3F06-3M	No pathological changes
	DK3F06-4M	No pathological changes
	DK3F07-1M	No pathological changes
	DK3F07-2M	No pathological changes
	DK3F07-3M	No pathological changes
	DK3F07-4M	No pathological changes
	DK3F09-1M	No pathological changes
	DK3F09-2M	No pathological changes
	DK3F09-3M	No pathological changes
	DK3F09-4M	No pathological changes
	DK3F10-1M	No pathological changes
	DK3F10-2M	No pathological changes
	DK3F10-3M	No pathological changes
	DK3F10-4M	No pathological changes

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 33 Gross pathology of male offspring (F1) at 14 days of age

Group	F1 No.	Findings
High	DK4F01-1M	No pathological changes
13F-OLE	DK4F01-2M	No pathological changes
200	DK4F01-3M	No pathological changes
mg/kg/day	DK4F01-4M	No pathological changes
	DK4F02-1M	No pathological changes
	DK4F02-2M	No pathological changes
	DK4F02-3M	No pathological changes
	DK4F03-1M	No pathological changes
	DK4F03-2M	No pathological changes
	DK4F03-3M	No pathological changes
	DK4F03-4M	No pathological changes
	DK4F05-1M	No pathological changes
	DK4F05-2M	No pathological changes
	DK4F05-3M	No pathological changes
	DK4F05-4M	No pathological changes
	DK4F06-1M	No pathological changes
	DK4F06-2M	No pathological changes
	DK4F06-3M	No pathological changes
	DK4F06-4M	No pathological changes
	DK4F08-1M	No pathological changes
	DK4F08-2M	No pathological changes
	DK4F08-3M	No pathological changes
	DK4F08-4M	No pathological changes
	DK4F09-1M	No pathological changes
	DK4F09-2M	No pathological changes
	DK4F09-3M	No pathological changes
	DK4F09-4M	No pathological changes
	DK4F10-1M	No pathological changes
	DK4F10-2M	No pathological changes
	DK4F10-3M	No pathological changes
	DK4F10-4M	No pathological changes

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 33 Gross pathology of female offspring (F1) at 14 days of age

Group	F1 No.	Findings
Control	DK1F01-1F	No pathological changes
Vehicle	DK1F01-2F	No pathological changes
0	DK1F01-3F	No pathological changes
mg/kg/day	DK1F01-4F	No pathological changes
	DK1F03-1F	No pathological changes
	DK1F03-2F	No pathological changes
	DK1F03-3F	No pathological changes
	DK1F03-4F	No pathological changes
	DK1F04-1F	No pathological changes
	DK1F04-2F	No pathological changes
	DK1F04-3F	No pathological changes
	DK1F04-4F	No pathological changes
	DK1F05-1F	No pathological changes
	DK1F05-2F	No pathological changes
	DK1F05-3F	No pathological changes
	DK1F05-4F	No pathological changes
	DK1F06-1F	No pathological changes
	DK1F06-2F	No pathological changes
	DK1F06-3F	No pathological changes
	DK1F06-4F	No pathological changes
	DK1F07-1F	No pathological changes
	DK1F07-2F	No pathological changes
	DK1F07-3F	No pathological changes
	DK1F07-4F	No pathological changes
	DK1F08-1F	No pathological changes
	DK1F08-2F	No pathological changes
	DK1F08-3F	No pathological changes
	DK1F08-4F	No pathological changes
	DK1F09-1F	No pathological changes
	DK1F09-2F	No pathological changes
	DK1F09-3F	No pathological changes
	DK1F09-4F	No pathological changes
	DK1F10-1F	No pathological changes
	DK1F10-2F	No pathological changes
	DK1F10-3F	No pathological changes
	DK1F10-4F	No pathological changes

General Footnote: Vehicle: 1.0 w/v% olive oil containing Tween 80

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 33 Gross pathology of female offspring (F1) at 14 days of age

Group	F1 No.	Findings
Low	DK2F01-1F	No pathological changes
13F-OLE	DK2F01-2F	No pathological changes
5	DK2F01-3F	No pathological changes
mg/kg/day	DK2F01-4F	No pathological changes
	DK2F02-1F	No pathological changes
	DK2F02-2F	No pathological changes
	DK2F02-3F	No pathological changes
	DK2F02-4F	No pathological changes
	DK2F03-1F	No pathological changes
	DK2F03-2F	No pathological changes
	DK2F03-3F	No pathological changes
	DK2F03-4F	No pathological changes
	DK2F04-1F	No pathological changes
	DK2F04-2F	No pathological changes
	DK2F04-3F	No pathological changes
	DK2F04-4F	No pathological changes
	DK2F05-1F	No pathological changes
	DK2F05-2F	No pathological changes
	DK2F05-3F	No pathological changes
	DK2F05-4F	No pathological changes
	DK2F06-1F	No pathological changes
	DK2F06-2F	No pathological changes
	DK2F07-1F	No pathological changes
	DK2F07-2F	No pathological changes
	DK2F07-3F	No pathological changes
	DK2F08-1F	No pathological changes
	DK2F08-2F	No pathological changes
	DK2F08-3F	No pathological changes
	DK2F08-4F	No pathological changes
	DK2F09-1F	No pathological changes
	DK2F09-2F	No pathological changes
	DK2F09-3F	No pathological changes
	DK2F09-4F	No pathological changes
	DK2F10-1F	No pathological changes
	DK2F10-2F	No pathological changes
	DK2F10-3F	No pathological changes
	DK2F10-4F	No pathological changes

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 33 Gross pathology of female offspring (F1) at 14 days of age

Group	F1 No.	Findings
Mid	DK3F01-1F	No pathological changes
13F-OLE	DK3F01-2F	No pathological changes
25	DK3F01-3F	No pathological changes
mg/kg/day	DK3F01-4F	No pathological changes
	DK3F02-1F	No pathological changes
	DK3F02-2F	No pathological changes
	DK3F02-3F	No pathological changes
	DK3F02-4F	No pathological changes
	DK3F02-5F	No pathological changes
	DK3F03-1F	No pathological changes
	DK3F03-2F	No pathological changes
	DK3F03-3F	No pathological changes
	DK3F03-4F	No pathological changes
	DK3F05-1F	No pathological changes
	DK3F05-2F	No pathological changes
	DK3F05-3F	No pathological changes
	DK3F05-4F	No pathological changes
	DK3F06-1F	No pathological changes
	DK3F06-2F	No pathological changes
	DK3F06-3F	No pathological changes
	DK3F06-4F	No pathological changes
	DK3F07-1F	No pathological changes
	DK3F07-2F	No pathological changes
	DK3F07-3F	No pathological changes
	DK3F07-4F	No pathological changes
	DK3F09-1F	No pathological changes
	DK3F09-2F	No pathological changes
	DK3F09-3F	No pathological changes
	DK3F09-4F	No pathological changes
	DK3F10-1F	No pathological changes
	DK3F10-2F	No pathological changes
	DK3F10-3F	No pathological changes
	DK3F10-4F	No pathological changes

Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats
by Oral Administration

Appendix 33 Gross pathology of female offspring (F1) at 14 days of age

Group	F1 No.	Findings
High	DK4F01-1F	No pathological changes
13F-OLE	DK4F01-2F	Spleen: Discoloration, pale
200		Stomach: Dilation
mg/kg/day		Retention, contents
		Liver: Focus, greenish brown, left lateral lobe, 10×5 mm
	DK4F01-3F	No pathological changes
	DK4F01-4F	No pathological changes
	DK4F03-1F	No pathological changes
	DK4F03-2F	No pathological changes
	DK4F03-3F	No pathological changes
	DK4F03-4F	No pathological changes
	DK4F05-1F	No pathological changes
	DK4F05-2F	No pathological changes
	DK4F05-3F	No pathological changes
	DK4F05-4F	No pathological changes
	DK4F06-1F	No pathological changes
	DK4F06-2F	No pathological changes
	DK4F06-3F	No pathological changes
	DK4F06-4F	No pathological changes
	DK4F08-1F	No pathological changes
	DK4F08-2F	No pathological changes
	DK4F08-3F	No pathological changes
	DK4F08-4F	No pathological changes
	DK4F09-1F	No pathological changes
	DK4F09-2F	No pathological changes
	DK4F09-3F	No pathological changes
	DK4F09-4F	No pathological changes
	DK4F10-1F	No pathological changes
	DK4F10-2F	No pathological changes
	DK4F10-3F	No pathological changes
	DK4F10-4F	No pathological changes

DAIKIN

CERTIFICATE OF ANALYSIS

DATE : 23 Feb 2016
SAMPLE NAME : C6OLF (13F-OLE)
CHEMICAL NAME : 3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluoro-octa-1-ene
Lot No. : C2160215

PURITY : 99.9477%
IMPURITIES :C6F13-I : under detection
:C6F13CH2CH2-I: under detection

EXPIRY DATE : 28 Feb 2018

ANALYTICAL METHOD
<GC> Analyzed as carboxylic acid 70%, Triethylamin 30% (additive)
Column : DB-1 (60 m, I.D.0.320mm, Film 5.00μm)
Carrier Gas : He 30cm/sec
Injection : Split 50:1, 250°C
Detection : FID, 250°C
Column Temp.: 40°C(5min) -> 20°C/min -> 250°C(5min)

DAIKIN INDUSTRIES.LTD.

H.Iwai

CERI

Receipt number	822-16-D-4089
Study number	X02-0302

FINAL REPORT

PHYSICOCHEMICAL STUDY OF "REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING TEST OF 13F-OLE IN RATS BY ORAL ADMINISTRATION"

March, 2017

Chemicals Evaluation and Research Institute, Japan, Hita

X02-0302

GLP COMPLIANCE STATEMENT

Chemicals Evaluation and Research Institute, Japan, Hita

Sponsor: Ina Research Inc.

Title: Physicochemical Study of "Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats by Oral Administration"

Study number: X02-0302

The study described in this report was conducted in compliance with the following GLP principle.

Concerning Standard of the Testing Facilities Conducting the Test Relating to the New Chemical Substances (March 31, 2011) on Japanese GLP [Notification 0331 No. 8 of the Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, Japan, No. 6 (March 29, 2011) of the Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry, Japan and No. 110331010 of the Environmental Health Department, Ministry of the Environment, Japan]

This final report accurately reflects the raw data and the test data are valid.

Study director: _ March 21, 2017
Date

X02-0302

CONTENTS

X02-0302

1. TITLE

Physicochemical Study of "Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats by Oral Administration"

2. ORIGINAL SPONSOR

Name DAIKIN INDUSTRIES, LTD.
Address 1-1 Nishi Hitotsuya, Settsu-shi, Osaka 566-8585, Japan

3. SPONSOR

Name Ina Research Inc.
Address 2148-188 Nishiminowa, Ina-shi, Nagano 399-4501, Japan

4. TESTING FACILITY

Name Chemicals Evaluation and Research Institute, Japan, Hita (CERI Hita)
Address 3-822 Ishii-machi, Hita-shi, Oita 877-0061, Japan

5. OBJECTIVE OF STUDY

The objective of this study is to confirm the concentration of the test article formulation for the subject study in the sponsor, which was consigned from the original sponsor.

Subject study: Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats by Oral Administration (Study number EF16149)

6. GLP COMPLIANCE

Concerning Standard of the Testing Facilities Conducting the Test Relating to the New Chemical Substances (March 31, 2011) on Japanese GLP [Notification 0331 No. 8 of the Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, Japan, No. 6 (March 29, 2011) of the Manufacturing Industries Bureau, Ministry of Economy, Trade and Industry, Japan and No. 110331010 of the Environmental Health Department, Ministry of the Environment, Japan]

7. STUDY SCHEDULE

Study Initiation	December 9, 2016
Experiment Initiation	January 5, 2017
Experiment Completion	March 2, 2017
Study Completion	March 21, 2017

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8. STUDY DIRECTOR

Section 2, CERI Hita

9. PERSONNEL CONCERNED WITH THE STUDY

Study staff

(Analysis of the test article)

10. STORAGE OF RAW DATA AND SPECIMENS

The original protocol, the original final report, raw data, other record documents and test article are retained in the archives of the testing facility. The storage period is 10 years after the study completion date.

After the termination of the retention period, any measures (continued storage, disposal or return) are done with the approval of the sponsor.

11. APPROVAL BY STUDY DIRECTOR

Study director: March 21, 2017

Date

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12. SUMMARY

The concentration of the test article (13F-OLE) formulation for the subject study was examined. Concentrations of the 40, 5 and 1 mg/mL formulations prepared in the subject study at first and final preparation were within the acceptable level. In the 0 mg/mL (control) formulation, no peak derived from the test article was detected.

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13. MATERIAL

13.1 Test article

a) Name, etc. (information provided by the sponsor)

Name	3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooct-1-ene
Other name	13F-OLE
CAS number	25291-17-2

b) Supplier and lot number (information provided by the sponsor)

Supplier	Ina Research Inc.
Lot number	C2160215

c) Structural formula, etc. (information provided by the sponsor)

Structural formula	$H_2C=\underset{H}{C}-CF_2CF_2CF_2CF_2CF_2CF_3$
Molecular formula	$C_8H_3F_{13}$
Molecular weight	346.09

d) Purity (information provided by the sponsor)

Purity	99.9477%

The test article was treated as 100% in purity.

e) Physicochemical property (information provided by the sponsor)

Appearance in ordinary temperature	Colorless clear liquid

f) Storage condition

The test article was put into a light shielding airtight container and stored at room temperature in the test article storage room (acceptable range: 10°C to 30°C).

g) Identity confirmation of test article

Identity of the test article was confirmed by comparing with IR spectra between measured in the testing facility and provided by the sponsor. The IR spectrum of the test article measured in the testing facility (Figure 1) was similar to that provided by the sponsor (Figure 2).

Instrument	IR spectrophotometer FT-720 (HORIBA)
Wavenumber	4000 cm^{-1} to 400 cm^{-1}
Pre-treatment	Potassium bromide neat

h) Handling

Chemically resistant gloves, a mask, a head cap, safety glasses and a lab coat were worn to avoid inhalation and contact with the skin and eyes.

14. METHOD

14.1 Concentration analysis of test article formulation

Concentrations of the test article formulation were measured once by gas chromatography (GC). Concentration analysis was conducted on the 40, 5, 1 and 0 (control) mg/mL formulations at first and final preparation in the subject study, which were delivered under cold condition from the sponsor.

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a) Analytical Method

1) Validation of Analytical Method

In validation of the analytical method performed under non-GLP at the testing facility, all results of specificity, linearity, accuracy and repeatability satisfied the criteria. Analytical method was determined according to the validation of the analytical method.

(1) Preparation of sample solutions for measurement

i) Standard solutions

The test article, 0.10049 g, was weighed, and brought to a volume of 50 mL with acetone (lot number 802H1830, for pesticide residue and PCB analysis, KANTO CHEMICAL) to prepare 2009.8 µg/mL standard stock solution. The standard stock solution was diluted with acetone to make 201 µg/mL standard solution (n=1). The 201 µg/mL standard solution was diluted with acetone to make 20.1, 40.2 and 80.4 µg/mL standard solutions (n=1 for each concentration).

ii) Vehicle

1.0 w/v% Olive oil containing Tween 80

Tween 80 (provided by the sponsor, lot number 802H1829, KANTO CHEMICAL)

Olive oil of Japanese Pharmacopoeia (provided by the sponsor, lot number UJ-08, KOZAKAI PHARMACEUTICAL)

Tween 80, 0.10048 g, of 1.0 w/v% of final volume was mixed with olive oil of Japanese Pharmacopoeia, and brought to a volume of 10 mL to prepare vehicle.

iii) Sample solutions for specificity

The 40.2 µg/mL standard solution was used. Acetone was used as solvent blank. The standard stock solution and vehicle were mixed, and diluted with acetone to make 40.2 µg/mL vehicle-containing standard solution (containing vehicle at 5 v/v%, equivalent of dilution rate of 20, n=1). Vehicle was diluted with acetone to make vehicle blank (containing vehicle at 5 v/v%, n=1).

iv) Sample solutions for linearity

The 20.1, 40.2 and 80.4 µg/mL standard solutions were used.

v) Sample solutions for accuracy and repeatability

The standard stock solution was diluted with acetone to make 20.1, 40.2 and 80.4 µg/mL standard solutions (n=3 for each concentration).

(2) Results of validation of analytical method

Concentrations of the test article in each sample were measured once by GC.

i) Specificity

The variation of detection value (peak area) of the test article between vehicle-containing standard solution and standard solution was 0.2%, and satisfied the criteria for specificity (within ±5%). In addition, neither solvent nor vehicle blanks showed background noises nor interfering peaks at any elution peak positions of the test article.

ii) Linearity

The calibration curve was made by using the concentration of the test article on the horizontal line and the detection value on the vertical line. The regression formula (y=250.353x) which was obtained from least square analysis passed through the origin of the coordinates, and the correlation coefficient (R) was 0.999. Therefore, it was confirmed that the result satisfied the criteria for linearity (0.999 or more).

iii) Accuracy and repeatability

Accuracy and repeatability were confirmed by the test article concentrations calculated from the regression formula obtained at the linearity.

Accuracies of 20.1 µg/mL standard solution were -4.0%, -4.5% and -3.5%, and repeatability was 0.5%.

Accuracies of 40.2 µg/mL standard solution were -1.0%, -0.7% and -1.0%, and repeatability was 0.1%.

Accuracies of 80.4 µg/mL standard solution were 1.2%, 0.9% and 0.5%, and repeatability was 0.4%.

It was confirmed that the results satisfied the criteria for accuracy and repeatability (accuracy: within ±10%, repeatability: 5% or less).

2) Pre-treatment

The sample was collected from the test article formulation that was mixed well using a tube mixer, and was exactly diluted as below.

Formulation (mg/mL)	Preparation	Dilution rate
40	Formulation 0.2 mL → 20 mL/acetone (A)	1000
	(A) 1 mL → 10 mL/acetone	
5	Formulation 0.2 mL → 25 mL/acetone	125
1	Formulation 0.2 mL → 5 mL/acetone	25
0 (control)	Formulation 0.25 mL → 5 mL/acetone	20

3) Preparation of standard solution

The standard stock solution and standard solutions were prepared each measurement day. The test article was accurately weighed, and was exactly diluted as below to make standard solution.

(1) For test article formulations at first preparation

Preparation	Concentration (µg/mL)
Test article 0.10004 g → 50 mL/acetone (standard stock solution)	2000.8
(Standard stock solution) 1 mL → 10 mL/acetone (A)	200
(A) 2 mL → 10 mL/acetone	40.0

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(2) For test article formulations at final preparation

Preparation	Concentration (μg/mL)
Test article 0.10289 g → 50 mL/acetone (standard stock solution)	2057.8
(Standard stock solution) 1 mL → 10 mL/acetone (A)	206
(A) 2 mL → 10 mL/acetone	41.2

4) Analytical conditions

(1) Instruments (GC4)

Gas chromatograph	GC-2010 (SHIMADZU)
Auto injector	AOC-20i (SHIMADZU)
Auto sampler	AOC-20s (SHIMADZU)
Data processor	GCsolution (SHIMADZU)

(2) Conditions

Column	HP-5ms (F.T. 0.25 μm, 0.25 mm I.D. × 30 m, Agilent Technologies)
Column oven temperature	30℃
Injection port temperature	200°C
Carrier gas	Helium
Control mode	Constant liner velocity
Liner velocity	24.5 cm/sec
Carrier gas flow rate	1.00 mL/min
Detector	FID
Detector temperature	250°C
Injection method	Split (split ratio 20:1)
Injection volume	2 μL

b) Data processing

1) Detection value and quantitative analytical method

A peak area was used as the detection value.

In the validation of analytical method, a straight line passing through the origin of the coordinates was obtained. Therefore, the concentrations of analytical samples were determined by one-point calibration method.

2) Calculation of test article concentration

Concentrations of the test article formulation (C: mg/mL) were rounded to three significant digits.

$$C = \frac{Cs \times A \times D}{As \times 1000}$$

Cs: concentration of standard solution (μg/mL)

As: detection value of standard solution

A: detection value of analytical sample

D: dilution rate of analytical sample

3) Calculation of rate to display concentration

Rate to the display concentration (R: %) was rounded to one decimal place.

$$R = \frac{\text{Actual concentration}}{\text{Display concentration}} \times 100$$

c) Criteria for judgment

R. is within the range of 99.0% to 110.0%.

In the 0 mg/mL (control) formulation, no peak derived from the test article is detected.

15. ENVIRONMENTAL FACTORS THAT MIGHT HAVE AFFECTED RELIABILITY OF STUDY RESULTS

There were no environmental factors that might have affected reliability of the study results.

16. TEST RESULT

16.1 Concentration analysis of test article formulation at first preparation

Rate to the display concentration of the 40, 5 and 1 mg/mL formulations were 92.0%, 91.6% and 92.6%, respectively. All results satisfied the criteria (Table 1).

In the 0 mg/mL (control) formulation, no peak derived from the test article was detected.

16.2 Concentration analysis of test article formulation at final preparation

Rate to the display concentration of the 40, 5 and 1 mg/mL formulations were 93.8%, 93.6% and 92.1%, respectively. All results satisfied the criteria (Table 2).

In the 0 mg/mL (control) formulation, no peak derived from the test article was detected.

17. CONCLUSION

Concentrations of the 40, 5 and 1 mg/mL formulations prepared in the subject study at first and final preparation were within the acceptable level. In the 0 mg/mL (control) formulation, no peak derived from the test article was detected.

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TABLES

Table 1 Concentration analysis of the test article formulation at first preparation

Date of analysis	Display concentration (mg/mL)	Actual concentration (mg/mL)	R. (%)
January 5, 2017	40	36.8	92.0
	5	4.58	91.6
	1	0.926	92.6

R: rate to the display concentration

Table 2 Concentration analysis of the test article formulation at final preparation

Date of analysis	Display concentration (mg/mL)	Actual concentration (mg/mL)	R. (%)
March 2, 2017	40	37.5	93.8
	5	4.68	93.6
	1	0.921	92.1

R: rate to the display concentration

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FIGURES

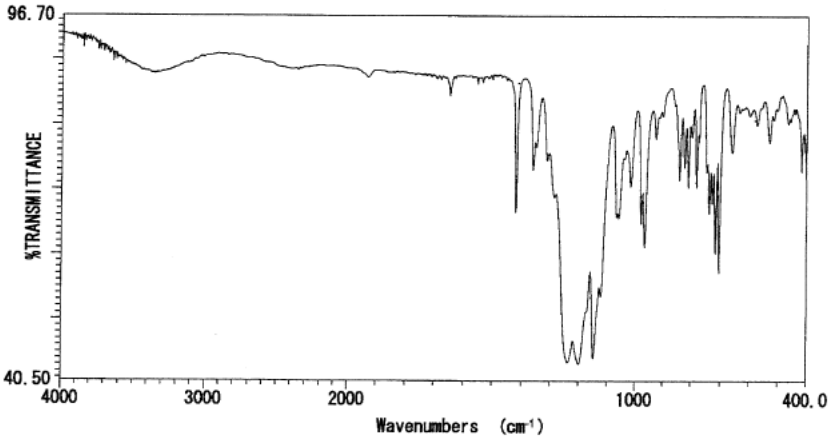

Figure 1 IR spectrum of the test article measured before in the testing facility

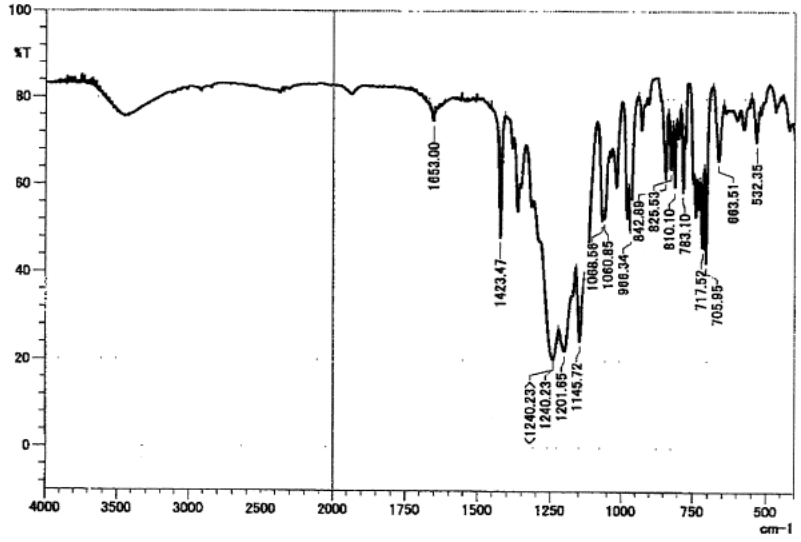

Figure 2 IR spectrum provided by the sponsor

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QUALITY ASSURANCE STATEMENT

Chemicals Evaluation and Research Institute, Japan, Hita

Sponsor: Ina Research Inc.

Title: Physicochemical Study of "Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats by Oral Administration"

Study Number: X02-0302

I assure that the final report accurately describes the test methods and procedures, and that the reported results accurately reflect the raw data of the study. The inspections of this study were carried out and the results were reported to the Study Director and the Test Facility Management by Quality Assurance Unit as follows.

Item of inspection	Date of inspection	Date of report
Study plan	December 12, 2016	December 12, 2016
Approval of study plan	December 15, 2016	December 15, 2016
Study plan amendment No. 1	January 5, 2017	January 5, 2017
Study plan amendment No. 2	January 5, 2017	January 5, 2017
Concentration analysis of test article formulation	January 5, 2017	January 5, 2017
Raw data and draft final report	March 14, 2017	March 15, 2017
Final Report	March 21, 2017	March 21, 2017

Date: March 21, 2017

Quality Assurance Manager:

Quality Assurance Statement

Study Title: Reproduction/Developmental Toxicity Screening Test of 13F-OLE in Rats by Oral Administration

Study No.: EF16149

This study was conducted in compliance with the Standard Concerning Testing Laboratories Implementing Tests for New Chemical Substances etc. (Notification No. 0331-8, Ministry of Health, Labour and Welfare, Notification No. Heisei 23.03.29-6, Ministry of Economy, Trade and Industry and Notification No. 110331010, Ministry of the Environment of Japan, Mar. 31, 2011) and in accordance with the protocol and standard operating procedures.

The final report provides accurate descriptions of the methods used in the study and the reported results accurately reflect the raw data generated during the study.

This study was inspected/audited by the Quality Assurance Unit (QAU) of Ina Research Inc. as described in the annex (Study-Based Inspections). Procedures common to many studies, including this study, are inspected periodically by the QAU to assure that the procedures are carried out appropriately (Process-Based Inspections).

Number of annexed sheets: 3

Total number of sheets: 4

August 1, 2017
Date

Quality Assurance Manager
Ina Research Inc.
2148-188 Nishiminowa, Ina-shi, Nagano 399-4501, Japan

Quality Assurance Statement (Annex)
- Study-Based Inspections -

Inspection/audit	Date of inspection/audit	Date of QA report to Study Director and Management
Protocol	Dec. 20-21, 2016	Dec. 21, 2016
Records related to animal receipt	Dec. 30, 2016	Dec. 30, 2016
Storage of the test materials	Jan. 3, 2017	Jan. 5, 2017
Shipment of dosing formulation samples	Jan. 3, 2017	Jan. 5, 2017
Computer protocol	Jan. 10, 2017	Jan. 11, 2017
Clinical observations	Jan. 10, 2017	Jan. 11, 2017
Identification of animals, cages and racks	Jan. 10, 2017	Jan. 11, 2017
Animal care	Jan. 10, 2017	Jan. 11, 2017
Body weights	Jan. 10, 2017	Jan. 11, 2017
Dosing	Jan. 10, 2017	Jan. 11, 2017
Preparation of dosing formulations	Jan. 10, 2017	Jan. 11, 2017
Mating ability	Jan. 25, 2017	Jan. 25, 2017
Organ weights	Feb. 8, 2017	Feb. 9, 2017
Gross pathology of males (F_0)	Feb. 8, 2017	Feb. 9, 2017
Blood sampling (for determination of T4 levels in males (F_0))	Feb. 8, 2017	Feb. 9, 2017
Shipment of biological samples (for determination of T4 levels)	Feb. 9, 2017	Feb. 9, 2017
Amendment(s) to the protocol-1	Feb. 9, 2017	Feb. 9, 2017
Measurements of the anogenital distance	Feb. 20, 2017	Feb. 21, 2017
Animal care (F_0, lactation period)	Feb. 20, 2017	Feb. 21, 2017
Clinical observations (F_0, lactation period)	Feb. 20, 2017	Feb. 21, 2017
Abnormalities or unforeseeable circumstances	Feb. 20, 2017	Feb. 21, 2017
Amendment(s) to the protocol-2	Feb. 22, 2017	Feb. 22, 2017
Count of the number of nipples/areolas	Mar. 1, 2017	Mar. 1, 2017
Identification of animals, cages and racks (F_1, 10 days of age)	Mar. 1, 2017	Mar. 1, 2017
Blood sampling (for determination of T4 levels in offspring (F_1))	Mar. 2, 2017	Mar. 2, 2017
Amendment(s) to the protocol-3	Apr. 10, 2017	Apr. 10, 2017
Histopathology of males (F_0)	Apr. 10, 2017	Apr. 11, 2017
Amendment(s) to the protocol-4	Apr. 27, 2017	Apr. 28, 2017
Amendment(s) to the protocol-5	May 16, 2017	May 16, 2017

Inspection/audit	Date of inspection/audit	Date of QA report to Study Director and Management
Raw data	May 16-Jun. 1, 2017	Jun. 1, 2017
Draft report (annexes)	May 16-Jun. 1, 2017	Jun. 1, 2017
Draft report (text)	May 29-Jun. 1, 2017	Jun. 1, 2017
Raw data (re-audit)	Jun. 7, 2017	Jun. 7, 2017
Raw data	Jul. 28-31, 2017	Aug. 1, 2017
Final report	Jul. 28-Aug. 1, 2017	Aug. 1, 2017

Quality Assurance Statement (Annex)
- Process-Based Inspections -

Inspection/audit	Date of inspection/audit	Date of QA report to Study Director and Management
Receipt, receiving inspection, and quarantine of animals	Oct. 11, 2016	Oct. 11, 2016
Histotechnology for light microscopy	Nov. 2-17, 2016	Nov. 17, 2016
	Feb. 17-Mar. 1, 2017	Mar. 1, 2017
Histopathology	Jan. 6, 2017	Jan. 6, 2017